University of Alberta

Supercritical and subcritical fluid technology utilization for the processing of flaxseed hull

by

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Dedications

To my family, thank you for the incredible support.

Most of all, thank you to Glaucia who gave me strength to overcome the obstacles in my path.

“Not everything that can be counted counts, and not everything that counts can be counted.”

William Bruce Cameron

Abstract

Flaxseed and Sacha inchi seed are among the highest omega-3 fatty acid sources in the world. Extraction of the oil and other value-added compounds (phenolics and carbohydrates) from the flaxseed hull using supercritical carbon dioxide (CO_2) and subcritical water technology utilizes alternative less toxic solvents than conventional methods of extraction. There are numerous studies on the phase equilibria and oil extraction of flaxseed oil. However, no studies are available on the phase behavior of Sacha inchi oil and CO_2. Also, flaxseed hull (a by-product comprising ~ 30% of the total weight of the seed) value-added components remain unexploited.

In this thesis, new data on phase equilibria for Sacha inchi seed oil in CO_2 have been measured using a variable volume cell phase equilibria system at temperatures of 303, 313 and 323 K and at pressures ranging from 4.3 to 27.7 MPa. The CO_2 mole fraction varied from 0.7488 to 0.9997. At the studied concentrations, phase transitions of vapor-liquid, liquid-liquid-vapor and liquid-liquid were observed.

Flaxseed hull oil was extracted by supercritical CO_2 at 10, 20 and 30 MPa and 313 and 333 K. A recovery of 80% w/w of the oil from the flaxseed hull was obtained at 30 MPa and 333 K within 330 min. Then, the commercial software SuperPro Designer® 6.0 was used to estimate the cost of manufacturing (COM) of flaxseed hull oil produced by supercritical CO_2 extraction process. The experimental overall extraction curve was used for

the COM prediction. The lowest COM estimated for flax hull oil extraction (US$ 10.50 kg^{-1}) was obtained for a semi-continuous supercritical CO_2 system with two $1m^3$ columns. At the condition studied, the main component of the COM was the operating labor (50.6%), followed by the capital investment (32.2%), raw material (11.2%), and utilities (5.9%). A factorial design with three factors was used to determine the best extraction conditions for removal of phenolics and carbohydartes from deffated flaxseed hulls using subcritical water. The factors were temperature (393, 423 and 453 K), pressure (1, 3 and 5 MPa) and flow rate (1, 2 and 3 mL min^{-1}) at a constant water:flaxseed hull ratio of 20:1 (v:w). Total phenolics and total carbohydrates quantifications were carried out utilizing the colorimetric Folin-Ciocalteau method with minor modifications and the phenol-sulphuric acid methodology, respectively. Recoveries of carbohydrates and phenolics utilizing subcritical water technology were successfully achieved. Furthermore, statistical analysis determined that temperature was the only significant effect on the recovery of carbohydrates and phenolic compounds from flax hull.

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List of Abbreviations

ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
CER	Constant extraction rate period
COL	Cost of operational labor
COM	Cost of manufacturing
CRM	Cost of raw material
CUT	Cost of utilities
CWT	Cost of waste treatment
DC	Diffusion-controlled rate period
EPA	Ecoisapentaenoic acid
EtOH	Ethanol
FCI	Fixed cost of investment
FER	Falling extraction rate period
GRAS	Generally recognized as safe
HPLC	High performance liquid chromatography
OEC	Overall extraction curve

PUFA	Polyunsaturated fatty acid
SFE	Supercritical fluid extraction

1. Introduction and Thesis Objectives

Flaxseed, flax or linseed is grown in approximately 50 countries. Canada is one of world's leading flax growers, with a total production of over 800 million kg per year [1]. Flax is an important source of bioactive ingredients for functional food production, since it is a rich source of soluble fibers, lignans and polyunsaturated fatty acids (PUFA), mainly omega-3 (ω-3) fatty acids, such as α-linolenic acid (ALA) [2]. ALA is an essential fatty acid and the precursor of long chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the human body. Studies on EPA and DHA in terms of their role in human health have shown promising results associated with a decrease in the risk of cardiovascular disease and protection against cancer, vision deficiencies and neurological disorders [3-4].

Flax hull is a low-value by-product. The amount of oil available in flax hull is around 25% of its weight, with approximately 45% of mono and polyunsaturated fatty acids [2]. Moreover, flax hull is a source of proteins, lignans, carbohydrates and phenolic compounds [2]. However, because of the high amount of unsaturated fatty acids, flax oil is susceptible to oxidation at high temperatures. Furthermore, conventional extraction methods for industrial oil extraction utilize organic solvents (usually hexane) [5], which extracts undesirable compounds from the hulls, such as waxes that confer turbidity and phospholipids with strong emulsifying properties, reducing the final product quality.

Supercritical fluid extraction (SFE) operates using a gas, usually carbon dioxide (CO_2), above its critical pressure (7.4 MPa) and temperature (304 K). At these conditions, CO_2 is neither a gas nor a liquid, but a supercritical fluid, which has unique gas-like diffusivity and liquid-like density. Moreover, with slight changes in temperature and pressure, it is possible to change the CO_2 selectivity for a more efficient separation. Although supercritical CO_2 extraction is a very effective method for the extraction of non-polar compounds, it has to be modified with the addition of a co-solvent, such as ethanol, for the extraction of polar compounds [6].

Subcritical water or pressurized hot water (PHW) extraction is an alternative method of extraction that uses mainly water as a solvent. At certain temperatures and pressures, its polarity can be varied close to those of some organic solvents. Therefore, PHW can improve the extraction of both polar and relatively non-polar compounds from various matrices [7], such as flax hull. Although the characterization and benefits of flax compounds have been largely discussed and studied, the viability of the utilization of its by-products, such as the hull, has not been well studied.

The cost of manufacturing (COM) estimation is important for the evaluation of a process industrial viability, especially when dealing with non-conventional technologies, such as supercritical or subcritical fluid technology. Moreover, the COM helps in studying and evaluating the use of new alternative by-products. Since Canada is the world’s largest producer of flax, innovation and research on flax and its by-products is of great interest to many areas, such as agricultural, pharmaceutical and food industries [8].

The main objective of this thesis was to obtain phase behavior data for the system Sacha inchi oil + CO_2 and on the extraction of bioactive compounds from flax hulls using supercritical and subcritical fluid technology and to estimate the COM of the extracted flax hull oil.

The specific objectives of this thesis were to:

- study the effects of pressure, temperature and CO_2 quantity on the solubility of Sacha inchi oil in CO_2, a rich source of ω-3 fatty acid (Chapter 3),
- find the best extraction parameters for flax hull oil using supercritical CO_2 extraction and evaluate its cost of manufacture (Chapter 4),
- extract carbohydrates and phenolic compounds from flax hull using PHW (Chapter 5).

The results obtained in this thesis should foster increased interest in the utilization of flax and flax by-products by industry. Moreover, the utilization and feasibility of supercritical and subcritical fluid processes, such as supercritical CO_2 extraction and subcritical water extraction are important to increase debates on the substitution of petrochemical solvent-based technologies for alternative methods of extraction, such as SFE and subcritical water. Therefore, this research could lead to an increase in the utilization of environmental friendly technologies and flax by-products for the development of new ingredients and products.

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2. Literature Review

2.1. Flaxseed

Flaxseed or linseed (*Linum usitatissimum*) is reported to have been used since 9000-8000 B.C. in the Middle Eastern countries, such as Turkey, Iran, Jordan, and Syria [1]. Nevertheless, its cultivation is indicated to have occurred around 7000-4500 B.C [1]. *Linum usitatissimum* means “very useful” in Latin. The term flaxseed is used when it is referred to human consumption while the term linseed is used when it is referred to industrial applications [2]. In 2012, the main producer of flaxseed in the world was Canada (489,000 tons), followed by China (350,000 tons), India (130,000 tons), and the United States (146,360 tons) [3].

The concern with human health has increased considerably in the last decades and consequently the search for healthy products, such as foods enriched with compounds that promote health benefits [4]. Flaxseed is an often used ingredient in healthy products and functional food. The reason why flaxseed is used in functional foods is because it contains healthy components, such as α-linolenic acid (ALA) (a type of omega-3) (ω-3) fatty acid, lignans, and fibers [1]. Although health benefits of flax protein are not well studied, some recent studies have demonstrated that its consumption has biological effects, such as anti-cholesterol, antioxidant, anti-diabetic and anti-inflammatory [5].

Flaxseed contains approximately 38-45% of oil. The main fatty acid present in the oil is ALA (50-62% of total fatty acids). However, fatty acid composition can vary depending on the cultivar, location, and oil extraction technique [1]. The other fatty acids present in flax oil are ~17% of linoleic acid (LA), ~19% oleic acid, ~3% stearic acid, and ~5% palmitic acid [6]. Essential fatty acids are those that should be acquired by the diet because humans cannot synthesize them. LA and ALA (main fatty acids present in flax oil) are essential fatty acids and are the parent omega-6 (ω-6) and omega-3 (ω-3) fatty acids, respectively. In other words, LA and ALA are converted to ω-6 long chain polyunsaturated fatty acid (arachidonic acid) and ω-3 long chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. However, in humans the conversion from ALA to DHA and EPA is low [7]. The appropriate LA:ALA ratio of consumption should be less than 4:1 [8], because these fatty acids compete for the same enzymes and transport system in the human body [9]. In general, the LA:ALA ratio in the diets of people in Western countries is approximately 20:1 due to the consumption of processed food and ready to eat meals. Because changing eating habits is very difficult, flax oil has been used as a key ingredient in ω-3 enriched foods, as its LA:ALA ratio is equal to 0.3:1. The health benefits associated with ALA consumption include the prevention of cardiovascular disease, diabetes, and obesity [6].

Another attractive component of flaxseed is secoisolariciresinol diglucoside (SDG). SDG is a type of lignan and flaxseed is the richest source of SDG among all food plants. There are some health benefits associated with SDG consumption, such as protection against cardiovascular disease, cancer, and diabetes. A minimum dose of 500 mg of SDG per day is recommended to have significant health effects. Nevertheless, SDG intake is not recommended for pregnant woman. Although SDG promotes health benefits, the mechanisms that provide such benefits in the human body are not currently well known [10]. SDG in flaxseed is mainly present in the hulls (30.9 mg/g) compared to 12.9 mg/g of the whole seed [2].

Flaxseed hulls contain not only SDG as a valuable component, but they also contain carbohydrates and phenolic compounds. The main carbohydrates are hemicellulose and cellulose, while the main phenolic compounds are ferulic, chlorogenic, gallic, and 4-hydroxibenzoic acids [11]. The hulls can also contain oil and the content (26-30% of its weight) depends on the dehulling process. Oil content in the hulls represents around 18% of the total seed oil and it is also rich in ALA [12].

Flax hulls are not only a source of high value components, but they are also a low-value by-product of flax processing. In order to extract value-added products from the hulls, it is necessary to use a dehulling process.

2.2. Sacha inchi Seed

Sacha inchi (*Plukenetia volubilis)* is an oilseed that contains ALA content very similar to flaxseed. However, interest in this oilseed started only in 1992 [13]. Sacha inchi is from the Peruvian Amazon and it grows in altitudes ranging from 200 to 1500 m. It is suggested that it was initially cultivated and consumed by the Incas and different parts of this plant, such as leaves and roasted seeds are still consumed by the Peruvian natives. Oil content varies from 35 to 60%. The oil is available in the market due to its high ALA content [14]. The seed also contains a high content of protein (24-27%) and high levels of essential amino acids (cysteine, threonine, tryptophan, and tyrosine) and essential minerals (magnesium, zinc, and calcium) [13,15].

Sacha inchi oil contains not only high levels of ALA, but also contains carotenoids (0.08 mg/ 100g) and tocopherols (3.8-6.3 mg/100 g). The presence of tocopherols makes the oil resistant to oxidation [13]. Because sacha inchi oil has been studied only in the last two decades, there are only a few studies reported in the literature. The main studies reported are on oil characterization and extraction techniques, such as cold pressing [16], Soxhlet [15,17], and supercritical carbon dioxide (SC-CO_2) [17]. Other studies of the oil include determination of thermal properties [15], toxicological effects [18], SC-CO_2 enzymatic hydrolysis [19], and development of a rapid test to detect adulteration of Sacha inchi oils [20].

2.3. Oil Extraction

Extraction is a separation process often used by the industry. The food industry utilizes extraction for the isolation of desirable components such as oils, antioxidants and flavors to be further added into functional foods. It is also used for the removal of undesirable components such as cholesterol [21].

Solid-liquid extraction is a separation process that transfers a solute from a solid matrix to a liquid solvent. The most utilized solvents in the food industry are water and organic solvents such as hexane and ethanol. A sequence of phenomena occurs during the interaction between the solute trapped in the solid matrix and the solvent. First, the solvent enters into the solid matrix. Then, the solutes are solubilized and transported outside of the solid matrix by the solvent. The solubilized solute migrates from the external surface of the solid matrix to the bulk. Finally, the solvent-solute mixture is separated from the solid matrix and the solvent is further recovered by flash distillation [21].

The choice of the solvent and to know its selectivity towards the solute is the first, and a very important decision when designing an extraction process. The solvent can be selected based on polarity, which should be similar to that of solute [21]. Nevertheless, it is important to note that some organic solvents are too toxic to be used in the food industry, such as n-hexane. Thus, substitution of toxic organic solvents by GRAS (Generally Recognized As Safe) solvents such as water, ethanol and carbon dioxide has been encouraged. As it will be explained in Section 4, SC-CO_2 is an environmentally friendly and non-toxic solvent that can be used in an extraction process for the food industry.

Method and selection of equipment to be utilized in an extraction process depends of the process objective, physical properties of the material to be extracted and the product [21]. It is important that industry optimize extraction process conditions. In other words, it should operate at the best conditions for its purpose, such as yield, quality, extraction time, and cost through appropriate techniques and methods [22].

2.4. Supercritical Fluid Technology

2.4.1. Supercritical Fluids

A gas becomes a liquid if it is compressed to a high pressure. On the other hand, “if a gas is heated beyond a specific temperature and the heated gas is compressed, it becomes a fluid with intermediate properties between a gas and a liquid”. This specific temperature is the critical temperature (T_c), while the specific pressure is the critical pressure (P_c). Critical temperature and pressure define the critical point, which is unique for each fluid. When values of pressure and temperature exceed the critical point, the fluid is referred to as a supercritical fluid [24]. This phenomenon is illustrated in Figure 2.1. In the supercritical region, the fluid can be considered as an expanded liquid or a compressed gas [23].

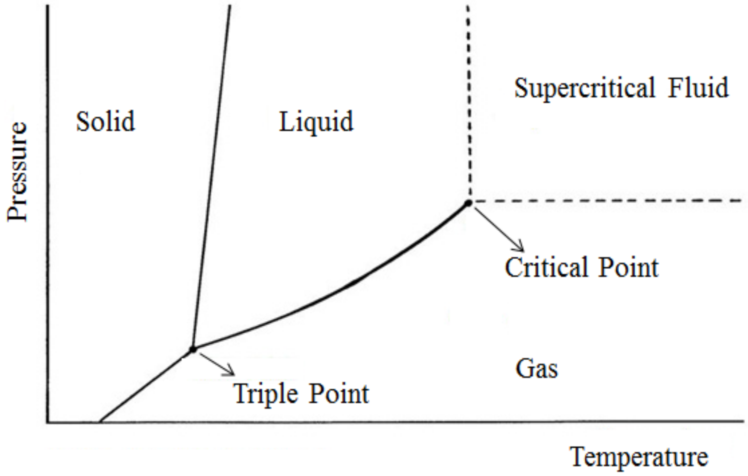

Figure 2.1 Phase diagram for a pure fluid

A fluid reaches its supercritical state above its critical pressure and temperature, as shown in Figure 2.1. Under supercritical conditions, there is only one phase with properties in between those of liquid and gas. Above the critical temperature, there is no phase transition, that is, the fluid does not become a liquid with a change in pressure. The density of supercritical fluids is similar to that of liquids while the viscosity is similar to that of gases. Diffusivity is intermediate between those of a gas and a liquid. Therefore, these properties allow good solvation power of the fluid and easier penetration and diffusion into the solid matrix. As a consequence, supercritical fluids present good properties to be used as solvents in extraction processes [24].

The solvent most often used in supercritical fluid extraction is carbon dioxide because of its mild critical properties (see Table 2.1), low cost, and high availability [24].

Table 2.1 shows critical properties of some pure fluids.

Table 2.1. Critical properties of pure fluids [25]

Fluid	**Critical Temperature (K)**	**Critical Pressure (MPa)**	**Critical Density (kg/m^3)**
Acetone	508.2	47.0	278.0
Water	647.4	22.1	322.6
Carbon Dioxide	304.2	7.4	467.6
Ethanol	513.6	6.1	276.0
Methanol	512.6	8.1	272.0
Ethylene	282.5	5.0	195.8

2.4.2. Applications of Supercritical Fluid Technology

The first supercritical fluid extraction industrial application process started in the 1930s with the deasphalting of heavy mineral oil fractions by means of dense propane in the petrochemical industry, even though the first supercritical literature report appeared in 1822. The first industrial plant (Hag AG Corporation) was installed in Germany in 1976 for decaffeination of coffee using SC-CO_2. Then, in 1982, a hops extraction plant was built in Munchester and later a tea decaffeination plant was also installed. SC-CO_2 extraction industrial plants started to be built in USA only in the year 1988 and they were also coffee decaffeination and hops extraction plants [26].

Nowadays, industrial plants are located in Europe, USA, Canada and Asia and all of them utilize SC-CO_2 as the solvent [26]. Particularly, supercritical fluid extraction used nowadays related to food processing are tea and coffee decaffeination, hops extraction, obtaining extracts from flavoring and condiments plants, extraction and fractionation of edible oils, fat removal from food, alcohol removal from wine and beer, removal of contaminants, etc [27]. Other industrial plants different from foods are removal of lipids from bones to be further used in orthopedic surgeries as grafting material, removal of oil from long quartz rods for further production of fibers for fiber optical transmission and denicotinization of tobacco [26].

Although extraction is the most commonly used application of supercritical fluids, there are other applications that have been studied and investigated. Such applications involve reactions in supercritical media, biodiesel production, impregnation, fractionation, chromatography and particle formation [28].

2.4.3. Phase equilibria

The utilization of supercritical fluids has greatly increased in the past years. Many high value products require several processing steps to achieve a high purity final product, which usually consume high amounts of energy and time. Supercritical fluids have attractive physical properties when compared to conventional solvents. The possibility of changing the solvent power by changing its density when temperature and pressure are varied is a great advantage over conventional extraction and separation methods [24].

Supercritical fluid applications in particle design have been a very attractive area of research in recent years. The supercritical encapsulation processes are based on creating a high degree of solution supersaturation that leads to a great number of nucleation sites and very small crystals. Several components participate during the encapsulation process, the encapsulating material, the solute to be encapsulated, the solvent and a co-solvent. In this context, the equilibrium between phases, solid-liquid, liquid-liquid (LL), and vapor-liquid (VL) is important information needed to design a process.

A multicomponent fluid mixture can have heterogeneous phases (VL or LL) or a homogeneous phase at supercritical conditions. The pressure-temperature diagram is useful to show the bubble and dew point phase transition curves and the mixture critical point. The region under the curve is heterogeneous, while a single phase is present above the maximum pressure at any temperature. Therefore, information on the properties and behaviors of pure components and their mixture is very important for design, simulation and optimization of chemical processes.

2.5.3. SC-CO_2 extraction

Supercritical fluid extraction can be considered as a potential technology for extraction of desirable compounds, such as carotenoids, essential oils, caffeine and others from vegetable matrices [29].

Supercritical fluid extraction can be defined as a unit operation by contact, where the fundamentals are based on equilibrium and physico-chemical properties of supercritical fluids, such as high solvation power, high diffusion coefficient, low surface tension and low viscosity [24].

Supercritical fluid extraction is advantageous if the supercritical fluid has low temperature, low vaporization enthalpy, high volatility and it is also inert, non-toxic and non-flammable. Furthermore, CO_2 is the most used solvent in supercritical fluid extraction [30]. CO_2 has limited capacity to dissolve polar molecules. In some cases, it is necessary to have co-solvent addition. Co-solvent has the objective to modify solvent characteristics like polarity. Thus, the use of co-solvent results in extracts with high content of polar compounds. However, when a co-solvent is used, it is necessary that pressure and temperature ensures that the binary mixture (co-solvent+supercritical fluid) is in the supercritical state [29, 31]. Nevertheless, co-solvents used in supercritical fluid extraction should be GRAS to guarantee the advantages of supercritical fluid extraction. Some examples of co-solvents are ethanol, isopropanol, and water [32]. Water presents some advantages compared to other organic solvents. This is because it has lower cost, less environmental issues, and it is easier to use. However, the vegetable matrix should be very dry, as there are strong interactions between water and cellulosic materials [29].

One of the most important characteristics of supercritical fluid extraction is that small variations in pressure and temperature alter solvent selectivity. Thus, considering phase equilibrium between the solvent under supercritical conditions and the components of the solid matrix, the extracts have different compositions depending of temperature and pressure conditions [29].

A general schematic of SC-CO_2 extraction is shown in Figure 2.2 and the steps are described: Extractor (E-1) is filled with the vegetable matrix (leaves, seeds, flowers, or hulls). The extractor (E-1) is then heated to the desired temperature condition. Afterwards, the solvent is transported from the reservoir (Sol-1) to the extractor (E-1) through a compressor or a pump in order to reach the extraction pressure. Then, the solvent is heated to the extraction temperature. When process conditions are reached, the pressure reducing valve (V-3) and micro metering valve (V-4) are opened to allow the flux of the solvent flow through the bed. Consequently, there is extraction of the extract. V-3 and V-4 should be heated in order to avoid Joule-Thompsom effect. T-1 and T-2 are thermocouples that monitor extractor and reducing pressure valve temperatures, respectively. Extract + CO_2 mixture separation occurs in the separator (S-1). Because there is pressure and temperature reduction in the separator (S-1), the extract precipitates in the separator (S-1) while the free solvent is recirculated through the system, with previous stages of cooling (R-2) and compression (B-2). Sometimes the solvent contains traces of residual extract [24].

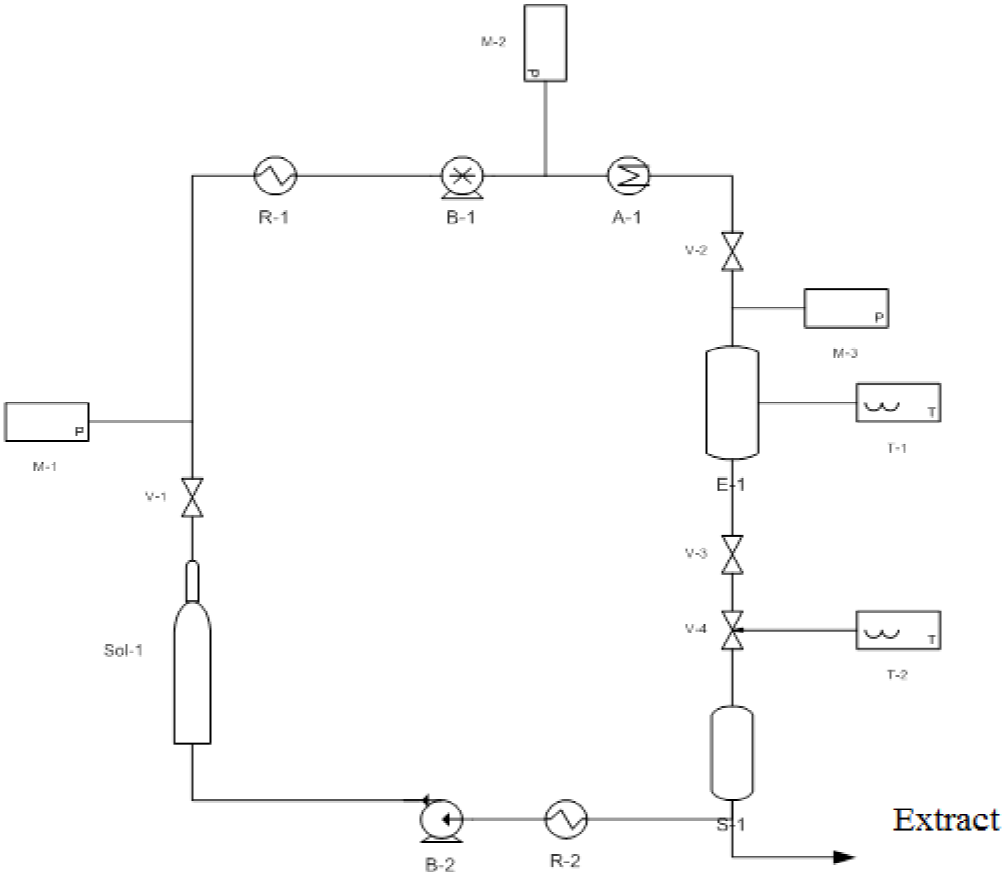

Figure 2.2. General schematic of supercritical fluid extraction, where Sol-1 is the CO_2 tank reservoir, V-1 is the reservoir valve, M-1 and M-3 are pressure gauges, R-1 and R-2 are coolers, B-1 is a pump, A-1 is an electrical heater, V-2, V-3 and V-4 are valves,T-1 and T-2 are the electrical heaters, E-1 is the extraction vessel, S-1 is the separator and B-2 is a compressor.

According to Quispe-Condori [30], supercritical fluid extraction presents advantages and disadvantages compared to other extraction techniques. The main advantages are highlighted as follows:

- Because CO_2 has mild critical temperature, the use of mild temperatures in the process results in extracts close to the initial characteristics, preserving thermal labile compounds
- Higher selectivity with small variations in pressure and temperature that allows extraction of different compound classes
- The low surface tension of supercritical CO_2 allows quick penetration of the solvent into the pores of the vegetable matrix which increases extraction efficiency
- Supercritical CO_2 has higher diffusion coefficient and lower viscosity than liquids
- CO_2 is easily separated from the product through pressure reduction

Some disadvantages are:

- Different vegetable matrices have unique structure with different chemical composition and physiological structure, therefore, it behaves differently at the same process conditions, thus, there is not a universal mathematical model to represent all matrices
- Common scale-up methods are not easily applicable for industrial process
- Extracts may have different characteristics such as color, odor and chemical composition when compared to those obtained

by traditional extraction methods, which can influence consumer preferences

- High initial capital investment cost due to high pressure operation

According to Pereira and Meireles [4], the first step in a supercritical fluid extraction is the optimization of pressure and temperature conditions to maximize the yield of compounds of interest. This is because the condition that presents the highest yield is not necessarily the same as that for the highest amount of the target compound. Thus, the determination of the global yield isotherms is the first step for supercritical fluid extraction of new vegetable matrices.

Global yield (Xo) is the quantity of soluble material that can be extracted from a vegetable matrix at a specific pressure and temperature. It is expressed as the mass of soluble material divided by the mass of solid substrate [29]. Figure 2.3 presents an example of global yields isotherms for a hypothetical vegetable matrix + CO_2 system.

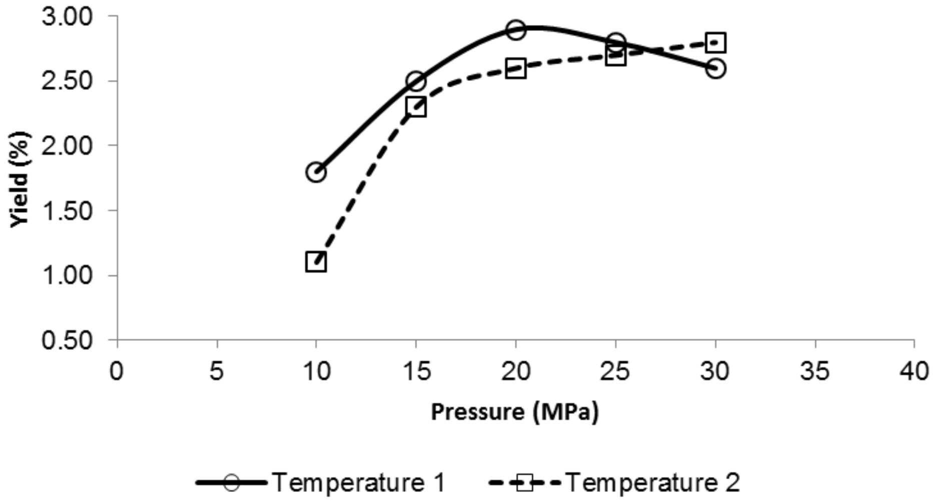

Figure 2.3. Global yields isotherms for a hypothetical SFE for a vegetable matrix + carbon dioxide system

Chemical composition and biological activity analyses along with the determination of global yield determine the best pressure and temperature conditions to obtain a high quality extract.

The next step after global yield determination is overall extraction curve (OEC) determination [4]. OEC represents the mass or yield of extract obtained as a function of extraction time [4]. Figure 2.4 shows an example of a typical OEC.

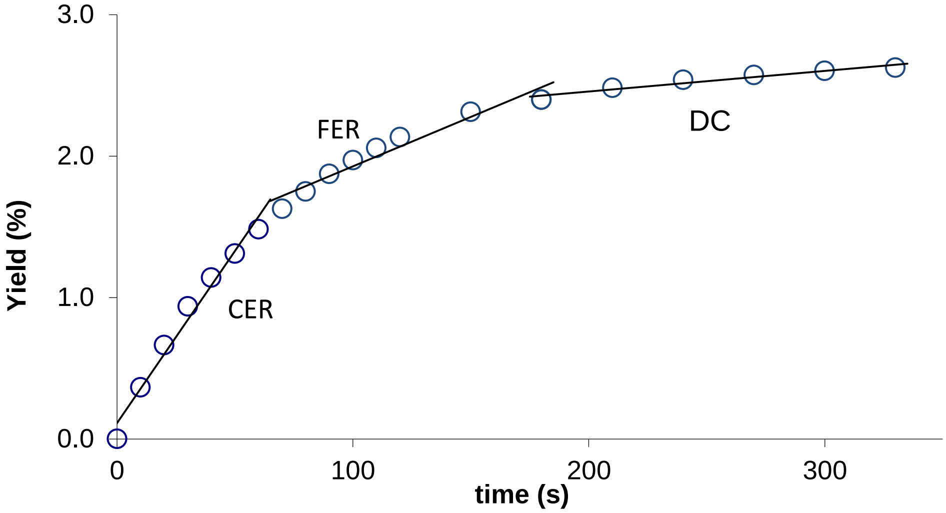

Figure 2.4. OEC of SFE process for an hypothetical system vegetable matrix + CO_2 where CER is the constant extraction rate, FER is the falling extraction rate and DC is the diffusion-controlled rate.

Constant extraction rate (CER) period: in this period, the most easily accessible solute from the solid matrix is extracted. The extraction of the solute takes place primarily governed by the convection phenomena, which leads the extraction to occur at a constant rate [4].

Falling extraction rate (FER) period: in this period, most of the surface solute has already been extracted and the solute trapped inside the particles start to be transferred by diffusion from inside to outside of the particles. This phenomena occurs because the vegetable cell walls were not broken uniformly during the grinding process, consequently, it slows down the extraction rate, although convection still occurs during this period [4].

Diffusion-controlled rate (DC) period: in this period, all superficial solute have been extracted, remaining only the solute trapped inside the cell walls. Therefore, diffusion is the only phenomena governing the extraction, which leads to a slow extraction rate [4].

When most of the extract (50-90%) is obtained in the CER period, the extraction should end just after this period. However, if the desirable compound is not extracted during CER period due to the low solubility in the solvent or because it is located inside the cell structures, the extraction ends later. Therefore, some important parameters to be determined are duration of the CER period (t_{CER}), duration of the FER period (t_{FER}), the yield of the CER period (R_{FER}), the mass transfer rate during the CER period (M_{CER}), and the mass ratio of the solute in the solvent at the extractor outlet (Y_{CER}) [4].

According to Meireles [29], natural extracts obtained by SFE have increased the interest of food and fine chemical industries because they are similar to the raw material. Thus, extracts obtained through SFE technology is recommended for the production of high value-added food components [33].

Supercritical CO_2 has been utilized to study the extraction of flax seed oil [21] and Sacha inchi seed oil [17]. Total flax seed oil recovery at 323 K and 30 MPa using SC-CO_2 (35%) was found to be higher than screw pressing (26%), but lower than Soxhlet (39%). Sacha inchi oil highest yield at 333 K and 40 MPa (50%) was also lower than the oil recovered using Soxhlet (54%). Soxhlet oil recovery is higher than using SC-CO_2 extraction because Soxhlet also extracts more polar compounds, such as waxes and phospholipids. Moreover, both studies showed that at higher pressures, the extraction time is reduced while the yield increased.

2.5. Subcritical water technology

Even though the polarity of SC-CO_2 can be modified through the addition of co-solvents, the extraction and solubilization of more polar compounds is still limited. Thus, subcritical water has received considerable attention in order to deal with more polar compounds.

Subcritical water (SCW) extraction is also known as hot pressurized water extraction, high temperature water extraction, superheated water extraction and hot liquid water extraction. SCW is defined as water at a temperature above its boiling point at a pressure high enough to keep it liquid and below its critical point [34].

SCW physical properties are temperature dependent and weakly pressure dependent. Dielectric constant is one of the most important properties that changes with temperature. Dielectric constant of water decreases with an increase in temperature. For example, the dielectric constant of water at room temperature is 80 while its value at 423 K is 27 [35]. As polarity of water decreases, its dielectric constant also decreases. Polarity is decreased because of thermal agitation as an increase in temperature weakens hydrogen bonds. As a consequence, SCW can extract non-polar organic compounds and can be a substitute for SC-CO_2 [36].

An increase in temperature also affects viscosity. Viscosity decreases with an increase in temperature. The decrease in viscosity increases mass-transfer, which is beneficial in mass-transfer limited reactions [35].

Although temperature increase provides beneficial changes to some properties of water, it also provides processing problems. Some examples are corrosion and thermal gradients. One of the problems related to corrosion is that reactor materials (stainless steel, or metal alloys) can release metal ions. Released metal ions can be soluble in SCW and act as homogenous catalysts during processing [35]. The heating rate is high in SCW processes. This is because within the temperatures conditions the reactions are carried out (usually 423-573 K). Therefore, thermal gradient results in higher temperature in the reactor wall, which leads to possible side-reactions. If the raw material is biomass, the side-reactions can result in carbonaceous material [35].

2.5.1. Conversion of biomass using SCW technology

The main application of SCW technology is conversion of biomass. Biomass is any residue generated during agricultural and food processing. Some examples include bagasse, oilseed and cereal meals, brewer waste, corn milling byproducts, and crop residues such as hulls, straw, stalks, stems, shells, seeds, etc. The main use of some byproducts is in animal feed. However, it has been found that most biomass contains value-added components. Thus, technologies to extract or convert these valuable components have been studied in the last decades. SCW is one of the technologies that have been investigated to convert biomass into more valuable products [34].

The main components of biomass are carbohydrates (75-80%) and lignins (20-25%) [34]. The main carbohydrates are cellulose, hemicellulose and starch. These carbohydrates are polysaccharides and the objective to treat biomass with SCW is hydrolyzing these polysaccharides into monosaccharides such as glucose, fructose, xylose, arabinose, galactose, etc. Monossacharides obtained can be further processed into liquid fuels through fermentation process or used as ingredients in food products, pharmaceuticals, cosmetics, etc [34]. Some examples of hydrolysis of biomass using SCW are black rice bran [36], rice bran [37], and flaxseed meal [38]. In all cases, SCW showed high efficiency in the hydrolysis of polysaccharides into monosaccharides. The hydrolysis of hemicellulose and cellulose into monosaccharides is possible by adding water to release individual sugar chains contained in these structures [36,38].

Lignin is a polyphenolic material composed of aromatic and C_3 chains. Lignin composition, molecular weight and amount differ between the different plants. Lignin decomposes under SCW conditions even though the mechanism is not known. The purpose of lignin hydrolysis is to obtain valuable phenolic compounds that can be further used as antioxidants in food products [34]. Examples of matrices where phenolic compounds were extracted from biomass are rice bran [37], potato peel [11], and pomegranate seed residue [39]. The rice bran study demonstrated that total phenolic content increases with an increase in temperature. The major phenolic compounds were protocatechuic and vanillic acids [37]. Phenolic compounds extraction increased with the increase of temperature and the major phenolic compound extracted was gallic acid [11]. Antioxidant activity and phenolic extraction from pomegranate seed residue also increased with the increase of temperature [39]. Carbohydrates have been successfully recovered using SCW from rice bran [37], flaxseed meal [38] and potato peels [11].

Some biomass, such as flaxseed hulls, contains oil. As it was discussed before, SCW can extract non-polar organic compounds by changing the dielectric constant of water. Extraction of non-polar organic compounds using SCW as a solvent was reviewed by Carr *et al*. [40].

Proteins are also present in biomass material and they can also be extracted using SCW. Some matrices examples include flaxseed meal [38], soy meal [41] and deoiled rice bran [41]. In all cases, proteins were extracted by SCW.

2.6. Cost of Manufacturing

SFE technology has shown some advantages compared to conventional extraction processes, such as the use of non-toxic solvent, etc. However, the best extraction process depends on each raw material. After determination of global yield and OEC, it is necessary to evaluate the economic feasibility of the process for each vegetable matrix [4].

Determination of cost of manufacturing (COM) for SFE process was introduced by Rosa and Meireles [42]. Their methodology is based on Turton’s methodology [43]. In this method, COM is a combination of three main costs: direct cost, fixed costs, and general expenses. Briefly, direct costs represent those costs associated with production such as raw material and labor. Fixed costs are not production rate dependent and should be considered even if the production is interrupted, for example taxes and depreciation. General expenses are those which keep the business running such as research and development, etc [4].

According to the *Association for Advancement of Cost Engineering International*, cost estimation is based on five distinguished classes.

Class 5 estimation is based on limited information. Class 5 estimation is prepared in a limited period of time and low effort is required. The level of definition for this class is 0 to 2% of the project cost. Class 4 is also based on limited information and they are used to determine availability, concept, and preliminary approval of the project. The level of definition required for class 4 is 2 to 5% of the total project. Class 3 is performed to obtain the base for quotation authorization. It should contain process design, layout, complete engineering process, and complete list of equipment. The level of definition required for class 3 is 10 to 40% of the total project. Class 2 estimation contains detailed information and should include process design, utilities design, mass and energy balances, tubing and equipment design, sales quotation, etc. For a class 2 project, 30 to 60% of the project definition is required. Class 1 is the closest of the total project definition. Class 1 is performed with as much detail as possible. The level of definition required for class 1 is 50 to 100% of the total project [44].

The methodology proposed by Rosa and Meireles [45] for SFE is within class 4 to 5. In order to obtain estimations close to the real project, a software was developed. SuperPro Designer is a commercial software used for cost simulation and is class 2 to 3 [45].

COM estimation of SFE for different vegetable matrices using SuperPro Designer has been reported. Some examples are mango leaves [46], grape seeds [47], and cashew leaves [48].

The first initial COM studies showed that investment costs were the predominant cost of SFE. Nevertheless, recent studies have demonstrated that the cost of raw material is the largest fraction of the COM (27 to 94%). Moreover, COM of extracts obtained by SFE technology was lower than for those obtained by traditional extraction process, such as steam distillation. This result demonstrates that SFE is indeed a competitive technology to obtain natural extracts [4].

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3. Phase Equilibrium Mesurements of Sacha Inchi Oil (*Plukenetia volubilis*) and CO_2 at High Pressures[1]

3.1. Introduction

Use of polyunsaturated fatty acids (PUFA) as a functional food ingredient and its consumption in dietary supplements have been experiencing a significant increase [1]. PUFA have been associated with a variety of health benefits such as decreased risk of cardiovascular disease and prevention of complications of lipotoxicity [2]. Fish and some vegetable oils such as flax are the common sources of PUFA [3]. Sacha inchi (*Plukenetia volubilis*), a Peruvian tree has been studied as an alternative source of PUFA due to its high oil content (35–60%) [4]. Sacha inchi is a millenarian legacy of the Inca civilization that has been cultivated to date by the native communities in the Peruvian Amazon. Sacha inchi seed oil is an excellent source of PUFA, composed mainly of linolenic (C18:3 ω-3) and linoleic (C18:2 ω-6) acids [4]. However, since this oil contains a high amount of PUFA, it is affected by low stability and rapid oxidation, which involves the formation of toxic products such as peroxides or undesirable off-flavor compounds [5].

Commercially, unrefined sacha inchi seed oil is produced by cold pressing and organic solvent extraction. Although cold pressing preserves its original composition on PUFA content, the yield is low

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(38.4%) compared to Soxhlet extraction (54.3%) [4]. However, during Soxhlet extraction, high temperatures are utilized. The effect of the utilization of high temperatures on unsaturated bonds present in the vegetable oils is to reduce the quantity of PUFA in the oil due to oxidation by high temperature exposure [5,6]. Supercritical fluid (SCF) extraction is an alternative process for the extraction of vegetable oils [7]. This extraction method has the advantage of having a high solvation power, low viscosity, and high diffusion coefficient offered by the supercritical fluid. Furthermore, carbon dioxide supercritical phase is achieved at low-mild temperatures (304.25 K), which preserves PUFA from oxidation during the extraction process.

Many vegetable oil phase equilibrium behaviors have been studied. For example, soybean or castor oil + supercritical CO_2 and fish oil + supercritical CO_2 have been reported earlier by Ndiaye et al. [8] and Riha and Brunner [9], respectively. These studies showed the pressure transitions and correlation of their data with equations of state (EoS). Some thermodynamic models are able to correlate and predict the phase behavior of oil systems in CO_2. These models are generally based on: (i) an equation of state approach like Peng-Robinson equation of state (PR-EoS), (ii) a density-based approach, or (iii) a solubility parameter approach [10-11].

Sacha inchi seed oil extraction has been reported using supercritical CO_2 at temperatures ranging from 313 to 333 K at pressures of 30 and 40 MPa [6]. However, phase equilibria of sacha inchi seed oil at high pressures have not been reported. Knowledge of phase equilibria at high pressures is crucial in any supercritical fluid process design such as SCF extraction, reaction and particle formation [12]. Therefore, the objective of this study was to measure the phase equilibria for sacha inchi seed oil in carbon dioxide at high pressures.

3.2. Materials and Methods

Cold pressed and unrefined Sacha inchi seed oil was obtained from Industrias Amazonicas (Lima, Peru). Sodium methoxide solutions of 0.5 M in methanol, methyl acetate and methyl heptadecanoate were purchased from Fluka Sigma Aldrich Co. (St. Louis, MO, USA). Hexane was acquired from Fisher Scientific (Ottawa, ON, Canada). Carbon dioxide with a purity of 99.98 mol% was supplied by White Martins (Maringa, PR, Brazil).

3.2.1. Fatty Acid Composition

The fatty acid (FA) composition of Sacha inchi seed oil was determined by using a modified fatty acid methyl ester (FAME) method.

Sacha inchi oil dissolved in hexane (100 µL) was transmethylated to FAME by heating at 323 K for 15 min with a mixture of 0.5 M sodium methoxide (80 µL) and methyl acetate (40 µL). In order to quantify sacha inchi FAME composition, methyl heptadecanoate was used as an internal standard at the concentration of 1 mg/mL as this compound was not present in the original sacha inchi seed oil. One milliliter of the standard solution was added to the mixture. To separate the FAME, 2 mL of water and 2 mL of hexane were added to the mixture and the solution was centrifuged (GLC-2B Sorvall Dupont Instruments, Wilmington, NC, USA) at 1237×g for 1 min. The supernatant was separated and 1 µL of the sample was injected to the gas chromatograph.

The FAME were analyzed using a gas chromatograph with a flame ionization detector (GC-FID) (Varian Model 3400, Agilent Technologies, Mississauga, ON, Canada) equipped with a fused silica capillary column (60 m × 0.32 mm, DB-5, J&W Scientific, Folsom, CA, USA). The carrier gas was helium. The temperatures of the injector and detector were 508 and 523 K, respectively. The column was heated to 343 K and held for 0.2 min, programmed to increase at a rate of 35 K/min to 483 K, then at a rate of 2 K/min to 508 K and held for 8.3 min.

3.2.2. Phase Equilibrium Apparatus and Procedure

Phase equilibrium experiments (cloud point) for the system Sacha inchi seed oil + CO_2 were carried out using the static synthetic method in a high pressure variable-volume view cell. The experimental apparatus and procedure have been well described in previous studies [12–14]. The experimental unit consists of a 25 mL variable-volume view cell with two sapphire windows for visual observation, an absolute pressure transducer (Smar, model LD 301, Sertãozinho, SP, Brazil) with an uncertainty of ±0.03 MPa, a portable pressure data acquisition system (Smar, model HT 201, Sertãozinho, SP, Brazil), and a syringe pump (ISCO, model 260D, Lincoln, NE, USA). The equilibrium cell contains a movable piston, which allows control of the pressure inside the cell. Phase transitions were identified visually through the manipulation system pressure using the syringe pump. Phase transitions were visually recorded as dew points. First, a known amount (depending on the desirable global composition) of sacha inchi seed oil was loaded into the equilibrium cell with a syringe weighed on a precision scale balance (Marte, model AM220, Santa Rita do Sapucaí, MG, Brazil) with an uncertainty of ±0.001. The cell was then flushed with low pressure CO_2 to remove any residual air. The amount of CO_2 remaining in the equilibrium cell is negligible ($< 10^{-5}$ mol) when compared to the total amount of CO_2 used to determine the experimental data. A known amount of CO_2 was loaded using the syringe pump (with an uncertainty of ±0.005 g). Then, the mixture inside the cell was continuously agitated with a Teflon-coated magnetic stirring bar. After achieving the desired temperature, the pressure was increased until visualization of one-phase in the cell. The system was kept at this point for at least 30 min to allow stabilization. Then, the pressure was slowly decreased at a rate of 0.1–0.3 MPa/min until a new phase was observed. The experiments were carried out at 303, 313 and 323 K. All experiments were conducted in triplicate.

3.3. Results and Discussion

3.3.1. Fatty Acid Composition

The fatty acid composition of Sacha inchi seed oil reported in Table 3.1 shows that this oil has a high level of unsaturated fatty acids (93%). Linolenic acid (C18:3, ω-3) was the major fatty acid, followed by linoleic (C18:2, ω-6), oleic (C18:1, ω-9), palmitic (C16:0) and stearic (C18:0) acids. The fatty acid composition of Sacha inchi seed oil found in this study is in agreement with the technical data provided by Industrias Amazonicas and data previously reported [4,6]. The fatty acid composition of Sacha inchi seed oil is similar to that found in flax seed oil [15]. The differences between these two oils are in the ω-6:ω-3 ratio. The World Health Organization recommends a ω-6:ω-3 ratio of 5:1 to 10:1, while a ratio between 1:1 and 4:1 is often considered as optimal [16]. The ω-6:ω-3 ratio for Sacha inchi seed oil is 0.75:1, a recommended value compared to the low ratios found in fish oils (cod liver, 0.04:1; salmon, 0.03:1; and sardine, 0.07:1), and high ratios of some seed oils (soya, 7:1; olive, 11:1; and sunflower 632:1) [3]. Due to this ratio, Sacha inchi seed oil could be used for human nutrition. Interestingly, fish oil contains important unsaturated fatty acids (eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids), which are not present in Sacha inchi oil. But, Sacha inchi seed oil contains linolenic acid, which can be converted to EPA and DHA by human metabolism [17]. Furthermore, Sacha inchi seed oil does not have an unpleasant taste, typical of fish oil.

Table 3.1. Fatty acid composition of Sacha inchi oil

Fatty acid	% Area				FA content (mg/g oil)	%w/w
	This Study	Technical data[a]	Follegatti-Romero *et al.*[6]	Hamaker *et al.* [4]		
Palmitic acid (C16:0)	4.08	3.65	4.24	4.50	38.95	3.90
Stearic acid (C18:0)	2.96	2.54	2.50	3.20	28.21	2.82
Oleic acid (C18:1 ω-9)	10.45	8.28	8.41	9.60	99.82	9.98
Linoleic acid (C18:2 ω-6)	35.34	36.80	34.08	36.80	337.36	33.74
Linolenic acid (C18:3 ω-3)	46.92	48.61	50.41	45.20	447.87	44.79
Gadoleic acid	0.25	–	0.16	–	2.34	0.23
Total	**100.00**	**99.88**	**99.8**	**99.30**	**954.55**	**95.46**
Saturated	7.04	6.19	6.74	7.70	67.16	6.72
Monounsaturated	10.71	8.28	8.57	9.60	102.16	10.21
Polyunsaturated	82.26	85.41	84.49	82.00	785.23	78.53

[a] Industrias Amazonicas, n.d. not determined, %w/w weight/weight (g FA/g oil × 100%)

3.4. High Pressure Phase Equilibria

Table 3.2 shows the experimental data measured for the CO_2 + Sacha inchi seed oil system for each temperature, T, pressure, P, and mole fraction of CO_2, x_{CO_2}. This table also reports phase transitions experimentally observed, where the CO_2 mole fraction varied from 0.7488 to 0.9997. With an increment of both CO_2 mole fraction and pressure, phase transitions were observed for each isotherm. For example, at 313 and 323 K, phase transitions from liquid-vapor (LV) to liquid-liquid (LL) were observed when the CO_2 mole fraction was increased from 0.8691 to 0.8948. Further increase in pressure and CO_2 mole fraction result in a CO_2 rich liquid phase (LL) for the three isotherms studied.

Table 3.2. High-pressure phase transitions (P.T.) and equilibria data for CO_2 + Sacha inchi oil

xCO_2	T (K)					
	303		313		323	
	P (MPa)	P.T.	P (MPa)	P.T.	P (MPa)	P.T.
0.7488	4.286	LV	5.061	LV	5.836	LV
0.7876	5.161	LV	6.161	LV	7.161	LV
0.8322	6.181	LV	7.471	LV	8.911	LV
0.8691	7.051	LV	9.199	LV	11.546	LV
0.8948	11.651	LLV	13.111	LL	15.521	LL
0.9145	22.560	LL	23.231	LL	24.531	LL
0.9994	27.720	LL	-	LL	-	LL
0.9994	24.740	LL	-	LL	-	LL
0.9995	24.291	LL	-	LL	-	LL
0.9996	24.180	LL	25.540	LL	27.110	LL
0.9996	22.230	LL	25.290	LL	27.390	LL
0.9997	20.860	LL	22.810	LL	24.881	LL

Figure 3.1 shows that at $x_{CO_2} = 0.8948$, liquid-liquid-vapor (LLV) is first observed in one experimental point at 303 K. At a constant pressure, increasing the CO_2 mole fraction, it was observed phase

transitions from liquid to LV or from liquid to LL. The LL phase is rich in CO_2, while the liquid phase is rich in oil. Figures 3.1 and 3.2 show dashed lines for visualization purpose of the phase transition limits observed experimentally.

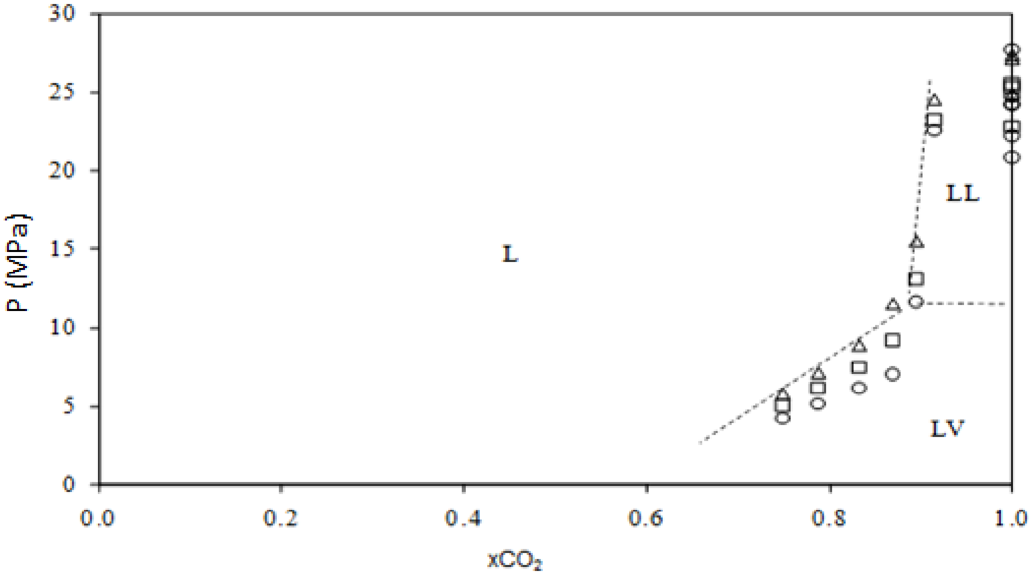

Figure 3.1. Phase diagram for the binary system supercritical CO_2 + Sacha inchi oil at 303 K (open circles), 313 K (open squares), and 323 K (triangles). Dashed lines indicate phase transition limits observed experimentally for Sacha inchi oil.

Figure 3.2 shows that the phase behavior of supercritical CO_2 + Sacha inchi seed oil is similar to that of supercritical CO_2 + soybean oil [8]. However, it can be observed that CO_2 is more soluble in soybean oil than in Sacha inchi seed oil at the same temperature and pressures below 12 MPa. Even though the molar masses of both oils are similar, soybean oil [8] contains more than 175 and 125% of palmitic and oleic acids, respectively than sacha inchi oil. The fatty acids, linolenic and stearic acids showed previously high absorption of CO_2 [18]. Therefore, the high solubility of CO_2 in the soybean oil can be due to the difference in fatty acid composition and its interactions with CO_2. Figure 3.2 also shows that the transition pressure values for Sacha inchi seed oil are higher than those obtained for soybean oil [8].

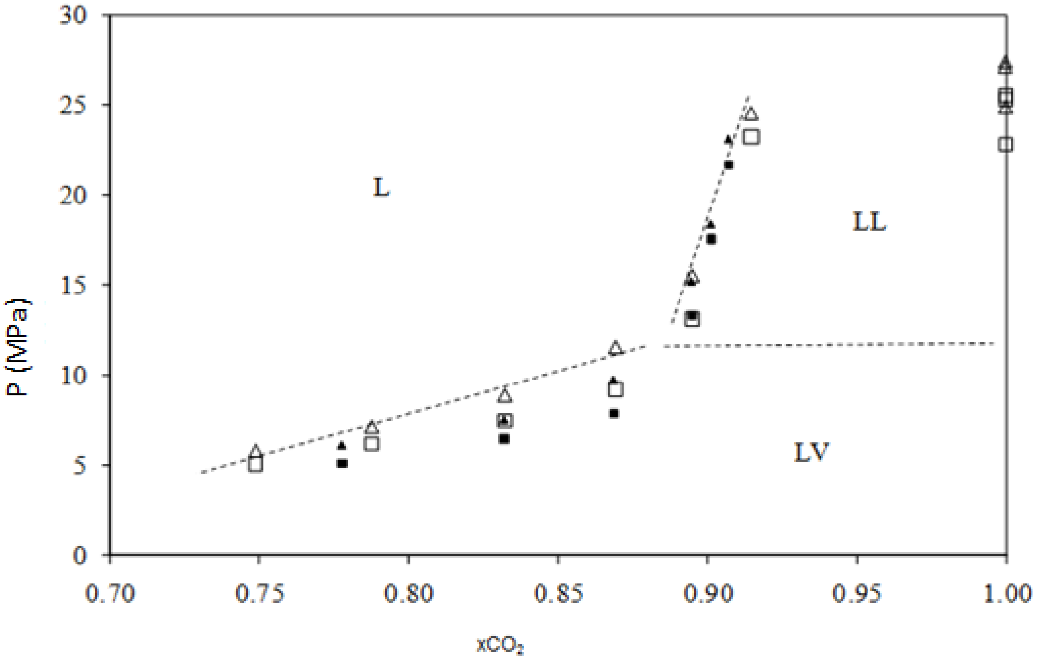

Figure 3.2. Phase diagram for binary systems of supercritical CO_2 + Oil. Sacha inchi seed oil at 313 K (open squares) and 323 K (triangles). Soybean oil at 313 K (filled squares) and 323 K (filled triangles) (data from Ndiaye et al. [8]). Dashed lines indicate phase transition limits observed experimentally

Figure 3.3 shows the pressure-temperature phase diagram at various mole fractions of soybean oil or Sacha inchi seed oil in supercritical carbon dioxide. Both systems exhibit a similar behavior at temperatures of 303, 313 and 323 K and at x_{CO2} = 0.9145 (for Sacha inchi seed oil) and at x_{CO2} = 0.9071 (for soybean oil).

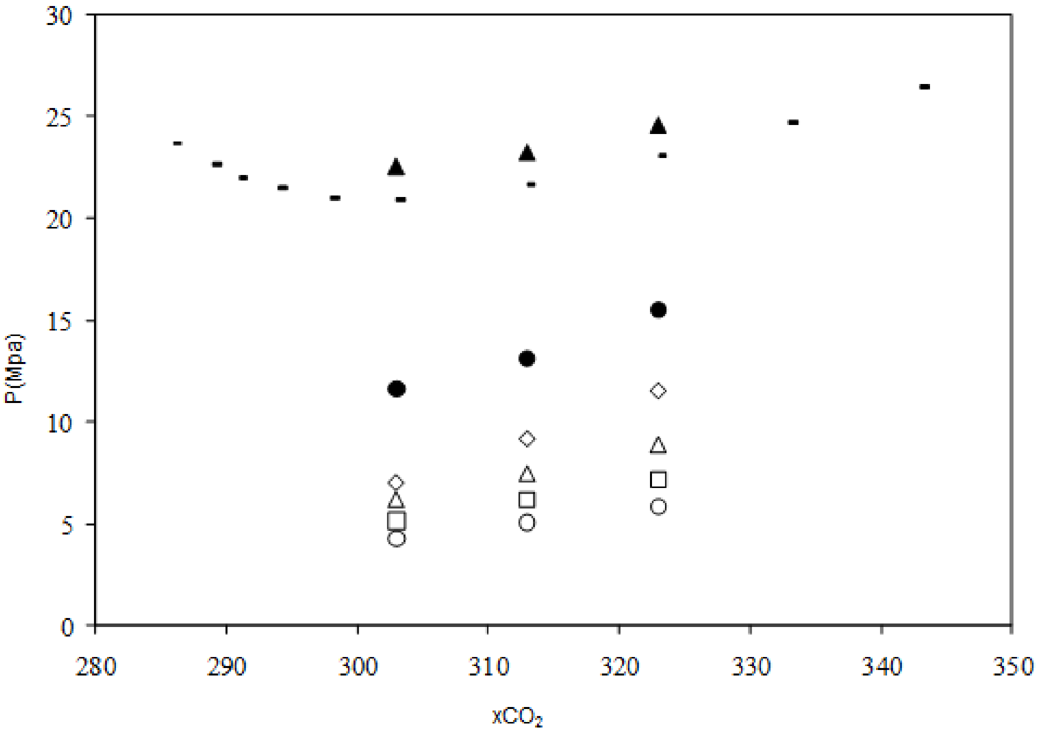

Figure 3.3. Pressure versus mol fraction diagram for CO_2 (1) + oil (2). Sacha inchi seed oil at x_1 = 0.7488 (open circles), x_1 = 0.7876 (open squares), x_1 = 0.8322 (open triangles), x_1 = 0.8691 (open diamonds), x_1 = 0.8948 (filled circles), x_1 = 0.9145 (filled triangles). Soybean oil at x_1 = 0.9071 (thick line) (data from Ndiaye et al. [8])

3.5. Conclusions

This study provided new experimental data on phase behavior of Sacha inchi seed oil in carbon dioxide at 303, 313 and 323 K, and pressures from 4 to 28 MPa. Sacha inchi oil has high contents of ω-3 fatty acid (46.92%) and PUFA (82.26%). The system CO_2 + Sacha inchi seed oil showed LV, LL and LLV phase transitions. Furthermore, CO_2 was more soluble in soybean oil than in Sacha inchi seed oil due to its different fatty acid composition and its interactions with carbon dioxide.

3.6. References

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4. Economical feasibility study for flaxseed hulls oil using supercritical CO_2

4.1. Introduction

Flaxseed (*Linum usitatissimum L.*), also known as linseed, is one of the oldest arable crops, having been cultivated in ancient Egypt. It has been consumed as a cereal for its medicinal qualities, while its oil was utilized as edible oil for food preparation and lamp oil [1].

Flaxseed grows in approximately 50 countries. Canada is the world’s leading grower of flaxseed with a total production of 861.1 million kg/year, followed by China (475 million kg/year) and Ethiopia (169.9 million kg/year) [2].

Furthermore, flaxseed has been emerging as an important source of bioactive ingredients for functional food production. It is one of the most important sources for functional products, since it is one of the richest sources of α-linolenic fatty acid (ALA), soluble fibers and lignan [3].

Studies showed numerous health benefits to humans of flaxseed oil consumption for cardiovascular diseases prevention [4,5], rheumatoid, arthritis and ulcerative colitis [6].

Flax hulls are a low valued by-product obtained after flaxseed dehulling process. It represents 22-36% of the total flaxseed weight, depending on the dehulling technology utilized [3]. Flaxseed hulls contain approximately 25% of protein [7] and 15-30% of oil (around 18% of the oil content in the seeds) with around 45% of mono and polyunsaturated fatty acids (PUFA), especially ω-3 fatty acids [8].

Extraction methodologies for oil removal are mostly based on mechanical pressing and organic solvent (usually hexane) processes. Organic solvent for lipid recovery is the single step for oil removal from hulls and second step of the oilseed processing performed after mechanical pressing. Despite the fact that mechanical pressing offers a relatively low initial operational cost and a product free from solvent contamination, its efficiency is quite low (<70%). An extraction process with a second step using organic solvent increases the recovery to approximately 99% of the initial lipid content [9]. However, utilizing flash distillation for solvent removal creates an additional step for the process and increases energy and time required for product recovery. Moreover, the organic solvent also extracts undesirable compounds from the hulls such as waxes and phospholipids, reducing the final product quality.

Supercritical fluid extraction (SFE) technology is an alternative technique to conventional methods of oil extraction. SFE technology utilizes typically CO_2 above its critical pressure and temperature with unique properties. As the solvent utilized is CO_2, it is completely removed from the product at the end of the process simply by decreasing the pressure of the system until ambient pressure [10]. The advantages of SFE technique are the use of a non-flammable, non-toxic and inexpensive solvent, preservation of thermolabile compounds as it operates at just above 304 K (critical temperature of CO_2). The main disadvantage of the SFE process is its initial investment for high pressure industrial equipment.

The cost of manufacturing (COM) estimation is important for project evaluation and its industrial feasibility. SuperPro Design 6.0® is an industrial process simulation software which utilizes the Turton based method [11] for the economical evaluation of industrial processes. The objective of this study was to evaluate the economic viability of flaxseed hull oil extraction utilizing supercritical CO_2.

4.2. Materials and Methods

4.2.1. Materials

Flaxseed hulls were kindly provided by the University of Alberta cereal crop laboratory (Edmonton, AB, Canada). Carbon dioxide bone dry (99.8% purity) and nitrogen (99.95% purity) were purchased from Praxair Canada Inc. (Mississauga, ON, Canada). Hexane with purity of 95% was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sodium methoxide 0.5M, methyl acetate and methyl heptadecanoate (99% of purity) were purchased from Sigma-Aldrich (St Louis, MO, USA).

4.2.2. Sample Preparation

Flaxseed hulls were ground (Model 197S, Emerson Industrial Controls, Grand Island, NY, USA) and sieved (W.S. Tyler Company of

Canada Ltd., St. Catharines, ON) into a final average particle size of 0.254 mm. Then, ground and sieved flax hulls were packed and stored at 255 K.

4.2.3. Proximate composition analysis

The moisture contents of flax hulls samples were determined by drying it in an oven (Model 5851, National Appliance Co., Portland, OR) at 398 K for 8 h. Ash content was determined according to AOAC Official Method 942.05 [12] using a muffle furnace (Model F-A1730, Thermolyne Corp., Dubuque, IA) set at 698 K overnight. Nitrogen content was determined using a nitrogen analyzer (Model fp-428, Leco Instruments Ltd., Mississauga, ON), which was then converted to protein content using a conversion factor of 6.25 [13]. Crude lipid content was determined using a Goldfisch extraction unit (Labconco, Kansas City, MI, USA) with petroleum ether extraction for 6 h. Carbohydrate content was calculated based on 100% minus the sum of moisture, ash, protein and lipid contents. Each proximate analysis determination was carried out in triplicate.

4.2.4. Supercritical CO_2 Extraction

A supercritical fluid extraction system from Supercritical Fluid Technologies Inc. (Newark, DE, USA) equipped with a 125 mL stainless steel vessel, shown in Figure 4.1 was used in this study. CO_2 from a gas cylinder at 5.5 MPa was fed to a CO_2 pump. The extraction temperature was achieved by heating both, the heat exchanger and vessel with an electrical device, and kept constant by a temperature controller. The extraction pressure was controlled by the pump, while the CO_2 flow rate was manually adjusted by opening or closing a needle-metering valve, which was heated and maintained at 353 K.

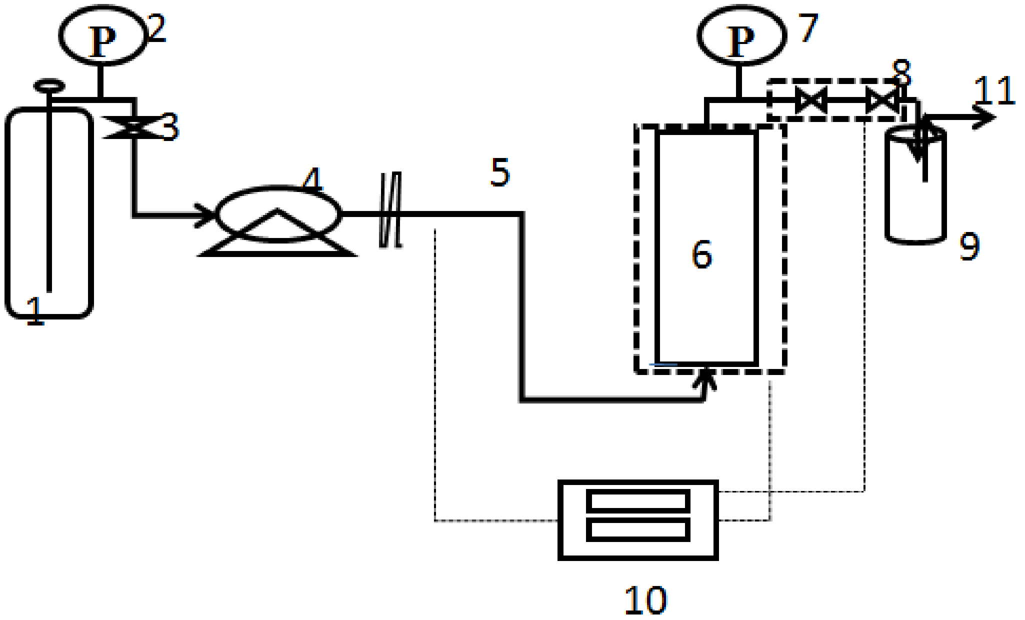

Figure 4.1. Supercritical fluid extraction equipment flow chart, where 1 is the CO_2 cylinder, 2 and 7 are pressure gauges, 3 is a valve, 4 is a high pressure pump, 5 is the line heater, 6 is the extraction vessel, 8 is a system containing a heated macro and micro-metering valves, 9 is the glass flask where the samples are collected, 10 is a temperature controller, and 11 is the CO_2 exhaust tubing.

The effect of pressure and temperature for flax hulls oil recovery was determined at three pressures (10, 20 and 30 MPa) and two temperatures (313 and 333 K). Flax hulls were weighed (≈ 5 g) and manually packed inside the extraction column. Furthermore, glass beads (diameter ≈ 3.0 mm) were utilized to fill the empty extraction column space, while a thin layer of glass wool was placed on both inlet and outlet sides of the extraction vessel to keep small particles away from the vessel filter, avoiding its clogging. The system was heated and pressurized to the experimental condition. Then, a static period of 15 minutes was used to allow the system supercritical CO_2+flax hulls oil to reach the equilibrium. Subsequently, CO_2 was pumped to the system at a flow rate of 3.15×10^{-5} $kg.s^{-1}$ for 120 min. Lastly, flax oil was recovered, weighed, flushed with nitrogen for air removal and stored at 269 K for further analysis.

Extraction kinetic curves were built at the best conditions of pressure and temperature obtained on the global yield determination experiments. Extractions were performed for 480 min at a CO_2 flow rate of 6.7×10^{-5} kg s^{-1}. Samples were collected each 15 min for the first 2 h of extraction, every 30 min from the second hour until the fourth hour of extraction and finally, each 60 min, when the weight of the extract recovered was within the balance error (0.0010 ± 0.0001g). Data reproducibility was verified by performing experiments in duplicate. Samples recovered were weighed, flushed with nitrogen for air removal and stored at 269 K for further analysis.

4.2.5. Fatty acid composition

The fatty acid (FA) composition of flax oil was determined using a modified fatty acid methyl esters (FAME) method described in Chapter 3, Section 3.2.1.

4.2.6. Economic Evaluation

The economic analysis of the SFE process was calculated in order to determine the process feasibility for flax hull oil recovery. The cost of manufacture (COM) of flaxseed hull oil was calculated utilizing a model developed by using the software SuperPro Designer®, which allows the mass and energy balance calculation for the entire process. Furthermore, it was utilized to generate economic reports, such as system mass and energy balances, depreciation and direct and indirect costs.

The COM is determined by the sum of the fixed capital investment (F_{CI}) and direct cost. This direct cost is composed of the cost of raw material (C_{RM}), operating labor (C_{OL}), utilities (C_{UT}), and waste treatments (C_{WT}), and it is directly dependent on the production rate. Expenses such as territorial taxes, insurance and depreciation are reported as fixed costs.

The process developed for COM estimation is shown in Figure 4.2. It consists of two extractors projected to simulate a semi-continuous operating system.

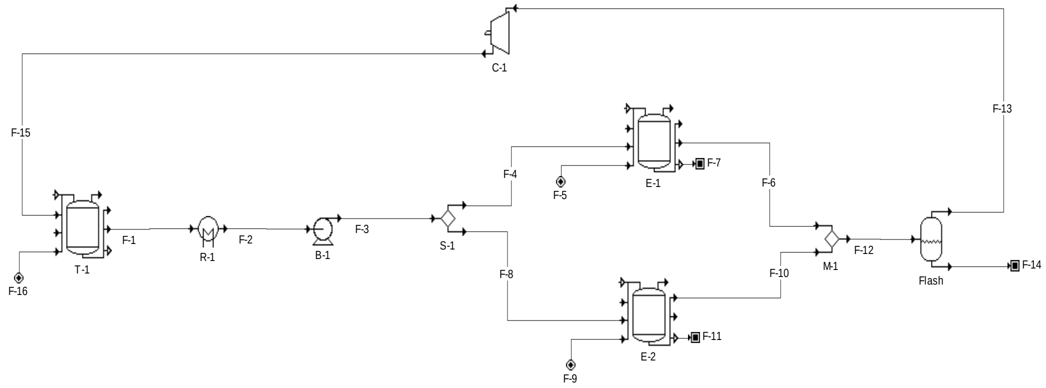

Figure 4.2. SFE model for a semi-continuous process, whereT-1 is CO_2 tank, R-1 is the electrical heater, B-1 is a high pressure CO_2 pump, S-1 is a stream splitter, E-1 and E-2 are the extractors, M-1 is the stream mixer, Flash is the recovery system and C-1 is a CO_2 compressor. F-16 is the CO_2 supply, F-8 and F-9 are the raw material inlets, F-7 and F-11 are the extraction by-products and F-14 is the flaxseed hulls oil stream.

The proposed process was designed to simulate a factory running the SFE equipment 24 h per day over 330 days per year, with a total of 7920 h of operation annually. The flaxseed hulls price was US\$ 200 ton^{-1} [2]. This price corresponds to the value received by the producer without any other treatment. The pre-processing cost (milling and sieving) estimated using the SuperPro Designer® software was US\$ 30.00 per metric ton of raw material. The CO_2 price considered in this study was US\$ 0.10 kg^{-1}, with a loss of 2% in each batch [15]. The flash tank pressure of operation was 4 MPa.

The COM for the SFE scale up of flax hull oil was performed for a pilot scale equipment (0.005 m^3) to a semi-industrial and industrial scale equipment with columns of internal volumes of 0.1 and 1 m^3, respectively.

According to Prado *et al.* [16] when scaling up a SFE process, the extraction yield remains constant once the solvent:feed material (S/F) ratio is fixed. Feed material and solvent quantities utilized were calculated for each equipment capacity. Moreover, the number of employees needed for the system operation was considered as: (*i*) 1 for the equipment with volume capacity of 0.005 m^3, (*ii*) 2 for 0.1 m^3 and (iii) 3 for the equipment equipped with vessels of 1 m^3 of internal capacity. The number of labor force used is in accordance with the estimates of Perrut [17]. The labor cost was estimated at US$ 25.00/h [18].

4.3. Results and Discussion

4.3.1. Proximate composition

The proximate composition of flax seed hulls is shown in Table 1. The values of ash, carbohydrates, lipids, protein and moisture were in agreement with previous studies [19-20].

Table 4.1. Proximate composition of flaxseed hulls[a]

Compound	**Proximate (%, w:w)**
Ash	3.03±0.12
Carbohydrates[b]	34.75±0.45
Lipid	26.00±0.33
Moisture	12.13±0.22
Protein	24.09±0.30

[a] Mean ±S.D.; [b] Sugars + Fibers

4.3.2. Supercritical Fluid Extraction

The global yield isotherms of flaxseed hulls oil are shown in Figure 4.3. For both temperatures studied (313 and 333 K), there is an increase in oil yield when the pressure increases. For low pressures, the effect of temperature is predominant in the amount of oil recovered. At 20 MPa, the two isotherms cross each other, indicating a change in the extraction behavior and the effect of pressure becomes more important for flax hull oil recovery. For pressures higher than 22 MPa, there is a positive influence of temperature on the solubility and extraction of the flax hull oil, indicating that the solubility of flax hull oil increases at higher supercritical CO_2 densities. For pressures lower than 22 MPa, the solvent density considerably decreases with an increase of temperature, leading to a lower solvation power. The best condition found for flaxseed hulls oil recovery via supercritical CO_2 extraction was 333 K and 30 MPa with a total yield of 17.56% on dry basis. The effect of pressure and temperature on the recovery of flaxseed hull oil was found to be similar to previous studies utilizing supercritical CO_2 to extract other sources rich in ω-3 fatty acids. The amount of oil recovered increased with pressure for extraction of canola flakes [20], canola seeds [21] and Sacha inchi seeds [24], while with an increase in temperature, the yield increased at high pressures but decreased at pressures lower than 25 MPa.

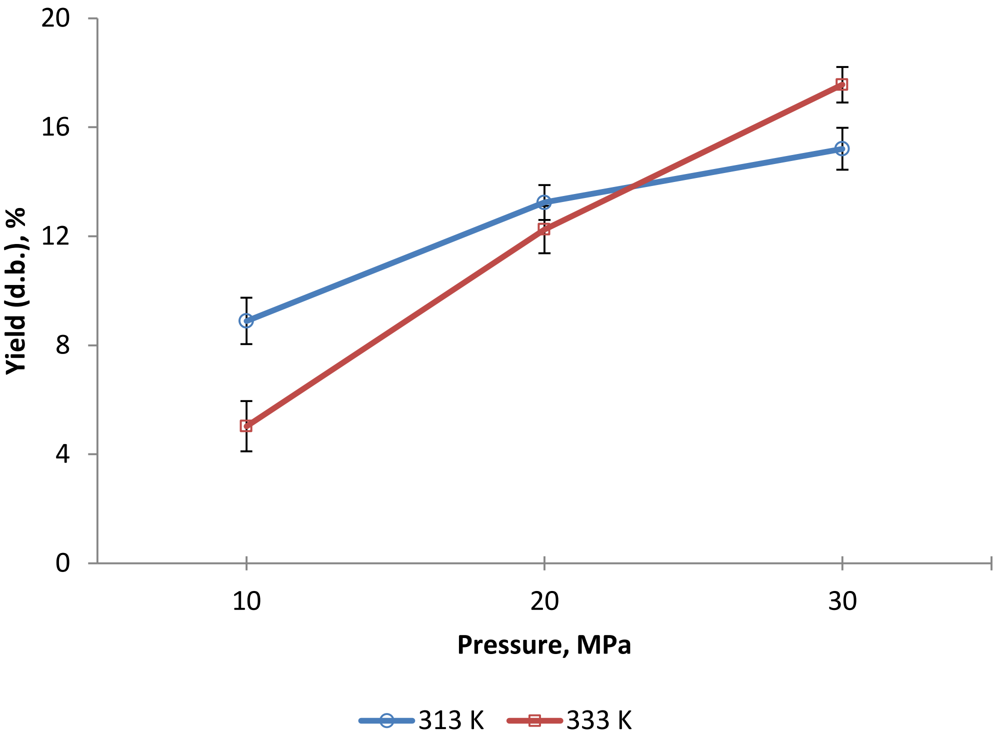

Figure 4.3 Global yield isotherms and error bars for the system flax hulls + CO_2 at 313 and 323 K and 10, 20 and 30 MPa (performed in duplicate)

According to the data obtained from the global yield experiments and previous studies [25-26], a lower extraction time would be possible with an increase in the extraction pressure. Lower extraction time would be possible because at higher pressures, supercritical CO_2 has higher density, therefore, the solubility power towards flax oil increases [26]. However, due to equipment limitations, the utilization of pressures higher than 30 MPa to test this hypothesis was not possible.

The SFE curve is not a linear function of time. A typical SFE extraction curve can be divided into three main regions [10]: (i) Constant extraction rate (CER) period, in which the main mass transfer mechanism is convection in the fluid phase; (ii) falling extraction rate (FER) period, where diffusion starts to increase its importance to mass transfer from matrix solute particles; and (iii) diffusional (DF) period, where the mass transfer is limited by the diffusion. In general, 50 to 90% of the total solute is obtained during the CER period. Therefore, process optimizations utilizing supercritical CO_2 as a solvent should be performed on this region.

The best extraction conditions found for the global yield isotherm experiments (333 K and 30 MPa) were utilized to build the overall extraction curve (OEC) of flaxseed hull oil as shown in Figure 4.4. The dehulling process followed by the grinding allowed the oil contained in the flaxseed hull to settle mostly on the surface. During the dehulling process, some cells containing flax oil are left attached to the hulls. But, after grinding, the cells are opened and the oil is exposed. Therefore, the CER period of extraction, which is governed by convection, shows to be predominant on the OEC, with a total time of 330 min. The maximum flaxseed hull oil yield extracted was 20.78% on dry basis after 480 min. Similar result (20.4%) was reported by Oomah & Sitter [8]. At the end of the CER period (330 min), the total amount of oil recovered was 91% of the total oil recovered by SC-CO_2 technology and 80% of the total oil present in the flax hull.

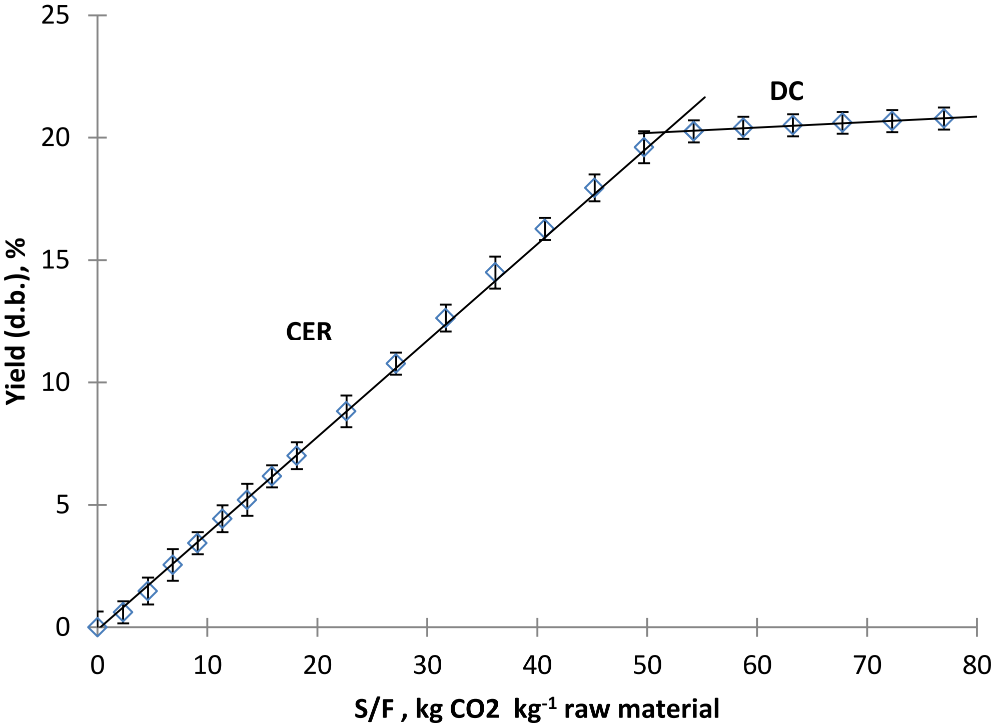

Figure 4.4. Overall extraction curve for the system flax hulls and CO_2 at 30MPa/333K/6.7×10^{-5} kg CO_2 s^{-1} for 480 minutes (S is solvent and F is the feed material)

The lower yield of oil recovered via SC-CO_2 extraction (21%) when compared with Soxhlet technology (26%) is due to the higher extraction of polar lipids with *n*-hexane. Sahoo *et al.* [17] reported that for oil extraction, usually hexane extracts contain a higher percentage of glycolipids and phospholipids in comparison to other processes, while SC-CO_2 extraction technology is ineffective for the extraction of such polar lipids because of the polarity of the CO_2.

4.3.3. Fatty acid composition

Table 4.2 presents the fatty acid composition of extracts obtained by SC-CO_2 global yield and kinetics experiments and by Soxhlet using ηhexane analyzed by gas chromatography. Ten fatty acids were identified in the extracts recovered, mainly linolenic (54%), linoleic (20%) and oleic (15%) acids. The results indicate that flaxseed hull oil and flaxseed oil have similar fatty acid profiles [21], thus, flax hulls are an important source of ω-3 fatty acids. In addition, Soxhlet and SFE processes showed similar fatty acid profiles and the composition did not alter significantly by changing pressure and temperature when using the SFE method (Table 4.2). Therefore, flaxseed hulls could be utilized as a source of ω-3 fatty acids for the food, nutraceutical and pharmaceutical industries in development of new products such as functional foods and food supplement.

Table 4.2. Fatty acid profile of flaxseed hull oil

Methodology	**Fatty acids (g/100g)***								
	16:0	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**	**20:0**	**22:0**	**22:6**
Soxhlet	6.97	0.10	1.87	20.17	14.70	52.19	0.12	0.07	0.14
SFE									
Global yield									
313 K, 10 MPa	6.33	0.09	1.92	20.22	14.91	53.33	0.09	0.07	0.08
20 MPa	6.45	0.09	1.82	20.15	14.83	53.89	0.09	0.08	0.07
30 MPa	6.44	0.10	1.90	20.21	14.75	53.78	0.11	0.11	0.08
333 K, 10 MPa	6.41	0.07	1.79	20.31	14.79	53.78	0.10	0.08	0.10
20 MPa	6.52	0.09	1.93	20.19	14.90	53.25	0.10	0.11	0.07
30 MPa	6.47	0.11	1.89	20.29	14.85	53.99	0.11	0.11	0.07
Kinetics	6.51	0.11	1.77	20.21	14.88	53.75	0.09	0.10	0.09

*Percentage of fatty acid present is the average of three analyses

4.3.4. Economic Evaluation

COM estimation was performed for flax hull oil recovery utilizing SFE experimental results obtained in this study. Usually, the time corresponding to half of the falling extraction rate period (FER) (FER/2) in a three step extraction curve and the end of CER period in a two-step OEC is responsible for the highest amount of extract recovery (≈80%) with lower COM. Therefore, the time to reach the end of CER period (330 min) was utilized for the COM calculations. Moreover, the S/F ratio used was 50, as it represents the S/F ratio for 330 min of extraction. The mass of flax hull particles loaded into the extraction columns were calculated based on the hull bulk density (333 $kg.m^{-3}$) and the columns internal volumes (0.005-1m^3).

Figure 4.5 shows the influence of equipment column internal volume on the manufacturing cost of flaxseed hull oil. The cost of manufacture decreases as the equipment column volume increases. The lower COM calculated was US\$ 10.50 kg^{-1} of oil produced for 1 m^3 column equipment.

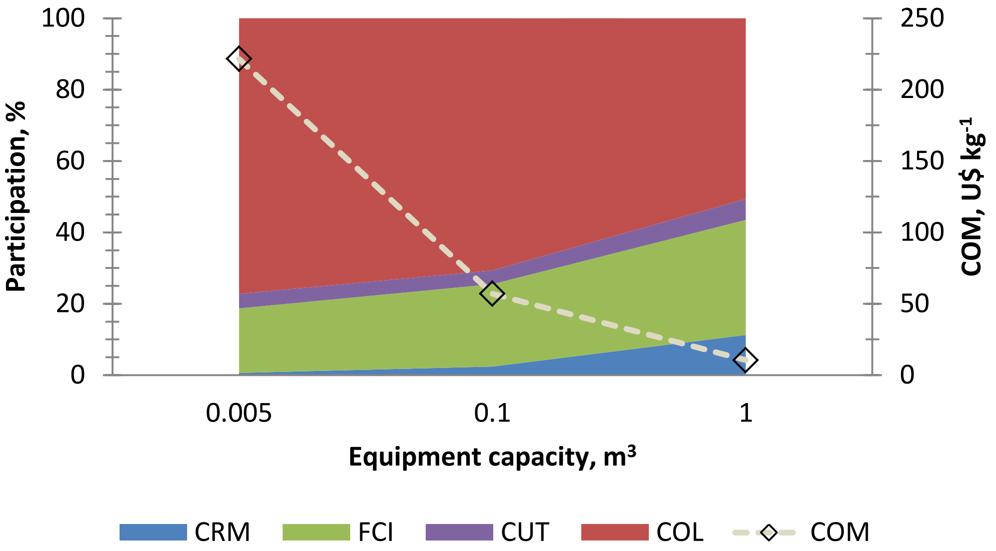

Figure 4.5. Cost of manufacture of flaxseed hull oil with supercritical CO_2 extraction for a semi-continuous apparatus with internal volumes of 0.005, 0.1 and 1 m^3. CRM is the cost of raw material, FCI is the fixed capital investment, CUT is the cost of utilities, COL is the labor cost and COM is the cost of manufacture

The C_{OL} has the highest participation for the flax hull COM, ranging from 77% for the 0.005 m^3 equipment to 51% for the 1 m^3 column internal volume. The C_{OL} high participation on the final extract cost can be explained by the high specialized labor force needed to run a SFE process industrial plant (US$ 20.00 – 30.00 per hour). In order to decrease the supercritical oil product COM, investments in countries with a lower C_{OL} could be a good strategy. Rosa and Meireles [15] estimated an operational labor cost of US$ 3.00 per hour in South American countries. The C_{OL} had a participation of less than 10% in the final COM of clove bud oil. Therefore, the production of supercritical extracts in these countries could be more viable when compared with countries with costly operating labor.

On the other hand, C_{RM} has a low participation on the flaxseed hulls oil COM at pilot scale and semi-industrial scale equipment, representing 1% for the apparatus equipped with an extraction column of 0.005 m^3 and 2.41% when the column internal volume was 0.1 m^3. For semi-industrial equipment, C_{UT} has the lower participation (4.25%) on the COM. The lowest participation of C_{RM} in the COM composition can be explained by the high extraction time and the flax hulls low cost. Once the extraction time needed to recover flax hull oil is high, the number of extractions performed per year is low, consequently, the amount of raw material utilized and its cost is low.

The F_{CI} participation on the COM increases with the equipment scale-up from 18.1% for 0.005 m^3 columns to 32.2% for columns having 1 m^3 of internal volume. The high participation of F_{CI} on the flax hulls oil COM can be explained by the fact that the direct costs related to raw material are low and the extraction time and initial investment needed for an industrial supercritical fluid plant are high.

The lowest COM for flax hull oil recovered by supercritical CO_2 technology was US$ 10.50 kg^{-1}. Although the flax hulls oil COM can be considered high, since the commercial flax oil price is around US$ 1,100 per ton [27], however, hulls by-product can also be utilized for the recovery of other major compounds such as mucilage, widely used in food applications as gum due to its water-holding capacity [28,29], proteins, used as emulsifiers [30], and minor compounds with high aggregated value such as lignans (linked to the reduction of breast cancer) [31-32]. This study was not focused on the recovery of these other valuable compounds because our major focus was supercritical CO_2 technology, which is ineffective for the recovery of the compounds mentioned. However, an industrial plant working on the recovery of these compounds could transform the proposed process feasibility.

4.4. Conclusions

Flaxseed hull oil was successfully recovered by supercritical CO_2 technology. The ideal extraction time found for the best extraction condition (30 MPa and 333 K) was 330 min with a recovery of 80% (w:w) of the oil from the flax hulls and 91% of the total oil extracted via supercritical CO_2. Moreover, the solubility of flax hull oil in supercritical CO_2 increased with the increase of the CO_2 density, consequently, the extraction time and the COM would also decrease at pressures above 30 MPa.

The methodology utilized to estimate the manufacturing cost of flaxseed hull oil by SFE technology showed that the extraction of flaxseed hull oil cannotbe economically feasible at the extraction condition considered since its COM value is higher than the bulk oil selling price. The lowest estimated flax hull oil cost of manufacture (US$ 10.50 kg^{-1} of flax hull oil) was obtained from a semi-continuous SFE equipment with two $1m^3$ columns. At the condition studied, the main component of the COM was the operating labor (50.6%), followed by the capital investment (32.2%), raw material (11.2%), and utilities (5.9%). Since defatted flaxseed hull is richer in lignans (a high value compound related to breast cancer control), mucilage and proteins important for the food industry, the commercialization of the by-products and the installation of the factory in regions with low cost operating labor and the increase on the process pressure could make this process feasible. Moreover, the amount, availability, process recovery cost and commercialization price of these compounds should be verified in order to consider the installation of a flaxseed hulls multi-compound recovery operation.

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5. Extraction of Total Phenolics and Carbohydrates from Flaxseed Hull by Subcritical Water

5.1. Introduction

Flaxseed (*Linum usitatissimum*) is one of the major Canadian crops, grown mainly for industrial applications. Flaxseed contains approximately 40% of oil, especially polyunsaturated fatty acids (PUFA), in particular alpha-linolenic acid (ALA), while its hull is a source of phenolics, carbohydrates and proteins [1].

Flaxseed is one of the leading sources of ALA (55-60%), an omega-3 (ω-3) fatty acid, which is essential for maintaining human health [1-5]. Although flaxseed is important for edible oil production, it is also considered as a source of phenolic compounds that are mostly present in the flaxseed hull. Phenolic compounds are natural antioxidants, which are believed to have protective effects against degenerative diseases in humans, such as cardiovascular diseases, cancer and diabetes mellitus [6-8].

While flaxseed oil composition and properties have been well studied, information on flaxseed hull composition is scarce. Flaxseed hulls are a low value product obtained from seed dehulling, accounting for roughly 30% of the total weight of the seed. Therefore, the recovery of oil, carbohydrates and total phenolics present in flaxseed hulls could contribute to the utilization of this by-product.

Total phenolics and carbohydrates extractions are mostly performed utilizing the conventional solid-liquid methodology [9-11]. Organic solvents, particularly ethanol and acetone have been used to extract phenolics, while proteins and carbohydrates have been extracted with NaCl solutions and water respectively [9-11]. The main advantages of this conventional solid-liquid extraction process are that it is a very well-known technology and it is simple. However, it is time consuming and requires large quantities of organic solvents [12]. Since the utilization of such organic solvents could be a potential hazard for human health, search for alternative technologies for the extraction of phenolics and polysaccharides from agricultural matrices has intensified.

An alternative extraction methodology for the recovery of carbohydrates and phenolics is the utilization of subcritical water or pressurized hot water (PHW). With this technology, water is used to replace organic solvents and can have its polarity adjusted by manipulating its temperature and pressure. As temperatures utilized are high, the pressure must be high enough to prevent water from vaporizing. Increasing the temperature from 293 to 523 K causes a similar change in solvent polarity, surface tension and viscosity as those achieved by mixing methanol or acetronitrile with water [13]. Therefore, PHW can improve the extraction of both polar and relatively non-polar compounds from agricultural matrices. Cacace and Mazza [14] and Ho et al. [15] showed the viability of the utilization of PHW for the extraction of lignans, carbohydrates and proteins from whole flaxseed and flax meal. However, there are no studies on the recovery of carbohydrates and total phenolics from flaxseed hulls. Therefore, the main objective of this study was to extract carbohydrates and phenolics from flaxseed hulls using PHW. Another objective was to compare the results obtained with PHW with those of the solid-liquid extraction method.

5.2. Materials and Methods

5.2.1. Materials

Flaxseed hulls were obtained from the farmland on the South Campus of University of Alberta (Edmonton, AB, Canada). The hulls were milled and passed through a 1.0 mm screen. Defatted flaxseed hull with supercritical CO_2 was obtained as explained in Chapter 4, sealed and stored in a freezer at 253 K. Folin-Ciocalteu (FC) reagent, sulfuric acid (99%), anhydrous sodium carbonate, petroleum ether, glucose (≥99.5%) and gallic acid (≥99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.2.2. Methods

Proximate composition of flaxseed hull

The flax hull proximate composition was determined as described in Chapter 4, Section 4.2.3.

Subcritical water extraction

Extractions were carried out using the subcritical water system previously described by Singh and Saldaña [19]. All extractions were carried out in a stainless steel cylindrical vessel of 50 mL volume. The vessel was equipped with 20 µm frit in the inlet and outlet to avoid hull micro-particles from leaving the extractor. The extraction vessel was filled with 3 g of defatted flaxseed hull obtained as described in Chapter 4, Section 4.2. Glass beads were used to fill the remaining reactor volume. The pressure was controlled by a back-pressure regulator (Tescom, Elk River, MN, USA).

The first step of the extraction procedure consists of degassing the deionized water. Then, the water was driven into the system by an HPLC pump (Gilson 305, Middleton, CT, USA), flowing through the preheating coil at a constant flow rate of 5 mL min^{-1} to fill the system and to increase the temperature and pressure up to the working pressure and temperature conditions. Once the extraction vessel reached its operation parameters, a 10 min static period was used to improve the contact of water and flaxseed hull. After the extraction was completed (20-60 min), the collection vial containing the extract was weighted, while the back-pressure regulator was opened to allow the extraction vessel depressurization. Extracts and flaxseed hull residues collected from each experiment were stored at 253 K for further analysis.

The water flow rate, system pressure and temperature were modified according to the experimental design described in Table 5.1. The ratio of water:feed material (20:1; v:w) was kept constant. Therefore, the extraction time was variable.

Experimental design

Response surface methodology with the face-centered design was used to investigate the extraction efficiency of total phenolics and total carbohydrates by subcritical water. The polynomial equation, analysis of variance (ANOVA), coefficient of determination, surface plot, and process conditions for maximum extraction were obtained using STATISTICA 7 (Stat Soft Inc., Tulsa, OK, USA). The variables studied were pressure (1-5 MPa), temperature (393-453 K) and water flow rate (1-3 mL/min). The ratio of water:feed material (20:1; v:w) was kept constant. The experimental design, predicted and experimental values are shown in Table 5.1. The central point was carried out in triplicate.

Analysis of total phenolics

Total phenolics of the extracts of flaxseed hulls were determined using the Singleton and Rossi methodology [20], with minor modifications. The extract (100 µL) was mixed with 7.9 mL of distilled water followed by 0.5 mL of Folin-Ciocalteu reagent addition. After 8 min of reaction at room temperature, 1.5 mL of sodium carbonate (Na_2CO_3, 20%) was added into the mixture. After 2 h of incubation at room temperature, the solutions were transferred to a cuvette and the absorbance was measured in a spectrophotometer at 765 nm. The standard curve for total phenolics quantification used gallic acid at different concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 1 and 2 mg/mL). Total phenolics were determined using linear regression.

Table 5.1. Full factorial experimental design

Run	Pressure X_1	Temperature X_2	Flow rate X_3
1	3 (0)	423 (0)	1 (-1)
2	3 (0)	453 (1)	2 (0)
3	5 (1)	453 (1)	1 (-1)
4	5 (1)	393 (-1)	3 (1)
5	1 (-1)	453 (1)	3 (1)
6	5 (1)	453 (1)	3 (1)
7	3 (0)	423 (0)	3 (1)
8*	3 (0)	423 (0)	2 (0)
9	1 (-1)	453 (1)	1 (-1)
10	1 (-1)	393 (-1)	3 (1)
11	1 (-1)	393 (-1)	1 (-1)
12	1 (-1)	423 (0)	2 (0)
13	5 (1)	393 (-1)	1 (-1)
14	3 (0)	393 (-1)	2 (0)
15*	3 (0)	423 (0)	2 (0)
16	5 (1)	423 (0)	2 (0)
17*	3 (0)	423 (0)	2 (0)

*Central point, P= 3 MPa, T=423 K, flow rate = 2 mL/min.

Analysis of total carbohydrates

Total carbohydrates were quantified in glucose basis using the phenol-sulphuric acid methodology adapted from Dubois et al. [21] for the determination of sugars and related substances. The extract was diluted until a final concentration of 10-50 µg of carbohydrates. An aliquot of 100 µL of the diluted extract was used for the analysis. Then, 0.5 mL of phenol 4% and 2.5 mL of sulfuric acid 96% were added for the reaction. The reaction was carried out in a water bath at 323 K for 20 min. The standard curve for carbohydrates used glucose solutions ranging from 5 to 100 µg mL^{-1}.

5.3. Results and Discussion

The average particle size of flaxseed hull was determined using a set of sieves ranging from 16 to 80 mesh. The average size of the flaxseed hull particle was 0.35 mm.

Proximate composition

Proximate composition data of flaxseed hulls is shown in Table 4.1 in Chapter 4, Section 4.3.1. Protein, lipid, ash, and moisture contents were 24, 26, 3, and 12%, respectively. Carbohydrates content (~35 %) was calculated by difference as the remaining portion in flaxseed hull. Although most of the flaxseed oil is recovered by dehulling the seeds, still 26% of the hull in weight is lipids. Carbohydrates content in flaxseed hull are much lower (35%) than in other cereals studied for subcritical water hydrolysis, such as barley hull (~80%) [22].

Table 5.2 shows the values for the recovery of carbohydrates and phenolics obtained using subcritical water. The effect of temperature on the recovery of carbohydrates can be explained by the increase of the solubility of the carbohydrates due to weakening of the bonds between the carbohydrates and the matrix [23]. Some studies showed a decrease on polysaccharides recovery at temperatures above 443 K [23-25]. However, in this study, temperatures up to 453 K showed to be beneficial for the carbohydrates recovery. This controversial effect could have been caused by the exposure time of the raw material under the conditions studied. Since previous studies used a contact time above 120 min, while this study utilized extraction times of up to 60 min [23-25].

Table 5.2. Results for carbohydrates and total phenolics recovery using subcritical water

Run	**Carbohydrates (mg g^{-1} of feed)**	**Total Phenolics (mg g^{-1} of feed)**
1	3.28	2.00
2	1.96	1.49
3	4.21	4.00
4	8.34	9.11
5	0.90	0.46
6	6.36	4.86
7	2.57	0.80
8*	6.01	3.60
9	2.67	0.70
10	4.76	1.17
11	1.64	7.85
12	1.00	0.25
13	8.18	7.85
14	0.90	0.67
15*	6.11	4.46
16	7.41	5.70
17*	6.06	4.17

*Central point: Total carbohydrates: 3.48±0.20 mg g^{-1}, Total phenolics: 4.26±0.26 mg g^{-1}

High phenolics recovery obtained by subcritical water (≈ 75% phenolics in flax meal [14-15] can be explained by the fact that at high temperatures, such as 453 K, the dielectric constant of water is similar to that of methanol [26]. Therefore, phenolics dissolve in PHW as much as they would dissolve in methanol. Moreover, water at such temperature hydrolyzes some lignin and cellulose [27, 14]. Lignin contains considerable amounts of phenolic compounds, which are bonded to carbohydrates and proteins. Therefore, besides improving the recovery of free phenolic compounds, PHW also releases some of the bonded phenolics [14, 15, 27].

Table 5.3. Regression coefficients for the statistical analysis for the total carbohydrates and total phenolic compounds extraction

Factor	**Sum of Square**	**Degree of Freedom**	**Mean Square**	**Std. Err.**	***p*-value**
		Carbohydrates			
Regression	96.75	9	10.75	0.94	0.042
x_1	7.16	1	7.16	0.70	0.1497
x_2	77.12	1	77.12	0.70	0.0011
x_3	0.05	1	0.05	0.70	0.9002
$x_1 x_2$	5.99	1	5.99	0.78	0.1825
$x_1 x_3$	0.05	1	0.05	0.78	0.8950
$x_2 x_3$	0.7	1	0.70	0.78	0.6293
		Phenolic Compounds			
Regression	72.31	9	8.03	0.94	0.0005
x_1	5.96	1	5.96	0.70	0.2395
x_2	55.37	1	55.37	0.70	0.0034
x_3	0.87	1	0.87	0.70	0.2679
$x_1 x_2$	1.22	1	1.22	0.78	0.2754
$x_1 x_3$	1.00	1	1.0	0.78	0.6429
$x_2 x_3$	0.12	1	0.12	0.78	0.9375

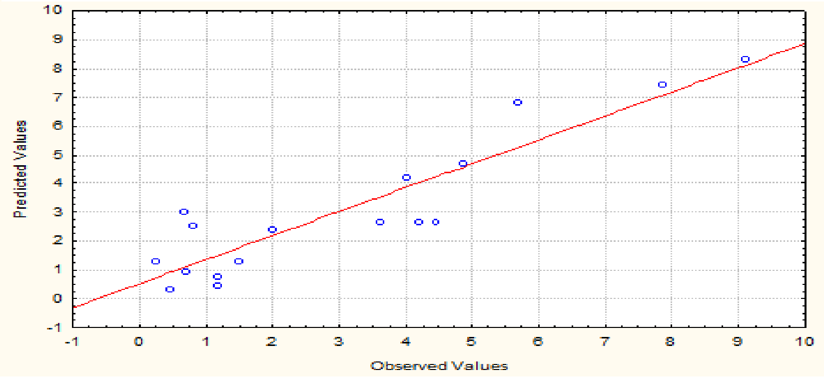
The experimental values of carbohydrates and phenolics obtained were adjusted to the following polynomial equations:

Carbohydrates (mg) = 4.21– 1.40 x_2

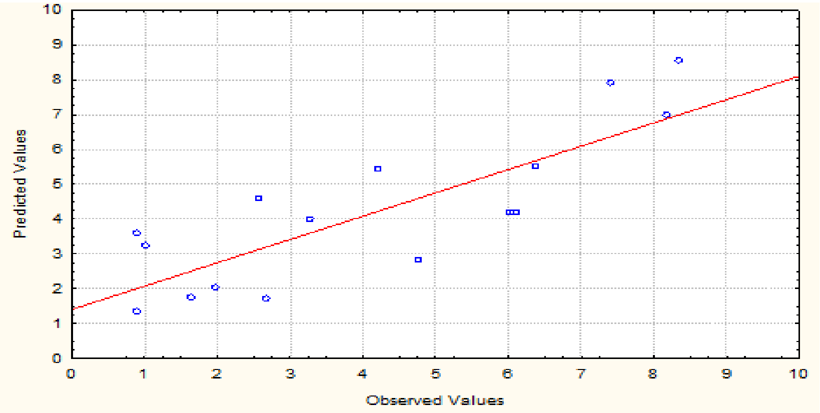
Total phenolics (mg) = 2.64 + 2.77 x_2

The comparison of predicted and experimental values (Figs. 5.1a and 5.1b) indicates a good fit of the model, as the relationship is linear.

Extraction of phenolics and carbohydrates were positively affected by the increase of temperature. However, there was no significant effect of pressure and flow rate (p>0.05) as reported in Table 5.3.



(a)



(b)

Figure 5.1. Comparison between predicted values and observed values of recovered total phenolics (a) and carbohydrates (b)

5.4. Conclusions

Carbohydrates and phenolics were extracted from flaxseed hull using PHW. Recovery of carbohydrates and phenolics improved by increasing the temperature up to 453 K. Based on the results of this study, PHW is a suitable method for the recovery of carbohydrates and phenolics from flaxseed hull. A high yield of phenolic compounds (≈ 80%) was obtained at 453 K. However, carbohydrates yield was low (≈ 5% of total carbohydrates). Results suggest that higher temperatures should be used to hydrolyze most of the carbohydrates present in the flax hull (cellulose and hemicellulose) and consequently increase the recovery of carbohydrates from flax hull.

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6. Conclusions and Recommendations

6.1. Conclusions

Flax and Sacha inchi seeds are among the most important sources of omega-3 fatty acids in the world. Super/subcritical fluid extraction using carbon dioxide (CO_2) and/or water as Generally Regarded as Safe (GRAS) solvents, show great potential New data on phase equilibria for sacha inchi seed oil in CO_2 have been measured using a variable volume cell phase equilibria system at temperatures of 303, 313 and 323 K and at pressures ranging from 4.3 to 27.7 MPa. The CO_2 mole fraction varied from 0.7488 to 0.9997. Sacha inchi oil contains 47% of omega-3 fatty acids, with a ratio of 0.76:1 for ω-6:ω-3, which is good for human health.

Flax oil was successfully recovered from its hulls by supercritical CO_2 technology. The ideal extraction time found for the best extraction condition (30 MPa and 333 K) within the range studied in this thesis was within 330 min with a recovery of 80% (w:w) of the total oil in flax hulls when compared to Soxhlet recovery. Then, these data were used to study the cost of manufacture (COM) to assess the economic feasibility of the technology. The methodology utilized to estimate the COM of flaxseed hull oil by supercritical CO_2 technology showed that the extraction of flaxseed hull oil is not economically feasible at the extraction conditions studied, since its COM value is higher than the bulk oil selling price. The lowest flax hull oil COM (US$ 10.50 kg^{-1}) estimated was obtained from a semi-continuous equipment with two 1m^3 columns. At the condition studied, the main component of the COM was the operating labor (50.6%), followed by the capital investment (32.2%), raw material (11.2%), and utilities (5.9%). Since defatted flaxseed hulls are rich in lignans, a high value compound related to breast cancer control, mucilage and proteins that are important for the food industry. The commercialization of the by-product and the utilization of higher pressures could make this process feasible, as the use of higher CO_2 density is related to high solvation power and consequently lower extraction time.

Subcritical or pressurized hot water is an alternative methodology of extraction in which water is used as an adjustable-polarity solvent by manipulating its temperature and pressure. Therefore, pressurized hot water can improve the extraction of both polar and relatively non-polar compounds from flaxseed hull. In this study, a factorial design with three factors was used to determine the best extraction conditions for removal of phenolics and carbohydrates from deffated flaxseed hulls. Phenolics and carbohydrates were extracted using pressurized hot water in a ratio of water:feed material (20:1; v:w) at different temperatures (393, 423 and 453 K), pressures (1, 3 and 5 MPa) and flow rates (1, 2 and 3 mL min^{-1}). Total phenolics and carbohydrates quantifications were carried out utilizing the colorimetric Folin-Ciocalteau method with minor modifications and the phenol-sulphuric acid methodology, respectively. The best extraction temperature was 453 K for the recovery of total phenolics (9.5 mg g^{-1}) and carbohydrates (8.2 mg g^{-1}). Temperature improved the recovery of carbohydrates and phenolics, while pressure, flow rate and their interactions had no significant effect.

Flaxseed and sacha inchi seed oil have high omega-3 fatty acid content, which aggregates value to flax. Using super/subcritical fluid technologies to process by-products, such flax hulls, creates new opportunities for the utilization of such by-products. This thesis shows a new perspective for the utilization of an important Canadian by-product.

6.2. Recommendations

Sacha inchi has only started to receive attention from the scientific community in the past few years. Because of the amount of omega-3 fatty acids present in its seed, a cost of manufacture study on Sacha inchi oil extraction would be interesting.

There are still many studies to be conducted on the utilization of flax hulls. The optimization of the recovery of phenolics and carbohydrates from flax hull utilizing subcritical water would be worthwhile. Moreover, it would be interesting to study the composition of the liquid extract and understand if it would be viable to use the liquid solution containing carbohydrates for the ethanol production. Ethanol production from renewable sources has been receiving enormous attention lately. However, literature lacks information on cost of manufacture of renewable energy, especially those produced utilizing technologies such as subcritical water. Therefore, a study on cost of manufacture for the production of ethanol utilizing subcritical water technology would be worthwhile.

Finally, a study on cost of manufacture using supercritical fluid extractors in different layouts such as the utilization of three extractors, or two sets of two extractors could lead to a lower cost of manufacture for supercritical extracts.