SUPERCRITCAL FLUID EXTRACTION, FRACTIONATION, AND CHARACTERIZATION OF WHEAT GERM OIL

By

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NOMENCLATURE

AMU	Atomic Mass Unit
ANOVA	Analysis of Variance
CO_2	Carbon Dioxide
ELSD	Evaporative Light-Scattering Detector
FAME	Fatty Acid Methyl Esters
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
MSD	Mass spectroscopy Detetctor
PA	Phosphatidic Acid
PDA	Photo Diode Array Detector
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
POC	Polycosanol
PI	Phosphatidylinositol
PL	Phospholipid
PS	Phosphatidylserine
PUFA	Poly unsaturated fatty acid
OC	Octacosanol
SC-CO_2	Supercritical Carbon Dioxide
SFE	Supercritical Fluid Extraction
SFF	Supercritical Fluid Fractionation
TG	Triglyceride

v/v	Volume to volume
WGO	Wheat Germ Oil
w/w	Weight to weight

Units

%	percentage
˚C	degree centigrade
g	gram
mg	milligram
mL	milliliter
min	minutes
nm	nanometer
µm	micro meter
µg	micro gram
µL	micro liter

Fatty acid structure	Systematic name	Common name
14:0	Tetradecanoic	Myristic
16:0	Hexadecanoic	Palmitic
16:1	Hexadecenoic	Palmitoleic
18:0	Octadecenoic	Steric
18:1	Octadecanoic	Oleic
18:2	Octadecadienoic	Linoleic
20:0	Eicosanoic	Arachidic
20:1	Eicosenoic	Gadoleic
18:3	Octadecatrienoic	Linolenic
22:0	Docosanoic	Behenic
22:1	Docosenoid	-
24:0	Tetracosanoic	Lignoceic

CHAPTER 1

INTRODUCTION

1.1 PROBLEM STATEMENT

Crude vegetable oil contains a number of compounds such as mono-, di- and triglycerides, free fatty acids, phospholipids, pigmented compounds, waxes as well as nutritionally beneficial compounds such as tocopherols, tocotrienols, phytosterols, squalene and policosanol. Crude oil needs to be refined to produce high quality and highly stable oils through eliminating undesirable compounds. Conventional edible oil extraction and refining processes involve several unit operations which have several disadvantages, including using large quantities of water and hazardous chemicals, generating large quantities of waste, as well as being energy intensive. A mixture of hexane isomers is commonly used for edible oil extraction. The amendments to the Clean Air Act listed normal hexane as a hazardous air pollutant. No new solvents have been cleared for commercial edible oil extraction to date. A significant portion of the nutritional oil components is lost during the conventional refining processes. There is a great need for development of new and environmentally benign processing techniques which will facilitate vegetable oil refining while sustaining the nutritional components naturally present in the edible oils while reducing the negative impact of oil processing on the environment.

1.2 HYPOYTHESIS

Supercritical fluid technology can be utilized to extract and process wheat germ oil to obtain high quality products that are comparable or superior to that of conventionally extracted and processed wheat germ oil.

1.3 OBJECTIVES

The main objective of this thesis is to examine the viability of supercritical fluid technology to extract and fractionate wheat germ oil. The specific objectives are as follows:

i. Chemical characterization of wheat germ oil samples that have been extracted and refined through conventional methods.
ii. Extraction and fractionation of wheat germ oil utilizing supercritical fluid technology and chemical characterization of the products.
iii. Comparison of composition of wheat germ oil processed with supercritical fluid technology to that of the conventional products.

CHAPTER 2

LITERATURE REVIEW

2.1 VEGETABLE OILS

Vegetable oils have been and will continue to be a vital part of human nutrition. Although they have been used in food applications for hundreds of years, the full potential of vegetable oils has yet to be achieved. It is well known that excessive consumption of fats and oils is linked to obesity, certain cancers, and high levels of cholesterol in blood with consequent cardiovascular disease. On the other hand, the numerous health benefits of vegetable oils consumed as part of a balanced diet are only beginning to be explored.

Lipids play essential roles in disease prevention and growth, serve as carriers for vitamins A, D, E, and K, and provide essential fatty acids such as linoleic and linolenic acids. Fats serve as thermal insulation for the body and protect internal organs. Lipids are also vital components of all cellular membranes, and precursors for compounds such as prostoglandins, steroid hormones, and bile acid. Vegetable oils are also carriers for other plant based biologically active compounds that have shown to be antioxidants, anti-cancer agents, and show potential for prevention of chronic disorders such as cardiovascular diseases and diabetes. Tocopherols, phytosterols, policosanol, and phosphatidylcholine (PC) are some of compounds naturally present in vegetable oils and posses disease treatment and prevention properties. Health benefits of these compounds will be discussed in detail later in this chapter.

Since many compounds in oil seeds already have proven nutritional benefits, there are great possibilities for using them to develop new functional vegetable oils. Vegetable

oils containing enhanced levels of beneficial active ingredients could have a substantial impact on human health considering the amount of cooking and salad oils consumed in most industrialized countries.

2.2 WHEAT

Wheat grain consists of endosperm, bran, and germ, which account for 81 to 84 %, 14 to 16%, and 2 to 3% of the grain, respectively (Atwell 2001). Commercial milling of wheat into flour aims at the maximum extraction of the endosperm with the minimum possible contamination by bran and the germ, which form the by-products of the flour milling industry.

Wheat germ is a unique source of highly concentrated nutrients. It offers three times as much protein of high biological value, seven times as much fat, fifteen times as much sugar, and six times as much mineral content when compared with flour from the endosperm (Atwell 2001). In addition, wheat germ is the richest known source of α-tocopherols (vitamin E) of plant origin and also a rich source of phytosterols, policosanols, thiamine, riboflavin, and niacin (Atwell 2001).

2.2.1 Wheat Milling

Before any of the advantages of wheat germ oil can be utilized, the germ must be separated from rest of the wheat kernel through a common process of dry milling. Three general operations are usually involved in this process: cleaning, tempering and milling. Cleaning removes unwanted materials; tempering softens the grain making it easier to

separate and grind; and milling involves grinding the wheat and isolating wheat components of specific sizes (Atwell 2001).

Cleaning starts when wheat is unloaded from a truck, rail car, or ship and conveyed into a mill elevator. Although numerous machines exist to clean wheat, they are all classified based on separation by size, shape, density, and magnetism.

Tempering is the addition of predetermined amounts of water to wheat during specific holding periods (usually 12-18h). It toughens the bran, making it easier to separate from germ and endosperm. Tempering also softens the endosperm, allowing it to break apart with less force. Temperatures lower than 50°C are employed during conditioning to ensure that the functionality of the flour components is maintained. (Atwell 2001).

At this point, the wheat is ready for milling and starts through the various systems in the mill Appendix I, (Figure 1). The first machine in almost every mill is the roller mill. A small distance called the "gap" separates two rolls, one rotating clockwise and the other counterclockwise. One of the rolls usually rotates faster than the other one. Consequently, at the nip, the rotation of the rolls is in the same direction and the wheat experiences a shearing action as well as a crushing action. The first roller mills are employed in the break system. This is the part of milling designed to remove the endosperm from the bran and the germ. The germ is pliable and tends to flatten when it goes through the rollers. Bran particles are usually in the form of low-density small flakes. These properties allow millers to separate the germ and bran fractions from the endosperm fraction. (Atwell 2001). In order to understand what is happening to the wheat germ and its oil, the storage conditions of the wheat prior to milling must be evaluated.

The moisture content of wheat varies with relative humidity and for practical purposes wheat stored during commercial distribution is maintained at 14% moisture or below. Usually grain is stored in silos or elevators, but modern practices also include the use of air tight bunkers, which can be flushed with carbon dioxide or nitrogen to reduce insect infestation and lower the rate of respiration (Atwell 2001).

2.2.2 Extraction

After the germ has been separated and collected the oil needs to be extracted. There are several methods for oil extraction that all have their advantages and disadvantages.

Mechanical expression (pressing) and organic solvent extraction are both being used for commercial extraction of wheat germ oil (WGO). Solvent extraction is by far the most widely used method to extract oil (Woerfel 1995). A mixture of hexane isomers containing about 60% *n*-hexane is the choice of solvent for commercial vegetable oil extraction. The residual oil content of solvent defatted wheat germ can be as low as 1 %, (w/w). In general, conventional methods have a tendency to generate crude extracts consisting of deteriorated constituents or to prematurely remove volatile components. Pressing recovers only 50 %, (w/w) of the WGO. Mechanical pressing of oil is considered only when very high purity wheat germ with very low bran contamination is available. The extract yield using various solvents are listed in Table 1 (Appendix II) (Barnes 1982). It is apparent from this data that yields are variable even when the same solvent is used: this is probably partly due to the degree of contamination of the germ

with bran, which usually contains not more than 5% oil, and partly to the variety of wheat (Barnes 1982).

2.2.3 Wheat Germ Oil Refining

Although WGO is often used in its crude form, refining improves the quality and stability of the oil. Degumming, neutralization, bleaching, and deodorization are typical refining steps of vegetable oil processing. Crude WGO often has very high phospholipid (PL) (1428 ppm PL) content (Wang and Johnson 2001). WGO needs to be degummed (phospholipids removal) at high temperatures and high shear for an extended time as compared to that for the typical vegetable oils to maximize PL hydration. Even though PL, specifically PC, has beneficial health effects for humans they are removed from the crude oil during the degumming process. PLs tend to precipitate out in the oil during the storage and have adverse effects on frying operations due to their emulsification properties (Wang and Johnson 2001).

The free fatty acid (FFA) content of the crude oil is usually very high and quite variable (5-25% is typical), depending upon conditions of germ separation, germ storage, and oil extraction. FFA often contributes to bitter and soapy flavor in food. With such high FFA content, physical refining (steam deacidification) would be appropriate provided that the phosphorous content could be reduced to a low level (<100 ppm). Chemical neutralization of FFA in WGO may need excess alkali treatment (Wang and Johnson 2001).

WGO is usually dark-colored and may have strong odor and flavor depending on the oxidative condition of the oil. WGO bleaching requires more bleaching earth than that

of the typical vegetable oil refining. The bleached WGO may contain higher amount of FFAs than that of the neutralized oil, because silica and bleaching earth used for bleaching process are acidic in nature, and residual soap left in the neutralized oil may be converted back to FFAs under these conditions. The acidic bleaching earth may also cause hydrolysis of triacylglycerides. The residual FFAs and odor compounds in the WGO are removed during the deodorization process. More FFAs are removed from WGO at higher deodorization temperatures and increased residence time (Wang and Johnson 2001). Tocopherol content of the WGO is significantly reduced during the high temperature deodorization process. A vegetable oil refining process that minimizes tocopherol removal is highly desirable.

2.3 SUPERCRITICAL FLUID TECHNOLOGY

2.3.1 Extraction

Supercritical carbon dioxide (SC-CO_2) extraction is a relatively new technique studied for oilseed processing. Although supercritical fluids (SCFs) were discovered more than 100 years ago, it wasn't until the 1970s that they were used commercially to decaffeinate coffee. Since then, SCFs have been used successfully to extract compounds from a variety of complex materials through manipulation of system pressure and temperature. A SCF can be defined as a substance that has undergone a phase change encountered upon conditions above its critical point for temperature and pressure. SCFs possess physical properties (density, viscosity and diffusivity) that are intermediate between liquids and gases. Near the critical point of a fluid, minute changes in pressure or temperature significantly alter the physicochemical properties of the SCF. This is

especially important for synthetic applications, in which reaction conditions (e.g., selectivity, rates) may be accurately manipulated. Such reaction control is impossible using traditional organic solvents. Furthermore, many organic solvents such as halogenated hydrocarbons (e.g., chloroform, dichloromethane) are being phased out of use and benign replacements are being developed because of their deleterious effects on the environment and/or health (Anonymous 2002).

The limiting property of SC-CO_2 is that it is only capable of dissolving non-polar solutes. However, the addition of small amounts of a co-solvent such as acetone or incorporation of emulsifiers into the supercritical phase has been shown to significantly improve the solubility of relatively polar solutes.

Several research studies reporting SC-CO_2 extraction of WGO have been published (Taniguchi and others 1985; Gomez and de la Ossa 2000; Dunford and Martinez 2003; Panfili and others 2003). Taniguchi et al. (Taniguchi and others 1985) reported that WGO solubility in SC-CO_2 was 0.35 % (w/w) at 40^{o}C and 200 bar. SC-CO_2 extracted oil has a lighter color and contains less phosphorus than that of the hexane extracted oil. The α- and β-tocopherols contents of SC-CO_2-extracted oil were found to be similar to those of hexane-extracted WGO (Taniguchi and others 1985; Dunford and Martinez 2003). Gomez and Ossa (Gomez and de la Ossa 2000) reported that optimum conditions for WGO extraction were 150 bar and 40˚C and solvent flow rate of 1.5 L/min (at standard temperature and pressure). Tocopherol content in the SC-CO_2 extracted oil was higher as compared to that of the hexane extracted oil. According to Panfili et al. (Panfili and others 2003) FFA content and peroxide value of the oils collected during the first 45 min of extraction were higher than that of the oil fractions collected at the later

stages of the process. Similar fractionation was also reported for tocopherols. WGO collected during the initial stages of SC-CO_2 extraction had a higher tocopherol content that that of the fractions collected at later stages of the extraction (Dunford and Martinez 2003). The most abundant carotenoid in SC-CO_2 extracted WGO was lutein, followed by zeaxanthin and β-carotene. A larger amount of carotenoids was extracted toward the end of SC-CO_2 extraction (Panfili and others 2003).

Extraction of wheat germ with liquid and SC-CO_2 (50-400 Bar) at relatively low temperatures (10-60^{o}C) indicated that pressure had a significant effect on the oil yields while the effect of temperature was insignificant (Taniguchi and others 1985). Dunford and Martinez (Dunford and Martinez 2003) studied the effect of pressure and temperature on the SC-CO_2 WGO extraction yields in the range of 100-550 Bar and 40-80^{o}C. Yields of SC-CO_2 extracts varied significantly with temperature and pressure in the 2 to 20 %, (w/w) range. Hot hexane (Soxhlet) extraction yielded 11%, (w/w) WGO. These results indicate that SC-CO_2 at high pressure extracts some of the wheat germ components, which are not soluble in hexane. At higher temperature and pressures moisture can be co-extracted with oil resulting in higher extraction yields (Dunford and Temelli 1996; Dunford and others 1998). The highest SC-CO_2 extraction yield was obtained at the highest pressure used (550 Bar). The temperature dependence of the extract yield was more pronounced at higher temperatures (60 and 80^{o}C) and the lowest pressure examined in that study (100 Bar). The fatty acid composition of the extracts was not affected by temperature, pressure and the extraction method (Dunford and Martinez, 2004). Supercritical carbon dioxide extracted oil samples had similar fatty acid composition to that of the Soxhlet extracted oil (Dunford and Martinez, 2004). These results indicated

that SC-CO_2 technology can be utilized for extraction and fractionation of WGO components to obtain products with high quality.

2.3.2 Supercritical Fractionation

Supercritical fluid extraction from a liquid phase can be carried out utilizing a vertical column. This process is also referred to as supercritical fluid fractionation (SFF). The liquid phase may be a liquid mixture, or a solution containing solutes. The process can be carried out in a semicontinuous or continuous mode. In general two fractions collected, one from the top of the column (extract and/or light phase) and the second fraction from the bottom of the column (raffinate and/or heavy phase) during the fractionation process. The advantage of an SFF is that it can be used as a continuous process, which tends to be more efficient than a batch process if properly designed.

The literature on utilization of SFF for vegetable oil processing is relatively limited as compared to many other topics that focus simply on the use of supercritical fluid extractions. The fundamentals and mathematical modeling of SFF technique was reviewed by Clifford (Clifford 1999). The studies designed to produce nutraceutical ingredients from vegetable oils and/or their by-products using SFF indicated viability of this technique. A U.S. patent describes enrichment of phytosterols in rice bran oil using a columnlar SC-CO_2 fractionation process (Dunford and King 2004). The SFF product contained free and fatty acid esters of phytosterols and oryzanol (Dunford and King 2001). When a continuous counter current SFF was used for deacidifaction of rice bran oil at 138 Bar and 80^{o}C, FFAs were effectively removed without any oryzanol loss in the extract fraction (Dunford and others 2002). Oryzanol and phytosterol fatty acid ester content of the raffinate fraction increased during the deacidification process. Hexane-

extracted RBO was used for these studies; however, oil extracted with SC-CO_2 can also be used as a starting material. When SC-CO_2 extracted corn fiber oil, which is also rich in phytosterol esters (especially oryzanol), was fractionated using SFF technique it was possible to obtain phytosterol-enriched TG fractions (>15% phytosterol content) (King and others 2000).

King and Dunford were also able to obtain oil fractions with 31% phytosterol and 30% TG from soybean oil deodorizer distillates using a pilot scale SFF unit (King and Dunford 2001). Fractionation of fish oil (Nilsson and others 1988), milk fat (Yu and others 1992), shark liver oil (Catchpole and von Kamp 1997), fatty acid ethyl esters (Fleck and others 1998) and separation of tocopherols from deodorizer distillates (Brunner and others 1991) using SFF technique have been also reported.

2.4 CHEMICAL COMPOSITION OF WHEAT GERM OIL

Wheat germ oil has a number of nutritional and health benefits such as reducing plasma and liver cholesterol levels, improving physical endurance/fitness, and possibly helping to delay effects of aging (Kahlon 1989). These effects are attributed to the high concentration of bioactive compounds present in the oil. Hexane extracted WGO consists of about 56 % linoleic acid (18:2 n6), which is an essential fatty acid (Dunford and Zhang 2003). Total unsaturated and polyunsaturated fatty acid (PUFA) content of WGO is about 81 and 64 %, respectively. WGO processing is very challenging because of its high PUFA content. Data on WGO PLs is scarce. It has been reported that PC represents about 40-60% of total PLs in dissected wheat germ. Phosphatidylethanolamine (PE) (9-15%)

and phosphatidylinositol (PI) (13-20%) are also present in significant amounts (Hargin and Morrison 1980).

Wheat germ oil is quite rich in unsaponifiable compounds, in particular phytosterols and tocopherols. Wheat germ oil is one of the richest natural sources of α-tocopherols. Wheat germ oil has been reported to improve human physical fitness and this effect is attributed to its high (policosanol) POC, specifically its high octacosanol (OC) content. The POC contents and compositions of wheat grain fractions were studied by Irmak and Dunford (Irmak and Dunford 2005; Irmak and others 2005). The PC content of wheat bran was higher than that of the germ, shorts and flour. Tetracosanol (C24), hexacosanol (C26) and OC (C28) were the major POC components in all the varieties. WGO contains higher amount of phytosterols than do the other common commercial oils (Itoh and others 1973). Sitosterol (60-70%) and campesterol (20-30%) are the two major phytosterols present in WGO (Anderson and others 1926; Itoh 1973; Itoh and others 1973).

CHAPTER 3

MATERIALS AND METHODS

3.1 OIL SELECTION AND SAMPLE PREPARATION

Commercial wheat germ oil (WGO) samples were donations from Vitamins, Inc. (Chicago, IL). Soybean oil (Oil S) was purchased at a local grocery store. All the oil samples were stored in sealed containers at 4^{o}C away from the light until further use. Four WGO samples consisted of crude (WGO A), two batches of refined (WGO B, WGO C) and concentrated WGO (WGO D). The hexane extracted crude WGO (A) was used without further purification except that it was centrifuged at 14 000 rpm, 4^{o}C for 30 min and vacuum filtered through a #2 Whatman filter paper before the analytical tests and SFF. The WGO B was hexane extracted and further processed by using conventional refining processes. The oil sample C was also hexane extracted and had undergone physical refining (molecular distillation). The last WGO sample (D) was concentrated in tocopherols. Wheat germ samples for SC-CO_2 extraction were supplied by ADM Milling Co. (Enid, OK, U.S.A.). Germ was obtained from milling of winter wheat (20% Kansas, 80% Oklahoma-grown winter wheat). The further details of the refining processes are propriety information and were not available to us. WGO samples were extracted and refined accordingly prior to our receiving them. Samples were used as is with no pretreatment unless stated otherwise.

3.2 SUPERCRITICAL FLUID PROCESSING

3.2.1 Extraction

The SC-CO_2 extraction of wheat germ was carried out at the USDA-Agricultural Research Services, National Center for Agricultural Utilization Research in Peoria IL. A picture and schematic diagram of the extraction unit are given in Picture 1 and Figure 1, respectively. The extraction was conducted in four parallel runs with three vessels (4 liters each) in series. Each vessel contained 3.3 lbs wheat germ. The total of 9.9 lbs of wheat germ was extracted at 80^{o}C and 10,000 psi using 35 lbs CO_2 (measured at atmospheric conditions). The CO_2 flow rate was 4 lbs/min. The extract was collected in a receiver maintained at 60^{o}C and 1600 psi. The recovery of the extract was accomplished by pressure drop across a back pressure relief valve followed by condensation and recovery of the precipitate. Extraction was conducted for a total of 45 min. The extraction was assumed complete when no more extract was collected in the receiver.

3.2.2 Fractionation

The SFF experiments were conducted at the Food and Agricultural Products Research and Technology Center's pilot plant facility on the Oklahoma State University campus. The SFF column which was designed in-house was 10 ft long and 1 inch inner diameter (Temco, Inc., Tulsa, OK). A schematic flow diagram and a picture of the SFF system are shown in Figure 2 and Picture 2. The fractionation column had a preheater and four independently controlled temperature zones. The temperature of the main column was maintained by a HS-4ZC Heating System (Temco, Inc., Tulsa, OK). The heating system consisted of a Watlow temperature controller which utilizes cascade style heating

(Watlow Electric Manufacturing Company. Winona, MN). The temperature of the column was maintained automatically at ±2°C of the set point by WatView Run-Time software (Watlow Anafaze, Inc., 1999-2002, Version: 2.3.7, Watsonville, CA) run on a Dell Inspiron 8100 laptop computer. The pre-heater set temperature was maintained by a PID type controller (Model TC-11-K Model, Watlow Electric Manufacturing Company. Winona, MN). The temperature sensing was from type-K thermocouples inserted inside and on the surface of the vessels. The main column was packed with protruded 316 SS packing material (0.16-inch Pro-Pak, Cannon Instrument Company, State College, PA). There were two separate ports for CO_2 and raffinate removal from the bottom of the column. The lower section of the column (about 12 inches) below the inlet was used for raffinate collection. The fractionation experiments were carried out in a continuous countercurrent mode of operation. Initially, column was pressurized with CO_2 (Research Grade, min purity 99.998%, Matheson Trigas, Houstan, TX) and allowed to equilibrate at desired temperature and pressure. An air driven gas booster pump, (Model ACT-62/152, Haskel Inc., Burbank, CA) was used to deliver CO_2 into the column. The pressure fluctuations in the column was minimized by placing a high pressure gas receiver (volume 34.8 cubic inches, Model # 157-12, Haskel Inc., Burbank, CA) and a backpressure valve (Model 26-2091B44S172, Tescom Corporation, Elk River, MN) after the booster pump and before the column. Carbon dioxide entered the column from the bottom of the column just above the raffinate reservoir. Oil was introduced from the top of the column by a syringe pump (ISCO model 100DX pump, Teledyne Isco, Inc. Lincoln, NB) controlled by an ISCO SFX 200 controller (Teledyne Isco, Inc. Lincoln, NB). Solute-laden SC-CO_2 rose upwards and recovered as the extract fraction from the

top of the column. Compounds with lower solubility in SC-CO_2 than that of the extract and/or components with larger molecular weight moved downwards and collected as raffinate from the bottom of the column. The extract and raffinate fractions were expanded through micrometering valves (Part no.30VRMM4812, Autoclave Engineers, Inc., Erie, PA) and the precipitate was collected into collection vials cooled by two Microban ICE-PAKs (Fisher Scientific, Pittsburgh, PA). Then CO_2 passed through a custom gas filtration device (a tube filled with glass wool), a flow indicator, and finally through a dry gas test meter (Model DMT-200A-3, American Meter Company, Philadelphia, PA) for recording the total amount CO_2 used for the fractionation process.

Fractionations of crude WGO were carried out at 2000 psi, 80^{o}C and 4 and 8 L CO_2/min flow rates. Oil was introduced to the column at a constant flow rate of 0.3 ml/min giving a solvent/feed ratio of approximately 25:1 and 50:1 (w/w). Fractionations of SC-CO_2 extracted WGO were performed under the same conditions but only at one flow rate, 8 CO_2 L/min (solvent/feed ratio = 50:1). The total run time for the fractionation experiments was 6. The system was allowed to reach steady state conditions for the first 4 h. The steady state conditions in the column were confirmed by attaining constant weight and composition of the extract fraction collected in 30 min intervals through several testing fractionations over various time intervals. The data on the fractionation experiments reported in this thesis were collected in 1 h intervals after steady state operation was established in the column (5^{th} and 6^{th} hour of the fractionation runs). These samples were characterized for their chemical composition. Insoluble oil components that had collected in the reservoir were drained every half an hour. After the completion of each experimental run the column was depressurized and residual oil was

drained off. The column was cleaned between runs at a pressure of 5000 psi and temperature of 80^{o}C by flowing CO_2 at 8-10 L/min flow rate for more than 6 h.

3.3 ANALYTICAL METHODS

3.3.1 Moisture Analysis

Moisture content of oil samples was determined by utilizing a Karl Fischer Titrator (758 KFD Titrino, Metrohm, Brinkman Instruments, Inc. Westbury, NY). The 34811 Hydranal Titrant-2 was used as a titrant and the 34812 Hydranol Solvent was the component solvent. Both solvents were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO).

3.3.2 Free Fatty Acid Determination

The FFA content of the oil samples were determined by utilizing a colorimetric method (Lowry and Tinsley 1976). Cupric acetate-pyridine solution was prepared by adjusting the pH of the filtered 5% (w/v) aqueous cupric acetate (99.9% purity, RJ Baker, Phillipsburg, NJ) solution to 6.0-6.2 using pyridine (99% purity, Fisher Chemicals, Fairlawn, NJ). About 0.03-0.05 g oil samples are weighed into a 5 mL volumetric flask and brought to 5 mL volume with benzene (ACS grade, EMD Manufacturing, Savannah, GA). Color development was initiated by addition of 1 mL cupric acetate-pyridine reagent into oil-benzene mixture. After mixing and centrifugation absorbance of the top layer was read at 715 nm using a UV/VIS spectrophotometer (Beckman DU 520, Fullerton, CA). FFA contents of the samples were determined from the calibration curve.

Oleic acid (90% purity, Aldrich, Milwaukee, WI) was used for the preparation of standard curve.

3.3.3 Fatty Acid Composition

Fatty acid compositions of the oil samples were determined by gas chromatography (GC). The GC unit was a HP 6890 Plus system equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE). Methylation of the fatty acids was carried out according to the AOCS Official Method Ce 2-66 (AOCS 1994). A Supelco SP-2560 fused silica capillary column with 100 m x 0.25 mm 0 0.2 μm film thickness (Supelco, Bellefonte, PA) was used for fatty acid analysis. The helium carrier gas flow rate was 19 cm/s. The injector temperature was maintained at 250^{o}C. A temperature program with total run time of 45 min was used. The initial column temperature 140^{o}C was maintained for 5 min. Then oven temperature was increased to 240^{o}C at a 4^{o}C /min ramp rate and kept constant at this temperature for 15 min. The detector conditions were as follows: temperature 260^{o}C, H_2 flow 40 mL/min, air flow 400 mL/min and make-up gas (He) 30 mL/min. Oil samples (1 μL) were injected by an autosampler (HP 7683, HP Company, Wilmington, DE). Peak areas were calculated and data collection was managed using an HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA). The split ratio was 150:1. Fatty acid peaks were identified using a standard 36 FAME mixture (Supelco 37 component FAME mix, Supelco, Bellefonte, PA).. Undecanoic acid (11:0) was used as an internal standard for quantification.

3.3.4 Tocopherols

Tocopherol content of the oil samples were analyzed by using an HPLC method (Katsanidis and Addis 1999). A normal phase HPLC column, Zorbax RX-SIL (5 μm particle size, 4.6 x 250 mm, Agilent Technologies, Santa Clara, CA) was used for separation of tocopherol isomers. Analytical separation of oil components on the column was achieved by using a mobile phase consisting of hexane: isopropyl alcohol (99:1 v/v) on isocratic mode. Total run time and flow rate were 15 min and 1.3 mL/min, respectively. The HPLC system (Alliance 2690 Waters Corp., Milford, MA) consisted of a separations module (Model 2695), a Photodiode Array Detector (PDA) (Model 2996, Waters, Milford, MA) and a Multi Wavelength Fluorescence Detector (FD) (Model 2475, Waters, Milford, MA). The oil samples were dissolved in hexane (0.025 mg/mL) and filtered through a 0.2 μm filter (Iso–Disc filter, Supelco, Bellefonte, PA). The fluorescence detector was set at 290 nm excitation wavelength and 400 nm emission wavelength. The fluorescence detector gain was set for 1. The column temperature was 35^{o}C. The injection volumes of the both, individual standards and the oil sample were 2 μL. An external calibration curve was prepared for each tocopherol standard (α, ß, γ and δ tocopherol standards, CN Biosciences Inc., La Jalla, CA) to calculate the amount of tocopherols present in the oil sample.

3.3.5 Policosanols and Phytosterols

Oil samples were hydrolyzed by refluxing with 100 mL of 1.0 N NaOH in methanol for 45 min. The mixture was cooled and deionized water was added. Then the solution was extracted with HPLC grade diethyl ether (Burdick & Jackson, Muskegon,

MI). The extraction was repeated three times using equal volumes of diethyl ether. The ethyl ether phase collected from three extractions was combined and washed with deionized water until neutrality. The ether extract was evaporated to dryness under nitrogen using a Reacti-Vap evaporation unit (Model 18780, Pierce, Rockford, IL) after drying over anhydrous sodium sulfate (ACS grade, EMD Chemicals Inc., Gibbstown, NJ). The residue was transferred to a 1 mL volumetric flask and 0.5 mL chloroform and 250 µL silylation reagent (MSTFA) were added. Then the solution was heated at 60°C for 15 min for derivatization. Chloroform was added to reach a total sample volume of 1 mL before analysis.

Trimethylsilyl derivatives of policosanols and phytosterols were analyzed using a HP 6890 Series Gas Chromatography (GC) system coupled with a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, USA). A fused silica capillary Equity-5 (30 m x 0.25 mm x 0.5 µm film thickness) from Supelco (Bellefonte, USA) was used for the analysis. Oven temperature was programmed from 150°C to 320°C with a 4 °C/min heating rate and maintained at 320°C for 15 minutes. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 300°C. Mass Spectrophotometer (MS) parameters were as follows: MS transfer line 280°C, ion source 230°C and MS quadruple temperature 150°C. The ionization energy was 70 eV. The scan range and rate were 100-600 AMU and 2 scans/sec, respectively. 1 µL of sample was injected into the GC-MS by an autosampler (HP 7683, HP Company, Wilmington, DE). The split ratio was 1:10. The data collection and analysis were managed using HP Chemstation (Enhanced Chemstation G1701 DA Version D.00.00.38, Agilent Technologies, Palo Alto, CA). The policosanol and phytosterol compositions of the

samples were identified by direct comparison of their chromatographic retention times and the mass spectra with those of the authentic compounds. The peaks were also confirmed with NIST/EPA/NIH Mass Spectral Library (Version 2.0).

3.3.6 Phospholipids

Phospholipid standards L-α-Phosphatidylcholic (PC) from soybean, 3-sn-Phosphatidic acid (PA) sodium salt from egg yolk lecithin, Phosphatidylserine (PS) from bovine, and L- α-Phosphatidylethanolamine (PE) were purchased from Sigma Inc. (St. Louis MO). Plant based Phosphatidylinositol (PI) was purchased from Matreya (State College, PA). Standards were dissolved in chloroform. All solvents utilized for HPLC mobile phase were HPLC grade and filtered using a GH Polypro (47 mm, 0.45 μm) hydrophilic polypropylene membrane filter (Pall Life Sciences, Ann Arbor, MI) before use.

A normal phase silica column, μPorasil 10 μm (3.9 mm i.d x 300 mm) from Waters (Milford, MA) was used for the analytical separation of the compounds. The mobile phase consisted of two mixtures: A: Hexane: Water: Isopropyl alcohol (40:58:2) and B: Hexane: Water: Isopropyl alcohol (40:50:10). The solvent gradient system was as follows: 100% A to 100% B in 7 min, then held for 6 minutes followed by returning to 100% A in 1min and held for 11 min. Total run time was 25 min. The detector system consisted of a Photodiode Array Detector (PDA) (Model 2996, Waters Milford, MA) in series with an Evaporative Light Scattering Detector (ELSD) (Model 2000, All tech associates Inc., Deerfield, IL). The ELSD set points were as follows: nitrogen flow rate 3.5 mL/min, impactor ON and drift tube temperature of 80^{o}C. The oil samples were

dissolved in chloroform and filtered through a 0.2 μm Iso Disc filters (Supelco, Bellefonte, PA) for further analysis. Identification and quantification of chromatographic peaks were based on external standard curves prepared for individual standards.

3.3.7 Analysis of Free and Fatty Acid Esters of Phytosterols

Analytical separation of triacylglycerides, FFA and free and fatty acid esters of phytosterols in oil samples were achieved by using an HPLC method developed by Moreau et al. (Moreau and others 1996). A LiChrosorb Diol, 5 μm, 100 x 3.0mm (Chrompack Inc., Raritan, NJ) column was used for the analysis. The mobile phase consisted of the following: A:Hexane: Acedic Acid (1000:1), B: 2-Propanol. The solvent gradient system was as follows, 100% A for 8 min, 100% A to 99% A (1% B) in 2 min, hold for 20 min, from 99% A to 100% A in 1 min, and hold for 29 min, resulting in 60 min total analysis time. The mobile phase flow rate was 0.5 mL/min. Oil samples were dissolved in HPLC grade hexane.

3.4 STATISTICAL ANALYSIS

All fractionation runs and analyses were carried out at least in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (Software Version 8.1. SAS Institute Inc., Cary, NC).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Fatty Acid Composition

The abbreviations used for oil samples were defined in Table 4.1. Fatty acid composition of WGO extracted and refined through various methods is shown in Table 4.2. Linoleic acid (18:2), which is an essential oil, consisted of 57% to 58% of the total fatty acids in WGO samples examined in this study. Although SFE WGO had significantly higher 18:2 content (59.7%) ($p<0.05$) than that of the other oils, the difference was not large enough to affect the oil quality for practical applications. Linoleic oil content of the soybean oil (54%) was significantly lower than the WGO oil. Palmitic, oleic and linolenic acids were also present in significant amounts in all the oils. Saturated fatty acid content of SFE WGO was lower (about 16%, w/w) than the other oils (>17%). Mono and polyunsaturated oil contents of SFE WGO were about 23% and 61%, w/w, respectively. Relatively lower saturated fatty acid and higher monounsaturated fatty acid content of SFE WGO indicates that SFE technique produces a product that is healthier and more stable as relative to the products obtained from conventional hexane extraction. The fatty acid composition of WGO reported in this study is in agreement with the data published in literature (Dunford 2005).

4.2 Free Fatty Acid Composition

Free fatty acid levels of WGO samples are given in Table 4.3. Hexane and SFE extracted WGO contained substantial amount of FFAs, 7.9% and 6.2%, respectively. Free fatty acids contribute to bitter and soapy flavor in foods hence they are undesirable in

edible oils. FFAs are removed during oil refining process. Both physical and chemical refining processes reduced FFAs content of the oil to 0.4%. Refined soybean oil had significantly lower FFA (0.03%) than that of the WGO. This result was expected because of two reasons: first, crude soybean oil contains only 1-2% FFA as compared to >6% FFA in WGO. The second reason is that a gentler refining is usually preferred for WGO to retain bioactive oil components in the final product.

4.3 Tocopherol Composition

Tocopherol compositions of WGO and soybean oil are listed in Table 4.4. All the WGO samples examined in this thesis contained significantly higher amounts of tocopherol than that of the refined soybean oil. The majority of the tocopherols in WGO was in the form of α-tocopherol (>90% of the total tocopherols). As expected WGO D showed the highest levels of tocopherols (109.7 mg/g) since it was a special product with high tocopherol content. β-Tocopherol was the second most abundant tocopherol in the WGO samples. SFE WGO contained significantly higher amount of tocopherols than those of the commercial WGO samples. γ-Tocopherol was the main isomer in soybean oil. Similar tocopherol contents and compositions were reported in the literature for WGO and soybean oil (Dunford 2005).

4.4 Phytosterol Composition

Phytosterol contents of oil samples are shown in Table 4.5. Hexane and SC-CO_2 extracted WGO contained similar amounts of total phytosterols (about 3.7 mg/g). Although refined WGO samples contained slightly lower phytosterol content than those

of the crude oil, differences were not statistically significant ($p>0.05$). Tocopherol enriched WGO (WGO D) and soybean oil had significantly higher and lower total phytosterol content, respectively, than both crude and refined WGO oils. β-Sitosterol was the most predominant (78-85% of the total phytosterols) phytosterol with campesterol being the second and stigmasterol being the least prevalent in all WGO samples. WGO D showed the highest levels of β-sitosterol (6.63 mg/g oil). SFE WGO also had significantly higher β-sitosterol amount (about 2.94 mg/g) than the other WGO samples except WGO D. Very high campesterol and β-sitosterol contents of WGO D indicate that the process used for tocopherol concentration also results in phytosterol enrichment in the final product. Campesterol (0.61mg/g) concentration of SFE WGO was similar to that of hexane extracted crude WGO (0.673 mg/g). However, SFE WGO did display the lowest level of stigmasterol of all the WGO samples. These differences might be due to processing techniques used for extraction and refining processes and/or wheat varieties used for processing. All WGO samples tested in this study showed greater levels of phytosterols than soybean oil.

4.5 Phospholipid Composition

Hexane extracted Crude WGO (WGO A) contained the highest amount of phospholipids among the samples tested in this study (Table 4.6). However, total phospholipids content of crude WGO was lower than the literature values (Wang and Johnson 2001). This is due to the fact that WGO A was stored in a cold room until the chemical tests. Significant amount of precipitate was formed during the cold storage. WGO A was centrifuged and filtered prior to chemical characterization. Hence a

significant portion of the phospholipids and wax components were removed with the precipitate. SFE WGO did not contain any detectable amount of phospholipids. Similar results have been reported in the literature for oils extracted with SC-CO_2 (Dunford 2004b). This is due to the low solubility of phospholipids in SC-CO_2. Phospholipid contents of refined oils were either very low or below the detection levels because these compounds are removed from the crude oil during the refining process (degumming step). Extraction of vegetable oils with SC-CO_2 simplifies the refining process by eliminating degumming step. Total phosphatidylinositol (PI) + phosphatic acid (PA) contents of oil samples were given in this study because the HPLC method used for the analysis of phospholipids did not separate these two components on the analytical column. Commercial WGO contained high amounts of PI + PA (>60% of total phospholipids). It has been reported in the literature that high PA content in crude vegetable oils may be an indication of poor seed handling and extraction conditions (Wang and Johnson 2001). PA is a nonhydratable phospholipid and separation of this compound by water degumming is very difficult during the refining process. Although crude WGO contained about 20% phosphatidylcholine (PC), refined WGO samples did not have any detectable amount of PC indicating that refining process was very effective at removing this compound.

4.6. Moisture Content

Moisture contents of oil samples were analyzed by using Karl Fisher method (Table 4.7). Water content of oil samples is an interest because it has been reported in the literature that SC-CO_2 may extract significant amount of water depending on the

extraction conditions and the moisture content of the feed material (Dunford and Temelli 1996; Dunford and others 1998). As can be seen in Table 4.7, SFE WGO had significantly higher moisture content than those of the commercial WGO including hexane extracted crude WGO. These results are in agreement with data reported in literature. During the industrial scale SC-CO_2 extraction of vegetable oils water would be separated in a high pressure separator prior to precipitation of lipids from CO_2.

4.7 CHARACTERIZATION OF EXTRACTS FROM SUPERCRITICAL FLUID FRACTIONATION PROCESS

Both WGO A and SFE WGO were used for SFF experiments. Chemical characterization of the SFF products were carried out on products that were collected at 5^{th} and 6^{th} h of the fractionation experiments after steady state was achieved in the system. Small amount of SFF extract was collected during the fractionation runs (0.04 g extract/1 h from WGO A at 4 L/min CO_2 flow rate and 0.08 g extract/1 h from WGO A and 0.07 g extract/1 h from SFE WGO at 8 L/min CO_2 flow rate) due to very low CO_2 and oil flow rates. Increasing CO_2 flow rate from 4 L/min to 8 L/min doubled the amount of extract collected under the same fractionation conditions, 2000 psi, 80^{o}C and 0.3 mL/min oil flow rate indicating that SC-CO_2 was saturated under the experimental conditions. Although, further increase in solvent/feed ratio above 50:1 would improve the FFA removal from crude oils, higher CO_2 flow rates were beyond our SFF system limits. Policosanol content of feed material and SFF products were below the detection limits of the method used for analysis. Policosanol in WGO A was removed during the centrifugation process before the SFF experiments.

4.7.1 Free Fatty Acids

The main objectives of SFF process was deacidification of crude WGO (removal of FFA) using SC-CO_2. This thesis also examined the effect of SFF on the bioactive oil components. The fractionation conditions (2000 psi and 80^{o}C) were chosen based on the previous research studies carried out with other vegetable oils (Dunford and King 2004). It was shown that SC-CO_2 was able to remove FFAs from crude oil very effectively when a fractionation column was used for processing. The extract fractions from SFF process contained over 77% FFA (Table 4.3). Increasing solvent (CO_2)/feed (oil) ratio from 25/1 to 50/1 did not improve the FFA removal efficiency significantly. This might be due to higher selectivity of SC-CO_2 for other oil components under the chosen processing conditions. The process efficiency can be improved by lowering the system pressure, i.e. 1500 psi. However, lower system pressure results in reduced extract amount. In this thesis our preference was to collect enough extract for chemical characterization of the product rather than maximizing FFA removal. Process optimization for SFF of WGO will be the subject of another research project.

4.7.2 Lipid and Phytosterol Compositions

Oil samples were analyzed for their free phytosterol, fatty acid esters of phystosterols, FFAs and triglyceride concentrations before and after SFF process (Table 4.8). FFAs consisted >90% of the lipids in the SFF extracts (fractions collected from the top of the column). Triglyceride content of the extracts was very low, about 2%, indicating that SFF process was very affective in removing FFAs while retaining triglycerides. Phytosterol contents (both free and fatty acid esters of phytosterols) of SFF

extracts were lower (2-4%) than that of the feed material (8-10%). These results indicate that phytosterols were retained in the raffinate fraction (product collected from the bottom of the column) with triglycerides. Higher solvent/feed ratio was beneficial for retaining phytosterols in the final product while removing FFAs with the extract fraction (Table 4.8). Increasing solvent/feed ratio from 25:1 to 50:1 resulted in significant reduction in phytosterol (both free and fatty acid esters) loss in the extract. Although there were statistically significant differences among the lipid and phytosterol compositions of SFF products from hexane and SFE extracted oils, the differences were not large enough to support a specific trend.

4.7.3 Tocopherols

Tocopherol compositions of extracts collected during SFF are shown in Table 4.4. SFF extracts contained very low amounts of tocopherols (0.05 mg/g). These results indicate that tocopherols were retained with the triglycerides in the raffinate fraction. It appears that solvent/feed ratio did not have a significant effect on the extract tocopherol concentrations. Compositions of hexane and SFE WGO were similar, containing very low amount of α-tocopherol and no γ- and β-tocopherols.

CONCLUSIONS

This study examined pilot scale extraction and fractionation of WGO utilizing SC-CO_2 as a solvent. The oil fractionation was carried out by using a pilot scale packed column. To best of our knowledge, this is the first study reporting SFF of WGO in the literature. Chemical composition of WGO products obtained from SFE and SFF processes were compared to those of commercial products. Soybean oil was used as a reference to demonstrate the potential of WGO oil as a functional food/oil containing a number of bioactive compounds with health benefits. This research study clearly demonstrated that both commercial and SFE WGO were rich in tocopherols and phytosterols and superior to soybean oil. It was also confirmed that nutritional composition of SFE WGO was at least similar (in some cases better) to that of the hexane extracted oil. SFE WGO does not contain phospholipids hence further refining of this product is simplified by elimination of degumming step. Furthermore, SFE WGO can be considered as high purity because of the lack solvent residues in the product. SFE WGO contained significantly higher amount of moisture than that of the hexane extracted oil. Moisture in the SFE WGO can easily be separated in a separator before precipitation of oil from CO_2 in a second vessel. This study also illustrated that SFF process was a viable process to remove FFAs efficiently from both hexane and SFE extracted WGO while retaining bioactive oil components in the final product. Wheat is a major commodity throughout the central plains states and with growing interest in nutritional health and functionality of foods, wheat germ may soon become not simply a by-product of wheat milling, but a high value specialty product itself.

FUTURE RESEARCH

The focus of this study was to evaluate the potential of SFE and SFF for WGO extraction and refining. Chemical compositions of SFE and SFF products were compared with commercial oils to evaluate the nutritional quality of the oils. SFE and SFF processes need to be optimized for WGO extraction and refining including retentions of the compounds of interest before they can be applied to industrial scale operations. Determination of economic feasibility of SFE and SFF of WGO requires further research on current WGO market supply and demand trends and equipment costs. It is also important that efficacy of WGO products enriched in bioactive compounds, such as tocopherols and phytosterols, in treating certain diseases and maintenance of good health is studied by using cell cultures, animal studies and clinical studies. Screening of various wheat varieties for their oil content and composition of bioactive compounds would help to choose a specific variety for a desired application.

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Table 4.1: Definition of the abbreviations used for wheat germ oil (WGO) samples processed utilizing different extraction and refining techniques.

Abbreviation	Sample Description
WGO A	Commercial hexane extracted crude wheat germ oil
WGO B	Commercial hexane extracted and refined (conventional chemical refining) wheat germ oil
WGO C	Commercial hexane extracted, and refined (physical refining, molecular distillation)
WGO D	Commercial hexane extracted, refined and concentrated in tocopherols
SFE WGO	Oil obtained from supercritical carbon dioxide (SC-CO_2) extraction of wheat germ at 80˚C and 10,000 psi
Soybean oil	Commercial hexane extracted and refined soybean oil
SFF 1	WGO A fractionated using supercritical fractionation (SFF) technique at 2000 psi, 80^{o}C and 25:1 solvent to feed ratio (extract)
SFF 2	WGO A fractionated using supercritical fractionation (SFF) technique at 2000 psi, 80^{o}C and 50:1 solvent to feed ratio (extract)
SFF 3	SFE WGO fractionated using supercritical fractionation (SFF) technique at 2000 psi, 80^{o}C and 50:1 solvent to feed ratio (extract)

Table 4.2: Fatty acid composition (%, w/w) of WGO samples extracted and refined through various methods[1] as compared to soybean oil.

Fatty Acid[2]	WGO A	WGO B	WGO C	WGO D	SFE WGO	Soybean Oil
14:0	0.09^{b}	0.09^{bc}	0.09^{bc}	0.23^{a}	0.09^{bc}	0.08^{c}
16:0	16.7^{a}	15.8^{b}	16.8^{a}	16.9^{a}	16.8^{a}	10.7^{c}
16:1	0.18^{b}	0.17^{b}	0.16^{b}	0.16^{a}	0.15^{c}	0.09^{d}
18:0	0.77^{b}	0.72^{b}	0.72^{b}	0.68^{b}	0.5^{c}	4.56^{a}
18:1	16.9^{d}	15.8^{c}	15.9^{c}	15.2^{b}	13.6^{e}	22.1^{a}
18:2	57.6^{b}	58.4^{b}	57.7^{c}	58.1^{d}	59.7^{a}	54.0^{e}
20:0	0.19^{c}	0.17^{c}	0.16^{bc}	0.15^{d}	0.11^{d}	0.36^{a}
20:1	1.7^{c}	1.6^{b}	1.6^{bc}	1.52^{a}	1.45^{d}	0.46^{e}
18:3	6.4^{b}	6.7^{b}	6.5^{c}	6.9^{d}	7.3^{a}	7.2^{a}
22:0	0.11^{b}	0.11^{b}	0.11^{b}	0.11^{b}	0.78^{c}	0.36^{a}
22:1	0.28^{bc}	0.27^{a}	0.26^{ab}	0.26^{ab}	0.23^{c}	0^{d}
24:0	0.10^{b}	0.10^{b}	0.10^{b}	0.09^{a}	0.06^{c}	0.12^{a}

[1] See Table 4.1 for the abbreviations.
[2] See the Nomenclatures section of this thesis for naming of the fatty acids (page XVI).
^{a,b,c,d,e}Means in the same row with the same letter are not significantly different at $p>0.05$.

Table 4.3: Free fatty acid composition (FFA) of WGO processed through various methods[1].

Sample	FFA (%, w/w)
WGO A	7.9[b]
WGO B	0.4[c]
WGO C	1.1[c]
WGO D	0.4[c]
SFE WGO	6.2[b]
Soybean oil	0.03[c]
SFF 1	77.9[a]
SFF 2	78.0[a]
SFF 3	78.7[a]

[1] See Table 4.1 for the abbreviations.
[a,b,c]Means in the same row with the same letter are not significantly different at $p>0.05$.

Table 4.4: Tocopherol compositions (mg/g oil) of WGO extracted and refined through various methods[1].

Sample	α-Tocopherol	β-Tocopherol	γ-Tocopherol
WGO A	13.9[c]	1.1[b]	0.08[cd]
WGO B	4.9[d]	0.70[c]	0.1[c]
WGO C	7.3[d]	0.6[c]	0.07[dc]
WGO D	109.7[a]	6.8[a]	0.7[a]
SFE WGO	25.6[b]	1.2[b]	0.06[d]
Soybean oil	n.d.	n.d.	0.2[b]
SFF 1	0.05[e]	n.d.	n.d.
SFF 2	0.05[e]	n.d.	n.d.
SFF 3	0.04[e]	n.d.	n.d.

[1]See Table 4.1 for the abbreviations.
n.d. not detected.
[a,b,c,d]Means in the same column with the same letter are not significantly different at $p>0.05$.

Table 4.5: Phytosterol compositions (mg/g oil) of WGO extracted and refined through various methods[1]

Samples	Campesterol	Stigmasterol	β-Sitosterol	Total Phytosterol
WGO A	0.67[b]	0.25[b]	2.77[b]	3.70[b]
WGO B	0.63[b]	0.21[c]	2.59[b]	3.05[b]
WGO C	0.52[c]	0.21[c]	2.70[b]	3.44[b]
WGO D	1.74[a]	n.d.	6.27[a]	8.01[a]
SFE WGO	0.61[bc]	0.20[d]	2.94[b]	3.75[b]
Soybean oil	0.034[d]	0.28[a]	0.29[c]	0.60[c]

[1]See Table 4.1 for the abbreviations.
n.d. not detected
[a,b,c,d]Means in the same column with the same letter are not significantly different at $p>0.05$.

Table 4.6: Phospholipid compositions (mg/g oil) of WGO extracted and refined through various methods[1].

Samples	PE[2]	PI+PA[3]	PS[4]	PC[5]
WGO A	3.5[a]	12.1[a]	3.3	0.9
WGO B	1.9[c]	0.6[b]	n.d.	n.d.
WGO C	2.1[b]	n.d.	n.d.	n.d.
WGO D	n.d.	n.d.	n.d.	n.d.
SFE WGO	n.d.	n.d.	n.d.	n.d.
Soybean oil	n.d.	n.d.	n.d.	n.d.

[1]See Table 4.1 for the abbreviations.
[2]PE: phosphatidylethanolamine; [3]PI+PA: phosphatidylinositol and phosphatic acid; [4]PS: phosphatidylserine; [5]PC phosphatidylcholine
n.d. not detected.
[a,b,c,]Means in the same column with the same letter are not significantly different at $p>0.05$.

Table 4.7: Water content of WGO extracted and refined through various methods[1].

Sample	Water content (%, w/w)
WGO A	0.49[b]
WGO B	0.06[c]
WGO C	0.07[c]
WGO D	0.04[c]
SFE WGO	4.4[a]
Soybean oil	0.05[d]

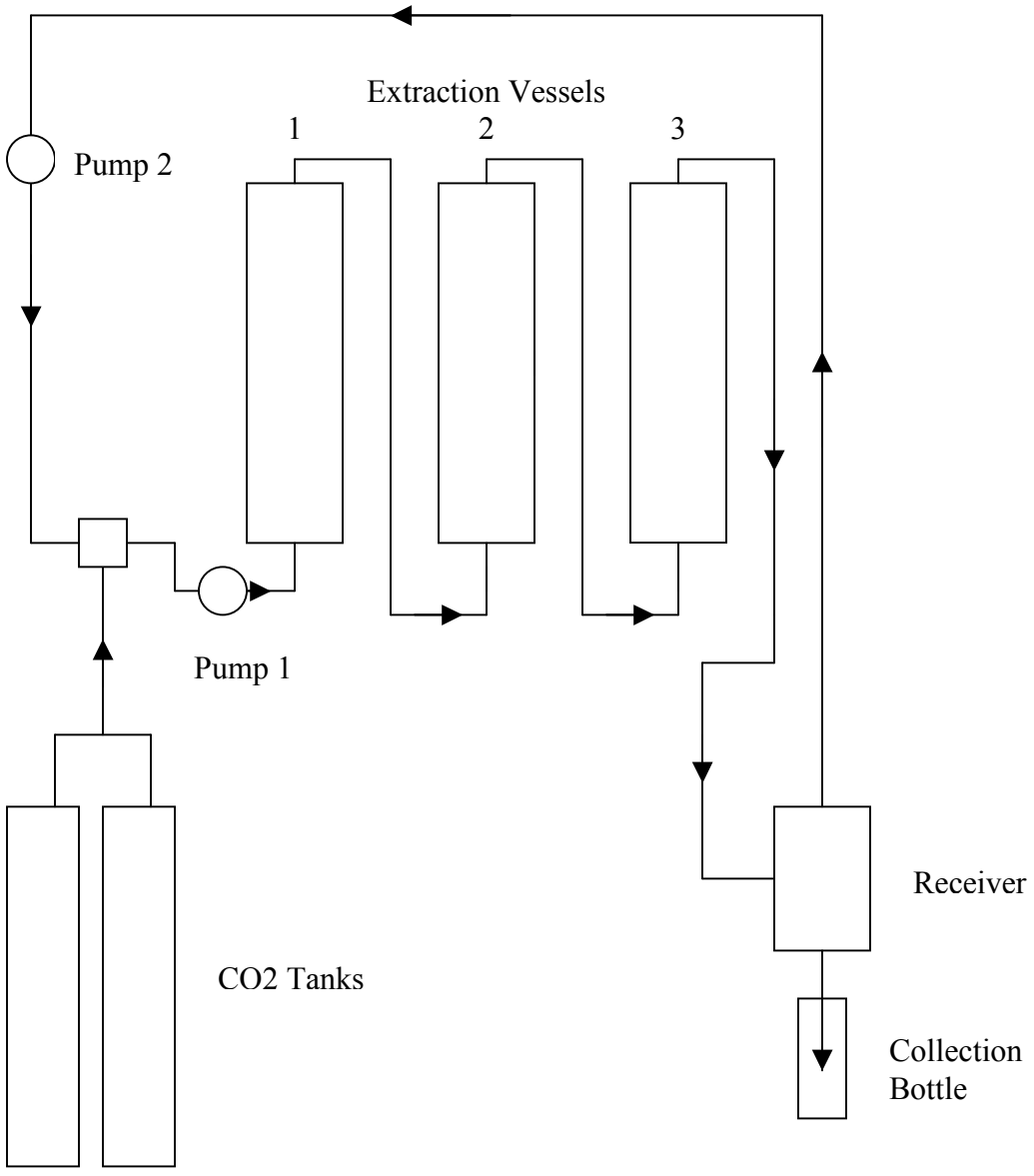
[1]See Table 4.1 for the abbreviations.
[a,b,c,d]Means in the same column with the same letter are not significantly different at $p>0.05$.

Table 4.8: Lipid and phytosterol compositions of wheat germ oil samples[1] (HPLC area %) before and after supercritical fluid fractionation process.

Lipid/Phytosterol	WGO A	SFE WGO	SFF 1	SFF 2	SFF 3
Phytosterol Esters	8.4[b]	10.1[a]	3.7[c]	2.2[d]	3.2[c]
Triglycerides	79.5[b]	81.5[a]	2.4[c]	2.7[c]	2.1[c]
Free Fatty Acids	11.4[c]	7.7[d]	92.7[b]	94.6[a]	93.9[a]
Free Sterols	0.8[b]	0.7[bc]	1.2[a]	0.5[c]	0.8[b]

[1]See Table 4.1 for the abbreviations.
[a,b,c,d]Means in the same row with the same letter are not significantly different at $p>0.05$.

Figure 1: A schematic diagram of the supercritical fluid extraction unit.



Picture 1: Supercritical fluid extraction unit.

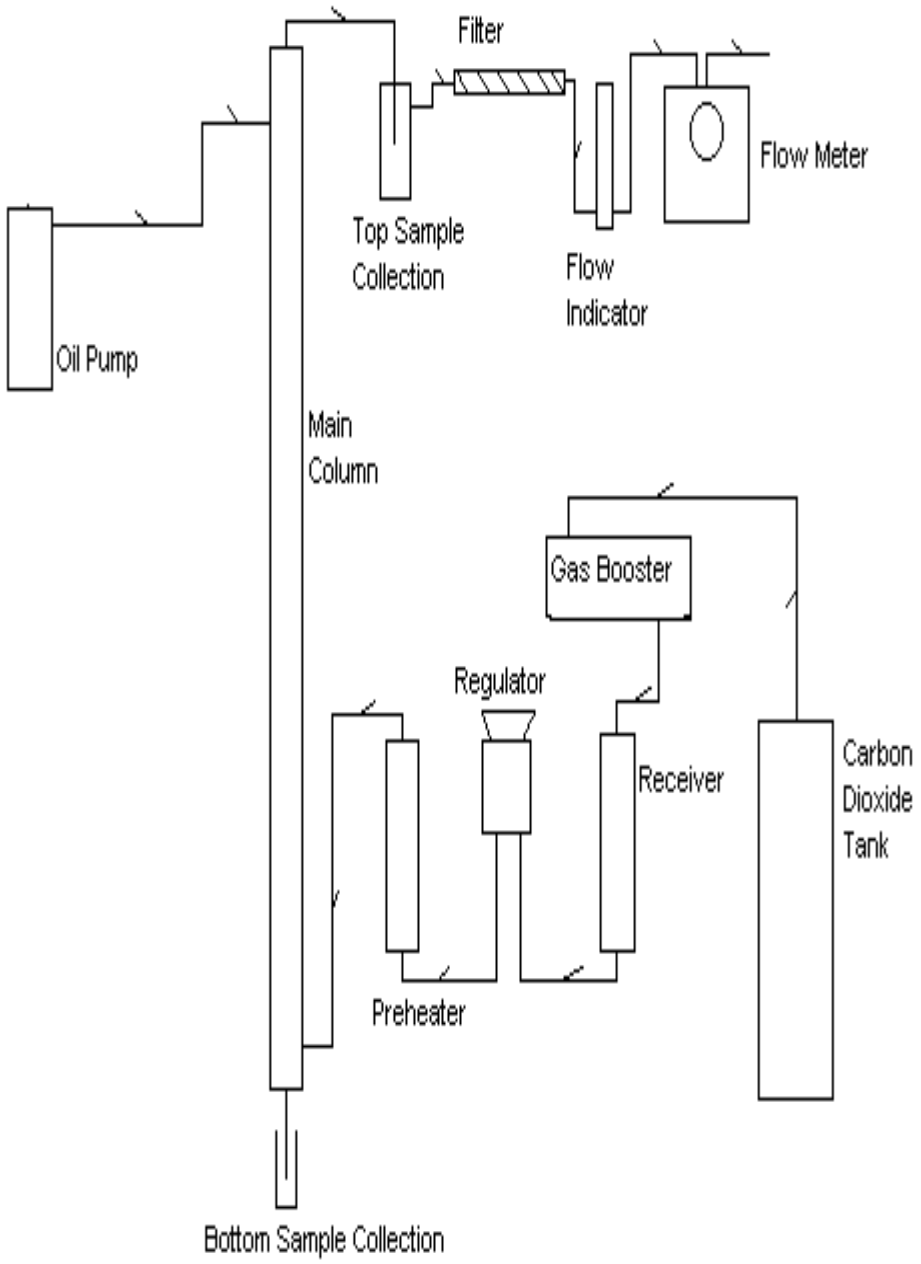
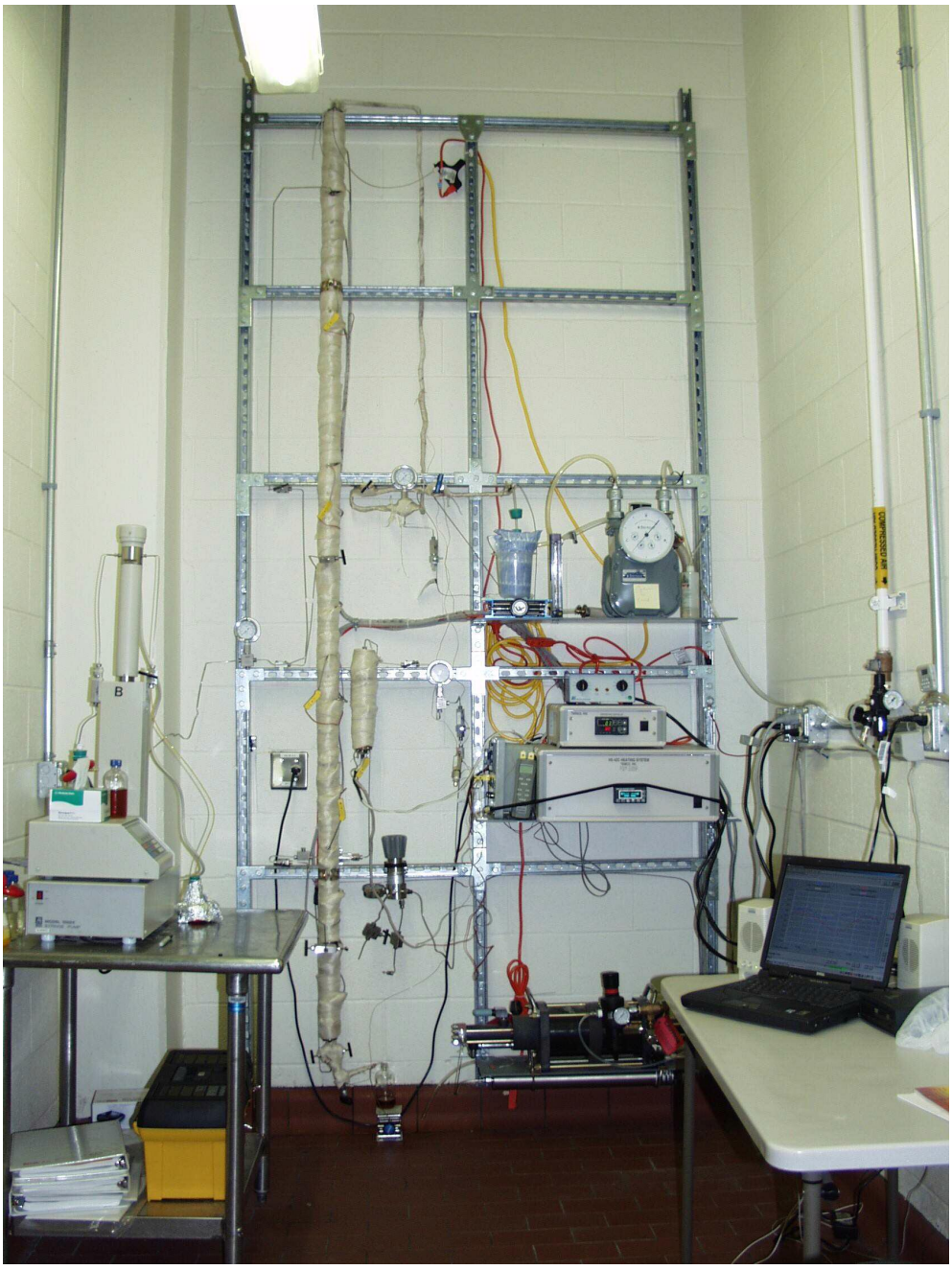


Figure 2: A schematic diagram of supercritical fluid fractionation unit.

Picture 2: Supercritical fluid fractionation unit.



APPENDIX I

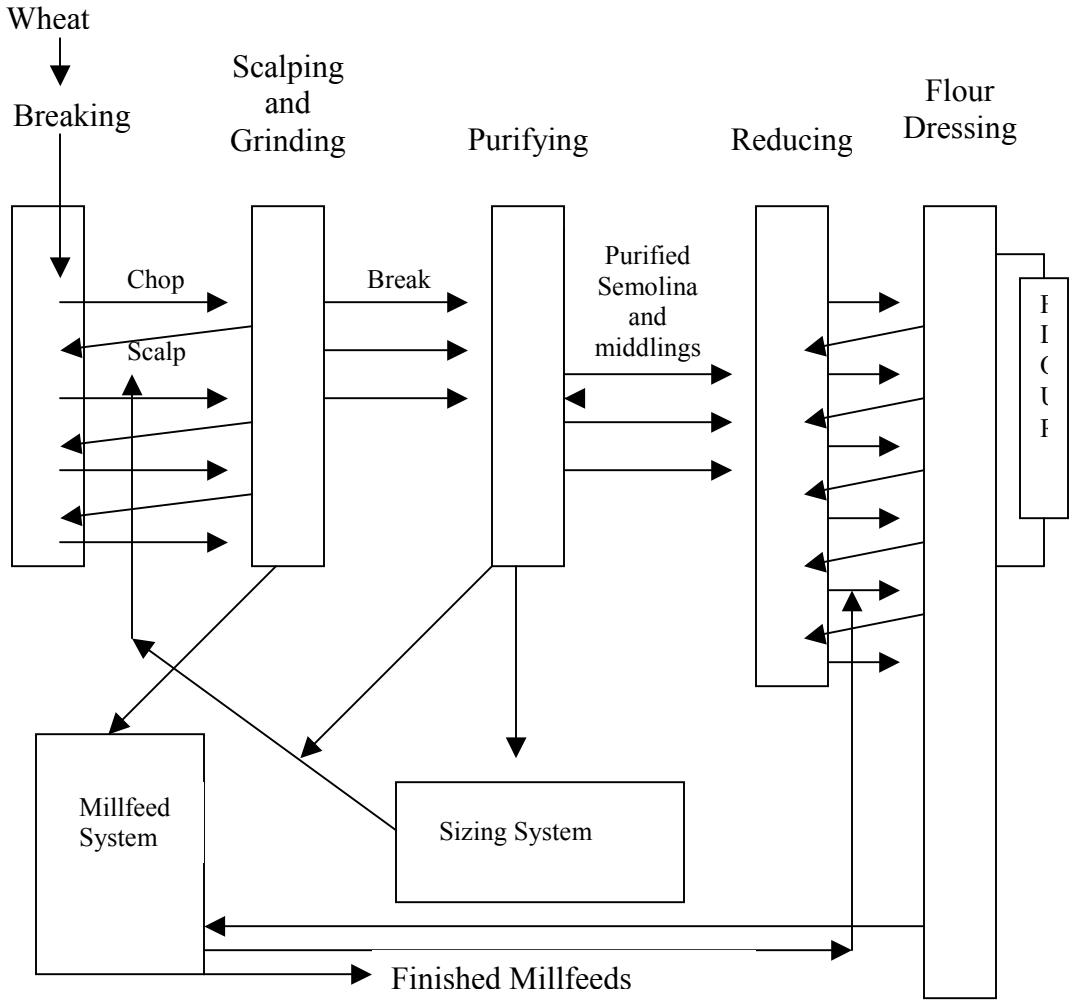


Figure 1. Simplified flow diagram of a flour mill (adapted from Atwell, 2001).

APPENDIX II

Table 1: Effect of solvent type and extraction method on wheat germ oil lipid composition (adapted from Barnes, 1982).

Extraction Method	Non-Polar Lipids kg/g (% of total lipids)	Glycolipids kg/g (% of total lipids)	Phospholipids kg/g (% of total lipids)
Chloroform-Methanol	254 (83.7)	6 (2.0)	44 (14.3)
Benzene-ethanol-water	67 (77.0)	4 (6.2)	11 (16.8)
Water, chloroform, methanol mix	104 (97.9)	1 (0.8)	1 (1.4)
Ethanol-diethyl ether	75 (89.8)	-	8 (10.2)
Butanol	91 (82.6)	19 (17.4)	
Hexane	93 (91.7)	8 (8.3)	
Pressed	(99.0)	(1.0)	

VITA

Michael Joseph Eisenmenger

Candidate for the Degree of

Master of Science

Thesis: SUPERCRITICAL FLUID EXTRACTION, FRACTIONATION, AND CHARACTERIZATION OF WHEAT GERM OIL

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Title of Study: SUPERCRITICAL FLUID EXTRACTION, FRACTIONATION, AND CHARACTERIZATION OF WHEAT GERM OIL

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Major Field: Food Science

Scope and Method of Study: The purpose of this study was to examine the ability of supercritical fluid technology to extract and fractionate wheat germ oil.

Findings and Conclusions: Supercritical carbon dioxide extraction yield was similar to that of conventional hexane extraction product. Supercritical fluid fractionation technique was effective in removing undesirable free fatty acids from crude wheat germ oil while retaining bioactive components.

ADVISER'S APPROVAL: Dr Nurhan Dunford