DEVELOPMENT OF A TWO-STEP EXTRACTION PROCESS FOR WHEAT GERM OIL RECOVERY

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DEVELOPMENT OF A TWO-STEP EXTRACTION

PROCESS FOR WHEAT GERM OIL RECOVERY

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Title of Study: DEVELOPMENT OF A TWO-STEP EXTRACTION PROCESS FOR WHEAT GERM OIL RECOVERY

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Scope and Method of Study: The main objective of this study was to optimize the mechanical extraction process parameters (cage temperature, germ pretreatment, shaft speed, back pressure and shaft arrangement) to increase wheat germ oil (WGO) yield without compromising oil quality. A two-step process: pre-pressing of wheat germ using a screw press followed by extraction of residual oil from the pressed cake by aqueous and aqueous enzymatic extraction techniques was also examined. In addition, mechanically extracted WGO quality was compared to that of hexane and supercritical CO_2 extracted oil by analyzing the free fatty acid (FFA), peroxide value (PV), *p*- anisidine value (AV), water content, phosphorus, tocopherols and phospholipids content.

Finding and Conclusion: The highest oil yield from the screw press, about 47.7%, was obtained under the following conditions: severe shaft arrangement, cage temperature of 107 ^{o}C, germ pretreatment at 82 ^{o}C, high back pressure, and shaft speed at 400 rpm. The aqueous extraction of wheat germ (WG) cake with boric acid–NaOH (pH 8) buffer using fine particle size at liquid solid ratio (LSR) of 20 and extraction time of 0.5 h resulted in the highest oil yield, 79.64%. The enzymatic extraction of WG cake with Alcalase 2.4L FG at LSR of 16.5, enzyme concentration of 4%, and extraction time of 5.25 h resulted in 76.7% oil yield which was slightly lower than the aqueous extraction. Mechanically extracted oil had better quality (lower FFA, PV, and AV values and higher α-tocopherol content) than that of the commercially hexane-extracted oil. A two-step process involving mechanical pressing of full fat WG followed by aqueous extraction of the residual cake from the mechanical press would result in 90% WGO recovery. This process can be an environmentally benign alternative to hexane extraction.

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NOMENCLATURE

ANOVA	Analysis of variance
CO2	Carbon dioxide
ELSD	Evaporative light-scattering detector
FAME	Fatty acid methyl esters
GC	Gas chromatography
HPLC	High-performance liquid chromatography
LSR	Liquid solid ratio
MPa	Mega pascal
PA	Phosphatidic acid
PDA	Photo diode array detector
PC	Phosphatidylcholine
PE	Phosphatidylethanolamin
PI	Phosphatidylinositol
v/v	Volume/volume
WG	Wheat germ
WGO	Wheat germ oil

w/w	Weight/weight
w.b.	Wet basis
Units	
%	percentage
^{o}C	degree centigrade
g	gram
h	Hour
mg	Milligram
mL	Milliliter
min	Minutes
mm	Millimeter
ppm	Parts per million
rpm	Rotation per minute
× g	Times gravity

CHAPTER I

INTRODUCTION

1.1 PROBLEM STATEMENT

Wheat germ, a by-product of the milling industry, is a unique source of highly concentrated nutrients such as α-tocopherol. Wheat germ contains about 10% oil or more, depending on the degree of contamination with bran and flour. The conventional wheat germ oil extraction method utilizes hexane as a solvent. This method has a yield higher than 95%, but hexane has been shown to be an environmental pollutant. Also, hexane is a flammable solvent, which can create an unsafe environment for the workers in the plants. Other alternative methods used to extract oil from wheat germ are supercritical fluid extraction, aqueous extraction and aqueous enzymatic extraction and mechanical pressing. Supercritical fluid extraction is environmentally friendly and does not leave solvent residue in the oil. However, the capital cost of setting up the system is quite high. Aqueous and aqueous enzymatic extractions are also environmentally benign processes which allow simultaneous recovery of oil and protein, but the main limitation of aqueous extraction is its low oil recovery efficiency. Enzymatic extraction requires utilization of expensive enzymes. Mechanical oil pressing utilizes mechanical pressure to force oil out of the germ. As a result, the final product can be considered natural and free of solvent residue.

In addition, the capital and operating costs of mechanical extraction are less than those for the solvent extraction method. Unfortunately, the oil yield from mechanical extraction is low. Only a small fraction of the available oil can be recovered mechanically. There is a need for an environmentally benign process for wheat germ oil extraction that enhances oil recovery and overcomes the disadvantages of existing extraction techniques.

1.2 HYPOTHESIS

Optimization of an environmentally benign wheat germ oil extraction process produces a high quality product with high oil yield.

1.3 OBJECTIVES

The main goal of this study is to optimize the mechanical extraction process parameters increasing wheat germ oil yield without compromising oil quality. The study will also investigate the potential of a two-step process: pre-pressing of wheat germ using a screw press followed by extraction of residual oil from the pressed cake by aqueous and aqueous enzymatic techniques. The specific objectives are as follows:

i) to study the effects of processing parameters (cage temperature, germ pretreatment, shaft speed, back pressure and shaft arrangement) on mechanical oil extraction yield.

ii) to optimize the process for maximum oil extraction yield.

iii) to examine the quality of mechanically extracted wheat germ oil from this study and compare it to that of the commercially hexane and supercritical CO_2 extracted oil.

iv) to examine the potential of a two-step process to improve wheat germ oil yield over mechanical pressing.

CHAPTER II

LITERATURE REVIEW

2.1 WHEAT

Wheat grain is usually between 5 and 9 mm in length and weighs between 35 and 50 mg (Šramková and others 2009). Wheat grains mainly contain carbohydrates (65-75% starch and fiber), proteins (7-12%), lipids (2-6%), water (12-14%), and micronutrients (Hemery and others 2007). The outer protective layers of the wheat kernel are called bran (Figure 1). Bran comprises about 14% of the kernel by weight. It is high in fiber and mineral content. Germ comprises only about 3% of the kernel. Most of the lipids and many of the essential nutrients in the kernel are concentrated in the germ. The remaining inner portion of the kernel (83% of the grain), endosperm, has high starch and moderately high protein content and provides energy and protein for the developing wheat plant (Atwell 2001).

Germ is composed of the embryo (1.2% by weight of the grain), which develops into the first roots and shoot of the new plant and scutellum (1.5% by weight of the grain), a layer of tissue lying between the embryo and the endosperm (Barnes 1982).

Commercial wheat germ composition is as follows: 6% moisture, 26% protein, 10% oil, 4% ash, 20% starch, 3% crude fiber, and 15% other substances (Barnes 1982). Wheat germ is considered a by-product of the wheat milling industry. It is one of the richest natural sources of α- tocopherol. Moreover, wheat germ is also rich in lysine, riboflavin, and thiamine (İbanoglu 2002). Because of its high content of polyunsaturated fatty acids and bioactive compounds, wheat germ processing presents challenges. These bioactive compounds are prone to oxidation and degradation under the conditions used for conventional extraction and refining methods (Dunford and Zhang 2003).

2.1.1 WHEAT MILLING

During wheat milling, about 70 to 75% of the grain becomes flour, and the remaining 25 to 30% is available as by-products which are commonly used as livestock feed (Blasi and others 1998). Milling is the separation of the bran and germ from the endosperm and the reduction of the endosperm to flour. This process is done by a sequence of breaking, grinding, and separation operations (Pomeranz 1988). During milling, the protective cell layers in grain cell structure are destroyed and the vitamins and polyunsaturated fatty acids are exposed to oxidation. Therefore, the germ is removed to provide white flour with a long shelf life. During the white flour milling process, bran is also removed. This process profoundly changes the baking properties and the taste of flour (Brandt and others 2005). Three steps are involved in the milling process: cleaning, tempering, and milling. Cleaning starts with screening to remove coarse and fine unwanted materials. In this step, grain is separated by size, shape, and weight (Pomeranz 1988; Blasi and others 1998). Conditioning or tempering is the addition of water to the cleaned wheat to increase its moisture content to about 15%. Then the grain is allowed to

stand for 2-24 h. The objective of conditioning is to toughen the bran coat and soften the endosperm so that large flakes can be removed during the milling step (Pomeranz 1988; Blasi and others 1998). At this point, the wheat is ready for milling. The milling step involves grinding the grain and fractionating wheat components of specific sizes (Atwell 2001). The grain is crushed gradually through the shearing action of four to six pairs of breaker rolls. The fines from each pair of breaker rolls are sifted, and the coarsest particles are transferred to successive breaker rolls. The germ and bran are removed by sieving or air separation (Blasi and others 1998).

2.2 CHEMICAL COMPOSITION OF WHEAT GERM OIL

Wheat germ oil (WGO) is a specialty product with high nutritional value. It has a number of nutritional and health benefits like reducing plasma and liver cholesterol levels, improving physical endurance/ fitness and delaying aging (Kahlon 1989). All these effects are due to the high concentration of bioactive compounds present in the oil. In addition, WGO is one of the richest natural sources of α-tocopherol (Eisenmenger and Dunford 2008). The major fatty acid in WGO is 18:2 (linoleic acid), which represents about 60% of the total fatty acids. About 80% of the total fatty acids are unsaturated. Palmitic acid comprises most of the saturated fatty acids and the content of stearic acid is below 2% (Barnes 1982). Eisenmenger and Dunford (2008) found that linoleic acid (18:2) consisted of 57-58% of the total fatty acid in commercial WGO. Also, supercritical-fluid-extracted WGO had slightly higher linoleic acid content (59.7%) than the commercially extracted and refined WGO. The difference might be due to the variations in the composition of wheat germ used for supercritical fluid extraction and commercial extraction. Tocopherols and tocotrienols are also referred to as tocols and

vitamin E. They are a group of fat-soluble antioxidants which are formed of a chromanol ring and a hydrophobic side chain. Individual tocopherols (α-, β-,γ - and δ-tocopherol) and the corresponding tocotrienols differ by number and positions of methyl substituents on the phenolic part of the chromanol. Tocopherols function as antioxidants by breaking up the radical formation chain reactions proceeding during oxidation in membranes as well as in foods. Because of their antioxidant properties at the molecular level, tocopherols are believed to reduce the risk of cardiovascular diseases and of certain types of cancer (Schwartz and others 2008). Eisenmenger and Dunford (2008) reported that supercritical fluid extracted WGO contained a significantly higher amount of tocopherols than those of the commercial WGO samples. This might be partly due to the compositional variations in wheat germ used for those studies. The majority of the tocopherols in WGO were in the form of α- tocopherol (90% of the total tocopherols). β-Tocopherol was the second most abundant tocopherol in the WGO samples. Piras and others (2009) reported that there was no difference in α- tocopherol content among oil samples obtained by different extraction procedures (supercritical CO_2, organic solvent extraction included hexane, and chloroform- methanol), except for oil from methanol extraction, which had the lowest amount of α- tocopherol.

Policosanols are a group of bioactive compounds found in WGO that are composed of a mixture of long chain (C24-C34) primary alcohols. Originally, they were isolated from sugar cane (Lin and others 2004). A study carried out by Irmak and others (2006) found that the solid fraction that precipitated at the bottom of the container containing crude WGO stored in refrigerated conditions had the highest amount of total policosanol among the extracts of wheat milling products (straw, bran, and germ). The

policosanol content of the clear WGO (oil above the precipitate) was lower than that of the WGO-solids/precipitate. This result was expected since policosanols belong to a group of compounds known as wax, which precipitates out of the crude oil during cold storage. Lin and others (2004) reported that the wheat germ policosanols consist of 8% hexacosanol, 67% octacosanol, 12% triacosanol, and 13% other long-chain alcohols. The composition of sugar cane policosanols is similar to that of wheat germ.

Phytosterols are also bioactive compounds present in WGO. They are cholesterol-like molecules found in highest concentrations in vegetable oils. Phytosterols lower serum low density lipoprotein (LDL) levels and are associated with a decreased risk of coronary heart disease (Ostlund 2002). Wheat germ oil contains significantly higher amounts of phytosterol than other common commercial oils (Eisenmenger and Dunford 2008). Dunford and others (2009) reported that wheat germ was a better source of phytosterol than wheat straw and bran because it has higher oil content than straw and bran and phytosterols are associated with oil. Hexane and supercritical fluid extracted WGO contained similar amounts of total phytosterols (about 3.7 mg/g oil) (Eisenmenger and Dunford 2008). β -Sitosterol was the most prominent (78–85% of the total phytosterols) while campesterol and stigmasterol being the second and the least prevalent phytosterols in WGO, respectively.

2.3 EXTRACTION OF WHEAT GERM OIL

After the germ has been separated during the milling process, it is subjected to an oil extraction process. Five methods can be used to separate the oil from the wheat germ:

organic solvent extraction, mechanical pressing, aqueous, enzymatic, and supercritical fluid extraction (Dunford 2001).

2.3.1 MECHANICAL EXTRACTION

In mechanical pressing mechanical pressure forces the oil out of the germ. The efficiency of this method is low. The main advantages of mechanical extraction are that it produces solvent/chemical free oil and is a safe process (Khan and Hanna 1983). In addition, the process is relatively simple and not capital-intensive. Furthermore, the operating costs are less than the solvent extraction method (Bachmann 2001). In large-scale oilseed processing facilities, oil recovery from high oil content seeds (i.e. canola, sunflower) is done in two stages. The first step is pre-pressing. This process leaves about 15-20% of the oil in the pressed cake, which is then extracted with an organic solvent, hexane (Kemper 2005). The main advantage of pre-pressing is that the pressed cake formed from the flaked seeds allows good solvent contact and reduces the amount of solvent required for oil recovery (Unger 1990). The key to full pressing, also known as high pressure pressing, is to apply maximum pressure to the oilseeds to squeeze out as much oil as possible (Kemper 2005). Low oil content seeds such as soybeans are directly solvent extracted without prepressing.

An oilseed screw press (Figure 2) has a horizontal main worm shaft that carries the worm assembly. The worm shaft revolves within a barrel or cage which consists of axially placed bars (barrel bars) contained within a metal frame. The two halves of the cage are held together by clamping frames. The barrel bars are locked into the cage frames and spaced apart by spacers (Ward 1976). The thickness of the spacing between

barrel bars is set depending on the type and preparation of the oilseeds to be extracted; for example, for cottonseed expeller processing, the spacing of the bars in the main barrel may be 0.2 mm in the feed section, 0.19 mm the center section, and 0.2 mm the discharge section (Board 2002). The main worm shaft and worms are designed to exert a pressure of 69 to 207 MPa on the oilseed that is being processed and, at the same time, to convey the oilseed through and out of the pressure chamber. Different worm shaft configurations may be applied depending on whether the operation is a prepress or full press and the material used (Board 2002). The screw shaft is designed so that the diameter increases from the inlet to the outlet of the barrel while leaving still some clearance between the shaft and barrel so the meal can move through the barrel and come out at the end. This increase of the shaft diameter and decrease in the clearance between the shaft and the barrel presses the material against the barrel interior, thus releasing the oil (Jacobsen and Backer 1986). After the oil is separated from the oilseed, it passes through the barrel bars and is collected in a trough under the screw, and the cake that is too large to exit through the narrow openings on the barrel is extruded through the large openings at the end of the press (Schumacher 2007). The basic steps involved in processing of oilseed by mechanical pressing are shown in Figure 3.

The theory of mechanical oil extraction suggests that the oilseed cells must be ruptured by a combination of physical (crushing) and thermal (cooking) pretreatments before oil expression can occur. The process of mechanical oil extraction from oilseeds is started by applying pressure to the oilseed. For mathematical modeling purposes it is assumed that oilseed is contained within an envelope which retains the oilseed solids but allows oil to escape across the envelope. During mechanical pressing oilseed solids are

forced to consolidate with the pressure in the barrel while oil flows through the cell wall pores into the inter-kernel voids through which it flows until it passes through the retaining envelope. This process can be divided into the following components: oil flow through the cell wall pores; oil flow in the inter-kernel voids; and consolidation of the oilseed cake (Mrema and McNulty 1985). Khan (1984) investigated the effect of temperature (22 to 60 °C), moisture (7.5 to 12%), pressure (35 and 45 MPa) and time of pressing (240 and 600 s) on oil recovery from soybean flakes. The following model was developed ($R^2 = 0.95$):

$$Y=199.16 + 2.81\ T_P + 32.26\ m + 1.40\ P + 1.23\ t - 0.007\ T^2_p - 1.200\ m^2 - 0.143\ T_p\ m - 0.013\ T_p\ p + 0.005\ T\ t - 0.076\ m\ P$$

where: Y = oil recovery (%); T_p = press temperature (°C); m = moisture content of the seed (% wet basis) (w.b.); t = time of pressing (min).

Singh and others (1984) developed models to predict the residual oil content in several forms of sunflower seeds including whole, dehulled, coarse and fine ground samples. The effect of moisture content (6 to 14% w.b.), temperature (20 to 80 °C), pressure (14 to 70 MPa) and pressing time (4 to 10 min) were studied. The equations for each seed form were as follows:

Whole seed: ($R^2 = 0.95$)

$$RO = -77 + 13.8\ m + 0.25\ P + 0.47\ T - 0.35\ m^2 - 0.0038\ p^2 + 0.0020\ T^2 - 0.0056\ mT$$

where:

RO = residual oil left in the cake (%), T = seed temperature before pressing (°C), P = pressure applied during the pressing (MPa), M = moisture content of the seed (% w.b.), R= multiple correlation coefficient = 0.97.

Dehulled seed: (R^2= 0.86)

$RO = 23 + 4.6\ m – 2.3\ t + 0.17\ T – 0.18\ m^2 – 0.0008\ p^2 + 0.1\ t^2 + 0.006\ mP + 0.09\ mt - 0.013\ mt$

Coarsely ground seed: (R^2 = 0.98)

$RO = -70 + 11.5\ m + 0.26\ P + 1.5\ t + 0.53\ T – 0.347\ m^2 – 0.0025\ P^2 + 0.13\ t^2 – 0.0014\ T^2 – 0.038\ mT – 00014\ PT.$

Finely ground seed: (R^2 = 0.96)

$RO = -10 + 4.5\ m + 0.29\ P – 1.7\ t + 0.13\ m^2 – 0.001\ T^2 – 0.011\ mP + 0.11\ mt – 0.012\ mT – 0.012\ Pt – 0.002\ PT + 0.017\ Tt$

For the whole seed, the model revealed that moisture content was the most important factor affecting residual oil content in the cake. Duration of pressing had no effect on cake residual oil content, therefore it did not appear in the model. However, for coarsely ground seed, all the factors and their second degree terms were significant. Moisture- temperature and pressure- temperature interactions were also significant. For the finely ground seed, the model indicated that all the independent variables and their interactions were significant.

The effect of moisture content (4.5, 5.9, 10.4, and 15.2 %, w.b.), roasting duration (5, 10, 15 and 20 min) and temperature of roasting (70, 90, 110 and 130 ^oC) on oil recovery from palm kernel (*Elaeis guineensis* Jacq*)* were examined. The following mathematical model describing the oil yield was developed.

$OY=22.174 - 4.333M+ 1.336RD\ 0.294RT + 0.219M - 0.006094RD^2 + 0.0005652RT^2 + 0.\ 002837MxRD - 0.01917MxRT - 0.01073RDxRT$ ($R= 0.927$, $R^2 = 0.859$ $S =4.1273$)

where:

OY = Oil Yield %; M = Moisture Content (% w.b); RD = Roasting Duration (min.); RT = Roasting Temperature (^{o}C); R = Regression Coefficient; S = Standard Error of Estimate

Moisture content was the most significant factor affecting oil yield. The oil yield increased with decreasing moisture content of the kernel (Akinoso and others 2006). The studies discussed above indicate that processing parameters have different effect on oil recovery from different oilseeds.

2.3.1.1 FACTORS AFFECTING OIL YIELD IN MECHANICAL EXTRACTION

2.3.1.1.1 HEAT TREATMENT

The purpose of the heat treatment of seeds prior to pressing is several-fold. The first reason is to coagulate the proteins in the walls of the oil-containing cells and make the walls permeable to the flow of oil. In addition, the flow of oil is assisted by the lowered viscosity of the oil at elevated temperatures. Furthermore, seed cooking/heat treatment decreases the affinity of the oil for the solid surfaces of the seed and increases the oil extraction yield (Board 2002). The cooking process also destroys mold and bacteria and improves the microbiological as well as chemical quality of the cake. The optimum cooking temperature and time for most oilseeds range between 105 and 130℃ and 30 and 120 min, respectively. The optimum conditions for cooking oilseeds depend on the initial moisture content, chemical and biochemical characteristics of the seed, cooking method, and method of oil extraction. Normal cooking (105-130℃) of oilseeds has little effect on the oil and improves the cake properties. However, over-cooking produces oil and cake of a dark color (Shukla 1992). Jacobsen and Backer (1986) reported that heating before extraction doubled the seed processing capacity and oil

output from a screw press. In addition, preheating reduced the solids in the oil (foots). A study by Moreau and others (2005) showed that the maximum oil yield from dry-milled corn germ was obtained by cooking the germ at 180 ^{o}C for 6.5 min in a conventional oven and 4.5 min in a microwave oven at 1500 watts before pressing. Singh and others (2002) found that oil recovery from crambe seeds increased with increasing cooking temperature and time. The maximum oil recovery was found to be 75.9% for seeds cooked at 100 ^{o}C for 12 min, 70.9% for uncooked seeds and 70.6% for seeds cooked at 120 ^{o}C and 20 min. Mwithiga and Moriasi (2007) reported that the oil yield increased with an increase in soybean temperature and reached the highest yield at 75 ^{o}C. Further increase in soybean temperature resulted in a rapid decrease in oil yield. In contrast, Soetaredjo and others (2008) observed that seed preheating resulted in lower Neem oil yield. The yield of Neem oil yield decreased from 32% at 30 °C to 18% at 80 °C. Olaniyan (2010) studied the effects of several process conditions (shelled or in-shell, ground or whole, heating temperature, and pressing time) on castor bean oil yield from a mechanical press. Maximum oil yield, 41.67%, could be obtained when crushed beans were heated at 90 °C and mechanically expressed for 12 min while the minimum oil yield, 2.70%, was obtained from unshelled samples heated at 30 °C and mechanically expressed for 8 min. These results indicate that seed cooking temperature needs to be optimized to achieve high oil yields.

2.3.1.1.2 MOISTURE CONTENT OF THE SEED

Moisture content of the seed is another factor that can affect the affinity between the seed and the oil. This factor can be controlled during the cooking operation. Very dry seeds cannot be efficiently freed from oil. The optimum moisture for cooked seeds varies depending on the type of the seed and the method used for extraction. For example, 5-6% moisture content in cottonseed is the best for hydraulic pressing, while about 3% is the best for expellers or screw presses (Board 2002). If the moisture content is higher than the optimal, it results in slippage of the material in the press (Shukla 1992). A study by Vadke and Sosulski (1988) showed that maximum press throughput and oil output from canola seeds were achieved at 5% seed moisture content. Fasina and Ajibola (1989) found that the oil yield of conophor nuts at any pressure was dependent on the moisture content of the sample after heating. A high oil yield was obtained from conophor with moisture content between 8 and 10% after heating. For the melon seed, Ajibola and others (1990) observed that the highest expression efficiency of about 80% could be achieved at 5% moisture content. Moreau and others (2005) reported that maximum oil yield could be obtained at 3% moisture content of the dry milled corn germ. The residual oil content in the cuphea seed press cake significantly decreased as the moisture content of the cooked flaked seed decreased. The oil recovery increased from 79.4 to 83.6% as the cooked cuphea seed moisture content decreased from 5.5 to 3.1% (Evangelista and Cermak 2007). Martínez and others (2008) reported that the highest oil recovery from walnuts was obtained at 7.5% moisture content. For wheat germ, there is no study examining the effect of moisture on mechanical oil extraction yield.

2.3.1.1.3 SCREW PRESS SHAFT SPEED

Screw press shaft speed is one of the important factors that affect oil yield during mechanical pressing. Higher shaft speed means more throughput and higher residual oil content in the press cake because the higher speed reduces seed residence time in the press; thus there is less time for oil to flow out of seeds (Beerens 2007). A study by Vadke and Sosulski (1988) demonstrated that lowering the shaft speed increased the back pressure in the press cage and reduced throughput, and residual oil in the cake. Olayanju (2003) reported that the best oil and cake qualities for sesame seeds were obtained when oil was extracted at 45 rpm shaft speed. For peanuts, no more than 90 rpm was the optimum shaft speed for efficient oil extraction using a screw press (Oyinlola and others 2004). Oil recoveries from two accessions of beniseed increased as the shaft speed increased from 30 to 45 rpm (Olayanju and others 2006). Effect of shaft speed and its interaction with moisture content on oil recovery were significant. The effects of different shaft speeds (21, 54, 65, and 98 rpm), nozzle sizes (6, 10, and 12 mm), and diameters of the shaft (8, and 11 mm) on *Nigella sativa L* seeds were examined (Deli and others 2011). In the latter study, a cylinder press was used. In this type of press the press cake is extruded through a nozzle attached to the end of the cylinder. Nozzle diameter is one of the factors affecting the pressure level in the expeller. Pressure increases with decreasing nozzle size. The highest oil yield was obtained under the following conditions: 21 rpm shaft speed, shaft diameter of 8 mm, and nozzle size of 6 mm. The studies discussed above clearly demonstrate the importance of shaft speed on mechanical oil extraction yield. The effect of shaft speed on WGO extraction yield has not been reported.

2.3.1.1.4 BACK PRESSURE

The pressure necessary to force the oil out of the cooked seeds is generated by a continuously rotating shaft equipped with a choke mechanism which controls the cake thickness and the back pressure in the barrel. During pressing, oilseeds are fed into a hopper and then transported and crushed by a rotating screw. As the feed section of a screw press is filled the seeds are compressed and broken and air trapped in the cake voids is removed (Beerens 2007). In addition, the screw is designed so that the volume displacement at the feed end of the press is greater than at the discharge end. Therefore, when the material is conveyed from the feed end to the discharge end, the pressure increases and oil is expelled (Khan and Hanna 1983). The compression ratio of a press is defined as the volume displaced per revolution at the feed end of the screw divided by the volume displaced per revolution at the discharge end (Khan and Hanna 1983). The compression curve (Figure 4) is split into feed, ram, and plug sections. The maximum radial pressure is generated at the feed end of the ram section. The axial pressure follows the radial pressure up to the beginning of the plug section and then falls in the axial direction toward the discharge end (Khan and Hanna 1983). A study on the effect of pressure on the oil yield of melon seed found that the highest oil yield, about 41%, was obtained at an expression pressure of 25 MPa (Ajibola and others 1990). A study by Mwithiga and Moriasi (2007) reported that the oil yield increased with increasing pressure. The effect of hydraulic press pressure (0.5, 1.0, 1.5 MPa) on the oil extraction yield of palm oil was examined (Owolarafe and others 2007). It was shown that increasing the pressure from 0.5 to 1.5 MPa increased the oil yield from 18% to 30%.

For the Neem seed the optimum pressure for mechanical pressing was 34.5 MPa (Soetaredjo and others 2008). Since there is no study on the effect of back pressure on mechanical WGO extraction, further research is needed on this topic.

2.3.2 AQUEOUS EXTRACTION

The aqueous extraction process was suggested as an alternative to the oil extraction with organic solvents in the 1950s (Rosenthal and others 1996). The process is safe and inexpensive and allows simultaneous recovery of oil and protein from most oil-bearing materials (Cater and others 1974). The hot water flotation method for oil extraction is a traditional method used in the rural areas of most developing countries. The aqueous extraction process uses the same principle as hot water flotation. The process includes heat conditioning of the seed, grinding, extraction by boiling, oil recovery, and drying (Rosenthal and others 1996). The advantages of the aqueous extraction compared with the organic solvent process are simultaneous recovery of oil and protein in the same process, lower protein damage during extraction, and lower risk of fire and explosion. However, the main limitations of this process are low oil extraction efficiency, and de-emulsification requirements to recover oil when emulsion is formed (Rosenthal and others 1996). Aqueous extraction of oil and protein from different oilseeds has been studied by several researchers. The maximum oil yield from palm kernel was obtained at 20 min of grinding with a Waring Blendor at 60 ^{o}C, extraction temperature of 45 ^{o}C, and pH 7.0. Increasing the grinding time and extraction temperature did not improve the oil yield (Kim 1989). Wang and others (2008) found that 72.5% of free oil yield was obtained when aqueous extraction of peanuts was carried out at pH 8.0 and temperature of 60 ^{o}C for 8 h. The aqueous extraction of WGO was

examined by Xie (2010). The following process parameters were used to optimize the extraction process; three buffers (0.1 M Tris-HCl pH 8.0, 0.12 M boric acid-NaOH pH 8.0, and 0.15 M citric-phosphate pH 5.0), five liquid solid ratios (LSR) (4, 7.5, 12, 16.5, and 20), and five extraction times (ET) (0.5, 5.25, 12.25, 19.25, and 24 h). In boric acid-NaOH buffer at pH 8.0, the oil extraction yields ranged from 15.62 to 47.7%, both of which were obtained at ET of 0.5 h, and LSRs of 4 and 20, respectively. The range of the oil yield (3.97 - 48.07%) was broader when Tris-HCl buffer at pH 8.0 was used for WGO extraction. The lowest and highest yields were observed at LSR of 12, and ETs of 24 and 0.5 h, respectively. In citric-phosphate buffer at pH 5.0 oil yields ranged from 2.07 to 17.07%, which were obtained at LSR of 20 and ET of 0.5 h, and LSR of 4 and ET of 12.25 h, respectively. In addition, the response surfaces model predicted that the highest oil extraction yield (70%) would be achieved at LSR of 20 and ET of 0.5 h for both boric acid-NaOH and Tris-HCl buffers.

2.3.3 AQUEOUS ENZYMATIC EXTRACTION

Aqueous enzymatic oil extraction is an emerging technology in the fats and oil industry. It can be defined as "simultaneous recovery of oil and protein from oilseeds by treating finely ground seeds with enzyme in water and then separating the dispersion by centrifugation into oil, solid, and aqueous phases" (Sharma and others 2002). Aqueous enzymatic extraction offers many advantages compared to conventional extraction, such as the elimination of organic solvent use and the need for crude oil degumming, lower risk of fire and explosion, and non-toxicity of the solvent used. In addition, high quality end products are obtained. The main limitation of this process is high cost of enzymes used in the process (Rosenthal and others 1996).

The oil globules found inside plant cells are associated with proteins and a wide range of carbohydrates. The cell contents are surrounded by a thick cell wall, which has to be ruptured for the protein and oil to be released. Enzymes used in the process hydrolyze the complex lipoprotein and lipopolysaccharide molecules into simple molecules and break up cell walls (Bargale 1997). The basic step in the aqueous enzymatic process is mixing the ground seeds with water before the enzyme is added. At this step, maintaining the pH of the solution is important because proper pH helps in separating the oil and protein from the liquid or solid phase. The other steps involved in the aqueous enzymatic process include incubation with an enzyme, separation of liquid and solid phases by centrifugation or filtration, and recovery of oil from the liquid phase.

2.3.3.1 FACTORS AFFECTING OIL YIELD DURING AQUEOUS AND AQUEOUS ENZYMATIC EXTRACTION

2.3.3.1.1 PARTICLE SIZE REDUCTION

Grinding breaks down the walls of the oil-containing cells and leads to efficient extraction of oil and protein (Cater and others 1974). In addition, a small particle size gives a large surface area, which not only allows better contact between oil-bearing material and solvent but also enhances enzyme diffusion rates (Rosenthal and others 1996). Two different types of grinding may be carried out: wet or dry depending on the initial moisture content and the chemical composition of the oilseeds (Rosenthal and others 1996). Although particle size is important in oil and protein extraction efficiency, only a few studies have been carried out on this topic. A study by Rosenthal and others (1998) showed that the particle size has a significant effect on protein and oil extraction from soybeans. Smaller particle size resulted in higher extraction yields. Gibbins and

others (2012) reported that during enzymatic extraction of safflower seeds by using protease and cellulase at pH 5 the amount of oil extracted increased by 3.5% and 4% when particle size was reduced from average of 0.6–1 mm and <0.6 mm, respectively. The studies discussed above clearly demonstrate the importance of particle size on enzymatic oil extraction yield. The effect of particle size on enzymatic WGO extraction yield has not been reported. Hence, further research is needed on this topic.

2.3.3.1.2 EXTRACTION PARAMETERS

In aqueous enzymatic extraction, the ground seeds are mixed with a buffer solution and then agitated to increase mass transfer. The main parameters that influence enzymatic extraction yield include enzyme type, enzyme concentration, LSR, temperature, and treatment time (Cater and others 1974). Maximum corn oil yield of about 80% was achieved using three different commercial cellulases, Multifect GC, Celluclast 1.5 L, and GC 220 (Moreau and others 2004). In another study the rapeseed slurry was treated with a mixture of pectinase, cellulase, and β-glucanase (4:1:1, v/v/v) at concentrations of 2.5% (v/w) for 4 h (Zhang and others 2007). This was followed by sequential treatments of seeds with an alkaline solution and then an alkaline protease (Alcalase 2.4L). The effects of pH (9.0, 10.0 and 11.0), enzyme concentration (0.5, 1.0 and 1.5%, v/w), and the duration of the hydrolysis (60, 120, and 180 min) were studied. Increasing the concentration of Alcalase 2.4L and the duration of the hydrolysis time significantly increased the yields of free oil which accumulated over the aqueous phase and protein hydrolysates while the extraction pH had a significant effect only on the yield of the protein hydrolysates. For peanut oil, Alcalase 2.4Lwas shown to be the most effective enzyme resulting in the highest oil yield (Wang and others 2008; Jiang and

others 2010). The effect of olive variety (Kroneiki, Iranian Native Oleaginous and Mission), enzyme type (Pectinex Ultra SP-L and Pectinase 1.6021) and concentration (zero, low, and high concentration) on the oil extraction yield was studied by Najafian and others (2009). The highest oil yield (72.13%) was obtained with the Koroneiki variety, using pectinex Ultra SP-L (pectinase) enzyme at the highest concentration. Five enzymes [Protex 7L (endoproteinase), Alcalase 2.4L (endoproteinase), Viscozyme L (carbohydrases), Natuzyme (mainly cellulose, xylanase, phytase, alpha-amylase, pectinase activities), and Kemzyme (mainly alpha-amylase, beta-glucanase, cellulase-complex, hemicellulase-complex, protease and xylanase activities)] were evaluated for their effectiveness in extracting the oil and protein from sesame seeds. Alcalase 2.4L was found to be the best for giving a high oil yield (57.4%), whereas, the maximum amount of protein (87.1%), was recovered in the aqueous phase with Protex 7L (Latif and Anwar 2011). Xie (2010) studied the aqueous enzymatic oil extraction of WGO. Two enzymes (Alcalase 2.4L FG and Multifect CX GC) were selected for optimization of processing parameters for maximum oil extraction yields by using Response Surface Methodology. The optimum extraction condition to obtain high oil yields (66.45%) was achieved with Alcalase 2.4L FG enzyme in Tris-HCl buffer (pH 8.0) at a LSR of 16.5 (w/w), enzyme concentration of 4% (w/w), and extraction time of 5.25 h. In a study by Li and others (2011) on aqueous enzymatic extraction of WGO, the extraction was carried out by using a multi-enzyme preparation consisting of cellulase, pentosanase, neutrase (protease), and fungal amylase (CPNF, 2:1:2:1 w/w/w/w). The enzyme preparation was added at 1.6% (w/w) level based on germ weight. The optimal set of extraction conditions was as

follows: water to wheat germ ratio 3.46 mL/g, pH 5.24, temperature 48℃, and time 6 h. The oil yield was 86.74% at the optimal conditions.

2.3.4 SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction of oilseeds is usually carried out by using CO_2 as a solvent. Carbon dioxide is non-toxic, non-explosive, relatively inexpensive and readily available and easily removable from the extracted product. This method is also as efficient as solvent extraction at removing triacylglycerides while yielding a high quality, gum-free, and light- colored crude oil (Bargale 1997). Use of supercritical fluids such as CO_2 for extraction of WGO has previously been reported by several research groups. Eisenmenger and others (2006) examined the supercritical CO_2 extraction and fractionation techniques to obtain WGO. Both commercial and supercritical CO_2 extracted WGO were rich in tocopherols and phytosterols. In addition, it was confirmed that the composition of supercritical CO_2 extracted oil was similar to that of the hexane extracted oil. Furthermore supercritical CO_2 extracted oil did not contain phospholipids leading to elimination of the degumming step during crude oil refining. Piras and others (2009) examined the supercritical fluid extraction of WGO. The effects of pressure (20-30 MPa at 40 ^{o}C) and extraction time on the oil quality and extraction yield were studied. The maximum WGO recovery, about 80%, was achieved with supercritical CO_2 at 30 MPa. The fatty acid and α-tocopherol composition of the extracts was not affected by pressure. Jiang and Niu (2011) optimized the WGO extraction by supercritical CO_2. A maximum oil yield of 10.46% (w/w) was obtained under the following conditions: wheat germ particle size 60-80 mesh, water content 4.37%, pressure 30 MPa, temperature 40 °C and extraction time 1.7 h. The low oil yield might be due to short extraction time.

CHAPTER III

MATERIALS AND METHODS

3.1 SOURCE OF WHEAT GERM AND OIL

Full fat wheat germ was purchased from ADM Milling Company (Enid, OK. USA). The germ was then stored in a walk-in cooler at 6 ^{o}C until further use. Commercially hexane extracted crude WGO was a donation from Vitamins, Inc. (Chicago, IL). Supercritical carbon dioxide (SC-CO_2) extracted WGO was provided by Dogal Destek Urunleri, Atburgazi, Soke, Aydin, Turkey. All the oil samples were stored in sealed containers at 4 ^{o}C away from light until further use. Hexane extracted crude WGO was used without further purification. However, SC-CO_2 extracted WGO was centrifuged at 25,673×g for 15 min before the analytical tests.

3.2 WHEAT GERM PRE-TREATMENT AND MECHANICAL EXTRACTION

The germ was heated to 82 ^{o}C in a steam-jacketed kettle (Model DM-US, Hamilton, Fairfield, Ohio). The cooked germ was screw-pressed using a heavy-duty laboratory screw press (Figure 2, Model L250, French oil mill Machinery Company, Piqua, Ohio). The press shaft was formed of 15 stainless steel rings (labeled A through Q) that can be arranged as desired (Figure 5). Two shaft arrangements (severe and mild) were examined.

Two types of rings, cylindrical and half-cone, were used to set up the shaft arrangements. For the severe arrangement the diameter of each ring was as followed: A- 63.5 mm , C- 63.5 mm, E- 66.67 mm, G- 69.85 mm, J- 69.85 mm, L- 76.20 mm, N- 79.37 mm and Q- 82.55 mm. For the half-cone rings front and back end diameters were as followed: B- 63.5 mm, D- 63.5 mm, F- 66.67 mm, H- 69.85 mm, K- 69.85 mm, M- 76.20 mm, P- 79.37 mm, and B- 66.67 mm, D- 69.85 mm, F- 76.20 mm, H- 76.20 mm, K- 82.55 mm, M- 85.72 mm, P- 85.72 mm, respectively. On the hand, the diameter of each ring in mild arrangement was as followed: A- 63.5 mm, B- 63.5 mm, C- 63.5 mm, E- 66.67 mm, G- 69.85 mm, J- 69.85 mm, L- 76.20 mm, N- 76.20 mm, Q- 76.20 mm. For the rest of the rings front and back end diameters were as followed: D- 63.5 mm, F- 66.67 mm, H- 69.85 mm, K- 69.85 mm, M- 76.20 mm, P- 76.20 mm, and D- 69.85 mm, F- 73.02 mm, H- 76.20 mm, K- 79.37 mm, M- 82.55 mm, P- 82.55 mm, respectively. The shaft had four sections, AA, BB, CC and DD, in which the screen bars were spaced by using 0.015, 0.010, 0.010, and 0.0070 mm shims from feed to discharge end, respectively. The main drive was powered by an electric motor (20 horsepower). The cone at the end of the screw shaft can be adjusted by using a 3-position directional valve (Model L-1057, Enerpac, Milwaukee, Wisconsin) and a hand pump (Model P 392, Enerpac, Milwaukee, Wisconsin) to increase or decrease back pressure at the press discharge. The cooked germ was loaded into the screw press hopper. The feed rate was controlled by a variable speed screw conveyor. Each experimental run was four hours. The preliminary tests showed that the system reached steady state after two hours, meaning that the amount of crude oil and press cake collected in 30 min intervals were similar. The samples collected during

steady state were used for further analyses. The press cake was analyzed for moisture and oil content. The oil extraction yield was calculated by the following formula:

$$\text{Oil extraction yield (\%)} = \frac{total\ oil\ in\ full\ fat\ wheat\ germ - oil\ in\ press\ cake}{total\ oil\ in\ full\ fat\ wheat\ germ} \times 100\% \quad \text{(Equation 1)}$$

A schematic flow diagram of the extraction process is shown in Figure 6.

3.3 OIL EXTRACTION FROM PRESS CAKE

HEXANE EXTRACTION

The residual WGO from press cake was extracted with hexane and used for oil quality tests. Ground WG cake (12 g) was mixed with 175 mL hexane in a 250 mL Erlenmeyer flask. The flask was covered and the mixture was stirred for 2 h, and then filtered through a Whatman #4 filter paper. The residual solids on the filter were rinsed once with 20 mL hexane. Hexane was removed from the oil by using a Rapid-Vap vacuum system (Model 7900002, Labconco, Kansas City, MO). The solvent free oil was immediately transferred to small amber glass vials and stored in a cooler (4 °C) until further analysis.

3.4 AQUEOUS AND AQUEOUS ENZYMATIC EXTRACTION

The full fat WG and WG cake from mechanical pressing were ground using a laboratory mill (Model 3600, Perten Instruments, Sweden) and a coffee grinder (Model CBG100W, Black & Decker, Towson, MD). The samples were kept in airtight plastic

containers at -20°C until further use for proximate composition analysis and extraction tests. The particle size distribution of both full fat WG and WG cake ground by laboratory mill and coffee grinder was determined by using a sieve tester (Model SS-15, Gilson Company, Inc, Lewis Center, Ohio). The instrument was run for 5 min. Two U.S.A. standard testing sieves with 150 and 500 μm openings and a pan (Seedburo Equipment Company, Chicago, Illinois) were used in the process. Weight of the material left on each sieve and pan as a percentage of the total material weight was reported.

3.4.1 ENZYME SELECTION

Two carbohydrases (Multifect CX GC and Multifect GC Extra) and one protease (Alcalase 2.4L FG) were used for the enzymatic extraction experiments. Selection of these enzymes was based on the chemical composition of WG and previous studies by Xie (2010). Multifect CX GC, an enzyme with cellulase activity, was provided by Genencor (Rochester, NY, U.S.A). This enzyme is derived from a selected strain of *Trichoderma reesei*, and has side activities including hemicellulase, xylanase, and β-glucanase. The declared activity is 3200 CMC/g. One CMC unit is defined as the amount of enzyme which produces 1 μmol glucose equivalent from carboxymethyl cellulose at pH 5.0 and 50 ^{o}C in one minute. This enzyme is effective at a pH of 2.7-5.7 and temperatures between 35 and 70 ^{o}C. Multifect GC Extra, an enzyme with cellulase activity, was also provided by Genencor (Rochester, NY,). It is produced from a selected strain of *Trichoderma reesei.* The enzyme, with a specified activity of 6200 IU/mL, has side activities including hemicellulase, xylanase, and β-glucanase. This enzyme is effective at a pH of 4.0- 6.0 and temperatures between 45 and 65 ^{o}C. Alcalase 2.4 L FG was donated by Novozymes (Bagsvaerd, Denmark). It is an alkaline endoproteinase

produced from a selected strain of *Bacillus licheniformis*. It has a declared activity of 2.4 AU/g. One unit is defined as the amount of enzyme that produces the equivalent of 1 μmol tyrosine per minute. This enzyme is active at a pH range between 7.5 and 8.5 and temperatures between 50 and 55 ^{o}C.

3.4.2 EXTRACTION EXPERIMENTS

Ground WG or WG cake was mixed with buffer in a 500 mL flask. The amount of WG and buffer used varied depending on the LSR. For Multifect CX GC and Multifect GC Extra 0.15 M citric-phosphate buffer at pH 5.0 and 0.1 M Tris-HCl at pH 8.0 was used for Alcalase 2.4 L FG. Two different buffers, 0.12 M boric acid-NaOH and 0.1 M Tris-HCl, at pH 8.0 were used for aqueous extraction. The mixture of WG and buffer was placed in a water bath shaker (Model C76, New Brunswick Science, Edison, NJ, USA), and heated to 50 ^{o}C with constant shaking at 200 rpm. Enzyme was added at a pre-determined concentration and mixture was incubated for the duration of desired extraction time. Then the mixture was centrifuged (Sorvall RC 5C, Thermo, Asheville, NC) at 25,673 ×g and 25 ^{o}C for 15 min. The liquid phase was drained off, and 180 mL deionized water was added to the centrifuge tube containing wet residual solids to wash away the oil which may remain on the wall of the centrifuge tube and in the solid matrix. The wet residue was well mixed with the deionized water, and subjected to a second centrifugation under the same conditions used before. The liquid phase was drained off once again. The wet residue was dried in a forced-air oven (VWR Science, Model 1370 FM, Bristol, CT) at 85 ^{o}C for 16 h. The dried residue was weighed and analyzed for oil content. The oil extraction yield is calculated by using equation 1. A schematic flow diagram of the extraction process for aqueous enzymatic extraction is shown in Figure 7.

3.5 ANALYTICAL METHODS

3.5.1 Moisture Content

The moisture content of the ground WG and WG cake from mechanical pressing was determined using AACC method 44-15A (AACC 1995). The sample was brought to room temperature prior to analysis. Aluminum dishes were pre-dried using a forced air oven (VWR Science Model 1370 FM, Bristol, CT) at 130 ^{o}C for 1 h before analysis and then cooled down to room temperature in a desiccator. Approximately 2 g of sample were weighed in the pre-dried aluminum dish and dried in the oven at 130 ^{o}C for 1 h. The moisture content of the sample was reported as the loss in sample weight as a percentage of the initial sample weight. The moisture content of WGO was determined using 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).

3.5.2 Ash Content

The ash content of the WG and WG cake from mechanical pressing was determined according to AOAC method 923.03 (AOAC 1995). The sample was brought to room temperature prior to use. Crucibles were pre-dried in a furnace (Fisher Science, Model 58 Isotemp® Muffle Furnace 600 Series, Fair Lawn, NJ) at 525 ^{o}C for 5 h and then cooled down to room temperature in a desiccator. About 2 g of the sample were weighed into the pre-dried crucible and ashed at 525 ^{o}C for 5 h. The percentage residual weight was reported as the ash content of the sample.

3.5.3 Oil Content

The oil contents of the WG and WG cake from mechanical pressing and the dried residue after aqueous and aqueous enzymatic extraction were measured by AOAC method 2003.05 (AOAC 2005). Samples were cooled to room temperature prior to use. About 1 g of sample was weighed in a cellulose thimble and extracted in a Soxtec extraction unit (Tecator, Model 1043 Extract Unit, Hoganas, Sweden) with 40 mL of petroleum ether for 1 h. The amount of oil extracted as the percentage of initial sample weight was reported as the oil content in the sample.

3.5.4 Protein Content

The protein content in the WG and WG cake was determined according to AOCS method Ba 4e-93 (AOCS 2004). Protein was measured as nitrogen on a Leco Truspec N (Truspec CN, Leco USA, St. Joseoh, MI). A factor of 5.7 was used to convert the amount of nitrogen to the amount of protein in the sample (Tkachuk 1969).

3.5.5 Free Fatty Acid Determination

The free fatty acid (FFA) content of the WGO samples was determined using a colorimetric procedure (Lowry and Tinsley 1976). A 5% (w/v) solution of copper acetate was prepared. Pyridine was added to this solution until the pH reached a range of 6.0-6.2. A 100 mg/mL stock standard solution of oleic acid (National Formulary/Food Chemicals Codex grade, Fisher Chemical, Fairlawn, NJ) was prepared by dissolving 100 mg of oleic acid in 1 mL of hexane. A standard curve was prepared by transferring 10, 20, 30, and 40 μL aliquots of stock standards to individual centrifuge tubes. A 5 mL benzene and 1 mL copper acetate solution was added to each tube and mixed for 2 min. Then the solution was centrifuged at 1380 ×g for 5 min. About 0.03-0.05 g of oil sample was prepared

using the same procedure mentioned above. Absorbance of the samples and the standards was read at 715 nm using a spectrophotometer (DU 520, Beckman Coulter, Inc, Fullerton, CA). A standard curve was prepared and used to calculate FFA content in the samples.

3.5.6 Peroxide Value (PV)

The PV of the WGO samples was determined according to AOCS official method Cd8- 53 (AOCS 2003). About 5 g of the WGO sample was weighed into a 250 mL flask. Then 30 mL of glacial acetic acid: chloroform (3:2, v/v) [both American Chemical Society (ACS) reagent grade and purchased from Fisher Chemical, Fairlawn, NJ], solution was added along with 0.5 mL of a saturated potassium iodide (ACS grade, Fisher Chemical, Fairlawn, NJ) solution. The solution was mixed and allowed to stand for 1 min. Then 30 mL of distilled water was added along with 2 mL of a saturated starch solution. The solution was then titrated with a 0.01 N sodium thiosulfate (ACS grade, Fisher Chemical, Fairlawn, NJ) solution until the color changed from dark blue to colorless. The PV was calculated using the equation,

PV = [(mL of titrant)*(0.01)*1000]/ (Sample weight).

3.5.7 *p*-Anisidine Value (AV)

p-Anisidine values for the WGO samples were measured according to AOCS official method Cd 18-90 (AOCS 2003). About 0.5 g of the WGO sample was dissolved in 25 mL isooctane (ACS reagent grade, Fisher Chemical, Fairlawn, NJ). The absorbance at 350 nm was measured using a spectrophotometer (DU 520, Beckman Coulter, Inc., Fullerton, CA). Five mL of the WGO isooctane solution was transferred into a test tube. Then 1 mL of 0.25 g/100 mL *p*-anisidine (99 %, ACROS Organics, Morris Plain, NJ)

solution in glacial acetic acid was added to the test tube. After shaking and resting the mixture for 10 min to produce a colored complex, the absorbance of the mixture was measured again at 350 nm. The AV was calculated using the following formula.

$AV = [25 * (1.28 * As - Ab)]/m$

Where; As = absorbance of the oil solution after reaction with the reagent, Ab = absorbance of the initial solution, and m = weight of the sample in g.

3.5.8 Phosphorus Content

Phosphorus content was determined by ashing the WGO sample in the presence of zinc oxide according to AOCS official method Ca 12-55 (AOCS 1998). The crucible after ashing was removed from the furnace and cooled down to the room temperature. Then 5 mL of distilled water and 5 mL of concentrated HCl were added to the ash. The crucible was covered with a watch glass and heated to a gentle boil for 5 min. The solution was filtered and cooled to room temperature and neutralized to a faint turbidity by drop wise addition of 50% KOH solution. Concentrated HCl was added drop wise until the zinc oxide precipitate is just dissolved, then 2 additional drops were added. The volume was diluted with distilled water to 100 mL. A10 mL of this solution was pipetted into dry 50 mL volumetric flask. Then 8 mL of hydrazine sulfate solution and 2 mL of sodium molybdate solution were added and heated for 10 ± 0.5 min in a boiling water bath. The volumetric flask was removed from the bath, cooled to room temperature and diluted to volume. The absorbance of the solution was measured at 650 nm using a spectrophotometer (DU 520, Beckman Coulter, Inc., Fullerton, CA), and the phosphorus content was determined by means of a standard curve using NaH_2PO_4 as a standard.

3.5.9 Fatty Acid Composition

Fatty acid compositions of the WGO samples were determined by gas chromatography (GC). The GC unit was an Agilent Technologies model 6890 system equipped with a flame ionization detector (FID). Methylation of the fatty acids was carried out according to AOCS Official Method Ce 2-66 (AOCS 2003). A Supelco SP-2560 fused silica capillary column with 100 m x 0.25 mm x 0.2 μm film thickness (Bellefonte, PA) was used for fatty acid analysis. The helium carrier gas flow rate was 20 cm/s. The injector temperature was held at 260 ^{o}C. A temperature program was maintained at 140 ^{o}C for 5 min, then increased at 4 ^{o}C /min to 240 ^{o}C 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) 45 mL/min. WGO samples (1 μL) were injected by an autosampler (7683B, Agilent Technologies, Palo Alto, CA). Peak areas were calculated and data collection was managed using HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA). The split ratio was 100:1. Fatty acid peaks were identified using a standard 37 FAME mixture (Supelco 37 component FAME mix, Supelco, Bellefonte, PA). Undecanoic acid (11:0) was used as an internal standard for quantification.

3.5.10 Tocopherols

Tocopherol of (α, ß, γ and δ) analysis was carried out by HPLC following the method of Katsanidis and Addis (1999). The WGO samples were dissolved in hexane (0.20 g/mL) and filtered through a 0.2 μm filter (Iso–Disc filter, Supelco, Bellefonte, PA). 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). A 2 μL sample or standard was injected into a normal phase HPLC column, Zorbax RX-SIL (5 μm particle size, 4.6 x 250 mm, Agilent Technologies, Santa Clara, CA). 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 fluorescence detector was set at 290 nm excitation wavelength and 400 nm emission wavelengths. The fluorescence detector gain was set for 1. The column temperature was 35 ^{o}C. An external calibration curve was prepared for each tocopherol standard (α, ß, γ and δ tocopherol standards, Sigma-Aldrich Corporation, St. Louis, MO) to calculate the amount of tocopherols present in the oil sample.

3.5.11 Phospholipids

The WGO samples were dissolved in chloroform: methanol (2:1, v/v) (0.5 g/mL) and filtered through 0.2 μm Iso Disc filters (Supelco, Bellefonte, PA) for further analysis. 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 A: chloroform and B: methanol/ water (95:5, v/v). The elution program for a binary gradient system was 99% of A and 1% of B for 15 min, then 75% of A and 25% of B for 5 min after that 10% of A and 90% of B for 10 min and finally 10% of A and 90% of B for 5 min. Total run time was 35 min and the flow rate was 1.0 mL/min. The detector system was 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. Identification and quantification of chromatographic peaks were based on external standard curves prepared for individual standards. Phospholipid standards L-α phosphatidylcholine (PC), L-α phosphatidic acid (PA) sodium salt, L- α-phosphatidylethanolamine (PE), and phosphatidylinositol (PI) sodium salt were purchased from Avanti polar lipids, Inc, Alabaster, Alabama. PC, PA and PE were isolated from eggs and PI was from soy lecithin.

3.5.12 Enzyme Activity Test

The activity of the Multifect CX GC and Multifect GC Extra enzymes was measured as CMC/g according to the method provided by Joint FAO/WHO Expert Committee on Food Additives JECFA (2003). Alcalase 2.4L FG activity was determined using the method provided by Megazyme (Wicklow, Ireland), and expressed as Unit/g (Megazyme 2006). One Unit is defined as the amount of enzyme that produces the equivalent of 1 μmol tyrosine per 19 min from soluble casein at pH 8.0 and 40 ^{o}C.

3.6 STATISTICAL ANALYSIS

The ranges of process variables examined in this study were as follows: shaft speed (400, 600, and 800 rpm), cage temperature (82 and 107 °C and no heating), back pressure plate position [high (57 mm away from the discharge end), medium (74 mm away from the discharge end) and no back pressure (89 mm away from the discharge end)], shaft arrangement (severe and combination of severe and mild arrangement), and germ pretreatment (heating at 82 °C and no heat). These factors were selected based on a review of literature and preliminary laboratory investigation. All mechanical extraction experiments and analytical tests were carried out in randomized order. A fraction of 2^{2}x 3^{3} factorial design was tested with 6 treatment combinations were replicated to measure

the error variability. In the first phase of this study, the mean of the best treatment combination was compared to the means of each of the other treatment combinations by using Dunnett's multiple comparison method. Analysis of variance (ANOVA) results from the five- way factorial experiments was performed using SAS 9.3 (SAS Institute Inc., Cary, NC).

Aqueous and aqueous enzymatic extractions were carried out at least in duplicate. In aqueous extraction, the effect of two starting materials (full fat WG and WG cake), two buffers (tris-HCl and boric acid-NaOH) and two particle sizes (fine and coarse) on oil yield were examined. A 2 x 2 x 2 full factorial design was used. In aqueous enzymatic extraction, the effect of three enzymes (Alcalase 2.4L FG, Multifect CX GC, and Multifect GC Extra), two starting materials (full fat WG and WG cake), and two particle sizes (fine and coarse) on oil yield were tested. A 3 x 2 x 2 full factorial design was used. In the second phase of this study, the means were compared using Tukey's adjustment. All statistical tests were performed at the 0.05 level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 CHARACTERISTICS OF WHEAT GERM

The high moisture content of the WG before heat pretreatment (about 10%) (Table 2) is due to the tempering, which is the addition of water to the whole wheat to increase the moisture content to about 15% prior to milling. The objective of tempering is discussed in section 2.1.1 of this dissertation. The moisture content of the WG was about 7% after heat pretreatment and prior to mechanical extraction. The oil content of the WG extracted by petroleum ether was about 12% (w/w, as dry basis) (Table 2). This result is in agreement with the data reported in the literature for WG (Barnes 1982; Dunford and Zhang 2003; Zhu and others 2006; Xie 2010; Hassan and others 2010). The lower oil content in the commercial WG as compared to the dissected germ (about 15-20%) is due to the contamination with bran and endosperm (Barnes 1982). According to the literature, WG is rich in protein, about 26- 36%, (Barnes 1982; Ge and others 2000; Zhu and others 2006; Xie 2010; Hassan and others 2010). WG used in this study had about 33% protein (Table 2), which is within the range of the data reported in the literature. The ash content of the WG was about 5% (Table 2). Similar WG ash content was reported in earlier studies (Barnes 1982; Xie 2010). Other components, which account for about 42% of

WG may be fiber, pentosans, and starch and non-starch carbohydrates, including free sugars such as sucrose and raffinose (Amadò and Arrigoni 1992).

About 85% of the WG ground by using a laboratory mill had a particle size between 150 and 500 μm compared to about 68% using a coffee grinder (Table 3). The laboratory mill produced smaller particle size (only 8.6% of the particles above 500 μm) than the coffee grinder (about 30% of the particles above 500 μm). Therefore the samples that were ground by using the laboratory mill was labeled as "fine" and the samples ground by using a coffee grinder as "coarse".

4.2 OPTIMIZATION OF THE MECHANICAL PRESSING PROCESS

Optimization tests were carried out using five factors: shaft speed, cage temperature, germ pretreatment, back pressure, and shaft arrangements (Table 1). According to the original experimental design, 72 experiments should have been carried out but only 42 experiments could be successfully performed because unheated germ did not move through the cage when the severe shaft arrangement was used without heating the press cage; thus no oil was obtained under these experimental conditions. This might have been due to the high moisture content (about 10%) of the seed and also the small clearance between the screw shaft and the cage in the severe arrangement. Moreau and others (2005) reported that during pre-pressing of dry-milled corn germ (about 13% moisture content), no oil was obtained with uncooked corn germ.

In this study the highest WGO yield, 47.7%, was obtained under the following conditions: severe shaft arrangement, cage temperature of 107^{o}C, germ pretreatment/heating at 82^{o}C, high back pressure, and shaft speed of 400 rpm. The lowest oil yield, about 2.8%, was obtained with the combination shaft arrangement (combination

of severe and mild), at cage temperature of 82^{o}C, no germ pretreatment/heating, no back pressure, and at shaft speed of 800 rpm (Table 4). The effect of shaft arrangement on oil yield was significant ($p < 0.0001$) at the available combinations of cage temperature, back pressure, and shaft speed examined in this study. Severe shaft arrangement resulted in higher oil yield (47.69-29.41%) than that for the combination of severe and mild arrangement (30.31-2.82%). This result was expected since the clearance between the shaft and cage in the severe arrangement was smaller than in the combination arrangement. It is expected that more pressure was exerted on WG at the severe arrangement releasing more oil.

There was a significant three way interaction of cage temperature x back pressure x shaft speed ($p = 0.0018$). Cage temperature had a significant effect on oil yield only at the following treatment combinations: high back pressure with 400 rpm ($p = 0.0007$) and with 600 rpm ($p = 0.0076$) shaft speeds; medium back pressure with 800 rpm ($p = 0.0017$); no back pressure with 600 rpm ($p = 0.0050$) (Table 5). Hughey and Tacoronte (2010) studied the effect of pressing temperature (50-70^{o}C) on the oil yield of roasted and unroasted shea nuts using a hydraulic jack press. For unroasted nuts, the highest oil yields occurred at 60.7^{o}C press temperature compared to 62^{o}C for roasted nuts indicating the effect of press temperature x seed pretreatment interaction on oil yield. High cage temperature does not always result in high oil yield. In this study, the back pressure had a significant effect on oil yield ($p < 0.0001$) at all cage temperatures and shaft speeds examined. High back pressure resulted in higher oil yield than that of medium and no back pressures (Figure 8 and 9). This result agrees with the findings reported in the literature with other oilseeds (Mwithiga and Moriasi 2007; Soetaredjo and others 2008;

Owolarafe and others 2007; Baryeh 2001). Khan and Hanna (1983) reported that pressure breaks the cell walls of the seed and release more oil. Adeeko and Ajibola (1990) found a significant increase in oil yield when the extraction pressure was increased from 10 to 20 MPa for finely ground groundnuts. Here, the effect of shaft speed on oil yield was significant ($p < 0.0001$) at all cage temperature and back pressure combinations examined. Low shaft speed, 400 rpm, resulted in higher oil yield than that of higher shaft speed 600 and 800 rpm (Figure 8 and 9). The effects of different shaft speeds on the oil yield are related to the duration of pressing. Lowering the shaft speed extends the pressing time and the seed heat treatment process in the press leading to higher oil yield (Evangelista 2009). Similar results were reported by Deli and others (2011), who found that the oil yield from *Nigella sativa L* seeds decreased with increasing shaft speed. Karaj and Müller (2011) also found that oil recovery from *Jatropha curcas L*. seeds decreased when shaft rotation speed was increased.

As expected oil yield increased as the back pressure increased even when combination shaft arrangement was used with WG pretreated at 82^{o}C and no cage heating (Figure 10). At constant back pressure and no cage heating oil yield decreased with increasing shaft speed. However, when press cage was heated at 82 ^{o}C, oil yield increased significantly ($p < 0.0001$) with increasing back pressure only at 400 and 800 rpm but not at 600 rpm (Figure 11). At 107 ^{o}C cage temperature, oil yield increased significantly ($p < 0.0001$) at 600 and 800 rpm with increasing back pressure but not at 400 rpm (Figure 12). Depending on the processing conditions WG is exposed to various shear and/or compaction forces during mechanical pressing. The capillaries in WG matrix through which oil is squeezed out, might be narrowing and even sealing/blocking

and affecting oil removal under extreme compaction forces (Ward 1976). Therefore, it is important to identify the optimum extraction conditions for oilseeds because higher pressure does not necessarily increase the oil yield.

4.3 CHARACTERISTICS OF WHEAT GERM CAKE

Moisture content of WG decreased significantly, from 7% to 6%, during mechanical pressing (Table 2). The reduction in the moisture content might be caused by the high temperature of press cage and heat generated by friction during mechanical pressing. The residual oil content in the cake was about 6% (as dry basis). This result is consistent with the oil yield obtained under the same conditions. Protein and ash contents in WG cake increased as compared to the original WG, as a result of decreased oil content (Table 2). Supercritical CO_2 defatted WG (Jiang and Niu 2011) had a similar composition to that reported in this study. No information is available in the literature on the proximate analysis of WG cake obtained by mechanical pressing. The laboratory mill used in this study produced smaller particle size (only 20% of the particles above 500 μm) than that of the coffee grinder (about 50% of the particles above 500 μm) (Table 6). Therefore the samples that were ground by using the laboratory mill was labeled as "fine" and the samples ground by using a coffee grinder as "coarse".

4.4 CHARACTERISTICS OF WHEAT GERM OIL

4.4.1 FATTY ACID COMPOSITION

Linoleic acid (18:2), which is an essential oil, makes up 55% to 57% of the total fatty acids in the WGO samples examined in this study (Table 7). Palmitic, oleic, and linolenic acids were also present in significant amounts in all WGO samples. About 19%

of the fatty acids in WGO were saturated and about 81% was unsaturated. Palmitic acid makes up about 90% of the saturated fatty acids, while linoleic acid comprises about 70% of unsaturated fatty acids in WGO. Although there were statistically significant differences in fatty acid composition among the oils, the variations were not substantial for practical purposes. These results are in agreement with data reported by other groups (Dunford 2001; Eisenmenger and Dunford 2008; Jiang and Niu 2011).

4.4.2 FREE FATTY ACID CONTENT

The free fatty acid (FFA) content of WGO is usually high and quite variable, between 5% and 25%, depending on conditions of germ separation, germ storage, and oil extraction methods used (Eisenmenger and others 2006). WGO H contained a significantly higher FFA content, 14.58%, than did WGO extracted by other methods (Table 8). This might be due to the extended heat exposure during commercial hexane extraction and poor WG handling and storage. The FFA content of WGO P was not significantly different ($p > 0.05$) than that of WGO N, 3.37% and 3.31% respectively. This result demonstrated that the heat pretreatment prior to mechanical extraction had no effect on the FFA content in the WGO. Heating coarsely ground groundnut at 70-160 ^{o}C increased the FFA content in the oil only 0.1-0.4% (Adeeko and Ajibola 1990). FFA content of WGO S was significantly lower than that of WGO P and WGO H (Table 8). The results obtained in this study are different from those reported by Eisenmenger and others (2006), who reported that hexane-extracted and supercritical fluid -extracted WGO contained similar amounts of FFA, 7.9% and 6.2%, respectively. Zacchi and others (2006) studied the effect of different extraction methods (solvent, supercritical CO_2, and press) on FFA content in WGO. Pressed oil had the highest FFA content among the

extraction methods examined. These variations in FFA content might be due to different conditions of germ separation and germ storage and germ quality. FFAs often contribute to bitter and soapy flavors in food; therefore, they need to be removed during the refining process for edible oils.

4.4.3 PEROXIDE VALUE (PV)

The PV is a measure of all peroxides and other lipid oxidation products that form during primary oil oxidation. High PV indicates low oil quality. WGO N and WGO C contained the lowest and highest PV among the samples examined in this study, 2.41 and 9.93 meq/kg, respectively (Table 8). The high PV indicates that oil was extracted and/or stored in improper conditions (Megahed 2011). Adeeko and Ajibola (1990) reported that increasing both seed temperature and time of seed heating increased the PV of the oil. Although the results indicate that WGO extracted by mechanical pressing had better quality because of its lower PV than WGO extracted with hexane and supercritical CO_2, it is important to note that oil quality cannot be directly attributed to the extraction technique used for these samples, because feedstocks from different sources were used to obtain the samples analyzed in this study. Igbo and others (2006) also found that solvent-extracted benniseed oil had higher PV than mechanically pressed oil. However, Tasan and others (2011) reported that full pressed sunflower oil had higher PV, 12.10 meq/kg than the pre-pressed and solvent-extracted oils. The reason for these variations might be due to different extraction and/or storage conditions.

4.4.4 *p*-ANISIDINE (AV)

p-Anisidine (AV) is a measure of the secondary oxidation products in oil (Megahed 2011). WGO N had the lowest AV among the oils tested in this study (Table

8). Lower PV and AV value of WGO N compared to the WGO P indicate that heat treatment prior to mechanical pressing had some adverse effect on the oil quality. Brevedan and others (2000) reported that pressed sunflower oil had lower AV content than hexane-extracted oils, due to generation and decomposition of hydroperoxides during solvent extraction. Hexane extracted WGO examined in this study, WGO H, also had the highest AV among the samples analyzed.

4.4.5 MOISTURE CONTENT

Water content of the oil samples is of interest for the following reasons: high moisture content in oil promotes microbial growth, hydrolysis during high temperature applications, phase separation, and cloudiness in the oil (Eisenmenger and Dunford 2008). All the oil samples had relatively low, moisture (<1%) (Table 8). Supercritical CO_2 extracted WGO, WGO S, had significantly higher moisture content (0.75%) than the other samples. This result agrees with the data reported in the literature by Eisenmenger and others (2006), who reported that WGO extracted by supercritical CO_2 had significantly higher moisture content than did commercial WGO, including hexane-extracted crude WGO. In contrast, Jiang and Niu (2011) reported that supercritical CO_2 extraction of WGO resulted in a moisture content of 0.47% compared to 0.68% using solvent extraction. These differences may be due to the variations in initial moisture content of the WG and extraction conditions. During the industrial scale supercritical CO_2 extraction of vegetable oils, water can easily be separated in a high pressure separator before precipitation of lipids from CO_2 at a lower pressure (Eisenmenger and Dunford 2008).

4.4.6 TOCOPHEROLS

WGO S had significantly higher total tocopherol content than did other oil samples examined in this study (Table 9). The most abundant tocopherol in WGO was α-tocopherol. Similar to the findings presented here, Eisenmenger and others (2006) reported that WGO from supercritical fluid extraction contained a significantly higher amount of tocopherols than did the commercial WGO samples. There was no significant difference between the α-tocopherol content of WGO P and WGO N. This result indicates that heat pretreatment of the germ prior to pressing did not affect the tocopherol content. However, WGO C had significantly lower α-tocopherol content (2.34 mg/g oil) than did the other oil samples. This is probably due to the longer heat exposure of the residual oil in the WG cake during mechanical pressing. Similar results were reported by Panfili and others (2003), who found that extraction of full fat WG by solvent produced WGO with a higher α-tocopherol content (1.6 mg/g oil) than the oil extracted from cake by solvent (0.2 mg/g oil). WGO P had significantly higher α-tocopherol than WGO H (Table 9). This result is similar to the result reported by Wang and Johnson (2001) who mentioned that cold-pressed WGO had higher α-tocopherol (3.5 mg/g) content than crude WGO extracted by solvent (1.8 mg/g). In contrast, Zacchi and others (2006) reported that the tocopherol content was higher for solvent- extracted oil than that of the pressed oil. This might be due to different extraction conditions used in these studies. β-Tocopherol is the second-most abundant tocopherol in the WGO samples. The variations in tocopherol content of WGO reported in the literature may be due to different conditions and extraction protocols and the quality of WG used in these studies.

4.4.7 PHOSPHOLIPID COMPOSITION

Phospholipids are naturally present in oilseeds and pass into oil during extraction. The HPLC method used in this study did not separate phosphatidylinositol (PI) from phosphatic acid (PA), hence the result is expressed as PI+PA. There were statistically significant differences in total phospholipid content among the oil samples. However, differences were not substantial for solvent extracted and mechanically pressed oils (Table 10). This finding was in contrast with the finding reported by Brevedan and others (2000), who pointed out that sunflower oil from solvent extraction had higher amount of phospholipids than that from mechanical pressing. This might be due to different extraction conditions. As expected WGO S did not contain any detectable amount of phospholipids. This result was also confirmed by the low amount of phosphorus found in this sample (Table 8). In general phosphorous content rather than phospholipid content of oils is reported. This is due to the simpler analyses of phosphorous than that of phospholipids which require expensive analytical instrumentation such as HPLC. A conversion factor which is calculated based on the phospholipid composition of oil is used to convert phosphorous to phospholipids (Smouse 1995). Zacchi and others (2006) reported that WGO extracted by supercritical CO_2 had lower phosphorus content (<16 mg/kg) compared to oil obtained by solvent extraction and mechanical pressing, 1100 and 1671 mg/kg, respectively. These results were expected because solubility of phospholipids in supercritical CO_2 is very low. It is important to note that hexane extracted oils (both commercial hexane extraction and hexane extraction from press cake) had higher PC content than the other oils. To our knowledge there is no study on the

selective extraction of PC with hexane in the literature. High PA content in crude vegetable oils may be an indication of poor seed handling and extraction conditions (Wang and Johnson 2001). In our study commercial hexane extracted oil had lower PI + PA than the oil mechanically pressed oil. This might be due to the differences in both WG quality and extraction conditions used in this study.

4.5 EXTRACTION OF RESIDUAL OIL FROM PRESSED WHEAT GERM CAKE

4.5.1 AQUEOUS EXTRACTION OF WHEAT GERM OIL

Considering that over 50% of the WGO still remained in the cake after mechanical pressing, the efficacy of the aqueous oil extraction for residual oil recovery was examined. The effect of buffer types (boric acid-NaOH and tris-HCl) and particle size (fine and coarse) on oil extraction yields from both full fat WG and WG cake from a screw press was investigated. A previous study by Xie (2010) showed that the highest aqueous oil extraction yield from WG, 70%, could be achieved at a LSR of 20 and an extraction time of 0.5 h with both boric acid-NaOH and Tris-HCl buffers. Hence, previous extraction conditions were used for the extraction experiments in this study (Table 11). The highest oil extraction yield, 79.7%, was obtained from WG cake in boric acid-NaOH using fine particles. On the other hand, the lowest oil extraction yield, 33.3%, was obtained from full fat WG in boric acid- NaOH using coarse particles. The effect of sample x buffer x particle size interaction on aqueous oil extraction yield was significant ($p = 0.0001$) (Table 12). Boric acid-NaOH buffer was more effective in extracting oil from press cake than Tris-HCl buffer at the same pH, pH 8.0. This result might be due to

the interaction of the buffer components with proteins. In both buffers fine particles resulted in higher oil extraction yield than that obtained using coarse particles. This result was expected since a smaller particle size gives a larger surface area, which provides better contact between the oil-bearing material and the solvent; thus more oil can be recovered (Rosenthal and others 1996). For the full fat WG, the oil extraction yield obtained with Tris-HCl at pH 8.0 with both particle sizes was significantly higher ($p < 0.05$) than that obtained with boric acid-NaOH at the same pH. The result obtained from this study was in contrast with the previous study which predicted that WGO extraction yield of 70% could be obtained with both buffer systems (Xie 2010).This might be due to the differences in particle size distribution used in two different studies. The oil extraction yields obtained from WG cake with both buffers and particle sizes were significantly higher than those obtained from full fat WG. This result might be due to the fact that during the mechanical pressing WG matrix is sheared and subsequent solvent extraction allowed better contact between solvent (water) and oil released more efficiently from a more open solid matrix (Unger 1990).

4.5.2 EFFECT OF ENZYME TYPE ON OIL EXTRACTION YIELD

For the three enzymes used in this study, Alcalase 2.4L FG, Multifect GC Extra, and Multifect CX GC, the measured and declared activities by the suppliers are presented in Table 13. The discrepancies between the measured and the declared enzyme activities may be due to the variations in analytical protocols used for the activity measurements. A study by Xie (2010) showed that the highest oil extraction yield from full fat WG using Alcalase 2.4L FG was obtained under the following condition: LSR of 16.5, 4% enzyme concentration and 5.25 h extraction time. For the Multifect CX GC enzyme, the previous

study showed that the highest oil extraction yield could be obtained at LSR of 4, 2.55% enzyme concentration and 12.25 h extraction time. Hence, these extraction conditions were used to process WG cake from mechanical pressing. Alcalase 2.4L FG resulted in a significantly higher oil extraction yield than Multifect CX GC and Multifect GC Extra (Table 14). This result agrees with results obtained with full fat WG (Xie 2010), rice bran (Hanmoungjai and others 2002), and soybeans (Rosenthal and others 2001), where proteases and carbohydrases were compared for their effects on oil extraction yields. No significant differences were observed among the oil extraction yields obtained with Multifect CX GC and Multifect GC Extra. Xie (2010) reported that no significant difference was observed among the oil extraction yield obtained by Multifect CX 13L, and Multifect CX GC. When extraction was conducted using Alcalase 2.4L FG, full fat WG produced a significantly lower oil extraction yield than that of WG cake. This result was expected since during the pressing the WG is exposed to high temperatures and pressure, which help to break down the WG structure; thus more oil can be released. Moreau and others (2005) reported that the high temperatures and pressure associated with pressing may make it easier to remove the residual corn oil from corn germ by aqueous enzymatic extraction methods. Table 18 also shows that when Multifect CX GC and Multifect GC Extra were used, full fat WG produced significantly higher oil extraction yields than that of WG cake. Mechanical and chemical disruption of cells and internal cell barriers is important for oil extraction (Campbell 2010). High-pressure high-temperature extrusion through a small opening (die) at the end of an extruder converts raw oilseeds into cooked and partially inflated particles. Although both flaking and extrusion are capable of achieving high degree of cellular disruption, extrusion appears to

create oil-protein complexes that prevent complete extraction of oil (Campbell 2010). Low oil yield with Multifect from WG cake obtained in this study could be explained by protein-oil complex formation during extrusion of WG while coming out of the press. However, protein-oil complex formation during mechanical pressing needs to be further examined in a future study. A significantly higher oil extraction yield was achieved with fine ground full fat WG as compared to coarse ground full fat WG (Table 14). However, when extraction was conducted using WG cake, particle size did not have a significant effect on oil yield. This result was expected since the WG cells were already disrupted by the high heat and shear during mechanical pressing. A mathematical model developed in a previous study predicted that increasing LSR from 16.5 to 25 and extraction time from 5.25 h to 24 h would increase the oil yield for full fat WG (Xie and others 2011). However, in this study the oil extraction yield decreased with increasing LSR and extraction time for both full fat WG and WG cake. This might be due to the lower enzyme (Alcalase 2.4L FG) concentration used in this study (Table 15).

The effects of sequential treatment of WG using two enzymes, Multifect GC Extra followed by Alcalase 2.4L FG, at LSR of 16.5 and two different extraction times, (12.25 h and 5.25 h), and (2 h and 3 h) respectively, on oil extraction yields were examined (Table 16). These treatments did not improve the oil extraction yields; in fact yield decreased compared to those using Alcalase 2.4L FG alone. This might be due to different buffer composition used with Alcalase 2.4 L FG. Similar results were reported for enzymatic peanut oil extraction. Sequential treatment of peanut with Alcalase and complex cellulase and AS1398 did not improve oil yields (Jiang and others 2010).

CHAPTER V

CONCLUSION

This study examined the efficiency of a mechanical press, aqueous, and enzymatic extraction processes for WGO recovery. The highest oil yield from the screw press, about 47.7%, was obtained under the following conditions: severe shaft arrangement, cage temperature of 107 ^{o}C, germ pretreatment at 82 ^{o}C, high back pressure, and shaft speed at 400 rpm. Mechanically extracted oil had better quality (lower FFA, PV, and AV values and higher α-tocopherol content) than that of hexane-extracted oil. Even after process optimization, only about half of the oil present in the WG could be recovered by mechanical pressing. Hence, a two-step extraction process was developed. Residual oil in the press cake obtained from mechanical extraction was recovered by aqueous extraction. The aqueous extraction of WG cake with boric acid –NaOH (pH 8) buffer using fine particle size at LSR of 20 and extraction time of 0.5 h resulted in the highest oil yield, 79.64%. The enzymatic extraction of WG cake with Alcalase 2.4L FG at LSR of 16.5, enzyme concentration of 4%, and extraction time of 5.25 h resulted in 76.7% oil yield which was slightly lower than the aqueous extraction.

Hence, the recommended process would be mechanical pressing of full fat WG followed by aqueous extraction of the cake obtained from a mechanical press. This two-step process would recover 90% of the oil present in full fat WG. The two-step process optimized in this study can be a viable environmentally benign alternative to hexane extraction and easily incorporated into a wheat biorefinery system that would produce flour and WGO which can be utilized in functional foods and nutraceuticals and add value to a byproduct, WG. The new process does not utilize hazardous chemicals, and is simple to operate. Hence, this technique can easily be adapted by small processors targeting niche markets and operated by farmers' cooperatives.

FUTURE WORK

In this study only one set of shims/spacers (0.015, 0.010, 0.010, and 0.0070 mm from feed to discharge end) was used to separate the cage bars in the mechanical press. The effect of spacer thickness in each cage section on WGO yield requires further research. Aqueous extraction of press cake was examined only at room temperature. The effect of higher aqueous extraction temperature on oil yield and quality should be examined. It is well known that WG contains other health beneficial compounds such as policosanols, lignans, organic acids and phenolic compounds. Oil and protein extracts from the new two-step extraction process should be further analyzed to determine the content and composition of these nutritional compounds. Determination of the economic feasibility of the new two-step WG processing technique requires further research on current WGO market supply and demand trends and equipment costs.

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Table 1: Levels of independent variables used to optimize mechanical pressing

Variables	Levels
Cage Temperature	3 Levels: 51^{o}C, 82^{o}C, and 107^{o}C
Pretreatments of Wheat Germ	2 Levels: Control (wheat germ as it is without any treatment), and indirect steam drying at 82^{o}C.
Shaft Speed	(3 Levels) 400 rpm, 600 rpm, 800 rpm
Back Pressure Plate	(3 Levels) All Plate In (57 mm), All plate Out (89 mm), Plate In the middle (74 mm)
Shaft Arrangement	(2 Levels) Severe, and Combination of severe and mild

Table 2: Proximate composition of wheat germ treated under different conditions. The data was presented as the germ dry weight basis.

Compounds	Full fat wheat germ (g /100g germ) before heat pretreatment	Full fat wheat germ (g /100g germ) after heat pretreatment	Wheat germ cake (g /100g germ)
Moisture	9.81 ± 0.18^{a}	7.07 ± 0.07^{b}	6.16 ± 0.09^{c}
Oil	11.91 ± 0.02^{a}	11.96 ± 0.11^{a}	6.34 ± 0.03^{b}
Protein	33.07 ± 0.07^{b}	33.48 ± 0.36^{b}	35.60 ± 0.20^{a}
Ash	5.11 ± 0.04^{b}	5.02 ± 0.04^{b}	5.33 ± 0.03^{a}
Other components (deduced by difference)	40.10 ± 0.13^{c}	42.47 ± 0.51^{b}	46.61 ± 0.42^{a}

^{a,b,c} Means ± SD in the same row with the same letter are not significantly different at $p>0.05$.

Table 3: Particle size distribution of full fat wheat germ ground using a laboratory mill and coffee grinder.

Particle size (µm)	Laboratory mill Weight percent (%, w/w)	Coffee grinder Weight percent (%, w/w)
>500	8.57 ± 0.42^{a}	29.51 ± 0.17^{b}
150-500	85.13 ± 7.35^{a}	68.27 ± 0.35^{a}
<150	4.89 ± 0.20^{a}	0.95 ± 0.07^{b}

[a,b] Means ± SD in the same row with the same letter are not significantly different at $p>0.05$.

Table 4: Oil yields from wheat germ pressed at different shaft arrangement, cage temperature, germ pretreatment, back pressure, and shaft speed

Shaft arrangement	Cage temperature (^oC)	Germ pretreatment (^oC)	Back pressure	Shaft speed (rpm)	Oil yield (%,w/w)
Combination	51	82	High	400	21.66
Combination	51	82	High	600	19.69
Combination	51	82	Medium	400	14.76
Combination	51	82	Medium	800	8.53
Combination	51	82	No	600	8.15
Combination	51	82	No	800	6.64
Combination	82	19	High	600	28.16
Combination	82	19	Medium	400	11.14
Combination	82	19	No	800	2.82
Combination	82	82	High	400	29.43
Severe	82	82	High	400	45.61
Severe	82	82	High	600	40.69**
Combination	82	82	High	800	17.40
Severe	82	82	High	800	37.02
Severe	82	82	Medium	400	42.53**
Combination	82	82	Medium	600	6.38
Severe	82	82	Medium	600	38.68
Combination	82	82	Medium	800	8.42
Severe	82	82	Medium	800	33.70
Combination	82	82	No	400	13.72
Severe	82	82	No	400	41.28
Combination	82	82	No	600	6.47
Severe	82	82	No	600	35.48
Severe	82	82	No	800	30.12**
Combination	107	19	High	400	14.90
Combination	107	19	Medium	800	6.66
Combination	107	19	No	600	7.82
Severe	107	82	High	400	47.69* & **
Combination	107	82	High	600	30.31
Severe	107	82	High	600	41.98
Combination	107	82	High	800	25.13
Severe	107	82	High	800	36.49
Combination	107	82	Medium	400	13.80
Severe	107	82	Medium	400	43.10
Combination	107	82	Medium	600	6.61
Severe	107	82	Medium	600	37.82

Table 4: Oil yields from wheat germ pressed at different shaft arrangement, cage temperature, germ pretreatment, back plate pressure, and shaft speed (continued from previous page).

Shaft arrangement	Cage temperature (^oC)	Germ pretreatment (^oC)	Back pressure	Shaft speed (rpm)	Oil yield (%,w/w)
Severe	107	82	Medium	800	35.46**
Combination	107	82	No	400	14.01
Severe	107	82	No	400	41.33
Severe	107	82	No	600	36.90**
Combination	107	82	No	800	7.80
Severe	107	82	No	800	29.42

* indicates the best treatment combination which was significantly differences from all other combination ($p < 0.05$)

** indicates that this combination was replicated.

Table 5: The effect of cage temperatures on oil yield as affected by back pressure and shaft speed at severe shaft arrangement and 82 ^{o}C germ pretreatment

Back Pressure	Shaft Speed (rpm)	Num DF	Den Df	F Value	Pr> F
High	400	1	6	40.44	0.0007
High	600	1	6	15.50	0.0076
High	800	1	6	1.99	0.2083
Medium	400	1	6	3.06	0.1309
Medium	600	1	6	5.18	0.0632
Medium	800	1	6	29.09	0.0017
No Pressure	400	1	6	0.02	0.9019
No Pressure	600	1	6	18.70	0.0050
No Pressure	800	1	6	4.60	0.0757

$p < 0.05$ indicates statistical significance.

Table 6: Particle size distribution of wheat germ cake ground using a laboratory mill and coffee grinder.

Particle size (µm)	Laboratory mill Weight percent (%)	Coffee grinder Weight percent (%)
>500	19.93 ± 0.46[a]	49.88 ± 2.95[b]
150-500	76.36 ± 1.03[a]	46.44 ± 0.35[b]
<150	2.66 ± 0.08[a]	2.10 ± 0.16[b]

[a,b] Means ± SD in the same row with the same letter are not significantly different at $p>0.05$.

Table 7: Fatty acid composition (%, w/w) of WGO samples*extracted through various methods

Fatty Acid[1]	WGO P	WGO H	WGO S	WGO N	WGO C
16:0	17.20±0.05^{b}	17.36±0.06^{a}	17.28±0.01^{b}	16.80±0.06^{c}	17.43±0.03^{a}
16:1	0.16±0.002^{b}	0.16±0.001^{b}	0.14±0.003^{d}	0.15±0.001^{c}	0.17±0.003^{a}
18:0	0.68±0.002^{c}	0.70±0.01^{a}	0.69±0.01^{b}	0.70±0.01^{a}	0.68±0.004^{c}
18:1	15.01±0.02^{a}	14.53±0.07^{d}	14.45±0.01^{e}	14.88±0.03^{c}	14.94±0.01^{b}
18:2	57.09±0.06^{a}	56.85±0.07^{b}	55.33±0.02^{d}	56.25±0.21^{c}	56.35±0.07^{c}
18:3	5.89±0.03^{d}	6.40±0.05^{b}	7.95±0.02^{a}	6.13±0.05^{c}	5.72±0.01^{e}
20:0	0.17±0.002^{b}	0.16±0.003^{c}	0.14±0.001^{d}	0.18±0.003^{a}	0.18±0.002^{a}
20:1	1.53±0.01^{a}	1.37±0.02^{c}	1.36±0.001^{c}	1.54±0.01^{a}	1.51±0.01^{b}
20:2	0.14±0.001^{a}	0.14±0.002^{a}	0.12±0.002^{c}	0.13±0.002^{b}	0.14±0.01^{a}
22:0	0.14±0.002^{d}	0.15±0.01^{c}	0.12±0.003^{e}	0.16±0.001^{b}	0.16±0.001^{a}
22:1	0.27±0.001^{b}	0.23±0.003^{c}	0.20±0.001^{d}	0.28±0.001^{a}	0.27±0.002^{b}
24:1	0.19±0.002^{b}	0.17±0.003^{c}	0.14±0.002^{d}	0.19±0.001^{b}	0.20±0.005^{a}
SAFA	18.21±0.04^{b}	18.37±0.07^{a}	18.23±0.01^{b}	17.83±0.06^{c}	18.44±0.03^{a}
MUFA	17.15±0.02^{a}	17.47±0.1^{c}	16.29±0.01^{d}	17.04±0.04^{b}	17.08±0.02^{ab}
PUFA	63.12±0.09^{b}	63.38±0.12^{a}	63.40±0.04^{a}	62.51±0.27^{c}	62.20±0.08^{d}

SAFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

*The sample abbreviations are as following:

WGO P : Crude WGO extracted by screw press at optimum condition (severe shaft arrangement, cage temperature of 107 ^{o}C, germ Pretreatment/heating at 82 ^{o}C, high back pressure, and shaft speed at 400 rpm); WGO H: Commercial hexane extracted crude WGO; WGO S:Commercial supercritical CO_2 extracted crude WGO; WGO N: Crude WGO extracted from screw press with no heat pretreatment of the wheat germ (combination between severe and mild shaft arrangement, cage temperature of 82 ^{o}C , high back pressure, and shaft speed at 600 rpm); WGO C: Crude WGO extracted by hexane from the optimum conditions wheat germ cake.

[1] Fatty acids names are as follows:

16:0 = Palmitic Acid; 16:1= Palmitoleic Acid; 18:0= Steric Acid; 18:1= Oleic Acid; 18:2= Linoleic Acid; 18:3=Linolenic Acid; 20:0=Arachidic Acid; 20:1=Gadoleic Acid; 20:2= Eicosadienoic Acid; 22:0= Behenic Acid; 22:1= Erucic Acid; 24:1= Nervoni Acid

[a,b,c,d,e]Means ± SD in the same row with the same letter are not significantly different at p>0.05.

Table 8: Characteristics of wheat germ oil

Sample*	FFA (%)	PV (meq / kg)	*p*-Anisidine (AV)	Water Content (%)	Phosphorus (ppm)
WGO P	3.37±0.07^{c}	2.88±0.05^{d}	2.12±0.07^{d}	0.49±0.01^{d}	1634.50±54.45^{ab}
WGO H	14.58±0.18^{a}	7.97±0.15^{b}	5.96±0.15^{a}	0.66±0.01^{b}	1578.50±17.68^{b}
WGO S	1.59±0.03^{d}	5.01±0.10^{c}	2.61±0.03^{c}	0.76±0.02^{a}	51.60±1.98^{d}
WGO N	3.31±0.07^{c}	2.41±0.04^{e}	1.01±0.03^{e}	0.55±0.02^{c}	1084.50±13.43^{c}
WGO C	3.88±0.07^{b}	9.93±0.08^{a}	3.39±0.15^{b}	0.45±0.01^{d}	1672.00±18.38^{a}

*Refer to Table 11 for sample abbreviations

^{a,b,c,d,e} Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Table 9: Tocopherol compositions (mg/g oil) of WGO samples extracted through various methods[1]

Sample	α-Tocopherol	β-Tocopherol	γ-Tocoherol	Total Tocopherols
WGO P	3.99±0.05[b]	0.92±0.01[b]	0.27±0.003[b]	5.18±0.06[b]
WGO H	3.16±0.24[c]	0.71±0.03[c]	0.67±0.02[a]	4.54±0.29[c]
WGO S	5.80±0.03[a]	1.14±0.01[a]	0.27±0.01[b]	7.21±0.04[a]
WGO N	4.31±0.01[b]	0.94±0.001[b]	0.05±0.001[d]	5.30±0.01[b]
WGO C	2.34±0.14[d]	0.92±0.03[b]	0.09±0.01[c]	3.35±0.17[d]

[1]Refer to Table 11 for sample abbreviation

[a,b,c,d]Means ± SD in the same column with the same letter are not significantly different at p>0.05.

Table 10: Phospholipid compositions (mg/g oil) of WGO samples extracted through various methods[1]

Sample	PE[2]	PI+PA[3]	PC[4]	Total phospholipids
WGO P	1.84±0.02[d]	13.37±0.08[a]	0.89±0.06[c]	16.10±0.16[a]
WGO H	2.22±0.04[c]	9.67±0.10[b]	2.31±0.15[b]	14.20±0.08[b]
WGO S	n.d.	n.d.	n.d.	n.d.
WGO N	2.89±0.05[a]	8.29±0.37[c]	1.12±0.05[c]	12.30±0.36[d]
WGO C	2.60±0.04[b]	7.31±0.22[d]	3.55±0.14[a]	13.46±0.01[c]

[1] Refer to Table 11 for sample abbreviation

[2]PE: phosphatidylethanolamine; [3]PI+PA: phosphatidylinositol and phosphatic acid; [4]PC: phosphatidylcholine

n.d. not detected.

[a,b,c,d]Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Table 11: Oil extraction yield (%, w/w) for non-enzymatic processes at LSR of 20 and extraction time 0.5h

Sample	Buffer	Particle Size	Oil Yield (%)
Wheat Germ Cake	Boric Acid-NaOH	Fine	79.64± 0.56[a]
Wheat Germ Cake	Tris-HCl	Fine	76.50 ± 0.69[b]
Wheat Germ Cake	Boric Acid-NaOH	Coarse	75.60 ± 0.28[b]
Wheat Germ Cake	Tris-HCl	Coarse	72.15 ± 0.43[c]
Full Fat Wheat Germ	Tris-HCl	Fine	65.05 ± 0.14[d]
Full Fat Wheat Germ	Boric Acid-NaOH	Fine	55.13 ± 0.02 [e]
Full Fat Wheat Germ	Tris-HCl	Coarse	36.04± 0.17[f]
Full Fat Wheat Germ	Boric Acid-NaOH	Coarse	33.32± 0.89[g]

[a,b,c,d,e,f.g] Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Table 12: Analysis of variance for non-enzymatic processes.

Source	**Df**	**SS**	**MS**	**F Value**	**Pr>F**
Sample	1	3268.7233	3268.7233	13692.9	<0.0001
Buffer	1	9.10048	9.10048	38.12	0.0003
Sample x Buffer	1	92.6387	92.6387	388.07	<0.0001
Particle size	1	876.4560	876.4560	3671.53	<0.0001
Sample x Particle size	1	450.0168	450.0168	1885.15	<0.0001
Buffer x Particle size	1	14.0493	14.0493	58.85	<0.0001
Sample x Buffer x Particle size	1	11.8277	11.8277	49.55	0.0001

$p < 0.05$ indicates statistical significance.

Table 13: Enzymes used in this study and their activities

Enzyme	**Activity [a]**	**Activity [b]**
Alcalase 2.4L FG	12.52 U/g	2.40 AU/g
Multifect CX GC	3071.73 CMC/g	3200.00 CMC/g
Multifect GC Extra	7844.40 CMC/g	6200.00 IU/ml

[a] Initial activity of the enzymes prior to test.
[b] Activity declared by the suppliers.

Table 14: Oil extraction yield (%) by different enzymes from enzymatic process

Sample	Enzyme Type	Particle Size	Oil Yield (%)
Wheat Germ Cake	Alcalase 2.4L FG	Fine	76.69 ± 1.42^{a}
Wheat Germ Cake	Alcalase 2.4L FG	Coarse	74.94 ± 0.04^{a}
Full Fat Wheat Germ	Alcalase 2.4L FG	Fine	65.30 ± 1.14^{b}
Full Fat Wheat Germ	Alcalase 2.4L FG	Coarse	45.79 ± 2.26^{c}
Wheat Germ Cake	Multifect CX GC	Fine	2.14 ± 0.01^{f}
Wheat Germ Cake	Multifect CX GC	Coarse	2.06 ± 0.10^{f}
Full Fat Wheat Germ	Multifect CX GC	Fine	20.59 ± 0.20^{d}
Full Fat Wheat Germ	Multifect CX GC	Coarse	11.57 ± 0.34^{e}
Wheat Germ Cake	Multifect GC Extra	Fine	2.12 ± 0.05^{f}
Wheat Germ Cake	Multifect GC Extra	Coarse	2.04 ± 0.05^{f}
Full Fat Wheat Germ	Multifect GC Extra	Fine	20.14 ± 0.94^{d}
Full Fat Wheat Germ	Multifect GC Extra	Coarse	10.79 ± 0.004^{e}

^{a,b,c,d,e,f} Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Table 15: Oil extraction yield (%) by Alcalase 2.4L FG at LSR of 25, enzyme concentration of 0.5% and extraction time of 24 h

Sample	Particle Size	Oil Yield (%)
Wheat Germ Cake	Fine	54.87 ± 2.10^{a}
Wheat Germ Cake	Coarse	50.62 ± 1.01^{a}
Full Fat Wheat Germ	Fine	44.63 ± 1.69^{b}
Full Fat Wheat Germ	Coarse	27.01 ± 1.06^{c}

^{a,b,c} Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Table 16: Oil extraction yield (%) using Multifect GC Extra followed by Alcalase 2.4L FG at liquid solid ratio 16.5 and two different extraction times.

Sample	Extraction Tim (h) (Multifect + Alcalase)	Oil Yield (%)
Full Fat Wheat Germ	12.25 + 5.25	54.52 ± 0.53^{a}
Wheat Germ Cake	12.25 + 5.25	50.17 ± 1.72^{b}
Wheat Germ Cake	2.00 + 3.00	42.27 ± 0.93^{c}
Full Fat Wheat Germ	2.00 + 3.00	40.21 ± 1.29^{c}

^{a,b,c} Means ± SD in the same column with the same letter are not significantly different at $p>0.05$.

Figure 1: Structure of Wheat grain (Fennema 1985).

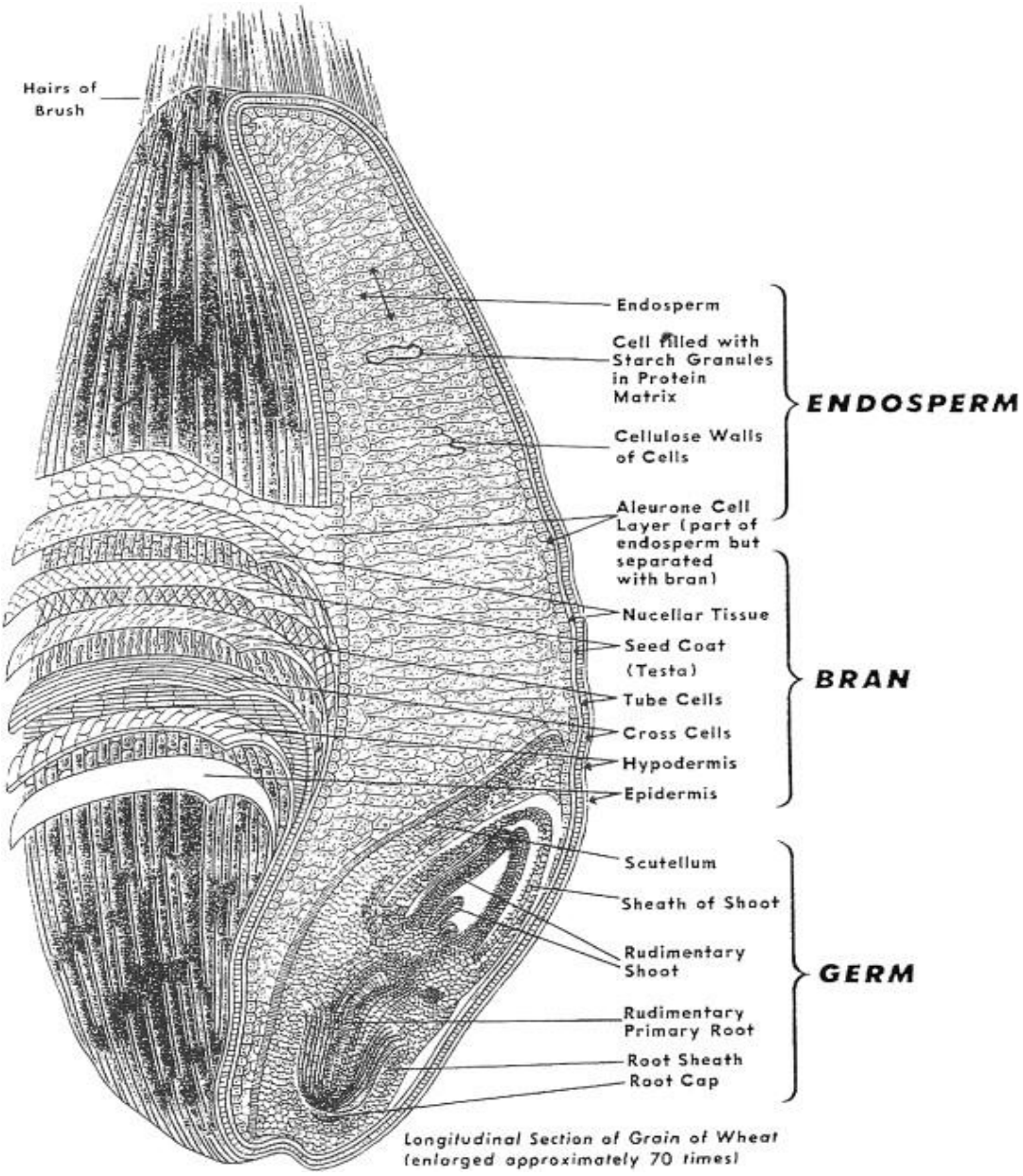


Figure 2: Heavy duty laboratory screw press (Evangelista and Cermak 2007).

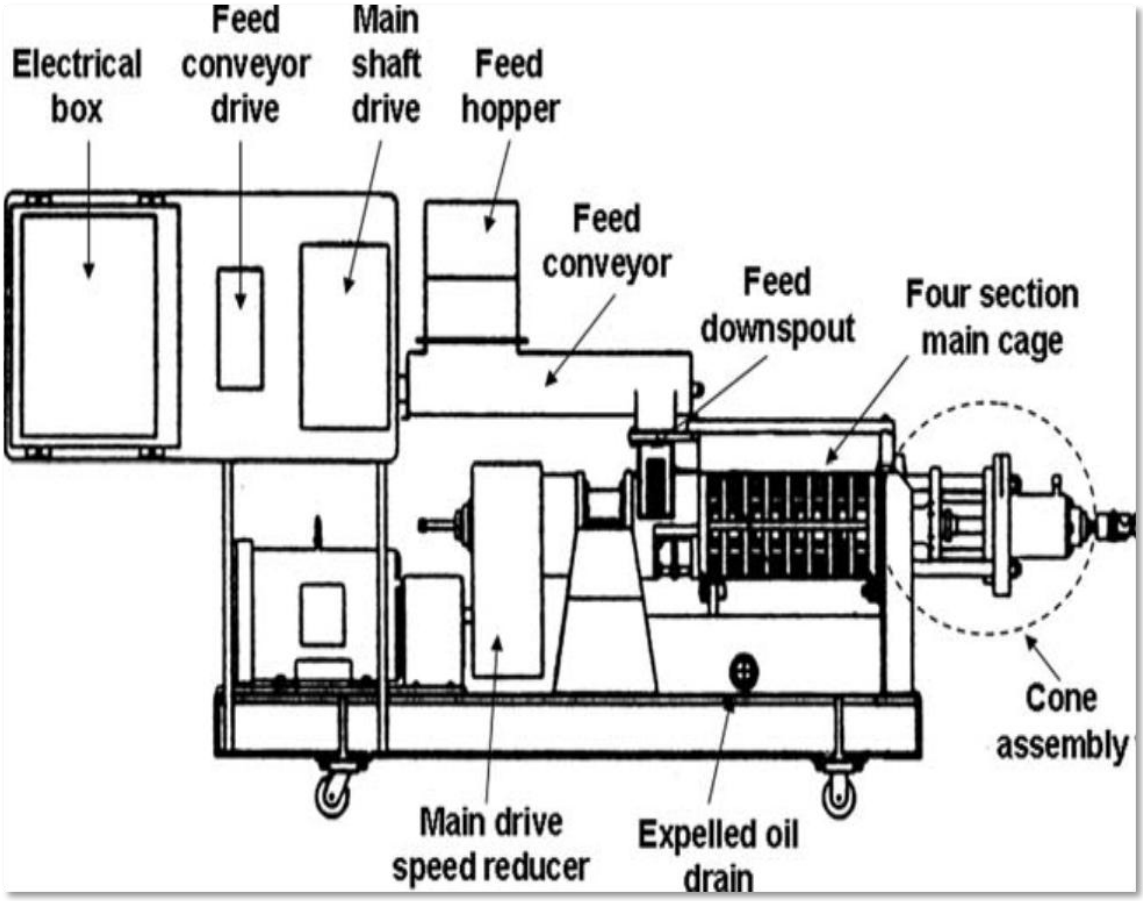


Figure 3: The basic steps involved in processing oilseeds by mechanical pressing

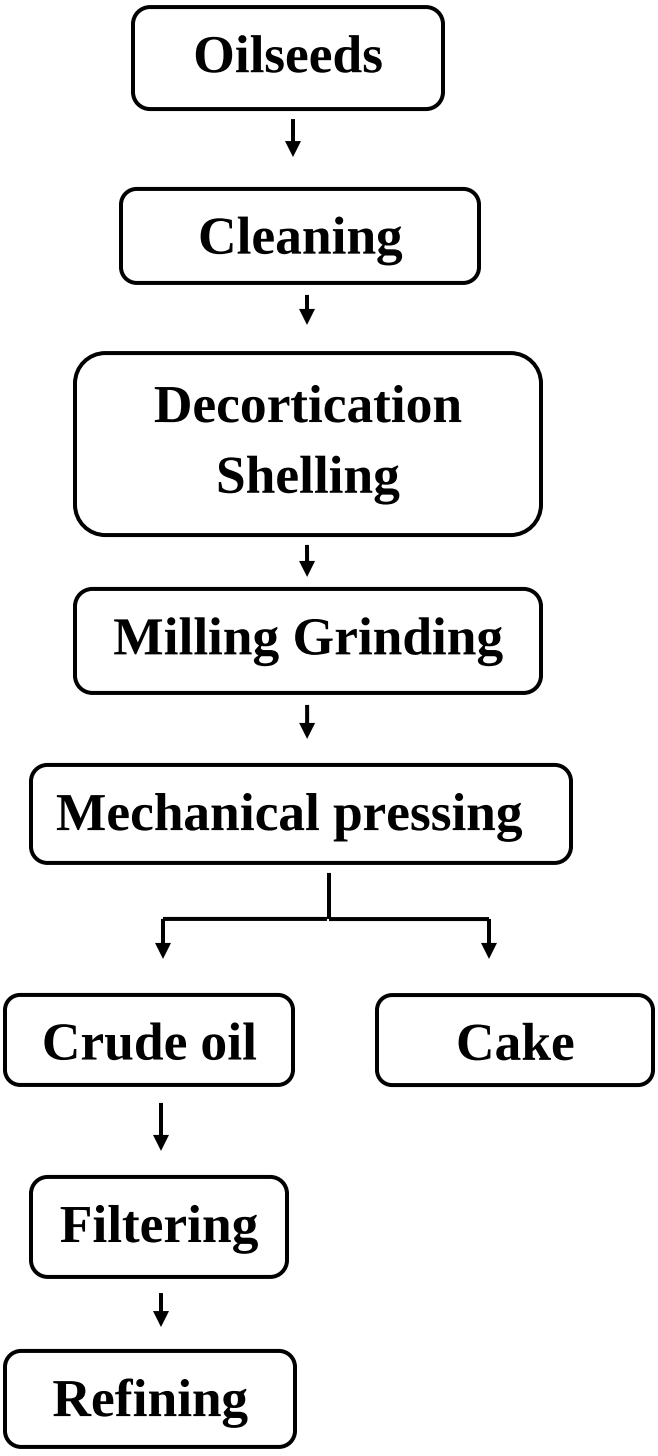


Figure 4: Compression curve of a screw press (Ward 1976)

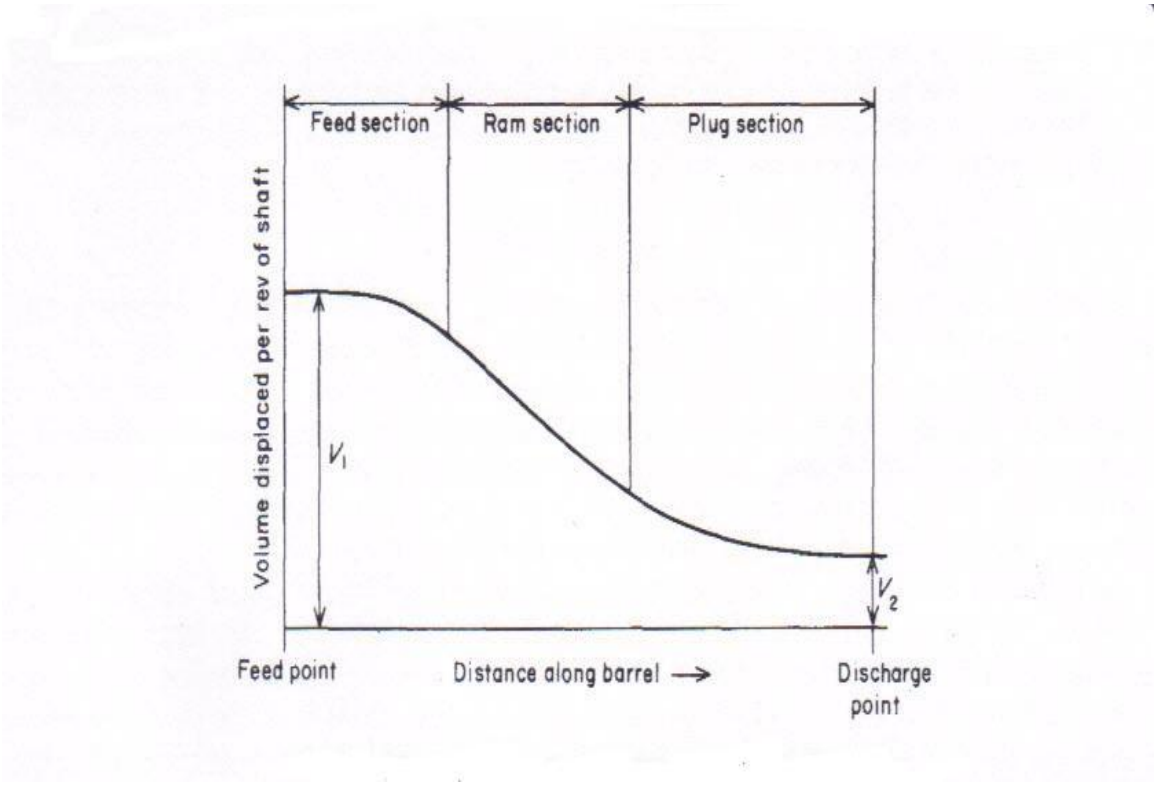


Figure 5: Diagram of the cage section inside the screw press (French Oil Mill Company Manual).

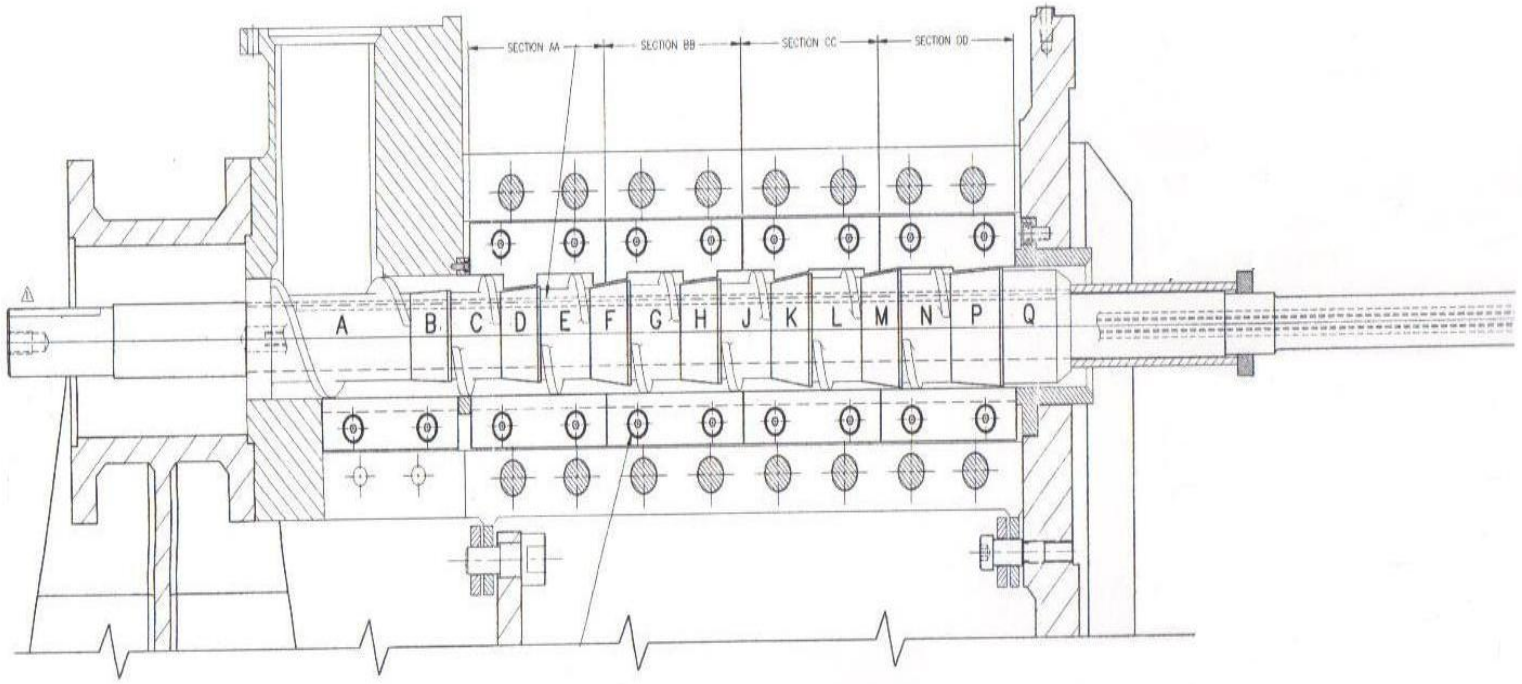


Figure 6: A schematic flow diagram of the mechanical extraction process for wheat germ

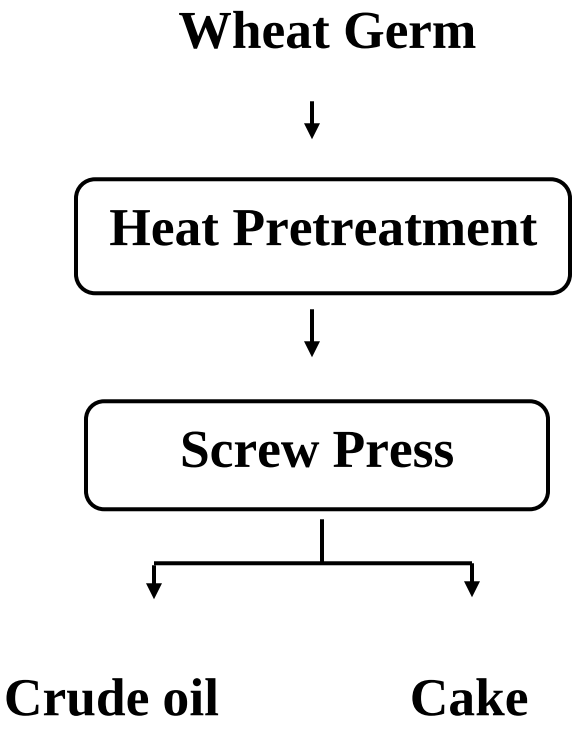


Figure 7: A schematic of aqueous enzymatic oil extraction procedure used in this study

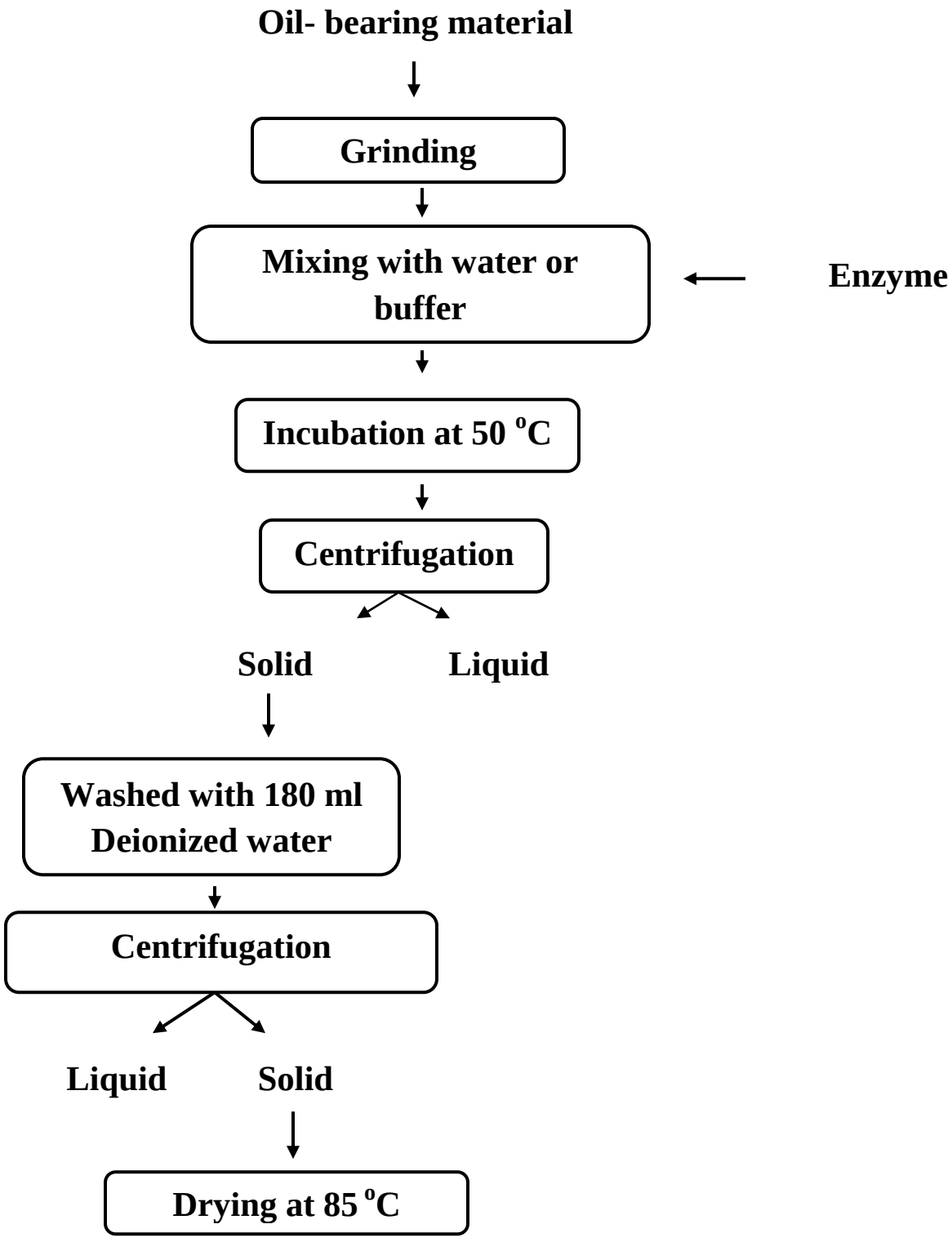


Figure 8: Effect of back pressure and shaft speed on oil yield from wheat germ expressed at severe shaft arrangement, 82 °C germ pretreatment, and cage temperature at 82 °C.

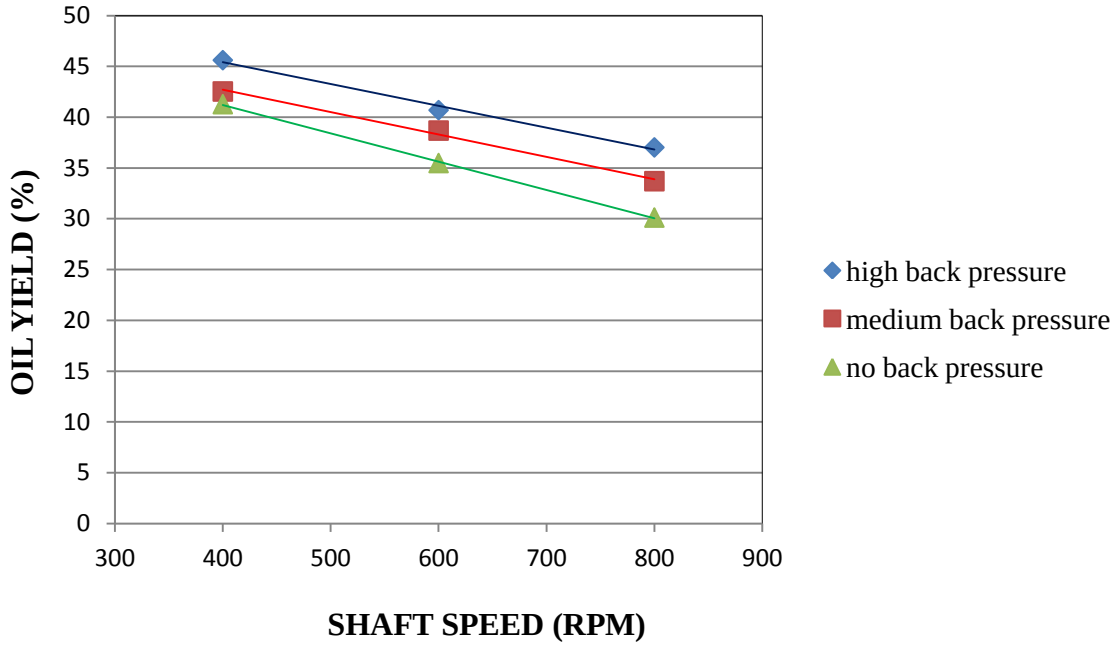


Figure 9: Effect of back pressure and shaft speed on oil yield from wheat germ expressed at severe shaft arrangement, 82 °C germ pretreatment, and cage temperature at 107 °C.

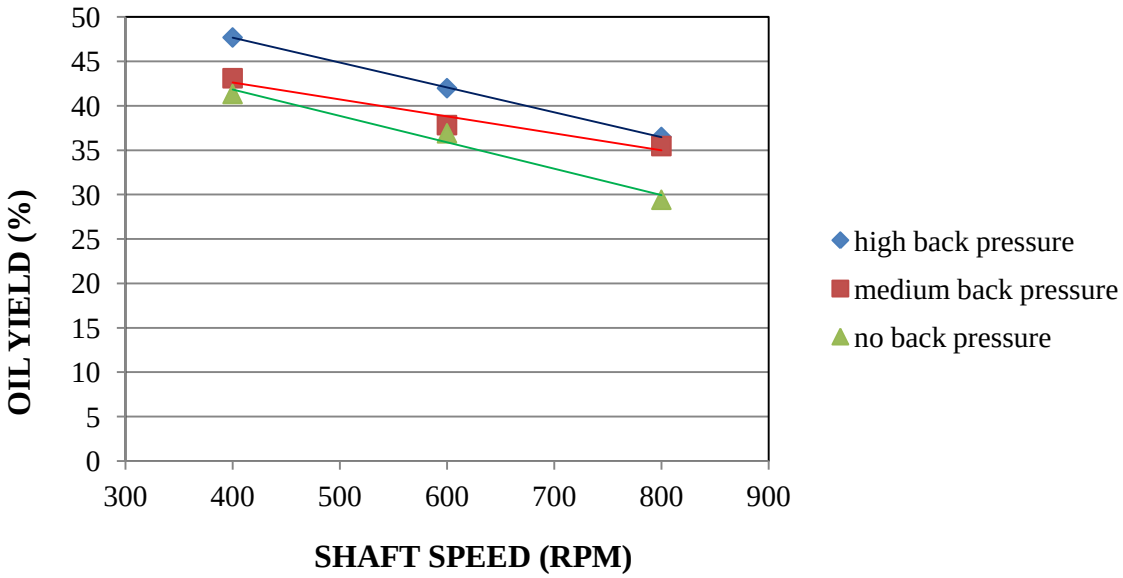


Figure 10: Effect of back pressure at each shaft speed on oil yield from wheat germ expressed at shaft arrangement combination of severe and mild, 82 °C germ pretreatment, with no heating cage temperature.

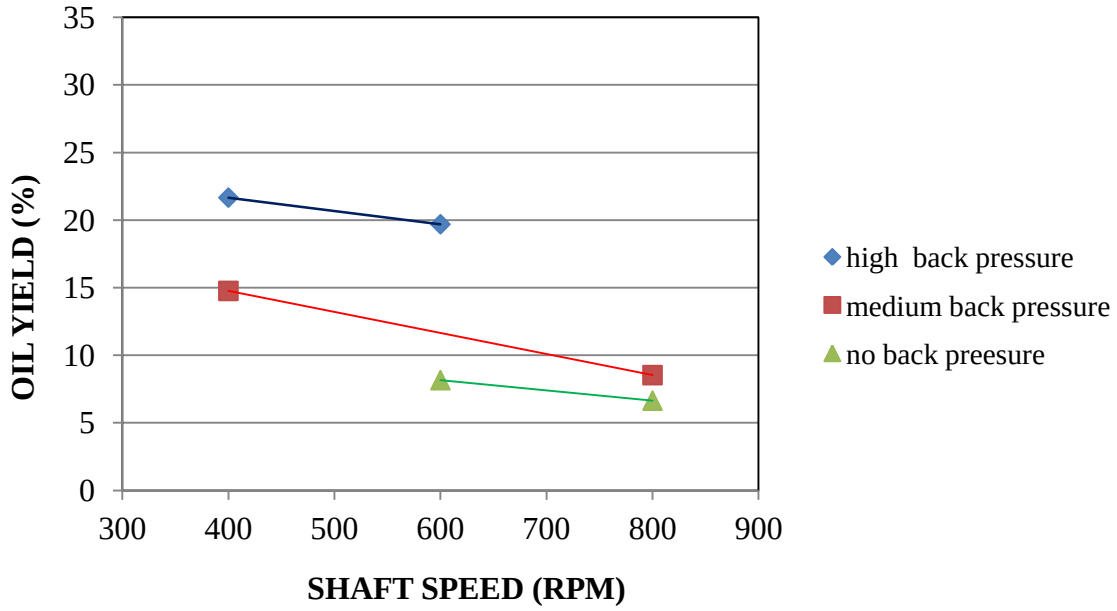


Figure 11: Effect of back pressure at each shaft speed on oil yield from wheat germ expressed at shaft arrangement combination of severe and mild, 82 °C germ pretreatment, and cage temperature at 82 °C.

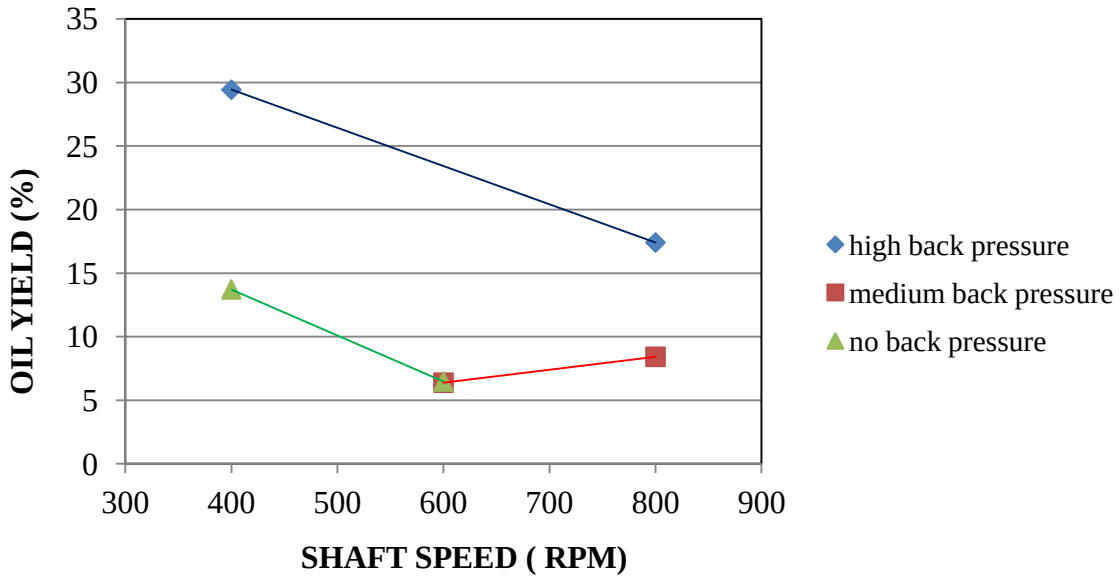
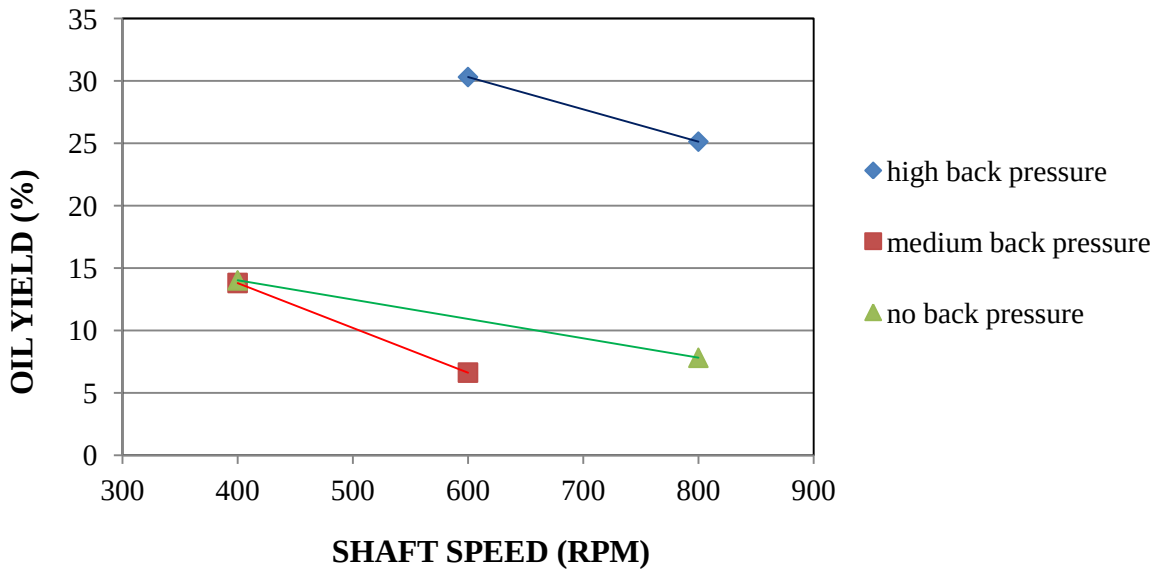


Figure 12: Effect of back pressure at each shaft speed on oil yield from wheat germ expressed at shaft arrangement combination of severe and mild, 82 °C germ pretreatment, and cage temperature at 107 °C.



VITA

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