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Effect of Extrusion Pre-Treatment on Physical Properties and Sugar Recovery of Cold Press and Solvent Extracted Canola, Camelina and Carinata Meal

Jasmeen Kaur

EFFECT OF EXTRUSION PRE-TREATMENT ON PHYSICAL PROPERTIES AND SUGAR RECOVERY OF COLD PRESS AND SOLVENT EXTRACTED CANOLA, CAMELINA AND CARINATA MEAL

BY

JASMEEN KAUR

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This thesis is approved as a credible and independent investigation by a candidate for the Master of Science in Agricultural & Biosystems Engineering degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Kasiviswanathan Muthukumappan, Ph.D.
Thesis Advisor Date

Van Kelley, Ph.D.
Head, Department of Agricultural & Biosystems Engineering Date

Dean, Graduate School Date

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CONTENTS

ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
WAI	Water Absorption Index
WSI	Water Solubility Index
CP	Cold Press
ASE	Accelerated Solvent Extraction
CM	Canola Meal
DM	Dry Matter
a_w	Water Activity
FPU	Filter Paper Unit
D	Thermal Diffusivity
K	Thermal Conductivity
C	Specific Heat Capacity
TD	True Density
BD	Bulk Density
DF	Degree of Freedom
SS	Sum of Squares

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ABSTRACT

EFFECT OF EXTRUSION PRE-TREATMENT ON PHYSICAL PROPERTIES AND SUGAR RECOVERY OF COLD PRESS AND SOLVENT EXTRACTED CANOLA, CAMELINA AND CARINATA MEAL

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Canola/rapeseed meal (CM) ranks second behind soybeans in global production of protein from oil cakes and meals. Commercial CM has been investigated as a feed ingredient in diets of a number of fish species. Camelina (*Camelina sativa*) has been a potential oilseed crop. The fat extracted meal contains over 40% protein. Camelina yields an average of 420–640 L/ha, and the protein and fiber content in its meal byproduct is comparable to that of soybean meal. Brassica carinata possesses many positive agronomic traits, and it can grow well in hot, dry, and semiarid climates and seed oil from these species and its byproduct (carinata meal) may have potential applications in the food, biofuel, and feed industries. The sugars present in the canola, camelina and carinata meal can be used as a source for production of feed for the fish: the sugars can be converted into a high protein diet with the help of fermentation. Due to comparatively low sugar content in oilseed meals, this issue needed to be resolved.

To solve this issue, the canola, camelina and carinata meal needs to increase their sugar level by using a pre-treatment. First of all, two different types of meals were prepared by using cold press oil extractor and accelerated solvent extractor. After extraction using two different methods, pre-treatment was done. Extrusion was used as a pre-treatment. The

canola, camelina and carinata meal were extruded using a total of 9 combinations of temperature and screw speed. Three different temperature including 80°C, 130°C and 180°C and screw speed of 50, 100 and 150 rpm were used. In order to measure the increased sugar recovery, enzymatic hydrolysis was done followed by the HPLC analysis. The enzymes were added at 50, 60, 70, 80 and 90 FPU/g cellulose in order to optimize the enzyme dosage. The sugar recovery was measured in terms of glucose, galactose and arabinose recovery. The extruder processing conditions were optimized based on the amount of sugar recovery. Enzyme dosage and extruder processing conditions played a major role in increasing the sugar recovery of canola, camelina and carinata meal. Higher sugar recovery was recorded for accelerated solvent extracted (ASE) canola meal versus to the cold press (CP) extracted canola meal. A significant increase in ASE galactose recovery and glucose recovery (CP&ASE) was observed due to extrusion temperature of camelina meal. It was observed that during extrusion, for camelina meal, temperature had a greater effect than screw speed. Higher sugar recovery was recorded for CP carinata meal than ASE carinata meal.

Physical properties can show characteristics of the transition and storage of these plant materials (canola, camelina and carinata meal). These physical properties, include moisture content, bulk density, true density, water absorption index (WAI), water solubility index (WSI), water activity and color and were measured. Thermal properties like thermal conductivity, thermal diffusivity and specific heat capacity were also measured. Extruder temperature had a significant effect ($p<0.05$) on WAI, water activity, color and bulk density of ASE canola meal. There was a significant increase ($p<0.05$) in WSI and thermal properties of CP camelina meal and WAI of ASE camelina meal.

Extrusion caused a significant ($p<0.05$) decrease in the values of WAI of CP camelina meal and bulk density, water activity and L (color) of ASE camelina meal. It was observed that during extrusion, temperature had a greater effect than screw speed. Extruder temperature had a significant effect ($p<0.05$) on WAI, WSI, water activity, color and moisture content at dry basis (MCdb) of CP and ASE carinata meal extrudates. No significant effect ($p>0.05$) of extruder temperature and screw speed was observed on bulk density and true density of CP and ASE carinata meal extrudates.

Overall, extruder temperature had a more significant effect versus screw speed on sugar recovery, physical properties and thermal properties of canola, camelina and carinata meal. Extrusion and enzyme dosage played a significant role in increasing the total % sugar recovery of canola, camelina and carinata meals.

CHAPTER 1
INTRODUCTION AND OBJECTIVES

1.1 Introduction

Historically fish meal has been the most important protein source in commercial feeds for fish. Protein quality, component concentration and palatability are some of the factors that make it important. Currently worldwide production of fish meal is approximately 6–7 million tons annually. The level of production is expected to remain stable over the next 10 years (New and Wijkstrom, 2002) and rapid expansion of aquaculture during this period will require the replacement of fish meal in aquafeeds with plant-based protein sources. There are certain problems of replacing fish meal with plant proteins, as the quality and concentration of proteins from plant sources is generally inferior to fish meal because the palatability of most plant proteins fish meal is low. However, the low cost of plant proteins and availability of plant proteins in abundant may allow processing of plant proteins to improve their nutritive value in fish.

The plant proteins must be compared to the fish meal in terms of protein quality for fish growth performance and health, and cost. Fish meal is produced either from fish that are harvested specifically for production of fish meal or from byproducts of fish destined for human consumption. Another important concern for replacing fish meal with plant based proteins is contamination of fish meal and oil with organochlorine compounds. Hites et al. (2004) reported that the concentration of organochlorine contaminants was higher in farmed salmon versus wild salmon. It was also reported that the high concentrations of organochlorine compounds in aquafeeds based primarily on fish meal and oil was the source of contamination. Replacement of fish meal and oil in aquafeeds might therefore

decrease the level of organochlorine compounds in farmed salmon and increase consumer acceptance. Thus increasing cost and potential contamination with organochlorines give a strong reason for the replacement of fish meal in aquaculture feeds.

After soyabean, canola/rapeseed meal (CM) ranks second in global production of protein from oil cakes and meals. The cost of CM protein is approximately half that of fish meal per unit protein basis (Sarwar et al., 1984). Investigations has been done for CM as a feed ingredient in diets of a number of fish species (Hilton and Slinger, 1986; Leatherland and Hilton, 1988; Satoh et al., 1998; Mwachireya et al., 1999; Soares et al., 2001). Lower growth performance of Chinook salmon was observed when fish meal was replaced by commercial CM (Satoh et al., 1998) but, the growth was not different than those fed with fish meal diet when the CM was extruded at 90 or 150 ◦C.

Camelina [Camelina sativa (L.) Crantz], also known as false flax, is a spring annual broadleaf oilseed herb of the Brassicaceae family that grows well in temperate climates. Several positive agronomic attributes were observed for camelina as compared to other oilseed crops. Some of them were low agricultural inputs, cold-weather tolerance, short growing season (85–100 days), compatibility with existing farm equipment, and grows well in semiarid regions and in low-fertility or saline soils. These qualities are unusual for an oilseed crop (Putnam et al., 1993; Retka-Schill, 2008b; Sawyer, 2008). Moreover, camelina, unlike soybean, can grow easily in cold regions and is nicely adapted to the more northerly regions of North America, Europe, and Asia. The seeds of camelina contain 28–40 wt.% of vegetable oil (Putnam et al., 1993; Budin et al., 1995), which is higher than what is found in soybeans (18–22 wt.%). Camelina has already been used for other purposes such as culinary oil, cosmetics, and animal feed (Sawyer, 2008).

Brassica carinata is also known as Ethiopian mustard and originated from a cross between *B. nigra* and *B. oleracea*. The plant has been widely used for oil extraction and is of great importance, economically and nutritionally. The defatted meal that is left after oil extraction is rich in protein. Because of some anti-nutritional components such as glucosinolates and phytates, and a high cellulose content, the defatted meal presents some problems for human consumption. For this reason, the Brassica defatted meal is only used for animal feeding and as an organic fertilizer (Duncan, 1991; Pedroche et al 2004).

To remove the oil from the oilseeds, there are different types of oil extraction methods. The cold-press procedure involves neither heat nor chemical treatments. Natural and safe food products can be obtained using cold press extraction method (Parker et al., 2003). Accelerated solvent extraction (ASE) technique uses pressure and temperature to extract solid samples (Ezzell, et al., 1995). The organic solvents that are used during extraction process works at temperatures above the normal boiling-point.

To utilize the oilseed meals more widely, it is important to consider the shelf life of these meals. The low bulk density is the key parameter that leads to the higher cost of handling and storage (Sokhansanj et al. 2006). Physical properties describe the basic characteristics of meals, which are indispensable for the handling and storage. Safe storage is important in supplying oilseed meals to conversion facilities. The surrounding conditions such as environment temperature and the relative humidity can influence the storage life of oilseed meals as they are directly related to the moisture content via moisture adsorption (Jamali, 2006). The storage life of biomass depends on the relationship of the moisture content of the oilseed meals, the temperature and relative

humidity of the environment and these factors can help to predict the stability of the oilseed meals and help to prolong the storage life of biomass.

Feed rate, feed moisture, screw speed and barrel temperature effect the various extrudate properties in significant way. Bulk density has significant effect on material handling and storage aspects and it depends on certain factors like moisture content, particle size, shape. So, it is necessary to find these characterizations which are important for the handling and storage of the oilseed meals and may lead to the possibility of cost reduction in aquaculture production and further develop the feed industry. As a result, there is a great need for this study.

1.2 Objectives:

The primary objective of this study will be to understand the influence of plant protein and extruder parameters on the sugar recovery from different biomasses (canola, camelina and carinata meal). Most of the literature was reported on the use of protein content present in the oilseed meals. No systematic carbohydrate hydrolysis of oilseed meals and pretreatment to increase the sugar recovery of oilseed meals was found in the literature. The objectives of the study were:

1. Understand the effect of extruder (barrel temperature and screw speed) and varying the enzyme dosage for maximizing sugar recovery
2. Determine the physical properties of cold press extracted and solvent extracted oilseed meals (canola, camelina and carinata meal)
3. Optimize the enzyme dosage for maximum sugar recovery from different oilseed meals

4. Optimize the extruder parameters to predict the maximum sugar recovery from different oilseed meals
5. To compare the effect of oil extraction methods on sugar recovery from different oilseed meals
6. To compare the physical properties of cold press extracted meals and solvent extracted meals

CHAPTER 2

LITERATURE REVIEW

2.1 Oilseed meals

The protein from vegetable origin can be an alternative to the animal protein for food and cosmetics applications, due to the availability, low cost, renewability of raw material and widespread and variety of sources (especially legumes, cereals and oilseeds). Some of the oilseed meals like soybeans, rapeseed, cottonseed, sunflower seed and peanut are the most abundant protein meal and it represents a 69%, 12.4%, 6.9%, 5.3% and 2.8% of world protein meal production (Ash & Dohlman, 2006). Different authors have studied the functionality of oilseed proteins like protein isolates in relation to food applications, the emphasis of their work being focused on different industrial crops (cereals, oilseeds, legume seeds) (Gueguen, Sanchez- Vioque, & Malabat, 1999; Lampart-Szczapa, 2001), soybean (Barraquio & Van de Voort, 1988), sunflower (Sosulski & Fleming, 1977), peanut (El-Zalaki et al., 1995), and legumes (Braudo et al., 2001; Singh, 2001).

2.1.1 Canola meal

Canola is the name given to the species produced by *Brassica napus* and *Brassica campestris* species and they are low in glucosinolates and erucic acid. Canola ranks second to soyabean for global production of protein (Higgs et al., 1996). Further, the chemical composition of the meal and other protein products was consistent and the cost of the meal is more economical on a per kilogram protein basis (Higgs et al., 1996).

Because of good balanced ratio of protein and amino acid, canola meal is considered as an important fodder crop (Uruakpa and Arntfield 2005a; Canola Council of Canada 2009). Canola protein concentrate (Anjou and Fecske, 1974) and rapeseed protein isolate (Gillberg and Tornell, 1977) may be useful for human consumption. Some previous studies have focused on utilisation of canola protein for production of bioactive peptides (BAPs) with the aims of improving human health or to supply nutritional benefits (Cumby, Zhong, Naczk, & Shahidi, 2008; Marczak et al., 2003).

Earlier studies with Brassica rapa (campestris) canola have determined that among different varieties for seeds (black and yellow), they differed significantly in their chemical composition such as oil, protein, and fiber contents, with yellow seeds containing more oil and protein and less fiber (Bell and Shires 1982). High amount of fiber is found in the canola meal due to the high content (30 %) of hull (Bell and Shires, 1982). The chemical composition of canola meal derived from black seeded canola (%DM) was: Crude protein – 43.8, Fat – 1.8, Non starch polysaccharide – 20.2, Dietary fiber – 30.1, Ash – 7.3 (Slominski et al., 2012).

The annual production of canola has been increasing and is measured as 15 million tonnes in 2015 (Canola Council of Canada 2009). As a result of increased oil extraction, more meal will be produced as the demand for canola meal is increasing as well. According to Australian Oilseeds Federation (2009), Australian oilseeds (canola) industry has grown in an excellent way. This leads to understand canola proteins in a better way which is the major constituents in the meal. The canola meal can be used as a substitution for fish meal because fish meal is very expensive and canola meal can prove as more economical for the industries.

Various studies has been done for the enzymatic hydrolysis of protein content of canola, in this study enzymatic hydrolysis of canola meal were done after pre-treatment with an objective to increase the sugar recovery of the canola meal and how the sugar recovery differs with different oil extraction methods i.e. cold press extraction and accelerated solvent extraction.

Table 2.1: Typical chemical composition of canola meal (12% moisture basis) (Newkirk et al., 2003a):

Component	Average
Crude protein (N*6.25%)	36
Rumen bypass protein (%)	35
Oil (%)	3.5
Linoleic acid (%)	0.6
Ash	6.1
Crude fiber (%)	12.0
Tannins (%)	1.5
Sinapine (%)	1.0
Phytic acid (%)	3.3
Glucosinolates (µmol/g)	7.2

This table shows nutrient composition for canola meal processed using the solvent extraction method. Canola meal will have 8-15% more fat and much higher energy values if processed using the double-press expeller method.

2.1.2 Camelina meal

Camelina, popularly known as false flax or gold of pleasure, belongs to the family *Brassicaceae* and grows in Mediterranean to Central Asia and is very adaptable to climate and soil types. Camelina is rich in linolenic acid and has a fatty acid profile very much similar to flaxseed. The fatty acid camelina oil profile contains 20 to 40% C18:3, 10 to 20% C18:2, 12 to 25% C18:1, 13 to 21% C20:1, and between 2 and 5% C22:1. Studies have shown that camelina could be used for applications other than food such as skincare products, soaps and soft detergents, production of lipopeptides and lipoaminoacids, and in paints (Bonjean and Le Goffic, 1999; Hurtaud and Payraud 2007). The protein-rich camelina pressed cake is also a valuable livestockfeed. The chemical composition of camelina meal is 10% oil, 13% fiber, 5% minerals, and 45% protein (Bonjean and Le Goffic, 1999; Hurtaud and Payraud 2007).

Compared to other crops, camelina has more advantages as it is much less weather dependent, has more consistent yields, and is cheaper to produce (Moloney et al., 1998). Camelina seeds yields an average of 420–640 L/ha and the chemical composition like protein and fiber in the meal byproduct can be compared to soybean meal (Retka-Schill, 2008b; Sawyer, 2008). The chemical composition of defatted camelina meal could be used for different applications like food, feed, and agricultural applications (Gugel and Falk, 2006; Zubr, 2010). Camelina seed meal contains chemical composition like 5–10% residual fat (which contains fairly high levels of omega 3 fatty acids), high-quality protein, and some potentially functional phytochemicals, which can be exploited to develop new feed and food uses (Gugel and Falk, 2006; Zubr, 2010).

Table 2.2: The chemical composition of camelina meal (Moloney et al., 1998):

Dry matter	23.8%
Crude protein	14.5%
Ash	3.0%

2.1.3 Carinata meal

Ethiopian mustard is the common name for Brassica Carinata (Nabloussi et al., 2008). It is derived from a cross between the species *Brassica oleracea* and *Brassica nigra* (Nabloussi et al., 2008). The average carinata seed yield for carinata is 0.92 MT/acre. The oil content present in carinata seed is around 40% (Agricultural Council of Saskatchwen).

Recently, interest has been shown in using this crop for production of biodiesel and solid biomass in mediterranean countries such as Spain, Greece, and Italy. In addition, the protein content present in the defatted meal resulting from oil extraction would increase the value of B. carinata crops. Enzymatic hydrolysis can increase the functional and nutritional properties of oilseed proteins, which are easily denatured during the process of oil extraction (Vioque et al., 2000; Pedroche et al., 2004). High glucosinolate content and high erucic acid content was recorded in carinata meal. Moreover, seed oil from these species and its byproduct (carinata meal) may have potential applications in the food, biofuel, and feed industries with the high erucic acid types, as well as zero erucic acid lines and zero erucic acid/high oleic acid lines, (Velasco et al., 2003). Inspite of many years of research on the breeding and agronomics of B. carniata, we still know little about its nutritive value and biodegradation behavior, (Singh et al., 1988; Barro et al., 2011; Gil-Humanes et al., 2011), which may allow for its use in animal diets.

Brassica carinata has many positive agronomic advantages, (Gugel, et al., 1990; Singh, et al., 1988; Malik, 1990) some of them are growing easily in hot, dry, and semiarid climates typical of the southern prairies of western Canada. Better agricultural performance was recorded for carinata seed than rapeseed. Proteins were found to be the main constituent of the oil cake, approx. 40 %, with a balanced content of the essential amino acids. The chemical composition of meal contained 32 % fibres, 9 % moisture, 6 % soluble sugars, 5 % glucosinolates and some other minor components (Pedroche et al., 2004).

Higher protein content and lower crude fiber concentration of B. carinata resulted due to larger seed size of carinata as compared to conventional canola seed (Getinet et al., 1996). Therefore, extensive research on breeding development and nutrition evaluation of this vigorous crop has been conducted in countries with semiarid climates such as western Canada (Rakow and Getinet, 1996; Warwick et al., 2006; Pan et al., 2012).

Table 2.3: Chemical composition of ASE carinata meal (Pedroche et al., 2004)

Protein	38.9±2.3
Ash	5.2±1.2
Moisture	8.9±0.5
Glucosinolates	5.1±1.1
Phytic acid	3.1±0.6
Fiber	31.8±4.2
Soluble sugars	5.7±0.7
Polyphenols	0.3±0.1

2.2 Oil extraction methods:

Oil extraction was done to remove oil from canola, camelina and carinata seeds. The meal obtained after extraction is rich in protein and the research was conducted with the objective of replacing the fish meal, which is rich in protein as well by these oilseed meals.

2.2.1 Cold press method

The cold-pressing procedure doesn't involve any heat or chemical treatments and is therefore popular among consumers for being safe and natural (Parker et al., 2003). Therefore, cold-pressed seed oil may retain more phytochemicals including natural antioxidants. Cold pressed oils could lead to better human health and prevention of certain diseases.

Over the last few years, increased interest in cold-pressed plant oils has been observed as these oils have better nutritive properties than those after refining. The advantages of cold press method is that the process is simple, ecological and does not require much energy. Low productivity and difficulties in obtaining a product of constant quality are some of the disadvantage of this process (Rotkiewicz et al., 1999). Various factors that affect the final chemical composition of plant oils are geographical location, species and processing technique (Beardsell et al., 2002). Other compounds that are present in plant oils are small amounts of free fatty acids, phenolic compounds, tocopherols, sterols, stanols, phospholipids, waxes, squalene and other hydrocarbons (Lecker and Rodriguez-Estrada, 2000).

Cold-pressed oils may have the potential for applications in the promotion of health and prevention of oxidative damages caused by radicals. Cold-pressed oils contain more polar phenols, the concentration of which varies from 18 to 99ppm caffeic acid equivalents (CAEs) (Koski et al., 2003).

2.2.2 Accelerated solvent extraction

Accelerated solvent extraction (ASE) technique uses pressure and temperature to extract solid samples (Ezzell, et al., 1995). Extraction time and solvent consumption is greatly reduced during the solvent extraction process. Automation or semi-automation can be achieved to extract analytes from different matrices. Static or dynamic extractions can be performed separately or in combination. The equipment is available commercially but the cost might be high for most laboratories. The solvent temperature that are used during the extraction process are above the normal boiling-point. The solvent is kept in the liquid phase owing to high pressure temperatures much above the boiling-point. The elevated temperature at which the extraction is conducted helps to solubilize the analyte. Due to increased temperature, the bonds between the analyte and the matrix begin to weaken, which further results in an increased extraction yield (Ezzell et al., 1996).

With ASE, a solid sample is enclosed in a sample cartridge that is filled with an extraction fluid and the sample is extracted under elevated temperature (50-200 °C) and pressure (500-3000 psi) conditions for short time periods (5-10 min). compressed gas is used to purge the sample extract from the cell into a collection vessel. Currently, ASE is applicable to solid or semisolid samples that can be retained in the cell during extraction

2.2.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) uses carbon dioxide as the extractant and is considered environmental safe because it replaces hazardous solvent consumption. Supercritical $C0_2$ has been used extensively because of advantages like low toxicity, high purity, and a good ability to solvate a range of organics. However, it is becoming increasingly apparent that by using pure $C0_2$ at "normal" extraction conditions (e.g., 400 atm at 50-80°C) it is difficult to efficiently extract many organics from heterogeneous environmental solids such as soils, sludges, and air particulate matter. However, there are several disadvantages to SFE such as CO_2 is nonpolar, which limits it uses; less stable extracted oils and subject to oxidation, and initial cost and maintenance cost of equipment are high (List et al., 1989; Calvo et al., 1994; Ganzler et al., 1986).

SC-CO_2 extraction can be used to extract isoflavones from soybean meals but it is more applicable to the extraction of acetylglucoside and aglycone. The results obtained were lower total isoflavone yield when compared to solvent extraction, (Kao et al., 2008). Yu et al., 2007 were able to produce isoflavone-rich soy protein isolate from SC-CO_2 defatted soy meal. The phospholipids present in a defatted soybean meal was completely extracted by 10% SC-CO_2/ethanol mixture (Montanari et al., 1997).

2.2.4 Gas-supported screw-pressing (GSSP)

GSSP is a recently developed process by Crown Iron Works (St. Paul, MN, USA) and Safe Soy Technologies (Ellsworth, IA, USA). CO_2 is injected into a screw press as a displacement fluid thereby achieving low residual oil contents in meal (3-6% db) and an increased oil yield. The CO_2 flashes when exiting the press to atmospheric pressure

cooling the meal to achieve low protein denaturation and high PDIs (>70). GSSP meal was used to produce soy protein isolate in high yields because of it's unique functional properties (Deak et al., 2008).

2.2.5 Microwave extraction

Microwave extraction reduces usage of solvent and generally affords complete extraction (Chen and Spiro, 1995). This method becomes labor-intensive because samples must cool before further processing. However, there are difficulties in automation of the process. In order to separate the solvent from the solid material, the analyte must then filter, centrifuge, or decant the samples. Time saved due to the fast microwave extraction is lost in the cooling process, re-extraction, and preparation of the analyte for further analysis.

2.3 Pre-treatment

The development of effective pretreatment methods to increase the susceptibility of cellulose to enzyme attack is a major technological challenge for process commercialization. In order to improve the rate of enzymatic hydrolysis and increase the yields of fermentable sugars from cellulose, different pretreatment methods can be used. Extrusion pre-treatment method was used in this research.

2.3.1 Extrusion pretreatment

Extrusion cooking is an popular food processing technique based on a high temperature/short time process to produce fiber-rich products (Gaosong & Vasanthan, 2000). In the extruder, due to high temperature and pressure, shear stress is generated in the screw-barrel assembly, which makes the food mix cook thermo mechanically. The

cooked melt is then texturized and shaped in the die (Arhaliass et al., 2003). The thermomechanical changes that occur during extrusion are gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-nutritional factors; all these actions take place in a shear environment, which results in a plasticized continuous mass (Bhattacharya & Prakash, 1994).

Extrusion processing has been evaluated as a method for preparation of aquaculture feed. Because extrusion processing is a high temperature process, the starch-based products form an elastic melt inside the barrel, which results in a more expanded final product (Ilo et al., 1996; Alves et al., 1999; Lin et al., 2000). However, a plastic melt that is formed during processing of protein based products results in a more porous and textured final product (Cumming et al., 1973; Gwiazda et al., 1987; Singh et al., 1991; Sandra and Jose, 1993). Moisture content (MC) of the mix and temperature gradient in the barrel are the two most important factors to be considered during extrusion processing as they affect the development of proper viscosity of the melt and final product characteristics (Kokini et al., 1992; Chevanan et al., 2007a, c). Aquaculture feeds require high proportions of both protein and starch, but very little information is currently available on extrusion processing of these types of ingredient blends.

Chevanan et al., 2009 studied the effect of extrusion while adding distillers dried grains with soluble (DDGS) and whey on aquaculture feed and concluded that an increase in moisture content, durability, and redness was observed by increasing the moisture content of the ingredient blends from 15 to 25% but a decrease of 9.8 and 5.6% was observed in brightness and yellowness of the extrudates.

Cruz-Suarez et al., 2001 assessed differently processed feed pea meals and canola meal in diets for blue shrimp and found out that extrusion cooking had no effect on growth and survival but significantly improved feed conversion and protein efficiency ratio. The micronized pea diet produced the highest feed intake and growth rate. Response to the diet containing extruded canola meal was similar to that of the control diet.

Muthukumarappan and Julson, 2007 conducted experiments in a twin screw extruder as biomass pretreatment method. Blue stem, big bluestem, switchgrass and indian grass (15-40% moisture content) were pretreated in a twin screw extruder at temperature of 25 and 100°C and screw speeds of 200 and 400 rpm. Fine ground big bluestem showed the highest (35.5%) glucose recovery at 25°C with 20% moisture content and screw speed of 200 rpm. Authors reported, of the four grasses, big bluestem yielded the highest glucose recovery followed by indian grass (31.7%), blue stem (27%) and switchgrass (24.5%)

2.3.2 Microwave pretreatment

Microwaves belong to the electromagnetic spectrum with wavelengths varying from 1 mm to 1 m with their corresponding frequencies between 300 MHz and 300 GHz. Within this portion of the electromagnetic spectrum the frequencies are used for various different applications like cellular phones, radar, and television satellite communications. The two most commonly used frequencies are 0.915 and 2.45 GHz. Recently, 0.9 to 18 GHz microwave frequency have been used for development of material processing (Lauf et al., 1993).

Over the years, a number of studies have been performed to recognize how microwaves affect the pretreatment process of biomass for producing bioethanol. For example, in the

earlier study of Ooshima et al., 1984 microwave irradiation pre-treatment was used by placing rice straw and bagasse with water in sealed glass vessels. They addressed that the materials were more accessible for enzymatic hydrolysis as the biomass was pretreated by microwaves. Zhu et al. (2005, 2006) employed microwaves and alkali to pretreat rice straw and wheat straw and compared with the alkali-alone pretreated processes. The results indicated that the biomass pretreated by microwaves and alkali had a higher hydrolysis rate and glucose contents in hydrolysate. Hu and Wen, 2008 highlighted the pretreatment of switchgrass using microwaves. The total sugar yield was 53% higher when given microwave-assisted alkali pretreatment than that obtained from conventional heating. Ma et al., 2009 used Box–Behnken design and response surface methodology to evaluate the optimal microwave pretreatment of rice straw. They found that the maximum efficiencies of cellulose, hemicellulose and total saccharification of rice straw were increased by 30.6%, 43.3% and 30.3%, respectively when the optimal conditions of microwave pretreatment were carried out.

2.3.3 Acid pre-treatment

Acid hydrolysis of biomass has been well known since the eighteenth century. This treatment could be done using dilute acid or concentrated acid. Acid pretreatments effectively solubilize hemicellulose and disrupt covalent bond, hydrogen bonds and vander wall forces that hold the cellulose, hemicellulose and lignin together in the biomass. This disruption can further result in separation of the lignin from the cellulose to some extent (Li et al., 2010; Sun and Cheng, 2005). Applying concentrated acids in moderate temperatures and dilute acid pretreatments at high temperature may result in achieving improved cellulose hydrolysis (McMillan, 1994).

Varga et al., 2004 reported that mixing the biomass with 2% (w/w) sulfuric acid for one night followed by steam treatment at 190°C for 2 min could result in 85% hemicellulose reduction in lignocellulosic feedstocks, such as corn stover. Lignin degradation is however not as extensive as with alkaline pretreatments (Sun and Cheng, 2002; Wyman et al., 2005). Relative to switchgrass, Torget et al., 1990 found that 92% solubilization of switchgrass xylan takes place with pretreatment of 0.5%(v/v) sulfuric acid at 140°C for 60min or at 160°C for 10 min.

2.3.4 Alkaline pretreatment

Alkali pretreatment of lignocellulosic materials results from the degradation of lignin content of the materials (Fan et al., 1987; McMillan, 1994). The alkaline hydrolysis disrupts intermolecular ester bonds that cross links together xylan hemicelluloses and other components, for example, lignin and other hemicellulose. As we remove the crosslinks, the porosity of the lignocellulosic materials increases (Tarkow and Feist, 1969). Swelling, increase in internal surface area, a decrease in the degree of polymerization, cellulose crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure are the changes that are experienced by treating the lignocellulosic materials with dilute NaOH (Fan et al., 1987). The digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 24–55% to 20%. However, no effect of dilute NaOH pretreatment was observed for softwoods with lignin content greater than 26%, (Millet et al., 1976). Hydrolysis of straws with relatively low lignin content of 10–18% were effected by dilute NaOH pretreatment (Bjerre et al., 1996). The combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark and peanut husk

was used by Chosdu et al., 1993. The glucose yield of corn stalk was higher than the cassava bark and peanut husk, the values recorded for corn stalk was 20% in untreated samples compared to 43% after treatment with electron beam irradiation at the dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively.

2.4 Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific (Beguin and Aubert, 1994). The products of the hydrolysis are usually reducing sugars including glucose, galactose, xylose and arabinose. The utility cost of enzymatic hydrolysis is lower as compared to acid or alkaline hydrolysis because it's usually conducted at mild conditions (pH 4.8 and temperature 45– 50°C) and does not have a corrosion problem (Duff and Murray, 1996). During hydrolysis of lignocellulosic materials, cellulases are produced by both bacteria and fungi. Cellulases are usually a mixture of several enzymes. The three major groups of cellulases involved in the hydrolysis process are: (1) endoglucanase attacking amorphous cellulose fiber and creating free chain-ends; (2) exoglucanase or cellobiohydrolase which leads to further degradation by removing cellobiose units from the free chain-ends; (3) β-glucosidase which hydrolyzes cellobiose to produce glucose (Coughlan and Ljungdahl, 1988). During the enzymatic hydrolysis, cellulases helps in the degradation of cellulose to reducing sugars which are fermented by yeasts bacteria to ethanol.

Substrates, cellulase activity, and reaction conditions (temperature, pH, as well as other parameters) are the factors that affect the enzymatic hydrolysis of cellulose. Research has

focused on the optimization of hydrolysis process and enhancement of cellulase activity to increase the yield and rate of the enzymatic hydrolysis (Cantwell et al., 1988; Durand et al., 1988; Orpin, 1988)

2.5 Physical properties:

2.5.1 Moisture content

Moisture content is one of many factors affecting biomass quality, material texture, microbial growth, and storage stability. In previous studies, the relationship of the wet based (w) and dry (d) bulk density of the samples is represented as a mixture equation in two forms of Eq. (1) (Peleg, 1988) or Eq. (2) (Hollenbach et al., 1982).

(1)

(2)

where ρ_b is the wet based bulk density of the samples (kg/m^3) at moisture content of *Mw*, ρ_d is the dry based bulk density (kg/m^3) at bone dry sample. M_w is the moisture content of the wet samples (decimal wet basis), ρ_w is the bulk density of water 1000 kg/m^3. A previous study mentioned that the pellet density of corn stover and switchgrass was significantly affected by moisture content (Mani et al., 2004). At the moisture content of 8%wb, the bulk densities of corn stover and switchgrass pellets were 147 kg/m^3 and 150 kg/m^3. It was concluded that the following mixture equation could be used for estimating bulk density of the same size material from particle moisture content

$$\frac{1}{\rho_b} = \frac{1 - M_w}{a x^b} + \frac{M_w}{\rho_w} \qquad (3)$$

where ρ_b is the wet bulk density of biomass at a moisture content of M_w (decimal fraction wet basis), x is particle length, a and b are biomass species constants, and ρ_w is the density of water (roughly 1000 kg/m^3) (Lam and Sokhansanj, 2007). For four different particle sizes, when the moisture content range of 8 to 60%, loose bulk density of switchgrass varied from 49.44 to 266.52 kg/m^3 (Kaliyan and Morey, 2009). A similar trend was also observed for the wheat straw (Lam, 2008).

2.5.2 Water Absorption Index (WAI) and Water Solubility Index (WSI)

Water absorption index (WAI) is the weight of gel obtained per gram of dry sample through a modification of the method described by Kite et al., 1957 for measuring the swelling power of starch. WAI shows the amount of water the sample can absorb. Water solubility index (WSI) shows the amount of dried solids recovered by evaporating the supernatant from the water absorption test, which is expressed as a ratio of dry solids got from the supernatant to the amount of original amount. WAI and WSI are influenced by the temperature and moisture content, and vary in different samples as well as different pretreated samples (Anderson, 1982). Jin et al., 1994 studied effects of soy fiber, salt, sugar and screw speed on physical properties and microstructure of corn meal extrudate. It was observed that with increasing screw speed, WAI decreased but increased WSI. WAI was decreased but WSI was increased by increasing the fiber content from 0-20%. The trends were reversed with further increases in fiber content. According to Ding et al.,

2005, extrudates with a higher density, lower expansion, higher WAI, lower WSI, higher hardness and lower crispness results with an increase in feed moisture content. During extrusion, it was observed that due to higher barrel temperature, lower WAI and higher WSI was observed (Ding et al., 2006).

2.5.3 Water activity

Water activity or a_w is developed to account for the intensity with which water associates with various non-aqueous constituents and solids. Simply stated, it is a measure of the energy status of the water in a system. The growth of bacteria, yeasts, and molds could be predicted with water activity (a_w). Water activity helps food designers to formulate products that are shelf stable. Water activity also helps in determining the shelf life of the product for example if a product is kept below a certain water activity, the shelf-life increases due to inhibition of mold growth. In the literature, food product design, shelf life and food product design are important considerations for determining the water activity (Igathinathane, 2009). Water activity is related to moisture content in a non-linear relationship known as a moisture sorption isotherm curve. These isotherms can be used for prediction of product stability during storage and are temperature specific.

2.5.4 Thermal properties

Thermal properties are related to heat transfer control in specified samples and can be classified as thermodynamic properties (enthalpy and entropy) and heat transport properties (thermal conductivity and thermal diffusivity). Thermal properties are mainly used in the research of food production. These properties play an important role in the

design and prediction of heat transfer operations during the handling, processing, canning, storing, and distribution of foods (Choi and Okos, 1983).

Thermal conductivity (κ), is the property of a material's ability to conduct heat, which represents the quantity of heat Q that flows per unit time through a food of unit thickness and unit area having unit temperature difference between faces. SI units for thermal conductivity are W/mK (Van der Held and Van Drunen, 1949).

Thermal diffusivity (usually denoted α) is the thermal conductivity divided by density and specific heat capacity at constant pressure. It has the SI unit of m^2/s.

Thermal diffusivity determines the speed with which the heat travels through a three-dimensional material or diffusion through the material (Ingersoll et al., 1954). Physically, it relates the ability of the material to conduct heat to its ability to store heat (Choi and Okos, 1983).

2.5.5 Bulk density and True density

Bulk density is an important characteristic of biomass that influences directly the cost of feedstock delivered to a biorefinery and storage cost (Sokhansanj and Fenton, 2006). Various factors that are affected by bulk density are storage requirements, material handling size system and material behavior during subsequent thermo-chemical and biological processes (McKendry, 2002). Bulk density and flow characteristics of feedstock affect the engineering design and operation of transport equipment, storages, and conversion processes (Woodcock and Mason, 1987). A recent study by Ryu et al., 2006 investigated the effect of bulk density on the combustion characteristics of biomass.

They found that the ignition front speed was inversely proportional to bulk density, while the burning rate tends to decrease linearly.

The important factors after grinding for downstream processing were moisture content, bulk density, true density, particle size and shape of biomass particles. Ebeling and Jenkins, 1985, determined the heating value and performed fuel proximate analysis for 62 kinds of biomass. Combustion characteristics of pelleted switchgrass were studied by Samson et al., 2001. They compared the combustion quality of switchgrass pellets with coal and natural gas. They reported that carbon dioxide emission from switchgrass pellets was very much lower than the fossil fuel.

2.5.6 Color

Color is associated with every aspect of our lives and influences many of our day-to-day decisions, including those involving food. The various factors affected by color are esthetics, safety, sensory characteristics, and acceptability of food. There have been a number of other studies that have shown an effect of color on other sensory characteristics, indicating an interrelationship that would account for its effect on food intake. Studies reviewed by Clydesdale, 1984 and other individual investigations (Gifford and Clydesdale, 1986; Gifford et al., 1987; Johnson and Clydesdale, 1982; Johnson et al., 1983; Johnson et al., 1982; Roth et al., 1988; Tourila-Ollikainen, 1982) found that other sensory characteristics such as sweetness, salt, and flavor was affected by color. Another study (Christensen, 1985) found that aroma and flavor were not affected by color in paired samples. Dubose et al., 1980 evaluated the effect of color on acceptability in soft drinks and cake. The overall acceptability of both the cherry and orange beverages was

significantly affected by color and flavor. It was found (Christensen, 1985) that once again the color in the cake significantly affected overall acceptability. Maga, 1973 evaluated the influence of color on potato chip sensory preference and found that when given a visual choice, dark chips were preferred less compared to the regularly colored chips most of the time.

CHAPTER 3

EFFECT OF EXTRUSION PRE-TREATMENT ON EXTRUDATE'S PHYSICAL PROPERTIES AND SUGAR RECOVERY OF COLD PRESS AND ACCELERATED SOLVENT EXTRACTED CANOLA MEAL

3.1 Abstract: The study on the effect of extrusion as a pre-treatment on the sugar recovery and physical properties of canola meal was performed. The oil from canola seed was extracted from cold press (CP) and accelerated solvent extraction (ASE) methods and the meal obtained from these processes were used for the extrusion pretreatment study. Meals were then extruded at temperature of 80, 130 and 180°C and at a screw speed of 50, 100 and 150 rpm. Temperature at feeding and barrel zone was kept constant (80°C). The physical properties like water activity, water absorption index (WAI), water solubility index (WSI), moisture content, bulk density, true density, thermal properties and color were measured. Sugar recovery following enzymatic hydrolysis was also determined. Enzyme dosage and extruder processing conditions played a major role in increasing the sugar recovery of canola meals. Higher sugar recovery was recorded for ASE canola meal. The highest total percentage of sugars observed for ASE canola meal was 17.89% at temperature of 80°C and 100 rpm screw speed and for CP canola meal, it was 15.62% at 80°C temperature and screw speed of 50 rpm. Extruder temperature had a significant effect ($p<0.05$) on WAI, water activity, color and bulk density of ASE canola meal.

3.2 Introduction:

The name canola was introduced in Canada in 1979 and was similar to rapeseed whose oil contains less than 2% erucic acid and less than 30 μmol/g meal of total glucosinolates

(Canola Council of Canada 1990). Typically, canola seed contains more than 40% oil (Kimber and McGregor 1995) and in Australia, the average oil content for the year 2008 was 41.8% (Seberry and others 2008). The annual worldwide growth of canola production was excellent and the production of canola for 2015 is 15 million tones.(Canola Council of Canada 2009). Australia is the world's 2nd largest exporter of canola seed after Canada with production rate of 1.5 million tones and contributing upto 96% of the total oilseeds production since 2000. The protein rich meal, extracted from the seed, is currently used as a protein source in livestock and aquaculture industries (Uruakpa and Arntfield 2005a; Canola Council of Canada 2009). It is known for it's high biological value (Campbell and others 1981) and good protein and amino acid composition (Sosulski 1983; Pastuszewska and others 2000).

The cold press method is becoming an interesting substitute for conventional methods because it doesn't involve any heat or chemical treatments (Parker et al., 2003). Cold press method is popular due to its simple technique and low energy requirement and consumer's desire for natural and safe food products. Some of the disadvantages of the process are difficulties in obtaining a consistent quality and low productivity (Rotkiewicz et al., 1999). Another method for oil extraction is accelerated solvent extraction (ASE). Pressure and temperature are used to extract oil in ASE (Ezzell et al., 1995). Reduced extraction time and less solvent consumption are some of the advantages of ASE. The extraction process can be done automated or semi automated.

Extrusion cooking is the process of forcing a material to flow under a variety of conditions through a shaped hole (die) at a predetermined rate to achieve various resulting products. Extruder is considered a high temperature-short time bioreactor that

transforms raw ingredients into modified intermediate and finished products. An extruder could be considered as a bioreactor in which thermal and shear energies are applied to raw food materials. The materials undergo many structural, chemical, and nutritional transformations (Camire et al., 1990; Camire, 2003; Singh et al., 2007; Riaz et al., 2009). Various extrusion pretreatment studies show a significant improvement on sugar recovery from different biomasses such as corn stover, miscanthus, switchgrass, big blueatem, prairie cord grass and Douglas fir wood (Dale et al., 1999; de Vrije et al., 2002; Karunanithy et al., 2009a-c; Lee et al., 2009; Jurusic et al., 2009). Soya white flakes and soyabean hulls had also been studied for extrusion pre-treatment (Karuppuchamy and Muthukumarappan, 2009). In the present work, extrusion is used as a pre-treatment to investigate how it affects the canola meal.

The oilseed meal has high protein and carbohydrate which makes them attractive raw material for various applications. The oilseed meals are largely available and are low in cost. In this research, cold press and accelerated solvent extraction was used to extract oil from the canola seeds and extrusion was done at different processing conditions. The effect of extrusion as a pre-treatment has been studied on the physical properties, thermal properties and sugar recovery of these meals.

Enzymatic hydrolysis of CP and ASE canola meal was done in order to breakdown the complex structure of carbohydrates. The sugars are desired in the form of simple carbohydrate structure so that they are easily accessible by the particular micro-organisms during fermentation to convert them into protein. This converted protein then can be used as fish feed. This study will give an insight view for the efforts that has been made as how the oilseed meals can replace the fish meal.

3.2.1 Objectives

1. Optimize the extruder parameters to predict the maximum sugar recovery in CP and ASE canola meal
2. Optimize the enzyme dosage for maximum sugar recovery in CP and ASE canola meal
3. Determine the physical properties, thermal properties and sugar recovery of CP and ASE canola meal

3.3 Materials and Methods:

3.3.1 Sample preparation:

The canola seeds were obtained from consumer supply distributing Co. (Oregon). Two methods were used to extract the oil: cold press extraction and accelerated solvent extraction. The cold press extraction (M70 oil press co.) consisting VFD motor (2HP, 1.5KW) was done at a temperature of 90°C, frequency of 20 Hz and die size of 0.22 inches. Time-temperature combination used for ASE was 100°C/90 min. The solvent used was hexane. Two different samples of 1.5 Kg were prepared according to two different oil extraction methods in order to compare the effect of different oil extraction methods on the sugar recovery and physical properties of canola meal.

3.3.2 Extrusion pre-treatment

Single screw extruder (Brabender Plasti-Corder, Model PL2000, South Hackensack, NJ) powered by a 7.5HP motor with an operating range of screw speeds from 0-210 rpms (0-22rad/sec) was used for extrusion of CP and ASE canola meal samples. The extruder had

a barrel with length to diameter ratio of 20:1 and a barrel diameter of 19 mm. ASE canola meal was moisture balanced to 20% db. Three different temperature and screw speed combination were used: 80°C, 130°C, 180°C and 50 rpm, 100 rpm, 150 rpm. A total of 9 combinations were extruded. The extrudates obtained were dried overnight. The sample size extruded at each condition was approximately 150g.

3.3.3 Grinding:

Grinding of extruded samples was done using a hammer mill (Speedy Jr, Winona Attrition Mill Co, MN). Particle size of 2 mm was obtained and then stored in zip lock bags in refrigerator at a temperature of 4°C. The samples were ground to a uniform size in order to obtain effective enzymatic hydrolysis and physical properties of canola meal.

3.3.4 Moisture content:

Moisture content of the control and extruded (CP and ASE) samples were measured according to the official methods of analysis (AOAC, 1984) using a laboratory oven (Thelco Precision, Jovan Inc., Wincester, VA).

3.3.5 Physical Properties:

Three replications were studied for each property.

3.3.5.1 Water activity:

Water activity of the samples was measured by using the AquaLab water activity meter (4TE, version 10, Decagon Devices, Inc., Pullman WA). The sample was placed in a plastic cup of the water activity meter and the lid was closed. The equipment showed the

water activity reading on the screen after 5 to 10 minutes. For each sample, three replications were done.

3.3.5.2 Water absorption index (WAI) and Water solubility index (WSI):

2.5 g sample was taken in a tarred 50 ml centrifuge tube. 30 ml of distilled water was added to it. This was stirred intermittently for a period of 30 min and centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was transferred into an aluminum dish, placed in the oven for 2 hr at 135^{o}C (AACC method 44-19, 1995), and then desiccated for 20 min before weighing the dry solids of the supernatant. The mass of the remaining gel was weighed, and WAI (-) was calculated as the ratio of gel mass to the original sample mass. WSI (%), on the other hand was determined as the ratio of mass of dry solids in the extract to the original sample mass.

3.3.5.3 Bulk density and true density:

True density of the samples was measured using Micromeritics Multivolume Pycnometer (No.1305, Micromeritics Instrument Corporation, Norcross, GA). The canola meal sample was filled up to the brim and then placed into the pycnometer. The unit works on the basis of helium gas replacement in the void space of the biomass sample. The values were measured in the form of P_1 when the knob was in the prep position and P_2 when the knob was in test position measured in psi. True density of the material was measured by the equation

$$TD = \frac{M_{sample}}{V_{cell} - \frac{V_{\exp}}{P_1 / P_2 - 1}}$$

Where TD is the true density, M_{sample} is the mass of the biomass sample that used to fill the alumnium cup, V_{cell} and V_{exp} are two constant numbers provided by the micromeritics multivolume pycnometer.

3.3.5.4 Thermal properties:

Thermal properties (thermal conductivity, thermal diffusivity, and specific heat capacity) were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, WA) that utilized the line heat source probe technique. The probe used was SH-1-00571.

3.3.5.5 Color:

The color was measured using a Spectrophotometer (CM-2500d, Minolta Co., Ltd., Japan). The color was described by value of 'L', 'a' and 'b' where L indicates intensity of color i.e. lightness which varies from L=100 for perfect white to L=0 for black. 'a' and 'b' are chromaticity dimensions which give understandable designations of color i.e. the value of 'a' measured redness when positive, grey when zero and greenness when negative and the value of 'b' measured yellowness when positive, grey when zero and blueness when negative.

3.3.6 Enzymatic hydrolysis:

The enzymatic hydrolysis was conducted in a hungate glass tube (Bellco glass, Inc, NJ, USA). The sample weight of canola meal extrudates taken was 2.0g. 8 ml of citrate buffer (0.1M, pH 4.8) was taken. Sodium azide (0.02g/l) was added to inhibit microbial contamination during incubation. CTec2 and HTec2 enzymes were added during enzymatic hydrolysis. The enzyme activity for CTec2 was 128 FPU/ml and for HTec2 was 4465 IU/ml. The enzyme dosage was optimized by adding varying enzyme dosage of

40, 50, 60, 70, 80, 90 and 100 FPU/g cellulase. CTec2 and HTec2 were added in the ratio of 9:1. Hydrolysis was carried out for 72 hrs at 50°C and 150 rpm. After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged at 13,000 rpm for 15 min and frozen and thawed. This process was repeated twice to remove impurities which contribute to the pressure increase in the HPLC system. The supernatant was then filtered into HPLC vials and then injected into the HPLC. Soluble sugars were quantified using HPLC (Agilent technologies, Santa Clara, CA; Bio-Rad Aminex 87H column, Hercules, CA) with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.6 ml/min at 65°C and a sample volume of 20µl as mentioned by Sluiter et al (2006). The sugar concentration obtained from the chromatogram was divided by the dry weight of biomass of pretreated material and multiplied by the total volume taken to get the percentage of that compound in the sample. The percentage was calculated by multiplying the value by 100.

3.3.7 Extruder processing parameters optimization

The extruder processing parameters were optimized by using design of expert8 (Version 8.0.7.1) software. General Factorial Design was applied to optimize the temperature and screw speed for maximum sugar recovery of cold press canola meal extrudaes and solvent extracted canola meal extrudates.

3.3.8 Statistical analysis

All the collected data were analyzed with SAS v.9 (SAS Institute, Cary, NC). The Proc GLM procedure was used to determine the main, treatment and interaction effects using a

Type I error rate (α) of 0.05. Post-hoc Duncan Multiple Range Test (DMRT) tests were used to identify where the significant differences occurred.

3.4 Result and discussion:

3.4.1 Water absorption index (WAI) and Water solubility index (WSI):

Extrusion of CP canola meal and ASE canola meal was done at different temperature and screw speed of 80°C, 130°C and 180°C; 50, 100 and 150 rpm. Fig. 3.1 and Fig. 3.2 represents the WAI and WSI of canola meal (CP and ASE) as affected by the extruder processing conditions. The control value of WAI & WSI for CP canola meal (3.95% & 23.18%) is higher than the ASE canola meal (3.27% & 21.11%). The values of WAI & WSI decreased after extrusion for CP canola meal. Several researchers suggested that any change in WAI can be due to structural modifications of the blend composition, such as starch gelatinization and protein denaturation (Badrie and Mellowes, 1991; Chevanan et al., 2007a; Rosentrater et al.,2009a, b). Unlike water absorption index, WSI indicates degradation extent of macromolecule components of a feed blend, mainly starch and protein molecules (Govindasamy, 1996; Colonna Mercier, 1983). Therefore, WAI is inversely related to WSI (Anderson et al., 1982). Almost similar values of WAI & WSI were observed during extrusion of CP canola meal. For ASE canola meal, WAI increased with extrusion and WSI decreased with extrusion. At the same temperature, WAI increased with screw speed. Temperature and screw speed had a significant effect ($p<0.05$) on WAI of ASE canola meal. The WSI for ASE canola meal decreased significantly ($p<0.05$) with increase in screw speed at the same temperature.

3.4.2 Color

Table 3.1 shows the color (L, a and b) of canola meal extrudates (CP and ASE) as affected by the extruder processing conditions. The control L value for ASE canola meal (65.98) is higher than the CP canola meal (59.84). Almost similar values of L were observed for extrusion at different temperature and screw speed. Screw speed had no significant effect ($p>0.05$) on the L values of CP canola meal. For ASE canola meal, temperature and screw speed both had a significant effect ($p<0.05$) on the L value. The L value of ASE canola meal extrudates is lower than the control value, which means the extrudates are darker in color than the control ASE canola meal. Change in color of extrudates can be an indication of nutrients degradation during extrusion processing (Bjorck & Asp, 1983). Almost similar 'a' values were observed for the control and extrudates of CP and ASE canola meal. The highest 'a' value for CP and ASE canola meal extrudates were 5.29 at temperature of 80°C and screw speed of 50 rpm and 6.12 at 130°C temperature and screw speed of 50 rpm. The CP canola meal (control and extrudates) had higher values of 'b' than ASE canola meal (control and extrudates). Extrusion didn't had a significant effect ($p>0.05$) on the 'b' value for CP canola meal extrudates. Almost similar 'b' values were observed for control and extrudates of CP canola meal. The 'b' values for ASE canola meal extrudates were quite lower than the control ASE canola meal.

3.4.3 MC, (db%) and water activity

The values observed for MC, (db%) and water activity of CP and ASE canola meal are shown in Table 3.2. The moisture content at dry basis MC, (db%) for control sample of

ASE canola meal (19.88%) was much higher than the control sample of CP canola meal (7.58%). For CP canola meal, keeping the screw speed constant during extrusion, as the temperature increased, the MC, (db%) decreased. At 50 rpm, the MC, (db%) of CP canola meal extrudates recorded at the temperature of 80°C, 130°C and 180°C were 6.39%, 5.77% and 5.27%. For ASE canola meal, the MC, (db%) decreased as the temperature and screw speed increased during extrusion. As for water activity, the ASE canola meal (control and extrudates) have higher values than the CP canola meal (control and extrudates). The control water activity value for CP and ASE canola meal were 0.41 and 0.77. The temperature and screw speed had a significant effect ($p<0.05$) on the water activity of CP and ASE canola meal extrudates. As the temperature and screw speed increased, the water activity values for ASE canola meal extrudates decreased.

3.4.4 Thermal properties

The values of thermal properties measured are shown in Table 3.3. The thermal properties were measured in terms of thermal diffusivity (D value), thermal conductivity (K value) and specific heat capacity (C value). The D value, K value and C value for control CP canola meal (0.77), (0.12), (1.14) was quite higher than the control ASE canola meal (0.10), (0.12), (1.12). Extrusion significantly ($p<0.05$) decreased the D value for CP canola meal extrudates as compared to the control value. Almost similar D values were observed for the control and ASE canola meal extrudates. As the temperature and screw speed increased, the K value increased for the CP canola meal extrudates. Heldman (2003) postulated that the k value of a heat processed material decreased due to the material changes, primarily because of protein and starch transformations. The presence of hydrophobic constituents in the feed blend affects the density and thus thermal

conductivity of the extruded feed (Mariama et al., 2008). There was not much difference among the K value of ASE canola meal extrudates. The highest and lowest K value recorded for the ASE canola meal extrudate was 0.13 at 130°C temperature and 50 rpm screw speed and 0.12 at 80°C temperature and screw speed of 150 rpm. Higher C values were observed for ASE canola meal extrudates as compared to the control value. As the extruder temperature increased, the C value for ASE canola meal extrudates decreased.

3.4.5 Bulk density and True density

Table 3.4 shows the bulk density and true density of CP and ASE canola meal (control and extruded). The ASE canola meal (control and extrudates) had a higher bulk density and true density values as compared to the CP canola meal (control and extrudates). The bulk density of canola meal extracted by CP and ASE method significantly ($p<0.05$) increased with extrusion. The highest bulk density values recorded for CP and ASE canola meal extrudates were 370.13 at 80°C temperature and a screw speed of 150 rpm and 559.37 at temperature of 180°C and screw speed of 100 rpm. The true density values for CP canola meal extrudates were lower than the control CP canola meal. For ASE canola meal extrudates, the true density values first decreased than the control value at temperature of 80°C and then increased at a temperature of 130°C and 180°C.

3.4.6 Interaction effects of independent variables on physical properties of canola meal

The interaction effects of independent variables on physical properties of canola meal are given in Table 3.5. The combined interaction of temperature and screw speed had the significant effect ($p<0.05$) on WAI of CP and ASE canola meal. Screw speed alone had a

significant effect ($p<0.05$) on WSI of ASE canola meal. The water activity, color (L value) and bulk density of CP and ASE canola meal was significantly affected ($p<0.05$) by the temperature, screw speed, combined interaction of temperature and screw speed.

3.4.7 Interaction effects of independent variables on thermal properties of canola meal

The interaction effects of independent variables on thermal properties of canola meal are given in Table 3.6. The thermal diffusivity (D value) and thermal conductivity (K value) of CP canola meal was significantly ($p<0.05$) effected by the temperature and combined interaction of temperature and screw speed. Only extruder temperature had a significant effect ($p<0.05$) on D value of ASE canola meal. The K value of ASE canola meal was not significantly affected ($p>0.05$) by extrusion.

3.4.8 Enzyme dosage optimization

The enzymes were added at 50, 60, 70, 80 and 90 FPU/g cellulose to control and extrudate of cold press and solvent extracted samples. The hydrolysis was done and each sample was measured for the sugar recovery using HPLC. The % total sugars was almost similar for the control CP sample at enzyme dosage of 50 (16.07%), 60 (16.53), 70 (16.61) and 80 (16.90). The % total sugars for CP control sample were not much increased for even a higher dosage of 90 FPU/g cellulose (17.41). The glucose recovery significantly increased ($p<0.05$) with extrusion for CP and ASE canola meal extrudates. The values obtained for CP extrudates were almost similar at 50, 60 and 70 FPU/g cellulose. For ASE canola meal extrudates, the %total recovery at 50, 60, 70, 80 and 90 FPU/g cellulose was 16.71, 17.37, 17.99, 18.18 and 18.33. Although sugar recovery was

higher at 90 FPU/g cellulose compared to other dosage, the optimized enzyme dosage for CP and ASE canola meal was 60 FPU/g cellulose. The reasons were that higher enzyme dosage will be costly and it might overcome the extrusion effect.

3.4.9 Sugar recovery

The effect of different extrusion conditions on sugar recovery of CP and ASE canola meal (control and extruded) is shown in Fig. 3.3. The screw speed and temperature had a significant effect ($p<0.05$) on the glucose recovery of ASE canola meal extrudates. The control CP canola meal (6.80%) had higher glucose recovery than control ASE canola meal (5.92%). The % increase in glucose recovery for ASE extrudates is higher than the CP extrudates. The glucose recovery for CP and ASE canola meal extrudates increased significantly ($p<0.05$) with extrusion. Karuppuchamy et al., 2011 also got the similar results for defatted soybean meal, with increase in temperature the glucose yield increased. The control galactose and arabinose recovery for ASE canola meal (8.22%; 1.23%) is higher than the control CP canola meal (7.68%; 0.91%). The temperature had a significant effect ($p<0.05$) on the galactose recovery of CP and ASE canola meal extrudates. The highest galactose recovery for CP and ASE canola meal extrudates were 7.49% at 80°C temperature and 50 rpm screw speed and 8.19% at 80°C temperature and screw speed of 100 rpm. The control arabinose recovery for CP and ASE canola meal were 0.91% and 1.23%. The screw speed and temperature had a significant effect ($p<0.05$) on the arabinose recovery of CP and ASE canola meal. As the temperature and screw speed increased, the arabinose recovery increased for CP and ASE canola meal extrudates.

The CP and ASE canola meal samples were also checked for the presence of any formaldehyde which might be present as a result of pre-treatment. The samples were run for duration of 60 minutes and no peaks were found after 20 minutes which shows the absence of any formaldehyde in the samples.

3.4.10 Interaction effects of independent variables on sugar recovery of canola meal

The interaction effects of independent variables on sugar recovery of canola meal are given in Table 3.7. Temperature and combined interaction of temperature and screw speed had a significant effect ($p<0.05$) on the glucose recovery of CP canola meal. A significant effect ($p<0.05$) on glucose recovery was observed for temperature, screw speed and combined interaction of temperature and screw speed in case of extrusion of ASE canola meal. The galactose recovery of CP and ASE canola meal was significantly ($p<0.05$) effected by the temperature, combined interaction of temperature and screw speed. Temperature, screw speed and combined interaction of temperature and screw speed had a significant effect ($p<0.05$) on the arabinose recovery of CP and ASE canola meal.

3.4.11 Extruder processing parameters optimization

For CP canola meal extrudates, the optimized extruder conditions obtained using the design of expert 8 were temperature of 80°C and screw speed of 150 rpm and a desirability of 0.955. The maximum sugar recovery obtained was 15.83%. For ASE canola meal extrudates, the optimized extruder conditions obtained were temperature of 180°C and screw speed of 50 rpm. The desirability obtained was 0.637 which is due to the reason that there was not much variation in the values of total sugar recovery for

different extruder processing conditions. The maximum sugar recovery value obtained for ASE canola meal extrudates was 17.3%.

3.5 Conclusions:

Extrusion was done at different temperature and screw speed in order to optimize these extruder process parameters for maximum sugar recovery. Screw speed, die temperature and oil extraction methods, affected the physical properties and thermal properties of canola meal. For enzymatic hydrolysis, the enzymes added at 60 FPU/g cellulose gave the best results for CP and ASE canola meal. The optimized conditions for CP extruded canola meal was observed at temperature of 80°C and screw speed of 150 rpm and for ASE extruded canola meal, the optimized conditions observed were 180°C die temperature and screw speed of 50 rpm. For physical and thermal properties, extruder temperature had a significant effect than the screw speed.

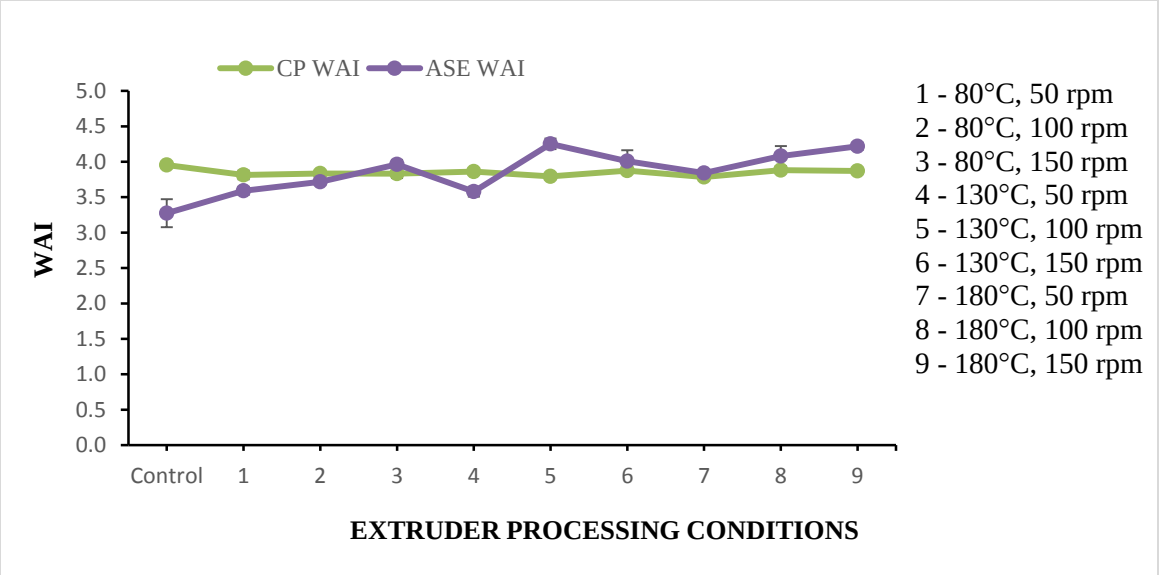

Figure 3.1. Effect of extruder processing parameters on WAI of CP and ASE canola meal extrudates

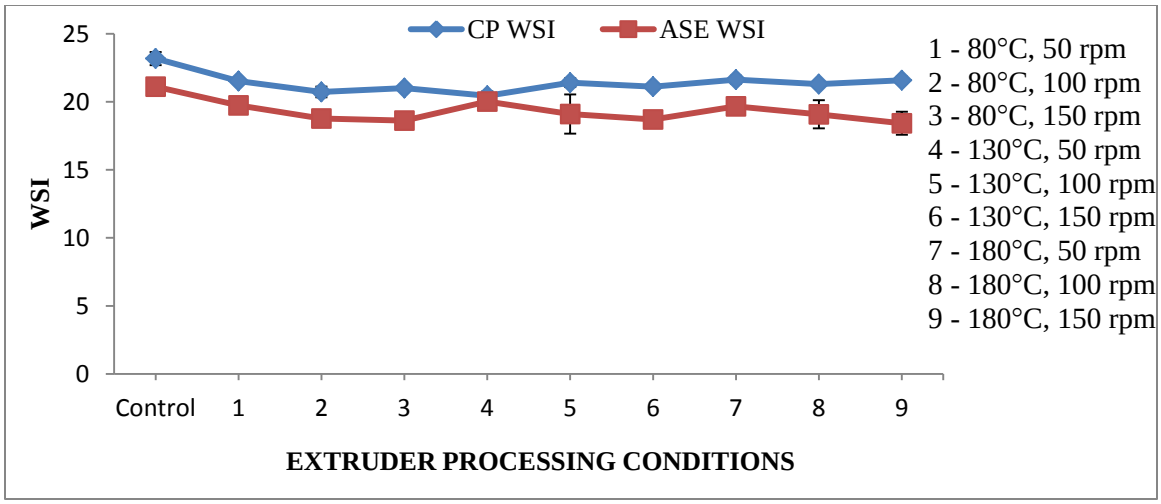

Figure 3.2. Effect of extruder processing parameters on WSI of CP and ASE canola meal extrudates

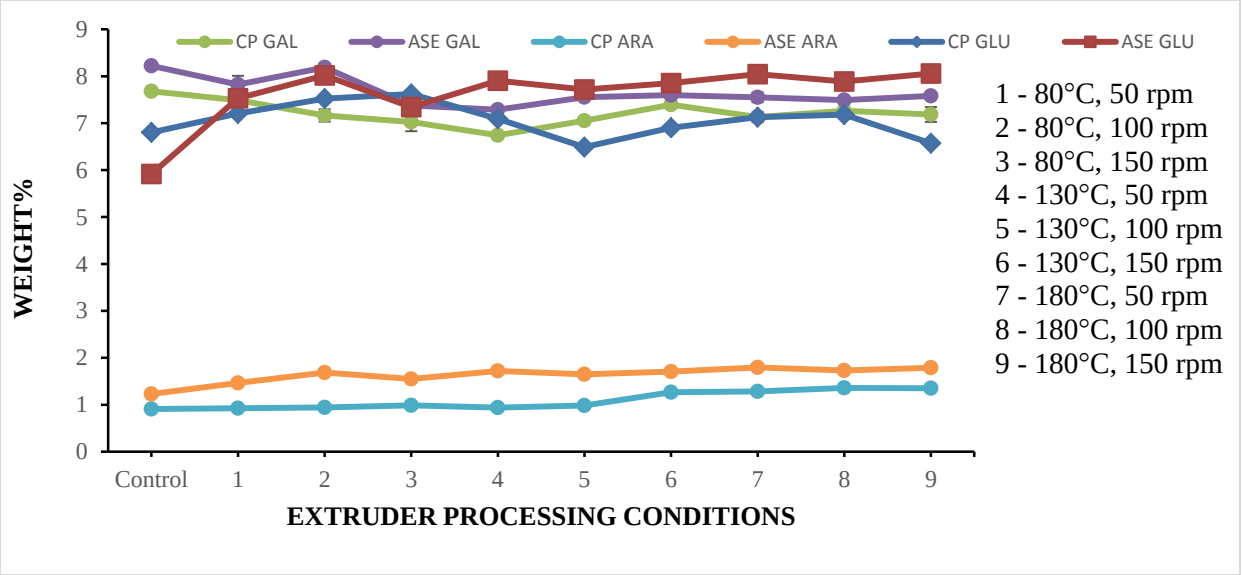

Figure 3.3. Effect of extruder processing parameters on sugar recovery (glucose, galactose and arabinose) of CP and ASE canola meal extrudates.

Table 3.1. Effect of extruder processing conditions on color (L, a and b) of CP and ASE canola meal extrudates

Processing conditions	CP			ASE		
	L	a	b	L	a	b
No Treatment	59.84^{b}	4.86^{d,c}	21.33^{b,a}	65.98^{a}	4.72^{c}	19.89^{a}
	(0.39)	(0.15)	(0.24)	(0.53)	(0.18)	(0.49)
80°C, 50 rpm	57.79^{c}	5.29^{a}	20.40^{c}	52.47^{e}	5.01^{c}	10.59^{f}
	(0.89)	(0.09)	(1.00)	(0.46)	(0.06)	(0.62)
80°C, 100 rpm	59.72^{b}	5.25^{a}	21.63^{b,a}	55.31^{d}	4.66^{c}	11.61^{f}
	(0.56)	(0.08)	(0.40)	(1.36)	(0.14)	(0.68)
80°C, 150 rpm	58.46^{c}	5.14^{b,a}	21.39^{b,a}	55.74^{d}	4.76^{c}	12.03^{f,e}
	(0.34)	(0.11)	(0.05)	(0.86)	(0.37)	(1.18)
130°C,50 rpm	59.77^{b}	4.65^{e}	20.99^{b,c}	58.94^{c}	6.12^{a}	17.11^{b}
	(0.95)	(0.14)	(0.45)	(0.07)	(0.05)	(0.11)
130°C,100 rpm	61.37^{a}	4.67^{e}	21.67^{b,a}	58.85^{c}	4.11^{d}	11.67^{f}
	(0.44)	(0.13)	(0.14)	(0.75)	(0.36)	(1.08)
130°C,150 rpm	59.90^{b}	4.97^{b,c}	21.68^{b,a}	58.45^{c}	4.84^{c}	13.33^{d,e}
	(0.49)	(0.14)	(0.55)	(1.33)	(0.24)	(0.75)
180°C,50 rpm	60.77^{b,a}	4.70^{d,e}	21.65^{b,a}	58.57^{c}	5.48^{b}	15.25^{c}
	(0.67)	(0.14)	(0.20)	(0.80)	(0.17)	(1.06)
180°C,100 rpm	59.82^{b}	4.78^{d,c,e}	21.36^{b,a}	56.79^{d}	4.19^{d}	11.45^{f}
	(1.05)	(0.11)	(0.40)	(0.90)	(0.39)	(0.64)
180°C,150 rpm	61.77^{a}	4.74^{d,e}	21.93^{a}	60.63^{b}	4.97^{c}	14.63^{d,c}
	(0.52)	(0.05)	(0.10)	(1.22)	(0.26)	(0.39)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 3.2. Effect of extruder processing conditions on MC, (db%) and water activity of CP and ASE canola meal extrudates

Processing conditions	CP		ASE	
	MC, (db%)	a_w	MC, (db%)	a_w
No Treatment	7.58^{a}	0.41^{g}	19.88^{a}	0.77^{a}
	(0.04)	(0.01)	(0.22)	(0.00)
80°C, 50 rpm	$6.39^{b,c}$	$0.43^{e,f}$	13.96^{b}	0.66^{b}
	(0.19)	(0.00)	(0.22)	(0.00)
80°C, 100 rpm	$6.16^{b,d,c}$	$0.44^{c,d}$	10.63^{c}	0.56^{c}
	(0.05)	(0.00)	(0.12)	(0.00)
80°C, 150 rpm	$6.67^{b,a,c}$	0.45^{b}	9.84^{d}	0.54^{d}
	(0.07)	(0.00)	(0.27)	(0.00)
130°C,50 rpm	$5.77^{d,c}$	$0.45^{c,b}$	$9.39^{e,d}$	0.51^{e}
	(0.74)	(0.00)	(0.55)	(0.00)
130°C,100 rpm	$6.94^{b,a}$	0.47^{a}	$8.78^{e,f}$	0.49^{f}
	(0.33)	(0.00)	(0.16)	(0.00)
130°C,150 rpm	$6.27^{b,c}$	0.47^{a}	$9.11^{e,d}$	0.48^{h}
	(0.19)	(0.00)	(0.32)	(0.00)
180°C,50 rpm	5.26^{d}	$0.44^{e,d}$	$9.36^{e,d}$	0.49^{g}
	(0.99)	(0.00)	(0.02)	(0.00)
180°C,100 rpm	$6.02^{b,d,c}$	0.42^{f}	8.27^{f}	0.47^{i}
	(0.68)	(0.00)	(0.89)	(0.00)
180°C,150 rpm	$5.92^{d,c}$	0.42^{f}	$8.65^{e,f}$	0.47^{i}
	(0.41)	(0.01)	(0.46)	(0.00)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 3.3. Effect of extruder processing conditions on thermal properties (D, K and C) of CP and ASE canola meal extrudates

Processing conditions	CP			ASE		
	D (m^2/s)	K (W/mK)	C (J/K)	D (m^2/s)	K (W/mK)	C (J/K)
No Treatment	0.77^{a}	$0.12^{e,d,c}$	$1.14^{b,c}$	$0.10^{b,a}$	0.12^{a}	1.12^{b}
	(0.58)	(0.00)	(0.03)	(0.00)	(0.00)	(0.05)
80°C, 50 rpm	0.10^{f}	$0.11^{e,f}$	$1.16^{b,c}$	$0.10^{b,a}$	0.13^{a}	$1.31^{b,a}$
	(0.00)	(0.00)	(0.02)	(0.01)	(0.03)	(0.19)
80°C, 100 rpm	$0.10^{e,f}$	$0.12^{e,d}$	$1.16^{b,c}$	0.10^{b}	0.13^{a}	1.35^{a}
	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.05)
80°C, 150 rpm	$0.10^{c,d}$	$0.13^{b,a,c}$	$1.24^{b,a}$	$0.10^{b,a}$	0.12^{a}	$1.21^{b,a}$
	(0.00)	(0.00)	(0.01)	(0.00)	(0.01)	(0.08)
130°C,50 rpm	$0.11^{c,b}$	$0.12^{b,d,c}$	$1.15^{b,c}$	0.10^{a}	0.13^{a}	$1.28^{b,a}$
	(0.00)	(0.01)	(0.10)	(0.01)	(0.02)	(0.10)
130°C,100 rpm	$0.10^{c,d}$	$0.13^{b,a}$	1.29^{a}	$0.10^{b,a}$	0.13^{a}	$1.28^{b,a}$
	(0.00)	(0.00)	(0.05)	(0.00)	(0.01)	(0.11)
130°C,150 rpm	0.10^{c}	$0.12^{e,d,c}$	$1.16^{b,c}$	0.10^{a}	0.13^{a}	$1.25^{b,a}$
	(0.00)	(0.00)	(0.08)	(0.00)	(0.01)	(0.03)
180°C,50 rpm	0.11^{b}	$0.13^{b,a,c}$	$1.21^{b,a}$	$0.10^{b,a}$	0.12^{a}	$1.23^{b,a}$
	(0.00)	(0.01)	(0.05)	(0.00)	(0.00)	(0.06)
180°C,100 rpm	$0.11^{c,b}$	0.13^{a}	$1.25^{b,a}$	0.10^{a}	0.13^{a}	$1.27^{b,a}$
	(0.00)	(0.01)	(0.09)	(0.00)	(0.00)	(0.02)
180°C,150 rpm	$0.10^{e,d}$	0.10^{f}	1.06^{c}	$0.10^{b,a}$	0.13^{a}	$1.24^{b,a}$
	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.05)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 3.4. Effect of extruder processing conditions on true density (TD) and bulk density (BD) of CP and ASE canola meal extrudates

Processing conditions	CP		ASE	
	TD (Kg/m^3)	BD (Kg/m^3)	TD (Kg/m^3)	BD (Kg/m^3)
No Treatment	1536.86[a]	313.17[e]	3201.65[d]	405.29[c]
	(95.25)	(2.74)	(43.19)	(7.44)
80°C, 50 rpm	1359.66[c]	402.05[a]	2866.07[d]	516.99[b]
	(55.24)	(6.90)	(24.88)	(9.14)
80°C, 100 rpm	1417.41[b]	343.74[c,b,d]	2813.76[d]	555.47[a]
	(10.06)	(7.02)	(18.48)	(8.06)
80°C, 150 rpm	1390.51[c,b]	370.13[b]	2851.90[d]	538.19[a]
	(48.12)	(35.42)	(34.21)	(3.75)
130°C,50 rpm	1417.17[b]	366.37[c,b]	2895.65[d]	507.84[b]
	(9.10)	(10.13)	(27.24)	(6.86)
130°C,100 rpm	1425.49[b]	359.26[c,b,d]	12002.40[d]	543.95[a]
	(8.66)	(0.23)	(15777.59)	(23.77)
130°C,150 rpm	1436.08[b]	334.27[e,d]	61507.22[b]	556.95[a]
	(19.65)	(4.70)	(6719.74)	(1.07)
180°C,50 rpm	1427.75[b]	338.96[c,e,d]	93881.39[a]	546.19[a]
	(10.72)	(8.49)	(14902.62)	(0.16)
180°C,100 rpm	1418.34[b]	345.20[c,b,d]	41700.09[c]	559.37[a]
	(7.14)	(3.76)	(3160.45)	(2.20)
180°C,150 rpm	1423.07[b]	336.16[e,d]	35288.94[c]	546.78[a]
	(11.34)	(19.65)	(6151.79)	(17.09)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 3.5. Interaction effects of independent variables on physical properties (WAI, WSI, water activity, L value and bulk density) of canola meal

Property	Extraction Method	Source	DF	Type SS	Mean Square	F Value	P value
WAI	CP	Temp	2	0.00	0.00	0.76	0.48
		SS	2	0.01	0.00	2.59	0.10
		Temp*SS	4	0.02	0.01	4.23	0.01
	ASE	Temp	2	0.39	0.20	18.06	0.00
		SS	2	0.83	0.41	38.25	0.00
		Temp*SS	4	0.30	0.08	6.99	0.00
WSI	CP	Temp	2	1.36	0.68	8.99	0.00
		SS	2	0.04	0.02	0.26	0.78
		Temp*SS	4	2.61	0.65	8.62	0.00
	ASE	Temp	2	0.31	0.16	0.35	0.71
		SS	2	7.02	3.51	7.82	0.00
		Temp*SS	4	0.24	0.06	0.13	0.97
a_w	CP	Temp	2	0.01	0.00	89.38	0.00
		SS	2	0.00	0.00	9.34	0.00
		Temp*SS	4	0.00	0.00	12.88	0.00
	ASE	Temp	2	0.06	0.03	18493.10	0.00
		SS	2	0.02	0.01	4417.70	0.00
		Temp*SS	4	0.01	0.00	1837.83	0.00
L	CP	Temp	2	22.73	11.36	24.31	0.00
		SS	2	3.51	1.75	3.75	0.04
		Temp*SS	4	12.67	3.17	6.78	0.00
	ASE	Temp	2	105.73	52.87	61.54	0.00
		SS	2	13.11	6.55	7.63	0.00
		Temp*SS	4	28.42	7.11	8.27	0.00
BD	CP	Temp	2	4615.10	2307.55	11.25	0.00
		SS	2	2674.74	1337.37	6.52	0.01
		Temp*SS	4	4273.91	1068.48	5.21	0.01
	ASE	Temp	2	1213.84	606.92	5.25	0.02
		SS	2	4338.78	2169.39	18.78	0.00
		Temp*SS	4	2108.07	527.02	4.56	0.01

Table 3.6. Interaction effects of independent variables on thermal properties (D and K) of canola meal

Property	Extraction Method	Source	DF	Type SS	Mean Square	F Value	P value
D	CP	Temp	2	0.00	0.00	29.33	0.00
		SS	2	0.00	0.00	0.63	0.54
		Temp*SS	4	0.00	0.00	15.24	0.00
	ASE	Temp	2	0.00	0.00	5.67	0.01
		SS	2	0.00	0.00	0.48	0.63
		Temp*SS	4	0.00	0.00	0.83	0.52
K	CP	Temp	2	0.00	0.00	4.96	0.02
		SS	2	0.00	0.00	6.69	0.01
		Temp*SS	4	0.00	0.00	16.27	0.00
	ASE	Temp	2	0.00	0.00	0.48	0.63
		SS	2	0.00	0.00	0.43	0.66
		Temp*SS	4	0.00	0.00	0.27	0.90

Table 3.7. Interaction effects of independent variables on sugar recovery of canola meal

Sugar	Extraction Method	Source	DF	Type SS	Mean Square	F Value	P value
Glucose	CP	Temp	2	1.92	0.96	67.65	0.00
		SS	2	0.06	0.03	2.28	0.13
		Temp*SS	4	1.61	0.40	28.34	0.00
	ASE	Temp	2	0.60	0.30	99.04	0.00
		SS	2	0.08	0.04	12.38	0.00
		Temp*SS	4	0.76	0.19	63.07	0.00
Galactose	CP	Temp	2	0.14	0.07	6.18	0.01
		SS	2	0.05	0.02	2.08	0.15
		Temp*SS	4	0.89	0.22	19.12	0.00
	ASE	Temp	2	0.52	0.26	35.20	0.00
		SS	2	0.28	0.14	19.21	0.00
		Temp*SS	4	0.88	0.22	30.11	0.00
Arabinose	CP	Temp	2	0.68	0.34	1281.28	0.00
		SS	2	0.11	0.05	206.38	0.00
		Temp*SS	4	0.10	0.02	90.75	0.00
	ASE	Temp	2	0.20	0.10	445.45	0.00
		SS	2	0.00	0.00	9.75	0.00
		Temp*SS	4	0.09	0.02	100.68	0.00

CHAPTER 4

EFFECT OF EXTRUSION PRE-TREATMENT ON EXTRUDATE'S PHYSICAL PROPERTIES AND SUGAR RECOVERY OF COLD PRESS AND ACCELERATED SOLVENT EXTRACTED CAMELINA MEAL

4.1 Abstract: The study on the effect of extrusion as a pre-treatment on the sugar recovery and physical properties of camelina meal was undertaken. The oil from camelina seed was extracted from cold press (CP) and accelerated solvent extraction (ASE) methods and the meal obtained from these processes were used for extrusion pre-treatment study. Meals were then extruded at temperature of 80, 130 and 180°C and at a screw speed of 50, 100 and 150 rpm. Temperature at feeding and barrel zone was kept constant (80°C). Physical properties like water activity, water absorption index (WAI), water solubility index (WSI), moisture content, bulk density, true density and color was measured. Thermal properties and sugar recovery following enzymatic hydrolysis was also determined. Extrusion had a significant effect ($p<0.05$) on the glucose and galactose recovery of CP camelina meal. No significant effect ($p<0.05$) of extrusion was observed for glucose and galactose recovery of ASE camelina meal. Extrusion had a significant effect ($p<0.05$) on the thermal properties of CP camelina meal. No significant effect ($p>0.05$) of extrusion was observed for WAI, WSI, color (L), bulk density of CP and ASE camelina meal. It was observed that during extrusion, temperature had a greater effect than screw speed.

4.2 Introduction:

Camelina, popularly known as false flax or gold of- pleasure is an annual or overwintering herb originating in the region from the Mediterranean to Central Asia and

is very adaptable to climate and soil type (Putnam et al.,1993). It presents a similar fatty acid profile to flaxseed and is rich in linolenic acid. The protein-rich cold pressed cake is also a valuable livestock food. This oilseed meal still contains 10% oil, 13% fiber, 5% minerals, and 45% protein (Bonjean and Le Goffic, 1999; Hurtaud and Payraud 2007). Camelina has several positive agronomic attributes: low agricultural inputs, cold-weather tolerance, short growing season (85–100 days), compatibility with existing farm equipment, and grows well in semiarid regions and in low-fertility or saline soils. These qualities are unusual for an oilseed crop (Putnam et al., 1993; Retka-Schill, 2008b; Sawyer, 2008). Moreover, camelina, unlike soybean, thrives in cool, arid climates and is nicely adapted to the more northern regions of North America, Europe, and Asia. As such, it may serve as a rotational crop for winter, which would facilitate disrupting undesirable weed and pest cycles (Retka-Schill, 2008b). Camelina yields an average of 420–640 L of oil/ha and the protein and fiber content in its meal byproduct is comparable to that of soybean meal (Retka-Schill, 2008b; Sawyer, 2008). Camelina has traditionally been used for relatively high value products such as culinary oil, cosmetics, and animal feed (Sawyer, 2008).

Because of consumers' desire for natural and safe food products, the cold-pressing procedure is becoming an interesting substitute for conventional practices as it involves neither heat nor chemical treatments (Parker et al., 2003). Cold pressing is simple, ecological and does not require much energy. Low productivity and difficulties in obtaining a product of constant quality are some of the disadvantage of this process (Rotkiewicz et al., 1999). Accelerated solvent extraction (ASE) technique uses pressure and temperature to extract oil (Ezzell, et al., 1995). Some of the advantages are greatly

reducing extraction time and solvent consumption. Automation or semi-automation can be achieved to extract analytes from different matrices. Static or dynamic extractions can be performed separately or in combination (Ezzell, et al., 1995).

A viable continuous pretreatment method might be found through extrusion. When the feed material passes through the extruder barrel, physico-chemical changes occur to the blend due to high shear, rapid mixing, heating, and so on. A few advantages of extruder are moderate temperature, short residence time, high shear, rapid heat transfer, ability to add or remove chemical or material during extrusion-all in a continuous process; no further conditioning is required as in acid or alkali pretreatment. Extrusion pretreatment studies (Dale et al., 1999; de Vrije et al., 2002; Karunanithy et al., 2008a-c; Lee et al., 2009; Jurusic et al., 2009) shows that sugar recovery significantly improved from different biomasses such as corn stover, miscanthus, switchgrass, big bluestem, prairie cord grass and Douglas fir wood. Extrusion pretreatment has also been studied for soya white flakes and soyabean hulls (Karuppuchamy and Muthukumarappan, 2009). In this work, extrusion is used as a pre-treatment to investigate how it affects the camelina meal.

The large availability and low cost make oilseed meals attractive raw materials, especially the proteins and carbohydrates, for various applications. Efforts have been made to utilize the co- products for various applications. In this research camelina meal has been extracted by cold press and accelerated solvent extraction and the effect of extrusion as a pre-treatment has been studied on the sugar recovery of these meals.

Enzymatic hydrolysis of CP and ASE camelina meal was done in order to convert the polysaccharides to monosaccharides. The sugars are desired in the form of

monosaccharides so that they are easily accessible by the particular micro-organisms during fermentation to convert them into protein. This converted protein then can be fed to the fish as a feed. This study will give an insight view for the efforts that has been made to replace the fish meal by the oilseed meals.

4.2.1 Objectives:

1. Optimize the extruder parameters to predict the maximum sugar recovery in CP and ASE camelina meal
2. Optimize the enzyme dosage for maximum sugar recovery in CP and ASE camelina meal
3. Determine the physical properties, thermal properties and sugar recovery of CP and ASE camelina meal

4.3 Materials and methods:

4.3.1 Sample preparation:

The camelina seeds were obtained from consumer supply distributing Co. (Oregon). The oil was extracted from the seeds using two methods: cold press extraction and accelerated solvent extraction. The cold press extraction (M70 oil press co.) consisting VFD motor (2HP, 1.5KW) was done at a temperature of 90°C, frequency of 20 Hz and die size of 0.22 inches. ASE was done at a time-temperature combination of 100°C/90 min. The solvent used was Hexane. approximately 1.5 Kg of meal was produced from two different oil extraction methods in order to compare the effect of different oil extraction methods on the sugar recovery and physical properties of camelina meal.

4.3.2 Extrusion pre-treatment:

The CP and ASE camelina meal samples prepared were extruded using single screw extruder (Brabender Plasti-Corder, Model PL2000, South Hackensack, NJ) which was powered by a 7.5HP motor with an operating range of screw speeds from 0-210 rpms (0-22rad/sec). The extruder had a barrel with length to diameter ratio of 20:1 and a barrel diameter of 19 mm. The extrusion was done at 3 different temperature and screw speed of 80, 130 and 180°C; 50, 100 and 150 rpm, respectively. In total, 9 combinations were formed from temperature and screw speed. The extrudates obtained were dried overnight. Approximately 150g was the sample size used for each combination.

4.3.3 Grinding:

The extruded samples were further grounded using a hammer mill (Speedy Jr, Winona Attrition Mill Co, MN) to mesh size of 2 mm and then stored in zip lock bags in refrigerator at 4°C. The samples were ground to a uniform size in order to obtain effective enzymatic hydrolysis and physical properties of camelina meal.

4.3.4 Moisture content:

Moisture content of the control and extruded (CP and ASE) samples were measured according to the official methods of analysis (AOAC, 1984) using a laboratory oven (Thelco Precision, Jovan Inc., Wincester, VA).

4.3.5 Physical properties

Three replications were studied for each property.

4.3.5.1 Water activity:

Water activity of the samples was measured by using the AquaLab water activity meter (4TE, version 10, Decagon Devices, Inc., Pullman WA) where the sample was placed in a plastic cup of the water activity meter and the lid was closed. After 5 to 10 minutes, the water activity value of the sample was shown on the screen. For each sample, three replications were done.

4.3.5.2 Water absorption index (WAI) and Water solubility index (WSI):

2.5 g sample was suspended in 30 ml of distilled water in a tarred 50 ml centrifuge tube. The centrifuge tube was placed in a laboratory oven (Thelco precision, Jovan Inc., Winchester, VA) at 30^{o}C. This was stirred intermittently for a period of 30 min and centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was transferred into an aluminum dish, placed in the oven for 2 hr at 135^{o}C (AACC method 44-19, 1995), and then desiccated for 20 min before weighing the dry solids of the supernatant. The mass of the remaining gel was weighed, and WAI (-) was calculated as the ratio of gel mass to the original sample mass. WSI (%), on the other hand was determined as the ratio of mass of dry solids in the extract to the original sample mass.

4.3.5.3 Bulk density and true density:

Micromeritics Multivolume Pycnometer (No.1305, Micromeritics Instrument Corporation, Norcross, GA) was used for the measurement of the true density of the samples. The biomass sample was filled full into an aluminum cup provided together with the pycnometer and then placed into the pycnometer. The unit works on the basis of helium gas replacement in the void space of the biomass sample. The values were measured in the form of P_1 when the knob was in the prep position and P_2 when the knob

was in test position measured in psi. True density of the material was measured by the equation

$$TD = \frac{M_{sample}}{V_{cell} - \frac{V_{\exp}}{P_1 / P_2 - 1}}$$

Where TD is the true density, M_{sample} is the mass of the biomass sample that used to fill the alumnium cup, V_{cell} and V_{exp} are two constant numbers provided by the micromeritics multivolume pycnometer.

4.3.5.4 Thermal properties:

Thermal properties of the materials were measured by using the KD2 thermal properties analyzer. Thermal properties (thermal conductivity, thermal diffusivity, and specific heat capacity) were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, WA) that utilized the line heat source probe technique. The probe used was SH-1-00571.

4.3.5.5 Color:

The color was measured using a Spectrophotometer (CM-2500d, Minolta Co., Ltd., Japan). The color is represented in terms of L, a and b, where L indicates intensity of color i.e. lightness which varies from L=100 for perfect white to L=0 for black. ‘a’ and ‘b’ are chromaticity dimensions which give understandable designations of color i.e. the value of ‘a’ measured redness when positive, grey when zero and greenness when

negative and the value of ‘b’ measured yellowness when positive, grey when zero and blueness when negative.

4.3.6 Enzymatic hydrolysis:

The enzymatic hydrolysis was conducted in a hungate glass tube (Bellco glass, Inc, NJ, USA) with 0.51g dry weight of pre-treated biomass in a solution of citrate buffer (0.1M, pH 4.8) and sodium azide (0.02g/l) to inhibit microbial contamination during incubation. The enzymes, CTec2 and HTec2, added during enzymatic hydrolysis were bought from Novozymes. The enzyme activity for CTec2 was 128 FPU/ml and for HTec2 was 4465 IU/ml. The enzyme dosage was optimized by adding varying enzyme dosage of 40, 50, 60, 70, 80, 90 and 100 FPU/g cellulose. CTec2 and HTec2 were added in the ratio of 9:1. Hydrolysis was carried out for 72 hrs at 50°C and 150 rpm. After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged at 13,000 rpm for 15 min and frozen and thawed. This process was repeated twice before injecting into the HPLC to remove impurities which contribute to the pressure increase in the HPLC system. Soluble sugars were quantified using HPLC (Agilent technologies, Santa Clara, CA; Bio-Rad Aminex 87H column, Hercules, CA) with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.6 ml/min at 65°C and a sample volume of 20µl as mentioned by Sluiter et al (2006). The sugar concentration obtained from the chromatogram was divided by the dry weight of biomass of pretreated material and multiplied by the total volume taken to get the percentage of that compound in the sample. The percentage was calculated by multiplying the value by 100.

4.3.7 Extruder processing parameters optimization

The extruder processing parameters were optimized by using design of expert8 (Version 8.0.7.1) software. General Factorial Design was applied to optimize the temperature and screw speed for maximum sugar recovery of cold press camelina meal extrudaes and solvent extracted camelina meal extrudates.

4.3.8 Statistical Analysis

All the collected data were analyzed with SAS v.9 (SAS Institute, Cary, NC). The Proc GLM procedure was used to determine the main, treatment and interaction effects using a Type I error rate (α) of 0.05. Post-hoc Duncan Multiple Range Test (DMRT) tests were used to identify where the significant differences occurred.

4.4 Result and discussion:

4.4.1 Water absorption index (WAI) and Water solubility index (WSI)

The effect of different extruder processing parameters on WAI and WSI is shown in Fig. 4.1 and Fig. 4.2. The WAI and WSI of control ASE camelina meal (17.16%); (18.47%) are higher than the control CP camelina meal (13.96%); (12.25%). Lower values of WAI were observed for ASE camelina meal extrudates than the control ASE camelina meal. No significant effect ($p<0.05$) of extrusion on WAI of CP and ASE camelina meal extrudates was observed. Almost similar values of WAI for CP and ASE camelina meal extrudates were recorded. Several researchers suggested that any change in WAI can be due to structural modifications of the blend composition, such as starch gelatinization and protein denaturation (Badrie and Mellowes, 1991; Chevanan et al., 2007a; Rosentrater et al.,2009a, b). Unlike water absorption index, WSI indicates degradation extent of macromolecule components of a feed blend, mainly starch and protein molecules

(Govindasamy, 1996; Colonna Mercier, 1983). Therefore, WAI is inversely related to WSI (Anderson et al., 1982). Generally, extrusion processing increases WSI (Menegassi et al. (2011); Anderson et al., 1982), which occurs due to the combination effects of high temperature, pressure, and shear forces on starch and protein degradations. The WSI values of CP camelina meal extrudates were higher than the control value of CP camelina meal. With increase in extruder temperature, the WSI values increased significantly ($p<0.05$). For ASE camelina meal, the extrudates had lower WSI values than the control ASE camelina meal. The extruder temperature and screw speed had a significant effect ($p<0.05$) on the WSI of ASE camelina meal extrudates. The highest and lowest value of WSI recorded for ASE camelina meal extrudates were 17.37% at 130°C temperature and 150 rpm screw speed and 13.82 at temperature of 80°C and screw speed of 50 rpm. Almost similar values were observed for control CP camelina meal and CP camelina meal extrudates. The lower WAI and higher WSI of extrudate with increasing screw speed might be related to the increased shear rate, resulting in the structural modification of starch (Diosady et al., 1985). Wen et al., 1990 indicated that screw speed had a direct effect on polysaccharide size distribution, and a higher screw speed resulted in more fragmentation than a lower screw speed.

4.4.2 Color

The color of CP and ASE camelina meal extrudates is shown in Table 4.1. The control L value of ASE camelina meal (73.73) is higher than the CP camelina meal (65.12). There is no significant effect ($p>0.05$) of extrusion on the L value, ‘a’ value and ‘b’ value of CP camelina meal extrudates. Almost similar L, ‘a’ and ‘b’ values were recorded for the control CP camelina meal and CP camelina meal extrudates. The highest L, a and b value

observed for CP camelina meal extrudates was 64.24 at temperature of 80°C and screw speed of 150 rpm; 6.86 at 180°C temperature and 100 rpm screw speed; 22.26 at 80°C temperature and 150 rpm screw speed. The L value for ASE camelina meal extrudates significantly ($p<0.05$) increased with extrusion. Change in color of extrudates can be an indication of nutrients degradation during extrusion processing (Bjorck & Asp, 1983). The extrudates became darker with increase in temperature. This may be due to fact that the temperature is increasing but the residence time remains same. The control L value for ASE camelina meal is higher than the ASE camelina meal extrudates, whereas the 'a' value for ASE camelina meal extrudates is higher than the control 'a' value of ASE camelina meal. Extrusion had a significant effect ($p<0.05$) on the 'a' value of ASE camelina meal extrudates. Almost similar values of 'b' were observed for ASE camelina meal extrudates.

4.4.3 MC, (db%) and Water Activity

Table 4.2 represents the water activity and MC, (db%) of CP and ASE camelina meal extrudates. For ASE camelina meal extrudates, lower MC, (db%) values were observed as compared to the control ASE camelina meal. As the temperature and screw speed increased, the MC, (db%) for ASE camelina meal extrudates decreased significantly ($p<0.05$).The water activity value for control ASE camelina meal (0.36) was higher than the control CP camelina meal (0.32). Extrusion had a significant effect ($p<0.05$) on the water activity of CP and ASE camelina meal extrudates. As the temperature and screw speed increased, the water activity values decreased significantly ($p<0.05$) for CP and ASE camelina meal extrudates. The highest and lowest water activity value recorded for CP and ASE camelina meal extrudates were 0.37 at 130°C temperature and 150 rpm

screw speed and 0.28 at 180°C temperature and 150 rpm screw speed; 0.25 at temperature of 80°C and screw speed of 50 rpm and 0.12 at temperature of 180°C and screw speed of 50 rpm. The ASE camelina meal extrudates had lower water activity than the CP camelina meal extrudates, this may be due to lower amount of oil content in the ASE meal and therefore it's easy to remove the moisture in the bonded form.

4.4.4 Thermal properties

The thermal properties for camelina meal were recorded in terms of thermal diffusivity (D value), thermal conductivity (K value) and specific heat capacity (C value). D, K and C values for CP and ASE camelina meal extrudates is shown in Table 4.3. Thermal properties were measured in terms of thermal diffusivity (D value), thermal conductivity (K value) and specific heat capacity (C value). Knowledge about the relation between the thermal properties of both raw ingredient blend and extruded feed allow us to anticipate the materials thermal behaviors, and thus to improve the feed formulation and control the process conditions better in order to achieve a high quality product (Blanche and Sun, 2004). Almost similar values of D were observed for the control and extruded samples (CP and ASE camelina meal). Extrusion had a significant effect ($p<0.05$) on CP camelina meal extrudates while no significant effect ($p<0.05$) of extrusion was observed on ASE camelina meal extrudates. Thermal conductivity of a material indicates its potential for transferring heat through itself due to conduction only, and the driving force is just the temperature gradient. Thermal conductivity of a substance varies depending on its temperature and density; therefore, the required times for post-extrusion drying and cooling processes could be predicted by determining the extrudate thermal conductivity during the heat transfer between the extrudate surface and center, the correspondent

temperature difference, and the void spaces of the extrudate (i.e. porosity) (Bouvier and Brisset, 2006). Thus, the k value of a material is also dependent on its composition. For example, the k value of air is 0.029 W/m.°C at 30°C, while reported values for starch and protein are 0.28 W/m.°C, and 0.25 W/m.°C, respectively (Choi et al., 1985). It is clear that, the more an extrudate expands, the lower thermal conductivity (Mariama, 2008). In another study, Heldman (2003) postulated that the k value of a heat processed material decreased due to the material changes, primarily because of protein and starch transformations. The presence of hydrophobic constituents in the feed blend affects the density and thus thermal conductivity of the extruded feed (Mariam, 2008). The K value of CP camelina meal extrudates was significantly increased ($p<0.05$) with increase in temperature. For ASE camelina meal extrudates, almost similar values of K were observed with increase in temperature and screw speed. Higher C value was observed for control CP camelina meal (1.07) as compared to the control ASE camelina meal (1.04). The extruder temperature had a significant effect ($p<0.05$) on the C value of CP and ASE camelina meal extrudates. The highest C value observed for CP and ASE camelina meal extrudates were 1.19 at 180°C temperature and screw speed of 100 rpm and 1.11 at 80°C temperature and screw speed of 150 rpm.

4.4.5 Bulk density and True density

The effect of extruder processing parameters (temperature and screw speed) on bulk density and true density of CP and ASE camelina meal extrudates is shown in Table 4.4. The bulk density and true density of control ASE camelina meal (515.68 and 2190.04) is quite higher than the control CP camelina meal (443.55 and 1505.81). Extrusion had no significant effect ($p>0.05$) on the bulk density of CP camelina meal extrudates. The ASE

camelina meal extrudates had higher bulk density values than the control ASE camelina meal. The extruder temperature had a significant effect ($p<0.05$) on the bulk density and true density of ASE camelina meal extrudates. Higher true density values were observed for CP and ASE camelina meal extrudates as compared to their control samples. The highest bulk density and true density values for CP and ASE camelina meal extrudates were 452.24 and 1525.93 at 80°C temperature and 100 rpm screw speed; 569.51 at 80°C temperature and 50 rpm screw speed and 2709.87 at 130°C temperature and 50 rpm screw speed.

4.4.6 Interaction effects of independent variables on physical properties of camelina meal

The combined interaction effects of independent variables on physical properties of camelina meal are shown in Table 4.5. The interaction of temperature and screw speed had no significant ($p>0.05$) effect on WAI, WSI and bulk density of CP and ASE camelina meal. The water activity for CP and ASE camelina meal was significantly ($p<0.05$) effected by the combined interaction of temperature and screw speed.

4.4.7 Interaction effects of independent variables on thermal properties of camelina meal

The combined interaction effects of independent variables on thermal properties of camelina meal are shown in Table 4.6. The combined interaction of temperature and screw speed had a significant ($p<0.05$) effect on the thermal conductivity and thermal diffusivity of CP camelina meal and a non significant ($p>0.05$) effect was determined for the thermal conductivity and thermal diffusivity of ASE camelina meal.

4.4.8 Enzyme dosage optimization

The enzymes were added at 50, 60, 70, 80, 90, 100, 110 and 120 FPU/g cellulose to control and extrudate of cold press and 50, 60, 70, 80 and 90 FPU/g cellulose to control and extrudate of solvent extracted samples. The hydrolysis was done and each sample was measured for the sugar recovery using HPLC. The % total sugars for CP camelina meal extrudates for 50 (14.6%) and 60 (14.7%) FPU/g cellulose was almost similar. Higher % total sugars were obtained at 70 (17.37%), 80 (17.34%) and 90 (21.38%) FPU/g cellulose for CP camelina meal extrudates. Almost similar values of % total sugar was obtained at 100 (21.93%) and 110 (21.82%) FPU/g cellulose. For ASE camelina meal extrudates, there was a significant increase ($p<0.05$) in % total sugars with increase in enzyme concentration. Although sugar recovery was increasing with increase in enzyme dosage, the optimized conditions for CP and ASE camelina meal was 70 FPU/g cellulose. The reasons for choosing this enzyme dosage were that higher enzyme dosage will be costly and it might overcome the extrusion effect.

4.4.9 Sugar recovery

Fig. 4.3 represents the effect of extruder temperature and screw speed on sugar recovery i.e. glucose, galactose and arabinose recovery of CP and ASE camelina meal extrudates. The sugar recovery for CP and ASE camelina meal was measured in terms of glucose, galactose and arabinose recovery. The glucose recovery for control ASE camelina meal (12.52%) is higher than the control CP camelina meal (12.04%). There was a significant ($p<0.05$) increase in the glucose recovery for ASE camelina meal extrudates with extrusion. Karuppuchamyet al., 2011 also got the similar results for defatted soyabean

meal, with increase in temperature the glucose yield increased. For CP camelina meal extrudates, glucose recovery values were similar to the control CP camelina meal. The highest glucose recovery value for CP camelina meal extrudate was 12.95% at temperature of 130°C and screw speed of 100 rpm. A significant increase ($p<0.05$) in galactose and arabinose recovery was observed for CP and ASE camelina meal extrudates with extrusion. The control galactose recovery for ASE camelina meal (9.18%) is higher than the CP camelina meal (6.22%). As the temperature increased during extrusion, the galactose and arabinose recovery increased for ASE camelina meal extrudates. The highest galactose and arabinose recovery for CP and ASE camelina meal extrudates were 7.69% and 1.70% at temperature of 80°C and screw speed of 50 rpm; 10.77% and 1.75% at 180°C temperature and 100 rpm screw speed.

The CP and ASE camelina meal samples were also checked for the presence of any formaldehyde which might be present as a result of pre-treatment. The samples were run for a duration of 60 minutes and no peaks were found after 20 minutes which shows the absence of any formaldehyde in the samples.

4.4.10 Interaction effects of independent variables on sugar recovery of camelina meal

The interaction effects of independent variables on sugar recovery of camelina meal are given in Table 4.7. The combined interaction of temperature and screw speed had a significant effect ($p<0.05$) on glucose, galactose and arabinose recovery of CP camelina meal. For ASE camelina meal, no significant effect ($p>0.05$) of temperature and screw speed was observed for glucose and galactose recovery. Arabinose recovery for ASE

camelina meal was significantly ($p<0.05$) effected by the combined interaction of temperature and screw speed.

4.4.11 Extruder process parameters optimization

For CP camelina meal extrudates, the optimized extruder conditions were temperature of 80°C and screw speed of 50 rpm at a desirability of 0.81 using the design of expert8 software. 21.001% was the maximum sugar recovery value obtained for CP camelina meal. For ASE camelina meal extrudates, the optimized extruder conditions were temperature of 180°C and screw speed of 50, with a desirability of 0.797. The desirability obtained was less, which might be due to the reason that the sugar recovery values obtained were almost similar for different extruder processing parameters. The optimized conditions selected for ASE camelina meal extrudates was 27.78%.

4.5 Conclusions:

Screw speed, die temperature and oil extraction methods, affected the physical properties, thermal properties and sugar recovery of camelina meal. ASE control camelina meal had higher values than CP control camelina meal for WAI, WSI, water activity, color (L), true density and bulk density. For enzymatic hydrolysis, the enzymes added at 70 FPU/g cellulose for CP camelina meal and 70 FPU/g cellulose for ASE camelina meal gave the best results. Optimized conditions recorded for CP extruded camelina meal was temperature of 80°C and screw speed of 50 rpm and for ASE extruded camelina meal, the optimized conditions observed were 180°C die temperature and screw speed of 50 rpm.

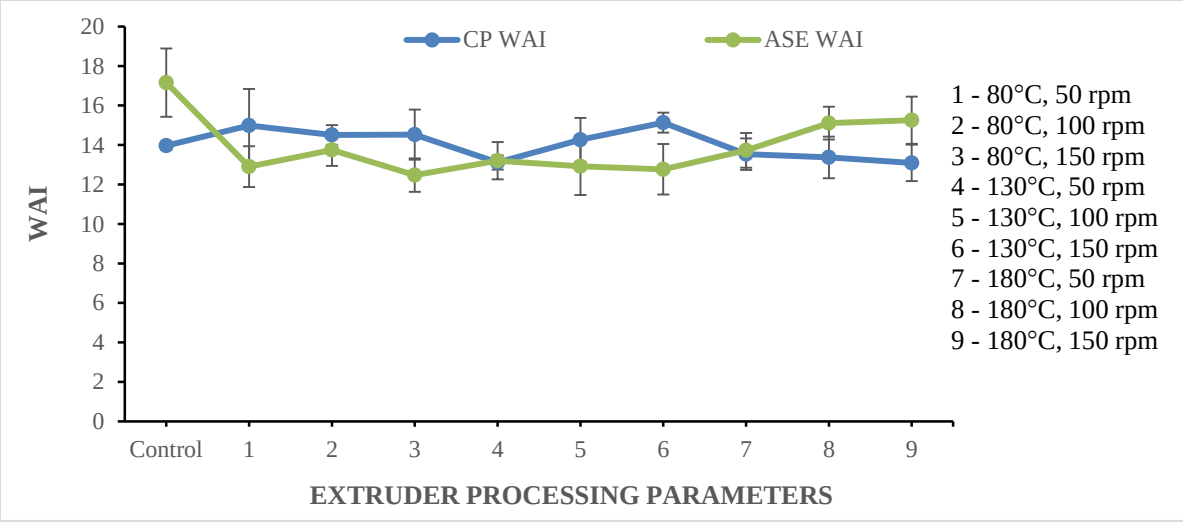

Figure 4.1. Effect of extruder processing parameters on WAI of CP and ASE camelina meal extrudates

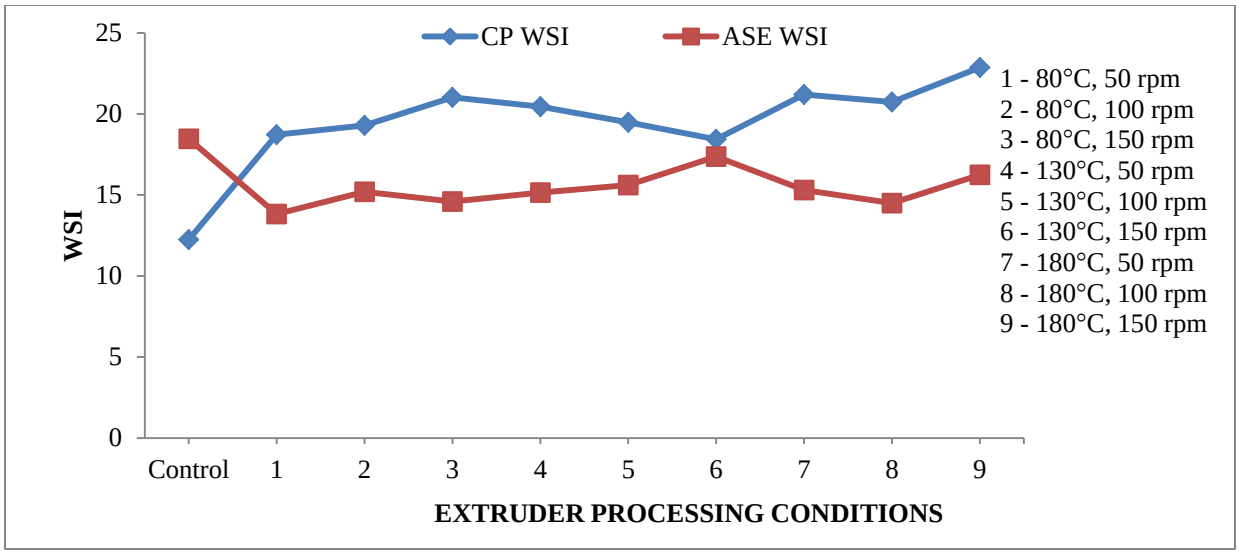

Figure 4.2. Effect of extruder processing parameters on WSI of CP and ASE camelina meal extrudates

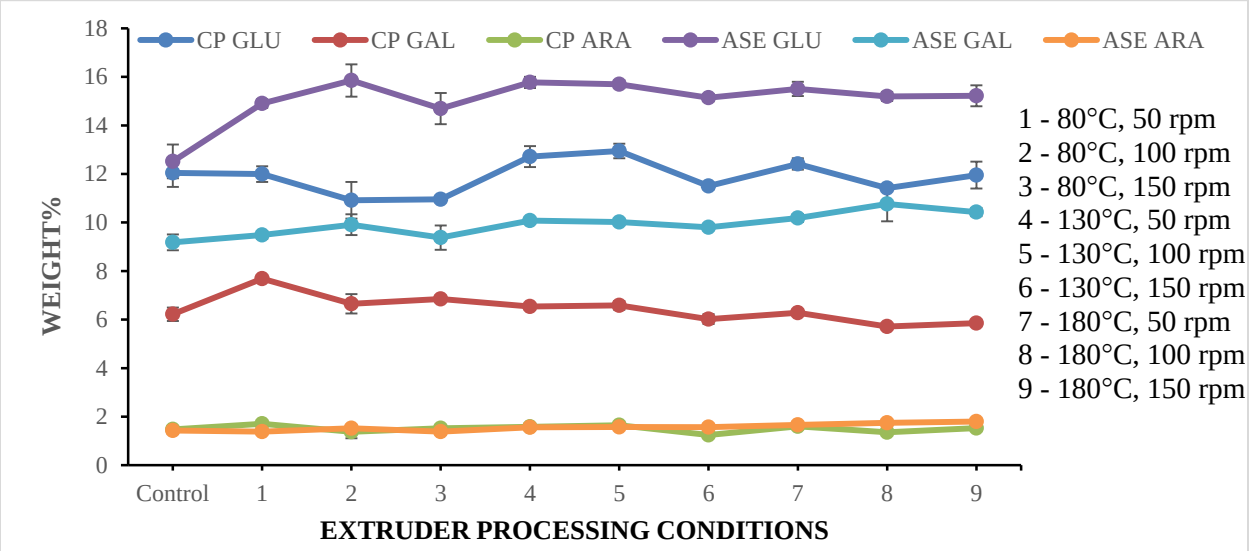

Figure 4.3. Effect of extruder processing parameters on sugar recovery (glucose, galactose and arabinose) of CP and ASE camelina meal extrudates.

Table 4.1. Effect of extruder processing conditions on color (L, a and b) of CP and ASE camelina meal extrudates

Processing conditions	CP			ASE		
	L	a	b	L	a	b
No Treatment	65.12^{a}	5.65^{b}	21.76^{b,a,c}	73.73^{a}	4.90^{d}	16.86^{b}
	(0.32)	(0.21)	(0.16)	(1.19)	(0.07)	(0.60)
80°C, 50 rpm	63.17^{b,c}	6.38^{a}	20.22^{d}	62.58^{e}	7.19^{a}	18.88^{a}
	(0.52)	(0.07)	(0.33)	(1.84)	(0.09)	(0.81)
80°C, 100 rpm	63.13^{b,c}	6.37^{a}	20.58^{d,c}	63.53^{e,d}	7.14^{a}	18.02^{b,a}
	(1.43)	(0.36)	(0.96)	(0.37)	(0.22)	(0.63)
80°C, 150 rpm	64.25^{b,a}	6.52^{a}	22.26^{a}	64.01^{c,e,d}	6.77^{b}	17.27^{b,a}
	(0.86)	(0.22)	(0.41)	(2.06)	(0.01)	(1.28)
130°C,50 rpm	63.16^{b,c}	6.50^{a}	21.14^{b,d,a,c}	64.88^{c,e,d}	7.07^{a}	18.10^{b,a}
	(0.36)	(0.17)	(0.54)	(1.45)	(0.18)	(0.86)
130°C,100 rpm	63.13^{b,c}	6.77^{a}	21.71^{b,a,c}	67.37^{b}	6.61^{b}	18.83^{a}
	(0.37)	(0.50)	(1.08)	(0.26)	(0.28)	(0.27)
130°C,150 rpm	63.00^{b,c}	6.77^{a}	21.34^{b,d,a,c}	66.09^{c,b}	6.76^{b}	18.65^{a}
	(0.53)	(0.33)	(0.64)	(1.04)	(0.09)	(0.92)
180°C,50 rpm	62.30^{c}	6.59^{a}	20.69^{b,d,c}	65.91^{c,b,d}	6.59^{b}	18.54^{a}
	(0.66)	(0.18)	(0.84)	(1.10)	(0.17)	(0.96)
180°C,100 rpm	63.40^{b,c}	6.86^{a}	21.91^{b,a}	67.36^{b}	6.27^{c}	18.60^{a}
	(0.50)	(0.20)	(0.34)	(0.78)	(0.12)	(0.74)
180°C,150 rpm	63.47^{b,c}	6.79^{a}	21.74^{b,a,c}	67.66^{b}	6.02^{c}	18.27^{a}
	(0.32)	(0.03)	(0.43)	(1.35)	(0.07)	(0.91)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 4.2. Effect of extruder processing conditions on MC, (db%) and water activity of CP and ASE camelina meal extrudates

Processing conditions	CP		ASE	
	MC db%	a_w	MC db%	a_w
No Treatment	7.60[a]	0.32[d,c]	9.06[a]	0.37[a]
	(0.15)	(0.00)	(0.03)	(0.01)
80°C, 50 rpm	7.54[a]	0.37[a]	6.77[b]	0.25[b]
	(0.12)	(0.01)	(0.62)	(0.00)
80°C, 100 rpm	7.50[a]	0.34[b]	5.64[d]	0.17[d]
	(0.10)	(0.01)	(0.08)	(0.00)
80°C, 150 rpm	7.23[a]	0.33[c]	6.23[c]	0.18[c]
	(0.46)	(0.01)	(0.08)	(0.00)
130°C,50 rpm	7.54[a]	0.36[a]	5.32[e,d]	0.15[e]
	(0.41)	(0.01)	(0.08)	(0.00)
130°C,100 rpm	7.34[a]	0.37[a]	4.92[e,g,f]	0.14[g]
	(0.15)	(0.01)	(0.01)	(0.00)
130°C,150 rpm	7.63[a]	0.38[a]	5.25[e,d,f]	0.15[f]
	(0.09)	(0.01)	(0.05)	(0.00)
180°C,50 rpm	6.48[b]	0.31[d]	4.35[h]	0.12[i]
	(0.21)	(0.00)	(0.06)	(0.00)
180°C,100 rpm	6.20[b]	0.29[e]	4.68[h,g]	0.12[i,h]
	(0.39)	(0.00)	(0.26)	(0.00)
180°C,150 rpm	6.11[b]	0.28[e]	4.83[g,f]	0.13[h]
	(0.07)	(0.00)	(0.22)	(0.00)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 4.3. Effect of extruder processing conditions on thermal properties (D, K and C) of CP and ASE camelina meal extrudates

Processing conditions	CP			ASE		
	D (m^2/s)	K (W/mK)	C (J/K)	D (m^2/s)	K (W/mK)	C (J/K)
No Treatment	0.10^{e}	$0.11^{d,e,f}$	$1.08^{c,b}$	0.10^{a}	$0.11^{b,a,c}$	1.04^{c}
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.02)
80°C, 50 rpm	0.10^{e}	$0.11^{d,e,f}$	$1.10^{c,b}$	$0.10^{b,a}$	$0.11^{b,a,c}$	$1.09^{b,a}$
	(0.00)	(0.00)	(0.04)	(0.00)	(0.00)	(0.05)
80°C, 100 rpm	0.10^{e}	$0.11^{e,f}$	$1.05^{c,d}$	$0.10^{b,a}$	$0.10^{b,c}$	$1.06^{b,a,c}$
	(0.00)	(0.00)	(0.01)	(0.00)	(0.01)	(0.04)
80°C, 150 rpm	0.11^{b}	0.11^{f}	1.00^{d}	$0.10^{b,a}$	$0.11^{b,a}$	1.11^{a}
	(0.00)	(0.01)	(0.04)	(0.00)	(0.00)	(0.02)
130°C,50 rpm	$0.11^{c,b}$	$0.11^{d,c}$	$1.09^{c,b}$	0.10^{b}	$0.11^{b,a,c}$	$1.07^{b,a,c}$
	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.02)
130°C,100 rpm	$0.10^{c,e,d}$	$0.11^{d,e}$	1.11^{b}	$0.10^{b,a}$	$0.11^{b,a,c}$	$1.07^{b,a,c}$
	(0.00)	(0.00)	(0.06)	(0.00)	(0.00)	(0.02)
130°C,150 rpm	0.11^{a}	0.13^{a}	1.19^{a}	$0.10^{b,a}$	0.11^{a}	1.10^{a}
	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.00)
180°C,50 rpm	$0.10^{c,e,b,d}$	0.12^{b}	1.19^{a}	$0.10^{b,a}$	0.10^{c}	1.02^{c}
	(0.00)	(0.00)	(0.02)	(0.00)	(0.00)	(0.04)
180°C,100 rpm	$0.10^{c,b,d}$	$0.12^{b,a}$	1.19^{a}	$0.10^{b,a}$	$0.10^{b,c}$	1.02^{c}
	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.03)
180°C,150 rpm	$0.10^{c,e,b,d}$	$0.12^{b,c}$	1.18^{a}	$0.10^{b,a}$	0.10^{c}	$1.03^{b,c}$
	(0.00)	(0.01)	(0.03)	(0.00)	(0.00)	(0.02)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 4.4. Effect of extruder processing conditions on true density (TD) and bulk density (BD) of CP and ASE camelina meal extrudates

Processing conditions	CP		ASE	
	TD (Kg/m^3)	BD (Kg/m^3)	TD (Kg/m^3)	BD (Kg/m^3)
No Treatment	$1505.82^{b,a}$	$443.55^{b,a}$	2190.04^{a}	515.68^{c}
	(28.13)	(3.60)	(125.38)	(11.09)
80°C, 50 rpm	1521.75^{a}	$435.15^{b,a}$	$2548.77^{b,c}$	569.51^{a}
	(14.74)	(11.80)	(109.06)	(21.80)
80°C, 100 rpm	1525.94^{a}	452.25^{a}	$2616.77^{b,a}$	$554.70^{b,a}$
	(34.80)	(20.96)	(150.81)	(7.78)
80°C, 150 rpm	$1473.72^{b,c,d}$	426.72^{b}	2706.18^{a}	$561.21^{b,a}$
	(7.33)	(2.42)	(28.44)	(3.61)
130°C,50 rpm	$1463.58^{b,e,c,d}$	449.68^{a}	2709.87^{a}	548.52^{b}
	(28.39)	(14.09)	(72.55)	(1.13)
130°C,100 rpm	$1459.97^{b,e,c,d}$	452.91^{a}	$2604.43^{b,a}$	$554.49^{b,a}$
	(21.08)	(10.79)	(119.82)	(7.54)
130°C,150 rpm	$1480.08^{b,c}$	$442.27^{b,a}$	$2533.76^{b,c}$	$557.45^{b,a}$
	(3.47)	(5.42)	(39.61)	(2.14)
180°C,50 rpm	$1436.70^{c,d}$	$439.67^{b,a}$	$2476.25^{b,c}$	541.37^{b}
	(19.47)	(7.91)	(76.15)	(16.30)
180°C,100 rpm	$1442.46^{e,c,d}$	$440.68^{b,a}$	2439.87^{c}	541.03^{b}
	(2.17)	(7.80)	(14.82)	(3.57)
180°C,150 rpm	1426.82^{e}	$445.04^{b,a}$	$2469.10^{b,c}$	541.51^{b}
	(3.67)	(1.59)	(42.93)	(0.72)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 4.5. Interaction effects of independent variables on physical properties of camelina meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
WAI	Cold press	Temp	2	8.24	4.12	4.11	0.03
		SS	2	0.62	0.31	0.31	0.73
		Temp*SS	4	6.24	1.56	1.55	0.22
	ASE	Temp	2	17.27	8.63	6.42	0.00
		SS	2	1.94	0.97	0.72	0.49
		Temp*SS	4	5.12	1.28	0.95	0.45
WSI	Cold press	Temp	2	25.11	12.55	3.58	0.04
		SS	2	4.20	2.10	0.6	0.55
		Temp*SS	4	18.06	4.51	1.29	0.31
	ASE	Temp	2	10.20	5.10	11.69	0.00
		SS	2	8.33	4.16	9.54	0.00
		Temp*SS	4	7.34	1.83	4.21	0.01
Aw	Cold press	Temp	2	0.02	0.01	186.34	0.00
		SS	2	0.00	0.00	11.18	0.00
		Temp*SS	4	0.00	0.00	7.83	0.00
	ASE	Temp	2	0.02	0.01	2711.84	0.00
		SS	2	0.00	0.00	505.83	0.00
		Temp*SS	4	0.00	0.00	342.07	0.00
L	Cold press	Temp	2	1.15	0.57	1.22	0.31
		SS	2	2.19	1.09	2.32	0.12
		Temp*SS	4	2.87	0.71	1.52	0.23
	ASE	Temp	2	63.66	31.82	19.4	0.00
		SS	2	14.51	7.25	4.42	0.02
		Temp*SS	4	3.25	0.81	0.5	0.73
BD	Cold press	Temp	2	483.98	241.99	2.18	0.14
		SS	2	525.28	262.64	2.37	0.12
		Temp*SS	4	717.08	179.27	1.62	0.21
	ASE	Temp	2	1914.32	957.16	9.11	0.00
		SS	2	61.34	30.67	0.29	0.75
		Temp*SS	4	393.98	98.49	0.94	0.46

Table 4.6. Interaction effects of independent variables on thermal properties of camelina meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
D	Cold press	Temp	2	4.0E-05	2.0E-05	8.56	0.00
		SS	2	5.0E-05	2.5E-05	10.74	0.00
		Temp*SS	4	6.5E-05	1.6E-05	6.89	0.00
	ASE	Temp	2	6.7E-06	3.4E-06	1.07	0.36
		SS	2	2.3E-06	1.2E-06	0.36	0.69
		Temp*SS	4	2.2E-05	5.6E-06	1.77	0.17
K	Cold press	Temp	2	1.0E-03	5.2E-04	37.83	0.00
		SS	2	8.0E-05	4.0E-05	2.9	0.07
		Temp*SS	4	5.9E-04	1.5E-04	10.69	0.00
	ASE	Temp	2	1.7E-04	8.5E-05	7.48	0.00
		SS	2	4.5E-05	2.2E-05	1.96	0.16
		Temp*SS	4	6.7E-05	1.7E-05	1.48	0.24

Table 4.7. Interaction effects of independent variables on sugar recovery of camelina meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
Glucose	Cold press	Temp	2	5.49	2.74	16.62	0.00
		SS	2	3.83	1.91	11.60	0.00
		Temp*SS	4	3.52	0.88	5.33	0.00
	ASE	Temp	2	0.68	0.34	2.22	0.13
		SS	2	1.48	0.74	4.83	0.02
		Temp*SS	4	1.69	0.42	2.77	0.05
Galactose	Cold press	Temp	2	5.68	2.84	95.2	0.00
		SS	2	1.88	0.94	31.61	0.00
		Temp*SS	4	1.04	0.26	8.76	0.00
	ASE	Temp	2	3.41	1.70	15.38	0.00
		SS	2	0.70	0.35	3.16	0.06
		Temp*SS	4	0.40	0.10	0.91	0.47
Arabinose	Cold press	Temp	2	0.01	0.00	0.57	0.57
		SS	2	0.20	0.10	10.15	0.00
		Temp*SS	4	0.33	0.08	8.47	0.00
	ASE	Temp	2	0.42	0.21	87.52	0.00
		SS	2	0.02	0.01	5.92	0.01
		Temp*SS	4	0.04	0.01	4.15	0.01

CHAPTER 5

EFFECT OF EXTRUSION PRE-TREATMENT ON EXTRUDATE'S PHYSICAL PROPERTIES AND SUGAR RECOVERY OF COLD PRESS AND ACCELERATED SOLVENT EXTRACTED CARINATA MEAL

5.1 Abstract:

The study on the effect of extrusion as a pre-treatment on the sugar recovery and physical properties of carinata meal was performed. The oil from carinata seed was extracted from cold press (CP) and accelerated solvent extraction (ASE) methods and the meal obtained from these processes were used for the extrusion pretreatment study. Meals were then extruded at temperature of 80, 130 and 180°C and at a screw speed of 50, 100 and 150 rpm. Temperature at feeding and barrel zone were kept constant (80°C). The physical properties like water activity, water absorption index (WAI), water solubility index (WSI), moisture content, bulk density, true density, thermal properties and color were measured. Sugar recovery following enzymatic hydrolysis was also determined. Enzyme dosage and extruder processing conditions played a major role in increasing the sugar recovery of carinata meals. Higher sugar recovery was recorded for CP carinata meal than ASE carinata meal. The total percentage of sugars observed for ASE carinata meal was 19.44% at 80°C temperature and 100 rpm screw speed and for CP carinata meal, it was 26.14% at 80°C temperature and 150 rpm screw speed. Extruder temperature had a significant effect ($p<0.05$) on WAI, WSI, water activity, color and moisture content on dry basis MC, (db%) of CP and ASE carinata meal extrudates. No significant effect ($p>0.05$) of extruder temperature and screw speed was observed on bulk density and true density of CP and ASE carinata meal extrudates.

5.2 Introduction

B. carinata (Ethiopian mustard) is related to rapeseed (*B. napus*) and originated from a cross between *B. nigra* and *B. oleracea. B. carinata* seeds are consumed in Ethiopia. Brassica carinata possesses many positive agronomic traits (Gugel et al., 1990; Singh et al., 1988; Malik, 1990), and it can grow well in hot, dry, and semiarid climates typical of the southern prairies of western Canada. Moreover, with the development of high erucic acid types for industrial uses, as well as zero erucic acid lines (Getinet et al., 1994) and zero erucic acid/high oleic acid lines, (Velasco et al., 2003) seed oil from these species and its byproduct (carinata meal) may have potential applications in the food, biofuel, and feed industries. The defatted meal resulting from oil extraction could represent an important source of protein, which would increase the value of *B. carinata* crops. Enzymatic hydrolysis can be used to improve the functional and nutritional properties of oilseed proteins, which are easily denatured during the process of oil extraction (Vioque et al., 2000).

Heat or chemical treatments can be avoided by using the cold press method and also it's safe and natural as per the consumer desire (Parker et al., 2003). The process is simple, ecological and does not require much energy. Some of the disadvantages of the system are low productivity and not having a consistent quality (Rotkiewicz et al., 1999). Pressure and temperature are two important parameters to extract oil in Accelerated Solvent Extraction (ASE) (Ezzell, et al., 1995). Extraction times are reduced in ASE. More information regarding oil extraction methods could be found in Section 3.2.

Extruder is a high temperature short residence time reactor where the feed material passes through the extruder barrel and due to high shear rate and heating, physico-chemical changes occur. A few advantages of extruder are moderate temperature, short residence time, high shear, rapid heat transfer. Chemicals can be added or removed during extrusion-all in a continuous process. More information on extrusion can be found in Section 3.2 and 4.2. Extrusion pretreatment studies (Dale et al., 1999; de Vrije et al., 2002; Karunanithy et al., 2009a-c; Lee et al., 2009; Jurusic et al., 2009) show a significant improvement on sugar recovery from different biomasses such as corn stover, miscanthus, switchgrass, big blueatem, prairie cord grass and Douglas fir wood. Extrusion pretreatment has also been studied for soya white flakes and soyabean hulls (Karuppuchamy and Muthukumarappan, 2009).

The large availability and low cost make oilseed meals attractive raw materials, especially the proteins and carbohydrates, for various applications. Efforts have been made to utilize the co products for various applications. In this research carinata meal has been extracted by cold press and accelerated solvent extraction and the effect of extrusion as a pre-treatment has been studied on the sugar recovery of these meals.

Enzymatic hydrolysis of CP and ASE carinata meal was done in order to convert the polysaccharides to monosaccharides. The sugars are desired in the form of monosaccharides so that they are easily accessible by the particular micro-organisms during fermentation to convert them into protein. This converted protein then can be fed to the fish as a feed. This study will give an insight view for the efforts that has been made to replace the fish meal by the oilseed meals.

5.2.1 Objectives:

1. Optimize the extruder parameters to predict the maximum sugar recovery in CP and ASE carinata meal
2. Optimize the enzyme dosage for maximum sugar recovery in CP and ASE carinata meal
3. Determine the physical properties, thermal properties and sugar recovery of CP and ASE carinata meal

5.3 Materials and methods:

5.3.1 Sample preparation:

The carinata seeds were obtained from consumer supply distributing Co. (Oregon). Two different methods were used to extract oil: cold press extraction and accelerated solvent extraction. The cold press extraction (M70 oil press co.) consisting VFD motor (2HP, 1.5KW) was done at a temperature of 90°C, frequency of 20 Hz and die size of 0.22 inches. ASE was done at a time-temperature combination of 100°C/90 min. The solvent used was Hexane. Two samples of 1.5 Kg each were prepared according to cold press extraction and accelerated solvent extraction in order to study their effect on sugar recovery, physical properties and thermal properties of carinata meal extrudates.

5.3.2 Extrusion pretreatment:

The CP and ASE carinata meal samples prepared were extruded using single screw extruder (Brabender Plasti-Corder, Model PL2000, South Hackensack, NJ) which was powered by a 7.5HP motor with an operating range of screw speeds from 0-210 rpms (0-22rad/sec). The extruder had a barrel with length to diameter ratio of 20:1 and a barrel

diameter of 19 mm. The extrusion was done at 3 different temperature and screw speed of 80, 130 and 180°C; 50, 100 and 150 rpm, respectively. In total, 9 combinations were formed from temperature and screw speed. The extrudates obtained were dried overnight. The sample size used for extrusion for every combination was approximately 150g.

5.3.3 Grinding:

The extruded samples were further grounded using a hammer mill (Speedy Jr, Winona Attrition Mill Co, MN) to mesh size of 2 mm and then stored in zip lock bags in refrigerator at 4°C. The samples were ground to a uniform size in order to obtain effective enzymatic hydrolysis and physical properties of carinata meal.

5.3.4 Moisture content:

Moisture content of the control and extruded (CP and ASE) samples were measured according to the official methods of analysis (AOAC, 1984) using a laboratory oven (Thelco Precision, Jovan Inc., Wincester, VA).

5.3.5 Physical Properties

Three replications were studied for each property.

5.3.5.1 Water activity:

Water activity of the samples was measured by using the AquaLab water activity meter (4TE, version 10, Decagon Devices, Inc., Pullman WA) where the sample was placed in a plastic cup of the water activity meter and the lid was closed. After 5 to 10 minutes, the water activity value of the sample was shown on the screen. For each sample, three replications were done.

5.3.5.2 Water absorption index (WAI) and Water solubility index (WSI):

Different authors used different procedures to determine the WAI and WSI. Jones et al., 2000 used the following procedure to determine the WAI and WSI. 2.5 g sample was suspended in 30 ml of distilled water in a tarred 50 ml centrifuge tube. The centrifuge tube was placed in a laboratory oven (Thelco precision, Jovan Inc., Winchester, VA) at 30^{o}C. This was stirred intermittently for a period of 30 min and centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was transferred into an aluminum dish, placed in the oven for 2 hr at 135^{o}C (AACC method 44-19, 1995), and then desiccated for 20 min before weighing the dry solids of the supernatant. The mass of the remaining gel was weighed, and WAI (-) was calculated as the ratio of gel mass to the original sample mass. WSI (%), on the other hand was determined as the ratio of mass of dry solids in the extract to the original sample mass.

5.3.5.3 Bulk density and true density:

Micromeritics Multivolume Pycnometer (No.1305, Micromeritics Instrument Corporation, Norcross, GA) was used for the measurement of the true density of the samples. The biomass sample was filled full into an aluminum cup provided together with the pycnometer and then placed into the pycnometer. The unit works on the basis of helium gas replacement in the void space of the biomass sample. The values were measured in the form of P_1 when the knob was in the prep position and P_2 when the knob was in test position measured in psi. True density of the material was measured by the equation

$$TD = \frac{M_{sample}}{V_{cell} - \frac{V_{\exp}}{P_1 / P_2 - 1}}$$

Where TD is the true density, M_{sample} is the mass of the biomass sample that used to fill the alumnium cup, V_{cell} and V_{exp} are two constant numbers provided by the micromeritics multivolume pycnometer.

5.3.5.4 Thermal properties:

Thermal properties of the materials were measured by using the KD2 thermal properties analyzer. Thermal properties (thermal conductivity, thermal diffusivity, and specific heat capacity) were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, WA) that utilized the line heat source probe technique. The probe used was SH-1-00571.

5.3.5.5 Color:

The color was measured using a Spectrophotometer (CM-2500d, Minolta Co., Ltd., Japan). The color was described by value of 'L', 'a' and 'b' where L indicates intensity of color i.e. lightness which varies from L=100 for perfect white to L=0 for black. 'a' and 'b' are chromaticity dimensions which give understandable designations of color i.e. the value of 'a' measured redness when positive, grey when zero and greenness when negative and the value of 'b' measured yellowness when positive, grey when zero and blueness when negative.

5.3.6 Enzymatic hydrolysis:

The enzymatic hydrolysis was conducted in a hungate glass tube (Bellco glass, Inc, NJ, USA) with 1.1g dry weight of pre-treated biomass in a solution of citrate buffer (0.1M, pH 4.8) and sodium azide (0.02g/l) to inhibit microbial contamination during incubation. The enzymes used during enzymatic hydrolysis were CTec2 and HTec2, were purchased from Novozymes. The enzyme activity for CTec2 was 128 FPU/ml and for HTec2 was 4465 IU/ml. The enzyme dosage was optimized by adding varying enzyme dosage of 40, 50, 60, 70, 80 and 90 FPU/g cellulose. CTec2 and HTec2 were added in the ratio of 9:1. Hydrolysis was carried out for 72 hrs at 50°C and 150 rpm. After hydrolysis, the samples were kept in boiling water for 10 min to inactivate the enzyme action. The supernatant was centrifuged at 13,000 rpm for 15 min and frozen and thawed. This process was repeated twice before injecting into the HPLC to remove impurities which contribute to the pressure increase in the HPLC system. Soluble sugars were quantified using HPLC (Agilent technologies, Santa Clara, CA; Bio-Rad Aminex 87H column, Hercules, CA) with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.6 ml/min at 65°C and a sample volume of 20µl as mentioned by Sluiter et al (2006). The sugar concentration obtained from the chromatogram was divided by the dry weight of biomass of pretreated material and multiplied by the total volume taken to get the percentage of that compound in the sample. The percentage was calculated by multiplying the value by 100.

5.3.7 Extruder processing parameters optimization

The extruder processing parameters were optimized by using design of expert8 (Version 8.0.7.1) software. General Factorial Design was applied to optimize the temperature and screw speed for maximum sugar recovery of cold press carinata meal extrudaes and solvent extracted carinata meal extrudates.

5.3.8 Statistical Analysis

All the collected data were analyzed with SAS v.9 (SAS Institute, Cary, NC). The Proc GLM procedure was used to determine the main, treatment and interaction effects using a Type I error rate (α) of 0.05. Post-hoc Duncan Multiple Range Test (DMRT) tests were used to identify where the significant differences occurred.

5.4 Result and discussion

5.4.1 Water absorption index and Water solubility index (WAI and WSI)

The effect of different extruder processing parameters on WAI and WSI is shown in Fig. 5.1 and Fig. 5.2. The control value of WAI and WSI is almost similar for CP (3.84% and 26.62%) and ASE (3.62% and 25.58%) carinata meal. As the temperature increased during extrusion, the WAI and WSI for CP carinata meal extrudates significantly ($p<0.05$) decreased. In case of ASE carinata meal extrudates, the temperature had a significant ($p<0.05$) effect on WAI and WSI. The highest WAI and WSI value observed for CP carinata meal was 3.84% at 80°C temperature and 50 rpm screw speed; 27.32% at 80°C temperature and screw speed of 150 rpm. Several researchers suggested that any change in WAI can be due to structural modifications of the blend composition, such as starch gelatinization and protein denaturation (Badrie and Mellowes, 1991; Chevanan et al., 2007a; Rosentrater et al., 2009a, b). Unlike water absorption index, WSI indicates degradation extent of macromolecule components of a feed blend, mainly starch and protein molecules (Govindasamy, 1996; Colonna Mercier, 1983). Therefore, WAI is inversely related to WSI (Anderson et al., 1982). Generally, extrusion processing increases WSI (Menegassi et al. (2011); Anderson et al., 1982), which occurs due to the

combination effects of high temperature, pressure, and shear forces on starch and protein degradations.

5.4.2 Color

The color of CP and ASE carinata meal extrudates is shown in Table 5.1. The color is represented in terms of L, a, and b, where L indicates intensity of color i.e. lightness which varies from L=100 for perfect white to L=0 for black. ‘a’ and ‘b’ are chromaticity dimensions which give understandable designations of color i.e. the value of ‘a’ measured redness when positive, grey when zero and greenness when negative and the value of ‘b’ measured yellowness when positive, grey when zero and blueness when negative. The control value of CP (70.95) and ASE (69.23) carinata meal extrudates had similar L values. The extruder temperature had a significant ($p<0.05$) effect on the L value of CP and ASE carinata meal extrudates. Change in color of extrudates can be an indication of nutrients degradation during extrusion processing (Bjorck & Asp, 1983). The highest L value recorded for CP and ASE carinata meal extrudates was 72.27 at 80°C and 150 rpm; 61.14 at 180°C and 50 rpm. The control ‘a’ value for ASE carinata meal (4.69) is higher than the CP carinata meal (1.30) and the control ‘b’ value for ASE carinata meal (25.24) is lower than the CP carinata meal (28.37). The extruder temperature had a significant ($p<0.05$) effect on the ‘a’ and ‘b’ values of CP carinata meal extrudates. No significant effect ($p>0.05$) of extruder temperature was observed for the ‘a’ and ‘b’ values of ASE carinata meal extrudates. The highest and lowest ‘a’ and ‘b’ values recorded for CP carinata meal extrudates were 2.72 at 180°C and 100 rpm and 1.51 at 80°C and 150 rpm; 30.76 at 130°C and 100 rpm and 27.91 at 180°C and 100 rpm.

5.4.3 MC, (db%) and Water activity:

Table 5.2 represents the MC, (db%) and water activity of CP and ASE carinata meal extrudates. The moisture content at dry basis MC, (db%) for control ASE carinata meal (20.37%) is quite higher than the control CP carinata meal (8.52%). Almost similar values of MC, (db%) were observed for CP carinata meal extrudates. For ASE carinata meal extrudates, keeping the screw speed constant as the temperature increased, the MC, (db%) decreased significantly ($p<0.05$). At 50 rpm screw speed, MC, (db%) was 14.32% at 80°C, 11.65% at 130°C and 9.74% at 180°C.

The water activity value of control (0.79) and extrudates of ASE carinata meal is higher than the control (0.42) and extrudates of CP carinata meal. Extruder temperature and screw speed had a significant effect ($p<0.05$) on the water activity of CP and ASE carinata meal extrudates. As the temperature and screw speed increased, the water activity values for ASE carinata meal extrudates decreased significantly ($p<0.05$). The lowest water activity value recorded for CP and ASE carinata meal extrudates were 0.32 at 80°C and 150 rpm and 0.55 at 180°C and 50 rpm.

5.4.4 Thermal properties:

The thermal properties for carinata meal were recorded in terms of thermal diffusivity (D value), thermal conductivity (K value) and specific heat capacity (C value). D, K and C values for CP and ASE carinata meal extrudates is shown in Table 5.3. The control value of D (0.09), K (0.12) and C value (1.16) for ASE carinata meal is higher than the D (0.09), K (0.08) and C value (0.84) for CP carinata meal. The extruder temperature had a significant effect ($p<0.05$) on the D, K and C value of ASE carinata meal extrudates.

Thermal conductivity of a substance varies depending on its temperature and density; therefore, the required times for post-extrusion drying and cooling processes could be predicted by determining the extrudate thermal conductivity during the heat transfer between the extrudate surface and center, the correspondent temperature difference, and the void spaces of the extrudate (i.e. porosity) (Bouvier and Brisset, 2006). Thus, the k value of a material is also dependent on its composition. It is clear that, the more an extrudate expands the lower thermal conductivity (Mariama, 2008). In another study, Heldman (2003) postulated that the k value of a heat processed material decreased due to the material changes, primarily because of protein and starch transformations. The extruder temperature and screw speed had no significant effect ($p>0.05$) on the K and C value of CP carinata meal extrudates. As the extruder temperature increased, the D value increased significantly ($p<0.05$) and C value decreased significantly ($p<0.05$) for ASE carinata meal extrudates. The D value and C value of ASE carinata meal extrudates observed at temperature of 80°C, 130°C and 180°C, keeping the screw speed constant at 50 rpm were 0.09, 0.10 and 0.11; 1.41, 1.35 and 1.26.

5.4.5 Bulk density and true density:

The effect of extruder processing parameters (temperature and screw speed) on bulk density and true density of CP and ASE carinata meal extrudates is shown in Table 5.4. The control bulk density value for CP carinata meal (496.13) was higher than the control ASE carinata meal (453.01). The true density control value for ASE carinata meal (4213.77) was higher than the control value of CP carinata meal (3013.04). Extrusion temperature and screw speed had no significant effect ($p>0.05$) on the bulk density and true density of CP and ASE carinata meal extrudates. Almost similar bulk density values

were observed for CP and ASE carinata meal extrudates at different extruder processing conditions. The highest bulk density and true density observed for CP and ASE carinata meal extrudates were 498.78 at 80°C and 100 rpm and 2977.79 at 130°C and 150 rpm; 569.15 at 130°C and 150 rpm and 3154.66 at 80°C and 50 rpm.

5.4.6 Interaction effects of independent variables on physical properties of carinata meal

The interaction effects of independent variables on physical properties of carinata meal are given in Table 5.5. The temperature and combined interaction of temperature and screw speed had the significant effect ($p<0.05$) on WAI of CP and ASE carinata meal. Temperature had a significant effect ($p<0.05$) on WSI of CP and ASE carinata meal. The water activity of CP and ASE carinata meal is significantly effected ($p<0.05$) by the temperature, screw speed, combined interaction of temperature and screw speed. The color of CP and ASE carinata meal extrudates was significantly effected ($p<0.05$) by the temperature and combined interaction of temperature and screw speed. There was no significant effect ($p<0.05$) of temperature and screw speed on the bulk density of CP and ASE carinata meal extrudates.

5.4.7 Interaction effects of independent variables on thermal properties of carinata meal

The interaction effects of independent variables on thermal properties of carinata meal are given in Table 5.6. The thermal diffusivity (D value) of CP and ASE carinata meal was significantly ($p<0.05$) effected by the temperature alone. The thermal conductivity (K value) of CP carinata meal was not significantly affected ($p>0.05$) by extrusion. For

ASE carinata meal, extruder temperature, screw speed and combined interaction of temperature and screw speed had a significant effect ($p<0.05$) on the thermal diffusivity.

5.4.8 Sugar recovery:

Fig. 5.3 represents the effect of extruder temperature and screw speed on sugar recovery i.e. glucose, galactose and arabinose recovery of CP and ASE carinata meal extrudates. The sugar recovery for CP and ASE carinata meal was measured in terms of glucose, galactose and arabinose recovery. The control glucose and galactose recovery of CP carinata meal (12.12% and 7.44%) was higher than the control ASE carinata meal (9.79% and 6.62%). The glucose and galactose recovery of CP carinata meal extrudates was significantly effected ($p<0.05$) by extrusion. Veeramani and Muthukumarappan, 2011 also got the similar results for defatted soyabean meal, with increase in temperature the glucose yield increased. On the other hand, extrusion had no significant effect ($p>0.05$) on the glucose and galactose recovery of ASE carinata meal extrudates. As the temperature increased, glucose and galactose recovery of CP carinata meal extrudates increased significantly ($p<0.05$). No galactose recovery was observed for ASE carinata meal extrudates. The highest glucose recovery for CP and ASE carinata meal extrudates was 21.87% at 180°C and 150 rpm and 16.72% at 80°C and 100 rpm. The control arabinose recovery for ASE carinata meal (2.59%) was higher than the control CP carinata meal (1.50%). As the extruder temperature and screw speed increased, arabinose recovery for CP carinata meal increased significantly ($p<0.05$). For ASE carinata meal extrudates, no significant effect ($p>0.05$) of extrusion was observed for arabinose recovery.

The CP and ASE carinata meal samples were also checked for the presence of any formaldehyde which might be present as a result of pre-treatment. The samples were run for duration of 60 minutes and no peaks were found after 20 minutes which shows the absence of any formaldehyde in the samples.

5.4.9 Interaction effects of independent variables on sugar recovery of carinata meal

The interaction effects of independent variables on sugar recovery of carinata meal are given in Table 5.7. Temperature, screw speed and combined interaction of temperature and screw speed had a significant effect ($p<0.05$) on the glucose, galactose and arabinose recovery of CP carinata meal. No significant effect ($p>0.05$) on glucose, galactose and arabinose recovery was observed for temperature, screw speed and combined interaction of temperature and screw speed in case of extrusion of ASE carinata meal.

5.4.10 Enzyme dosage optimization:

The enzymes were added at 50, 60, 70, 80 and 90 FPU/g cellulose to control and extrudate of cold press and solvent extracted samples. The hydrolysis was done and each sample was measured for the sugar recovery using HPLC. The % total sugars for CP carinata meal extrudates for 50 (18.37%), 60 (17.82%) and 70 (18.33%) FPU/g cellulose was almost similar. Higher % total sugars were obtained at 80 (21.71%) and 90 (23.61%) FPU/g cellulose for CP carinata meal extrudates. For ASE carinata meal extrudates, almost similar values of % total sugars were obtained for 50 (17.05%), 60 (16.28%), 70 (16.06%), 80 (17.62%) and 90 (17.85%) FPU/g cellulose. The glucose yield for CP carinata meal extrudates significantly increased ($p<0.05$) with increase in enzyme concentration. The glucose yield values for ASE carinata meal extrudates were almost

similar at different enzyme concentrations. Although sugar recovery was higher at 90 FPU/g cellulose compared to other dosage, the optimized enzyme dosage for CP and ASE carinata meal was 50 FPU/g cellulose. The reasons were that higher enzyme dosage will be costly and it might overcome the extrusion effect.

5.4.11 Extruder processing parameters optimization

For CP carinata meal extrudates, the optimized extruder conditions were temperature of 80°C and screw speed of 150 rpm and for ASE carinata meal extrudates, the optimized extruder conditions were temperature of 140°C and screw speed of 88 rpm. The desirability obtained for CP and ASE carinata meal extrudates were 0.75 and 1.0. The maximum sugar recovery at the optimized conditions for CP and ASE carinata meal extrudates were 25.09% and 19.62%.

5.5 Conclusions

Extrusion was done at different temperature (80°C, 130°C and 180°C) and screw speed (50, 100 and 150 rpm) in order to optimize these extruder process parameters for maximum sugar recovery and to study the effect of extrusion on physical properties of CP and ASE carinata meal. Screw speed, die temperature and oil extraction methods, affected the physical properties and thermal properties of carinata meal. For enzymatic hydrolysis, the enzymes added at 50 FPU/g cellulose gave the best results for CP and ASE carinata meal. The optimized conditions for sugar recovery of CP extruded carinata meal was temperature of 80°C and screw speed of 150 rpm and for ASE extruded carinata meal, the optimized conditions were 140°C die temperature and screw speed of

88 rpm. For physical and thermal properties, extruder temperature had a more significant effect ($p<0.05$) than the screw speed.

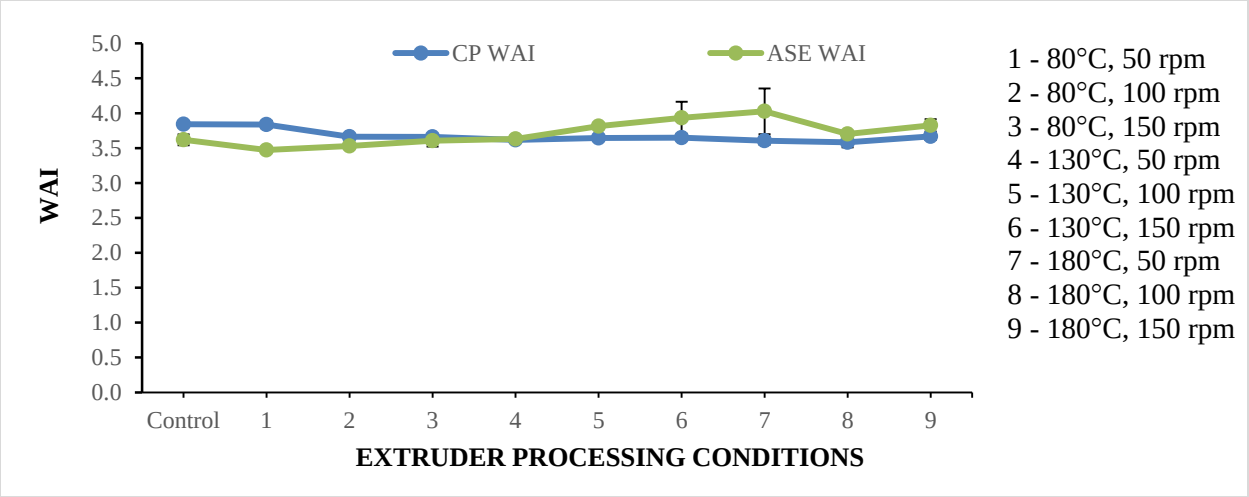

Figure 5.1. Effect of extruder processing parameters on WAI of CP and ASE carinata meal extrudates

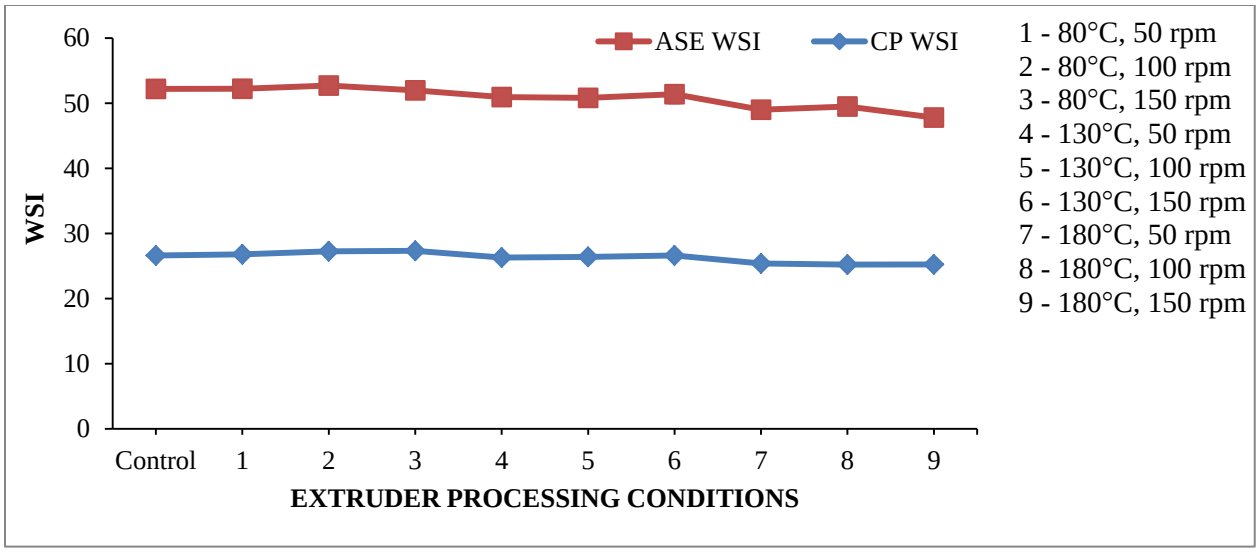

Figure 5.2. Effect of extruder processing parameters on WSI of CP and ASE carinata meal extrudates

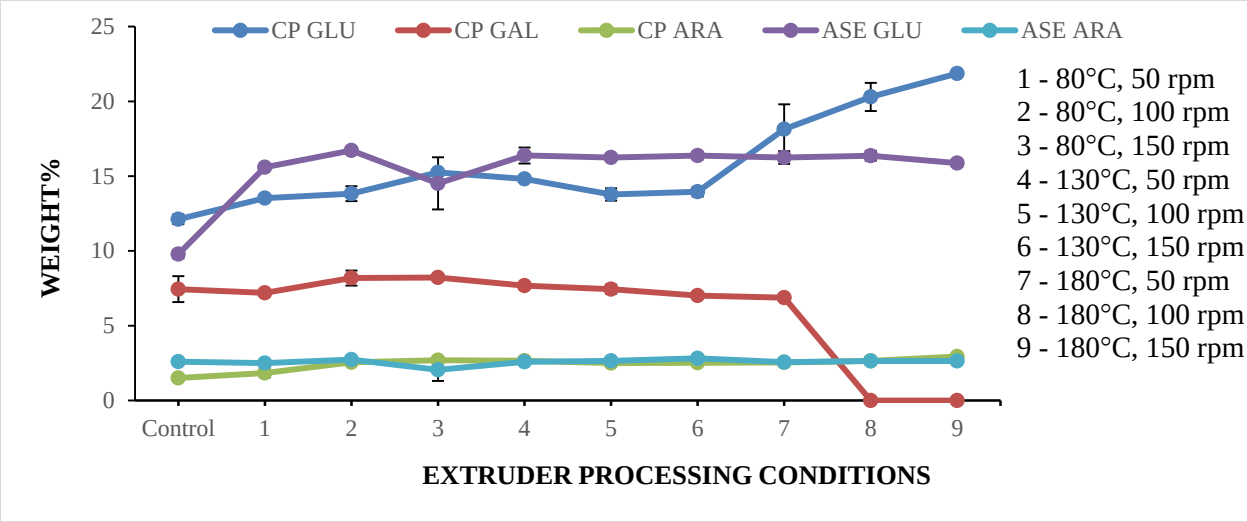

Figure 5.3. Effect of extruder processing parameters on sugar recovery (glucose, galactose and arabinose) of CP and ASE carinata meal extrudates

Table 5.1. Effect of extruder processing conditions on color (L, a and b) of CP and ASE carinata meal extrudates

Processing conditions	CP			ASE		
	L	a	b	L	a	b
No Treatment	70.95[b,a]	1.30[d]	28.37[c]	69.23[a]	4.69[c]	25.24[a]
	(0.95)	(0.21)	(0.85)	(0.50)	(0.11)	(0.09)
80°C, 50 rpm	70.12[b,c]	1.83[c,b]	28.98[c]	59.43[c,b,d]	6.79[b,a]	20.29[c,b]
	(1.32)	(0.39)	(0.42)	(1.13)	(0.29)	(0.69)
80°C, 100 rpm	70.62[b,c]	1.92[c,b]	29.19[b,c]	56.04[e]	7.11[b,a]	17.44[d]
	(0.33)	(0.17)	(0.40)	(1.37)	(0.43)	(0.95)
80°C, 150 rpm	72.27[a]	1.51[c,d]	30.34[b,a]	59.07[c,b,d]	6.82[b,a]	21.20[c,b]
	(0.07)	(0.07)	(0.23)	(0.51)	(0.24)	(0.77)
130°C,50 rpm	72.56[a]	1.61[c,b,d]	30.31[b,a]	58.69[c,d]	7.39[a]	19.23[c,b,d]
	(1.42)	(0.25)	(0.91)	(1.56)	(0.79)	(2.05)
130°C,100 rpm	71.50[b,a]	1.73[c,b,d]	30.76[a]	60.58[c,b]	6.77[b,a]	18.90[c,d]
	(0.85)	(0.21)	(0.27)	(0.81)	(0.45)	(0.84)
130°C,150 rpm	69.68[c]	2.04[b]	29.18[b,c]	57.23[e,d]	7.12[b,a]	17.06[d]
	(0.58)	(0.19)	(0.35)	(0.80)	(0.33)	(0.85)
180°C,50 rpm	68.98[c]	2.56[a]	28.69[c]	61.14[b]	6.89[b,a]	19.24[c,b,d]
	(0.55)	(0.53)	(1.12)	(1.58)	(0.22)	(1.64)
180°C,100 rpm	68.98[c]	2.72[a]	27.91[c]	59.62[c,b]	6.58[b]	17.90[d]
	(0.32)	(0.20)	(0.85)	(0.99)	(0.18)	(1.47)
180°C,150 rpm	69.05[c]	2.71[a]	28.92[c]	60.05[c,b]	6.87[b,a]	21.54[b]
	(1.11)	(0.08)	(1.01)	(1.57)	(0.44)	(1.39)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 5.2. Effect of extruder processing conditions on MC, (db%) and water activity of CP and ASE carinata meal extrudates

Processing conditions	CP		ASE	
	MC db%	a_w	MC db%	a_w
No Treatment	8.52[a]	0.42[a]	20.38[a]	0.79[a]
	(0.35)	(0.01)	(0.21)	(0.00)
80°C, 50 rpm	7.58[b]	0.36[b]	14.33[c]	0.70[d]
	(0.07)	(0.00)	(0.18)	(0.00)
80°C, 100 rpm	7.44[c,b]	0.33[e,f]	14.79[c,b]	0.71[b]
	(0.04)	(0.00)	(0.06)	(0.00)
80°C, 150 rpm	7.19d[e]	0.32[f]	15.11[b]	0.71[c]
	(0.05)	(0.01)	(0.28)	(0.00)
130°C,50 rpm	7.26[c,d,e]	0.34[e,d]	11.65[d]	0.62[e]
	(0.10)	(0.01)	(0.49)	(0.00)
130°C,100 rpm	7.36[c,d]	0.36[c,b]	10.80[e]	0.58[g]
	(0.08)	(0.01)	(0.05)	(0.00)
130°C,150 rpm	7.42[c,b]	0.35[c,d]	11.51[d]	0.61[f]
	(0.04)	(0.01)	(0.51)	(0.00)
180°C,50 rpm	6.93[f]	0.34[e,d]	9.74[f]	0.55[i]
	(0.19)	(0.01)	(0.29)	(0.00)
180°C,100 rpm	7.08[f,e]	0.33[e,f]	10.64[e]	0.56[h]
	(0.07)	(0.00)	(0.29)	(0.00)
180°C,150 rpm	7.22[c,d,e]	0.34[c,d]	9.84[f]	0.56[h]
	(0.17)	(0.00)	(0.04)	(0.00)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 5.3. Effect of extruder processing conditions on thermal properties (D, K and C) of CP and ASE carinata meal extrudates

Processing conditions	CP			ASE		
	D (m^2/s)	K (W/mK)	C (J/K)	D (m^2/s)	K (W/mK)	C (J/K)
No Treatment	0.09^{b}	0.08^{b}	0.84^{b}	$0.10^{b,a,c}$	0.12^{d}	1.16^{e}
	(0.00)	(0.01)	(0.08)	(0.00)	(0.01)	(0.02)
80°C, 50 rpm	0.10^{b}	$0.10^{b,a}$	$1.03^{b,a}$	$0.09^{d,c}$	$0.13^{b,a}$	1.41^{a}
	(0.00)	(0.02)	(0.15)	(0.00)	(0.00)	(0.03)
80°C, 100 rpm	0.10^{b}	$0.09^{b,a}$	$0.99^{b,a}$	0.09^{d}	$0.12^{d,c}$	$1.32^{b,c,d}$
	(0.00)	(0.02)	(0.18)	(0.00)	(0.00)	(0.01)
80°C, 150 rpm	$0.10^{b,a}$	$0.11^{b,a}$	$1.05^{b,a}$	$0.10^{b,d,c}$	$0.13^{b,c}$	$1.33^{b,c,d}$
	(0.00)	(0.00)	(0.06)	(0.00)	(0.00)	(0.02)
130°C,50 rpm	$0.10^{b,a}$	$0.10^{b,a}$	$1.00^{b,a}$	$0.10^{b,d,c}$	$0.13^{b,a}$	$1.35^{b,a}$
	(0.00)	(0.01)	(0.08)	(0.00)	(0.00)	(0.05)
130°C,100 rpm	$0.10^{b,a}$	$0.10^{b,a}$	$1.03^{b,a}$	$0.10^{b,d,c}$	0.14^{a}	$1.39^{b,a}$
	(0.00)	(0.02)	(0.14)	(0.00)	(0.00)	(0.02)
130°C,150 rpm	0.10^{b}	$0.10^{b,a}$	$1.00^{b,a}$	$0.09^{b,d,c}$	$0.13^{b,c}$	$1.34^{b,c}$
	(0.00)	(0.01)	(0.09)	(0.00)	(0.00)	(0.03)
180°C,50 rpm	$0.10^{b,a}$	0.11^{a}	1.14^{a}	0.11^{a}	$0.13^{b,a}$	1.26^{d}
	(0.00)	(0.01)	(0.09)	(0.01)	(0.01)	(0.05)
180°C,100 rpm	$0.10^{b,a}$	$0.10^{b,a}$	$1.04^{b,a}$	$0.10^{b,d,c}$	$0.13^{b,c}$	$1.28^{c,d}$
	(0.00)	(0.01)	(0.13)	(0.00)	(0.00)	(0.01)
180°C,150 rpm	0.10^{a}	0.12^{a}	1.15^{a}	$0.10^{b,a}$	$0.12^{d,c}$	1.19^{e}
	(0.00)	(0.00)	(0.01)	(0.00)	(0.01)	(0.08)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 5.4. Effect of extruder processing conditions on true density (TD) and bulk density (BD) of CP and ASE carinata meal extrudates

Processing conditions	CP		ASE	
	TD (Kg/m^3)	BD (Kg/m^3)	TD (Kg/m^3)	BD (Kg/m^3)
No Treatment	3013.05[a]	496.13[a]	4213.78[a]	453.01[c]
	(165.97)	(26.82)	(1179.04)	(6.07)
80°C, 50 rpm	2854.81[a]	493.07[a]	3154.66[b]	550.53[a]
	(14.10)	(7.56)	(818.41)	(29.47)
80°C, 100 rpm	2781.35[a]	498.78[a]	2858.08[b]	509.10[d]
	(105.99)	(19.79)	(88.54)	(15.54)
80°C, 150 rpm	2704.11[a]	481.36[a]	2788.15[b]	521.39[d,c]
	(128.08)	(20.64)	(36.01)	(8.46)
130°C,50 rpm	2910.35[a]	474.08[a]	2731.01[b]	537.52[b,d,c]
	(140.34)	(5.84)	(41.56)	(10.40)
130°C,100 rpm	2702.35[a]	496.62[a]	2700.29[b]	552.13[b,a,c]
	(8.65)	(0.36)	(39.87)	(4.27)
130°C,150 rpm	2977.80[a]	483.40[a]	2627.68[b]	569.15[b,a]
	(426.33)	(24.39)	(34.30)	(9.67)
180°C,50 rpm	2679.82[a]	495.17[a]	2797.99[b]	529.37[b,d,c]
	(4.61)	(1.54)	(61.35)	(16.92)
180°C,100 rpm	2680.11[a]	476.42[a]	2701.49[b]	539.42[b,d,c]
	(139.52)	(15.32)	(15.22)	(3.84)
180°C,150 rpm	2764.04[a]	472.67[a]	2861.68[b]	524.55[d,c]
	(59.64)	(15.05)	(22.56)	(2.81)

*Means with different letters in a column within each extraction method are significantly different ($p<0.05$)
Means with same letters in a column within each extraction method are not significantly different ($p>0.05$)

Table 5.5. Interaction effects of independent variables on physical properties of carinata meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
WAI	CP	Temp	2	0.05	0.02	13.30	0.00
		SS	2	0.01	0.00	3.75	0.04
		Temp*SS	4	0.05	0.01	7.67	0.00
	ASE	Temp	2	0.50	0.25	13.53	0.00
		SS	2	0.05	0.02	1.46	0.25
		Temp*SS	4	0.27	0.06	3.68	0.02
WSI	CP	Temp	2	15.54	7.77	153.33	0.00
		SS	2	0.24	0.12	2.43	0.11
		Temp*SS	4	0.47	0.11	2.36	0.09
	ASE	Temp	2	13.66	6.83	19	0.00
		SS	2	2.69	1.34	3.75	0.04
		Temp*SS	4	3.28	0.82	2.29	0.09
a_w	CP	Temp	2	0.00	0.00	6.47	0.00
		SS	2	0.00	0.00	4.11	0.03
		Temp*SS	4	0.00	0.00	17.68	0.00
	ASE	Temp	2	0.10	0.05	7583.82	0.00
		SS	2	0.00	0.00	11.89	0.00
		Temp*SS	4	0.00	0.00	116.19	0.00
L	CP	Temp	2	27.37	13.68	19.44	0.00
		SS	2	0.25	0.12	0.18	0.83
		Temp*SS	4	20.05	5.01	7.12	0.00
	ASE	Temp	2	20.52	10.26	7.37	0.00
		SS	2	5.87	2.93	2.11	0.14
		Temp*SS	4	35.49	8.87	6.37	0.00
BD	CP	Temp	2	1332436.77	666218.38	2.02	0.15
		SS	2	699515.67	349757.83	1.06	0.36
		Temp*SS	4	2178583.56	544645.89	1.65	0.20
	ASE	Temp	2	1786550.92	893275.46	2.98	0.07
		SS	2	837113.90	418556.95	1.4	0.27
		Temp*SS	4	6619952.52	1654988.13	5.52	0.00

Table 5.6. Interaction effects of independent variables on thermal properties of carinata meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
D	CP	Temp	2	0.00	0.00	3.8	0.04
		SS	2	0.00	0.00	2.25	0.13
		Temp*SS	4	0.00	0.00	1.4	0.27
	ASE	Temp	2	0.00	0.00	12.35	0.00
		SS	2	0.00	0.00	1.36	0.27
		Temp*SS	4	0.00	0.00	1.32	0.29
K	CP	Temp	2	0.00	0.00	2.91	0.07
		SS	2	0.00	0.00	0.88	0.43
		Temp*SS	4	0.00	0.00	0.44	0.77
	ASE	Temp	2	0.00	0.00	3.68	0.04
		SS	2	0.00	0.00	8.07	0.00
		Temp*SS	4	0.00	0.00	3.25	0.03

Table 5.7. Interaction effects of independent variables on sugar recovery of carinata meal

Property	Extraction method	Source	DF	Type SS	Mean Square	F Value	P value
Glucose	CP	Temp	2	209.54	104.77	235.88	0.00
		SS	2	11.07	5.53	12.47	0.00
		Temp*SS	4	16.81	4.20	9.46	0.00
	ASE	Temp	2	2.57	1.28	3.25	0.06
		SS	2	3.32	1.66	4.18	0.03
		Temp*SS	4	4.39	1.09	2.77	0.05
Galactose	CP	Temp	2	171.75	85.87	1215.58	0.00
		SS	2	26.60	13.30	188.33	0.00
		Temp*SS	4	70.53	17.63	249.59	0.00
	ASE	Temp	2	1.21E-27	0.00	0	1
		SS	2	1.21E-27	0.00	0	1
		Temp*SS	4	2.35E-27	0.00	0	1
Arabinose	CP	Temp	2	0.54	0.271	22.94	0.00
		SS	2	0.63	0.31	26.74	0.00
		Temp*SS	4	0.93	0.23	19.78	0.00
	ASE	Temp	2	0.32	0.16	2.63	0.09
		SS	2	0.32	0.16	2.63	0.09
		Temp*SS	4	0.32	0.16	2.63	0.09

CHAPTER 6

CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1 Conclusions

Enzyme optimization:

1. Canola meal: 60 FPU/g cellulose gave the best results for CP canola meal and ASE canola meal
2. Camelina meal: 70 FPU/g cellulose gave the best results for CP camelina meal and ASE camelina meal
3. Carinata meal: 50 FPU/g cellulose gave the best results for CP carinata meal and ASE carinata meal

Extruder processing parameters optimization:

1. Canola meal: Maximum sugar recovery for CP extruded canola meal was observed at temperature of 80°C and screw speed of 50 rpm and for ASE extruded canola meal, 80°C die temperature and screw speed of 100 rpm
2. Camelina meal: CP extruded camelina meal showed maximum sugar recovery results at temperature of 80°C and screw speed of 50 rpm and for ASE extruded camelina meal, the optimized conditions observed were 80°C die temperature and screw speed of 100 rpm.
3. Carinata meal: Temperature of 80°C and screw speed of 150 rpm showed the maximum sugar recovery for CP extruded carinata meal and for ASE extruded carinata meal, the optimized conditions recorded were 80°C die temperature and screw speed of 100 rpm.

Physical properties and Thermal properties:

1. Canola meal: Extruder temperature had a significant effect ($p<0.05$) on physical properties and thermal properties of CP and ASE canola meal than the screw speed. Extruder temperature had a significant effect ($p<0.05$) on WAI, water activity, color and bulk density of ASE canola meal.
2. Camelina meal: Extrusion had a significant effect ($p<0.05$) on the thermal properties of CP camelina meal. No significant effect ($p>0.05$) of extrusion was observed for WAI, WSI, color (L), bulk density of CP and ASE camelina meal. It was observed that during extrusion, extruder temperature had a greater effect than screw speed.
3. Carinata meal: For physical and thermal properties of carinata meal extrudates, extruder temperature had a more significant effect ($p<0.05$) than the screw speed. Extruder temperature had a significant effect ($p<0.05$) on WAI, WSI, water activity, color and moisture content on dry basis MC, (db%) of CP and ASE carinata meal extrudates. No significant effect ($p>0.05$) of extruder temperature and screw speed was observed on bulk density and true density of CP and ASE carinata meal extrudates.

6.2 Future recommendations

1. Physical properties of cold press extracted and solvent extracted canola, camelina and carinata meal extrudates should be compared
2. Sugar recovery of cold press extracted and solvent extracted canola, camelina and carinata meal extrudates should be compared

3. Pre-treatment of oilseed meals using microwave treatment should be studied
4. Fermentation of pre-treated oilseed meals (canola, camelina and carinata) using particular micro-organisms to convert the sugars into protein should be investigated
5. Feeding the prepared fish feed of canola, camelina and carinata meal to different fish and how they react to the feed should be studied

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