Extraction, Modification, and Chemical Characterization of Protein and Dietary Fiber from Camelina Sativa

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Dedication

"Plus j'étudie la nature et plus je suis émerveillé par les travaux de Notre Créateur."

– Louis Pasteur

Abstract

Camelina sativa, a sustainable short-season cover crop, is an oilseed (35% oil) gaining interest due to the increasing global demand for sustainably sourced ingredients. Camelina provides numerous agricultural benefits—low production cost, low nitrogen requirements, drought resistance, cold weather tolerance, and short growing season—in addition to being high in protein (20%) and dietary fiber (30%), which are two of the fastest growing segments of the food ingredient market. In order to create functional, market-viable ingredients from camelina, the following need to be explored: efficient means of protein extraction, evaluation of protein functional properties, and chemical characterization of the dietary fiber constituents.

The objectives of this study were as follows: (1) determine the impact of oil pressing conditions and protein extraction protocol on protein yield and content; (2) characterize structural differences in proteins extracted following salt precipitation and pH solubilization; (3) determine the impact of structure and enzymatic modification on the functionality of the different protein extracts; (4) isolate, quantify, and characterize the insoluble and soluble dietary fiber fractions of defatted camelina meal (DCM) prepared by two different oil pressing conditions.

Protein extraction by pH solubilization and salt precipitation was tested and optimized. Camelina meal obtained from hot and cold press was further defatted by hexane and analyzed for protein content. Protein from DCM was extracted following degumming and pH solubilization at pH 12, separating non-protein material by centrifugation, acidifying the supernatant to pH 5 to precipitate out the protein, neutralizing and desalting. Protein from DCM was also extracted following salt precipitation, first by solubilizing the protein using 0.05 M phosphate buffer (pH 8, 1 M NaCl), followed by precipitation using 85% saturated ammonium sulfate solution, and desalting. To produce protein hydrolysates, extracted proteins were subjected to hydrolysis with *Aspergillus oryzae* protease by pH-stat methodology to a degree of hydrolysis less than 8%. Protein purity of the extracts was analyzed, and mass balances were tracked in order to evaluate extraction yields. The denaturation state, protein profile, and surface hydrophobicity of the protein extracts were determined using DSC, SDS-PAGE, and a fluorometric assay, respectively. Functionality

was evaluated by determining protein solubility as well as emulsification, foaming, and gelation properties.

Total dietary fiber (TDF) from DCM was determined following the AOAC method 2011.25, and three fractions —insoluble dietary fiber (IDF), soluble dietary fiber that precipitates in 78% ethanol (SDFP), and soluble dietary fiber that is soluble in 78% ethanol (SDFS) — were isolated preparatively. IDF and SDFP were analyzed spectrophotometrically for pectin content. The monomers of IDF and SDFP fractions were determined by alditol acetate formation and measured by GC-FID. Degree of pectin methylation (DM) of SDFP was determined by ^{1}H NMR. The degree of polymerization (DP) of saccharides in the SDFS fraction (DP 2 – DP 7) was determined by liquid chromatography-ESI-mass spectrometry (LC-MS) using a ligand-exchange stationary phase and quantified by high performance anion exchange chromatography coupled with a pulsed amperometric detector (HPAEC-PAD). Disaccharides in DCM were differentiated and quantified spectrophotometrically following standard enzymatic assays.

Compared to camelina protein concentrates (CPC) produced by alkaline pH extraction, CPC produced by salt extraction were less denatured and more functional. The functionality of the salt extracted CPC was comparable and sometimes better than that of soy protein isolate (SPI). Specifically, the solubility of the salt extracted CPC at pH 3.4 was significantly ($P < 0.05$) higher than that of SPI. Additionally, salt extracted CPC had significantly higher emulsification capacity and foaming capacity than SPI. On the other hand, the gelation property of CPC was inferior to that SPI, an observation attributed to the molecular size of camelina protein compared to SPI. Upon hydrolysis of CPC with *Aspergillus oryzae* protease, a limited benefit to solubility was noted at pH 7 post thermal treatment.

TDF of DCM averaged 51.2% (45.3 – 49.1% IDF, 2.00 – 5.98% SDFP, 1.1 – 1.2% SDFS). The SDFS fraction was comprised mainly of stachyose and raffinose, which is in line with other Brassicaceae crops. The chief disaccharide present in DCM was verified to be sucrose (2.43 – 3.36%). Free glucose and fructose were also present in the SDFS fraction. Of the pectic polysaccharides measured in SDFP, low methoxyl pectin represented the major constituent, with a DM of 12.5 – 14.5%. Based on alditol acetate analysis, glucose was the main monomer in the IDF fraction. Other monosaccharides

detected in the IDF fraction were xylose, arabinose, mannose, and galactose. The monosaccharide composition indicated the presence of cellulose, xyloglucans, galactomannans, and arabinoxylans in the IDF fraction. In SDFP, the monosaccharides rhamnose, arabinose, galactose, and mannose were evenly distributed. Monomer composition of the SDFP fraction indicated the presence of pectin and galactomannans.

Results show that camelina meal contains a significant amount of protein and dietary fiber that can be isolated into functional ingredients. This is the first study to provide a comprehensive evaluation of protein and dietary fiber from camelina as potential alternatives to traditional ingredients. Further work is needed to understand how isolated camelina ingredients interact in various food matrices.

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1 Chapter 1: Literature Review

1.1 Introduction

The demand for plant-based, sustainably-sourced food ingredients is increasing, as concerns about traditional farming practices, dwindling agricultural land, and the pressure to feed an increasing population arise. Ancient crops that have yet to be developed for commercial use are being explored for their potential as ingredient sources to replace traditional higher input, higher cost food materials. *Camelina sativa* L. Crantz, an oilseed in the Brassicaceae family, is one example of a crop that has been cultivated for thousands of years as a source of oil for fuel, cooking, and medicinal uses (Zubr 2010). Historically, the residual meal from oil pressing was channeled as waste or left for animal feed, at best (Obour et al. 2015). Camelina meal, however, is generating renewed interest as a potential source for protein and dietary fiber ingredients.

Demand for high protein products has been increasing over the past decade—a trend that is expected to be sustained among consumers (Grand View Research 2018). The food industry is facing challenges with traditional protein sources (dairy, egg, soy): cost, allergenicity, increasing popularity of exclusion diets such as vegetarianism and veganism, and the use of genetic modification to increase agricultural productivity, which brings up safety concerns among some groups of consumers (Roberts 2017). Along with a desire for high protein products, consumers are interested in new sources of protein, particularly from plants, as they want their purchasing habits to match their environmental and health-conscious convictions. Soy protein has the majority of the plant protein market, however the food industry is searching for alternatives, as soy is associated with some of the aforementioned challenges: soy is one of the "Big 8" allergens and is a GMO crop (Frost & Sullivan 2018). When added to food products, soy protein contributes key functional properties that any alternative protein must match or outperform in order to be competitive in the marketplace (Singh et al. 2008). These functional properties include protein solubility, emulsification and foaming capabilities, and gelling properties, all of which are governed by the structural characteristics of the proteins (Foegeding and Davis 2011).

Although there is some research on the structural characteristics of camelina proteins, their functional properties have yet to be evaluated.

Traditionally, plant proteins have demonstrated inferior functionality compared to animal proteins due to their globular nature and low molecular flexibility (Martínez et al. 2007; Wanasundara 2011). In efforts to improve functionality, limited and controlled enzymatic hydrolysis has been performed in order to produce peptides with unique and improved physiochemical characteristics (Barac et al. 2006). Hydrophilicity, for example, is a physiochemical characteristic generally associated with better functional properties. Modification in any way has not be explored with regards to camelina protein.

Camelina seeds are also a rich source of dietary fiber (Zubr 2010). In 2012, it was estimated that less than 3% of Americans consumed the recommended amount of dietary fiber (Clemens et al. 2012). Because of this, dietary fiber has been classified as an "under-consumed nutrient of public health concern" by the Dietary Guidelines Advisory Committee (Clemens et al. 2012). Large efforts to ameliorate this problem through education and public awareness have led to fiber ingredients being one of the fastest growing segments of the food and beverage ingredient industry, and digestive health being a top consumer health and wellness trend (Frost & Sullivan 2012). In order to meet the needs of the food and beverage industry and to address public health concerns regarding dietary fiber intake, more research is needed to investigate the composition of dietary fiber from different plant sources such as camelina.

There are knowledge gaps surrounding camelina protein functionality, dietary fiber composition, and its potential to address needs among consumers and in the food industry. Therefore, camelina merits deeper investigation.

1.2 Hypothesis and Objectives

We hypothesize that isolating camelina protein by different extraction methods will have a structural impact on the proteins, which will impact functionality. Furthermore, we anticipate that protein modification by targeted and controlled enzymatic hydrolysis will increase solubility and thus impart better functional properties. In regard to dietary fiber, we hypothesize that soluble fiber isolated from camelina seeds will have a structural

composition that lends to desirable food ingredient properties—water holding, gelling, and humectant capabilities.

The overall objective of the project was to evaluate methods to isolate protein and dietary fiber from camelina, analyze functional characteristics of the proteins, and determine properties isolated protein and fiber could contribute to a food product. Specific objectives were:

1. Determine the impact of oil pressing conditions and protein extraction protocol on protein yield and content.
2. Characterize structural differences in proteins extracted following pH solubilization and salt precipitation.
3. Determine the impact of structure and enzymatic modification on the functionality of the different protein extracts.
4. Quantify total dietary fiber in defatted camelina meal.
5. Characterize the chemical composition of three dietary fiber fractions:
    - Insoluble dietary fiber (IDF)
    - Soluble dietary fiber that precipitates in 78% ethanol (SDFP)
    - Soluble dietary fiber that is soluble in 78% ethanol (SDFS)

1.3 Need for Novel, Sustainably Sourced Ingredients

As the world population is anticipated to reach over 9 billion people by 2050, we face a global challenge to produce more food with a fixed number of resources (Department of Economics and Social Affairs 2015). Because arable land is a shrinking commodity, farmers are expected to increase productivity of existing agricultural practices. Two main routes for increased productivity include increasing crop yield and increasing crop intensity (Food and Agriculture Organization 2003). Crop intensity involves the implementation of multiple cropping—harvesting cover crops (plants that replenish and protect the soil) during off seasons between primary crops—as well as decreasing the length of fallow periods (shortening resting times between plantings) (Clark 2015).

In addition to addressing agricultural pressures, the food industry is looking to replace traditional, animal-sourced ingredients with plant-sourced ingredients that require lower energy, labor, fertilizer, and water inputs (Obour et al. 2015). Plant-based ingredients

are generally less expensive to produce, have a smaller environmental impact, and easily integrate into vegetarian, vegan, and kosher diets (González et al. 2011). These exclusion diets are gaining popularity. It is estimated that nearly 8 million Americans are vegetarians or vegans, with an additional 13% of the population claiming to be "flexitarian" or semi-vegetarian, abiding largely to a plant-based diet (Ginsberg 2018). Furthermore, a recent poll showed that 50% of consumers are seeking out more plant-based foods, and 40% of respondents said they are willing to reduce their traditional meat consumption (Watson 2017). The shift in consumer behavior necessitates ingredient development to match this trend.

Protein ingredients and high protein products have become a top consumer priority over the last decade, a trend that is anticipated to continue for the foreseeable future. One of the drivers for this continued growth are the health benefits associated with protein rich diets. Protein is essential for the building and maintenance of muscle and bone, as well hormones, enzymes, and numerous other biological structures (USDA 2016). Additionally, protein consumption has been linked to increased satiety and healthy weight management (Paddon-Jones et al. 2008). Soy protein consumption in particular has been associated with heart health, showing reductions in total cholesterol, LDL cholesterol, and serum triglycerides (Anderson et al. 1995). Consumers have become more aware of these benefits, which have motivated this trend. In 2017, the global protein ingredient market was valued at $36 billion and is projected to reach over $50 billion by 2022 (Frost & Sullivan 2018). Plant proteins accounted for 24.2% of this market, of which soy protein claimed 59% (Frost & Sullivan 2018). Despite being the plant protein market leader, demand for soy protein is declining as new, novel plant proteins are introduced to the market. Additionally, more consumers are avoiding soy, as it is a "Big Eight" allergen—one of eight foods that are responsible for 90% of food allergies in the US—and it is a genetically modified (GM) crop (Roberts 2017). A study by Mintel on "Free-from" food trends reported that 24% of respondents are avoiding soy and 43% of consumers are avoiding GM ingredients (2017). No matter the motivating factors that drive these avoidances, the result is market space for sustainably sourced, plant-based, non-allergenic, non-GM food ingredients.

Among the crops that have the opportunity to meet these environmental and nutritional needs is *Camelina sativa* (L.) Crantz. Camelina is an oilseed and member of the

Brassicaceae family. It is known by other monikers such as false flax, gold of pleasure, linseed dodder, and Siberian oilseed (Fleenor 2011). Related crops include many mustard seed varieties (*Brassica juncea*), several varieties of canola (*Brassica napus L., Brassica rapa subsp. Oleifera, Brassica campestris*), and rapeseed (*Brassica napus*) (Waraich et al. 2013; Obour et al. 2015). Camelina seeds contain approximately 35% lipids, 20% protein, 35% carbohydrates, 5% moisture, and 5% ash, with variability contributed by growing conditions (Toncea et al. 2013; Li et al. 2014). Camelina is a winter annual cover crop that fixes nitrogen in the soil, sequesters carbon so as to limit contributions to the greenhouse gas cycle, and maintains moisture in the soil (Shonnard et al. 2014; Clark 2015). These agricultural attributes allow for more productive primary crop yields, which has been demonstrated by camelina's use in soybean rotations. Camelina increased soybean revenue by 11.6%, showing that it is an effective companion crop to soy, creating greater revenue than a mono-cropping system (Clark 2015; Wyse 2017). This crop rotation system can strengthen the agricultural economy by adding diversity of crop offerings, as well as by increasing productivity of current farming practices. The beneficial role camelina can play in agricultural systems demonstrates the opportunity for it to be a sustainable, novel crop for food use.

Camelina seeds have largely been disregarded for food use, as they have mainly been grown for their oil. Oil is isolated from the seeds by pressing, which results in the collection of residual seed material, the press cake, a byproduct that gets diverted to animal feed (Murphy 2016). Recently, there has been interest in using appropriate processing technologies to create higher value, food ingredients from the press cake rather than use in animal feed. As a food ingredient, camelina has attractive properties that align with current consumer trends; it is a non-allergenic, non-GM source, as of yet. It is also less expensive than animal ingredients and aligns with vegan diets. Nevertheless, camelina is sparsely cultivated, as there is not an established market for camelina use (Berti et al. 2016). As camelina is investigated for food use and applied as a source of functional ingredients in food matrices, this will incentivize greater production of camelina (Arnason 2016).

1.4 Camelina Sativa Production

1.4.1 Camelina History and Production

As an oilseed, camelina seeds contain 30 – 40% oil on a dry basis (Fleenor 2011). Because it is a rich source of oil, camelina has been grown and processed as early as 3000 BCE. It originated in the northern climates of Europe and Russia and grew as far south as central Asia (Gugel and Falk 2006; Waraich et al. 2013). In modern history, camelina growth spread to Canada and northern parts of the US, particularly Montana, which currently boasts the highest camelina production in the US (Obour et al. 2015). It is speculated that camelina was introduced to North America as a weed among flax crops; nevertheless it was controlled and cultivated for the oil as an industrial lubricant (Fleenor 2011; Li et al. 2014; Johnson 2016). Camelina prevalence was more substantial prior to WWII, but its use diminished as it was replaced with other higher yielding, more prolific oilseeds such as canola and soybeans (Bouby 1998; Shonnard et al. 2014; Obour et al. 2015).

Currently, camelina production in Montana peaked at 22,500 acres planted in 2007 when the USDA began tracking production (Sommer and Chard II 2016). Since then, production has declined significantly as other primary oilseed crops have been heavily subsidized by the government (Zeman 2007). In comparison, 1,714,000 acres of canola and 90,000,000 acres of soybeans were planted in the US in 2016 and 2017, respectively (US Canola Association 2017; White and Honig 2018). These subsidies favoring higher-yield crops combined with a lack of a market for products using camelina have hindered the growth of camelina (Obour et al. 2015). In 2015, Montana's production dropped to 500 acres—a mere 2% of 2007 production levels (Sommer and Chard II 2016). In contrast, Canada has seen a greater need for camelina and anticipates an increase in demand; Saskatchewan reported 5,000 acres of camelina grown in 2015, a figure that was expected to double in 2016 (Arnason 2016). The aquaculture industry is driving this growth, as they are moving towards marine-free diets for trout and salmon farming (Arnason 2016). Camelina oil has a similar fatty acid profile to traditionally used fish oil but is a much cheaper alternative for use in fish food (Arnason 2016). This camelina oil application has yet to spur on American camelina production.

Despite the decline in US camelina production in recent years, the overall market for oilseeds has the potential to grow. In 2010, it was mandated that in accordance with the US Renewable Fuels Standard, 36 billion gallons of renewable transportation fuel will be produced in America per year by 2022, 0.5 billion gallons of which will be contributed from oilseeds including soy, canola, and camelina (USDA 2010).

1.4.2 Agronomic Benefits of Camelina Production

Camelina boasts agronomic traits that are beneficial to growers. Being native to northern climates, camelina is a cold weather hardy crop, germinating at temperatures as low as 12°F (Fleenor 2011). It requires low fertilizer and pesticide inputs (about half the nitrogen requirements of canola) and is drought resistant, thus it is adaptable to many growing environments (Gugel and Falk 2006; Arnason 2016; Berti et al. 2016). Furthermore, it is a fast-growing crop, reaching maturity in 85-100 days, and thus can be used as a cover crop, rotating between other primary crops (Berhow et al. 2014). It is often implemented in a relay-cropping system, in which camelina seeds are planted before traditional spring crops (in fall or late winter) (Ehrensing and Guy 2008; Wyse 2017). Camelina is harvested in late spring, allowing the primary crop to continue growing until fall. This overlap process has shown higher yields and more revenue than mono-cropping (Berti et al. 2016; Wyse 2017). Growers have capitalized on these traits by incorporating camelina into a fallow-cropping system, planting it between wheat crops. Camelina used as a fallow crop allows for retention of moisture and nutrients in the soil for the next wheat crop boosting the yield and revenue, decreases the incidence of infestation compared to maintaining the same crop type each year, and generates a new profitable crop for farmers to sell for further processing as a source of oil, protein, and fiber (Shonnard et al. 2014).

1.5 Camelina Oil

1.5.1 Cold and Hot Press Oil Processing

Grown primarily for its oil use, traditional protocol involved crushing camelina seeds with substantial force until the oil was expelled. Similar processes are used today to collect the oil with the addition of hexane treatments to extract residual oil remaining after pressing (Moreau 2010; Broaddus 2017). When seeds are not subjected to any heat

treatment and are pressed at ambient or refrigerated temperatures, it is designated as cold press oil processing. The resulting oil from cold pressing is generally of higher quality, as the volatile flavor compounds characteristic of the seed that are sensitive to heat are left unaltered (Hamilton 2016). Heat can destroy flavor components or detrimentally change them to unfavorable tastes and aromas (Shahidi et al. 1997). Alternatively, seeds can be heated to more readily liberate oil from oil bodies (or oleosomes), which are the storage organelles for lipids in plants materials (Moreau 2010). Oil bodies are surrounded by a phospholipid monolayer that protects the oil during seed development stages (Moreau 2010). Additionally, oil bodies are protected by an exterior protein layer comprised of oleosins, small (15 – 26 kDa) proteins that resist the action of phospholipases on the oil body (Moreau 2010). Heat can disrupt the oleosin structure, allowing the release of lipids from the oil bodies, and thus improving oil extraction yields (Shahidi et al. 1997; Moreau 2010). This process is called hot press oil processing. Pre-heated seeds (50° to over 130°C) are forced through an oil expeller or expresser, in which an auger system uses both pressure and heat to break the seeds, compact them into pellets, and press out the oil to be collected (Pradhan et al. 2011; Hamilton 2016; Broaddus 2017). Although there are no regulations in the US regarding oil pressing temperatures, Europe designates that cold press oil cannot reach temperatures greater than 50°C (Broaddus 2017).

1.5.2 Oil Applications

Historically camelina oil has been put to use in a variety of ways—as cooking oil, a personal hygiene product, and lamp fuel (Iskandarov et al. 2014). Currently, the predominating use of camelina oil is that of a biodiesel feedstock. The pressed oil is derivatized to fatty acid methyl esters (FAMEs) via a traditional transesterification reaction with methanol and sodium hydroxide. The FAMEs are purified for biodiesel distillation after separation from glycerin produced during the reaction (Environmental Resources Management 2008). One of the smaller markets for camelina oil applications includes cosmetic products. High levels of unsaturated fatty acids in camelina oil allow it to act as a skin emollient, preventing transepidermal moisture loss and reducing epidermal inflammation (Zielińska and Nowak 2014). Given these favorable properties, camelina oil has been added to lotions and soaps. Another niche market for camelina oil is as an edible

oil, particularly in Europe (Fleenor 2011). The cold pressed, unrefined oil carries attractive nutty and mustard-like flavors, however the antinutritional factors native to camelina, such as glucosinolates and erucic acid, limit its widespread use in human food products (Section 1.8.1) (Waraich et al. 2013).

1.5.2.1 Residual Press Cake from Oil Processing

After pressing, the compacted seed cake remains. Depending on the pressing method, fat content can vary; cold press cakes typically contain more oil (approximately 20%) compared to hot press cakes (approximately 10%) (Heuzé et al. 2017). Depending on the desired application, the cake can be used as is or further defatted by solvent extraction (hexane is a common choice used in soy and canola ingredient production), resulting in the residual meal (Tan et al. 2011; Wanasundara 2011).

1.5.2.2 Applications for Residual Meal

The residual meal from camelina oil pressing has been mainly used for animal feed in a variety of species such as fish, poultry, swine, cattle, and sheep (Berti et al. 2016; Murphy 2016). Supplementing feed with camelina meal and oil has resulted in higher levels of polyunsaturated fatty acids in animal products including α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Berti et al. 2016). The aquaculture industry has unique challenges that camelina composition aptly addresses. The demand for carnivorous fish – eg. salmon, trout, tuna, halibut—is increasing, which necessitates fish meal used as feed in commercial operations. This supply is dwindling, so supplementation with plant feed sources must be explored. Due to camelina's high content of polyunsaturated fatty acids (PUFA) (see Section 1.5.3), it provides a promising substitute for fish feed. In laying hens, low levels of camelina meal (up to 10%) added to feed resulted in increased egg production and egg yolks with higher ALA concentrations (Berti et al. 2016). In broiler chickens, camelina meal supplemented in feed (6% of the diet) caused increased ALA levels in breast meat without significant flavor changes in the meat and resulted in reduced plasma cholesterol levels (Jaśkiewicz et al. 2014). However, replacing fish oil with excessive amounts of camelina oil (80% of original fish oil amount) in aquaculture feed resulted in decreased PUFA content in the fish. Furthermore, high

inclusion rates of camelina meal in aquaculture feed showed decreased fish weight, which was attributed to antinutritional factors, like glucosinolates (see Section 1.8.1) (Berti et al. 2016; Bischoff 2016).

1.5.3 Composition and Health Benefits of Camelina Oil

Although camelina was used as a source of edible oil for centuries, it was not until 2016 that camelina oil received GRAS approval in the US. Canada approved the oil for food use in 2010, and in Europe it has been approved since the 1990s (Johnson 2016). Nevertheless, camelina has maintained a small but steady position as a healthful, edible oil, especially in European markets. Most of the oil in camelina seeds is unsaturated (85.3% - 89.4%), meaning the fatty acids contain at least one double bond (Toncea et al. 2013). Of the unsaturated fatty acids, over 50% are PUFA, including 15% linoleic acid, a major omega-6 fatty acid, and 38% ALA, a major omega-3 fatty acid, which are the two dietary essential fatty acids (Zubr 2003a). Flaxseed, the most abundant source of ALA, has slightly more ALA (45.1%) than camelina oil, but camelina provides much more ALA than soybean, canola, and sunflower oil—7.2%, 6.6%, 0.0%, respectively (Li et al. 2014).

These PUFA are critical for proper metabolic function. Higher intakes of PUFA have been associated with positive health effects (NIH Office of Dietary Supplements 2018). With respect to routine biological processes, ALA and linoleic acid are required for constructing phospholipids, which in turn become the building blocks of cellular membranes (NIH Office of Dietary Supplements 2018). ALA and linoleic acid also play important functions in synthesizing eicosanoids, which act as signaling molecules in a variety of systems in the body (NIH Office of Dietary Supplements 2018). Additionally, these essential fatty acids have been linked to lowering cholesterol levels, subsequently reducing the risk of cardiovascular disease and strokes (Zubr 2003a). ALA and linoleic acid also play a key role in proper brain and eye development, and they are associated with proper maturation of the central nervous system (Uauy et al. 2001). Although most Americans show adequate intake of omega-3s and omega-6s, health professionals suggest that higher PUFA intake could promote beneficial health outcomes (NIH Office of Dietary Supplements 2018).

Another unique feature of camelina oil is its high vitamin E (tocopherols) content (Ehrensing and Guy 2008; Agricultural Marketing Resource Center 2017). This may allow for longer shelf stability of camelina oil, given the antioxidant properties imparted by vitamin E (Shahidi and De Camargo 2016; Agricultural Marketing Resource Center 2017). Previous reports suggest that camelina oil is more stable than flax and fish oil, but less stable than corn, olive, and sunflower oil (Murphy 2016). Having high ALA content coupled with protective antioxidant effects of tocopherols puts camelina oil at an advantage over flaxseed, canola, and soybean oil (Zubr 2009; Shahidi and De Camargo 2016). These traits of camelina oil classify it as potentially beneficial for human diets in addition to its long-established position as a useful industrial oil.

1.6 Camelina Protein

Camelina seeds contain approximately 20 – 30% protein by weight depending on variety and growing conditions (Toncea et al. 2013). After pressing and defatting, defatted camelina meal contains 35 – 45% protein. Similarly, canola seeds are reported to have 20 – 24% protein and 37 – 41% in the defatted meal, while soybeans have about 40% protein and 53% in the defatted meal (Singh et al. 2008; Wanasundara et al. 2016).

1.6.1 Amino Acid Composition and Structure of Camelina Protein

The amino acid profile of camelina is similar to that of canola (genetically modified rapeseed) and rapeseed, containing 5% or more of the following essential amino acids: phenylalanine, valine, threonine, leucine, and lysine (***Table 1***). The limiting amino acid in camelina is tryptophan, as it was present at the lowest concentration in the meal (1.15%) (Zubr 2003). Tryptophan was not reported by Li et al. (2014) or by Tan et al. (2011) due to limitations of their analytical method; tryptophan is destroyed by acid hydrolysis and therefore was not accounted for in the final results. Of the remaining eight essential amino acids, methionine is present in low concentrations, 1.7% (Zubr 2003a; Li et al. 2014). The percentage of essential amino acids in camelina meal is 40%, which is slightly lower than that of soy (49%) and canola (42%) (Li et al. 2014). The considerable percentage of basic amino acids—arginine (8.15%), lysine (4.95%), and histidine (2.60%)—indicates that

camelina holds a basic isoelectric point (pI). Li et al. (2014) have demonstrated this characteristic by showing that camelina protein had the highest solubility at pH 12 (2014).

Table 1. Amino acid compositions for camelina and related Brassicaceae crops

	Zubr 2003				Li et al. 2014		Tan et al. 2011	
Amino Acid	**Camelina**	**Rapeseed**	**Soy**	**Flax**	**DCM**	**Canola meal**	**Yellow canola**	**Brown canola**
Alanine	4.61	4.0	4.8	5.5	5.68	5.40	4.33	4.32
Arginine	8.15	6.7	7.5	11.1	--^	--	5.51	5.50
Aspartic acid	8.71	6.6	12.7	12.4	9.84	8.44	6.95	6.27
Cystine	2.12	3.0	1.3	4.3	--	--	2.17	2.61
Glutamic acid	16.4	18.1	19.0	26.4	19.94	22.37	17.07	17.48
Glycine	5.44	4.7	4.5	7.1	6.40	6.07	4.87	4.59
Histidine*	2.60	3.1	3.2	3.1	2.90	3.32	2.95	3.00
Isoleucine*	3.96	4.1	3.1	5.0	4.94	5.80	4.05	4.11
Leucine*	6.63	6.3	7.3	7.1	8.32	8.86	6.79	6.72
Lysine*	4.95	6.5	6.1	4.3	5.93	5.85	5.61	5.53
Methionine*	1.72	1.7	1.3	2.5	1.70	1.74	1.90	1.92
Phenylalanine*	4.19	3.5	5.0	5.3	5.08	5.00	5.89	5.65
Proline	5.09	6.0	6.0	5.5	--	--	6.16	6.57
Serine	5.04	4.0	5.6	5.9	6.09	5.47	4.78	4.69
Threonine*	4.25	4.5	4.2	5.1	5.26	5.45	4.01	3.82
Tryptophan*	1.15	--	1.3	1.7	--	--	--	--
Tyrosine	3.04	2.4	3.9	3.1	3.73	3.23	3.04	2.98
Valine*	5.42	6.0	3.2	5.6	5.63	6.05	4.88	4.86

*Denotes essential amino acid
^Value not reported

The Brassicaceae crops have two major storage proteins: cruciferin (11S), a legumin-type globulin, and napin (2S), a napin-type albumin (Wanasundara 2011). Cruciferin, the most abundant storage protein in camelina (about 60% of total protein in Brassicaceae, depending on variety), is a hexameric protein made of two trimer units (Wanasundara 2011). The hexamer is held together in a trigonal antiprism conformation by non-covalent interactions, namely hydrophobic, van der Walls, hydrogen bonds, and electrostatic interactions (Fahs and Louarn 2013; Wanasundara et al. 2016). The trimers

are composed of three protomers, each with heavy (~30 kDa), acidic and light (~20 kDa), basic subunits linked by disulfide linkages (Fahs and Louarn 2013). The molecular weight of each protomer is approximately 51 to 56 kDa, totaling about 300 kDa for the cruciferin structure (Wanasundara 2011). With respect to secondary structure, cruciferin has a low percentage of α-helices (~10%) with a higher proportion of β-sheet conformation (50%) (Tan et al. 2011). Cruciferin has a alkaline pI, which can vary depending on the subunit, and is insoluble at low pH values (pH 3.5 – 5.7) (Wanasundara 2011).

Canola contains similarly structured cruciferin proteins, differing slightly in the molecular weight distribution of the acidic and basic subunits (~40 kDa and ~20 kDa, respectively) (Tan et al. 2011; Wanasundara 2011). Cruciferin proteins in canola are also held together by similar means—disulfide linkages in addition to non-covalent interactions—as confirmed by protein profiling (Tan et al. 2011). Glycinin, the 11S soy globulin protein, is the most similarly structured protein to cruciferin outside of the Brassicaceae family but has a larger molecular weight, averaging about 350 kDa (Badley et al. 1975; Wanasundara 2011). Glycinin pI varies based on the subunit (pI 4.8 – 5.4 for the acidic subunits, pI 8 – 8.5 for the basic subunits) (Mo et al. 2006).

Napin is the smaller, less abundant storage protein in camelina (about 20% of total protein in Brassicaceae, depending on variety) with short (4.5 kDa) and long (10 kDa) subunits linked by two disulfide linkages and is highly basic with a pI in the range of 10.89 to 12.16 (Wanasundara 2011). Three-dimensional modeling has confirmed that napin from canola exhibits a different secondary structure to cruciferin: higher α-helix structure (40 – 46%) than β-sheet conformation (12%) (Tan et al. 2011). Another characteristic of napin is its ability to trigger an immunologic response; although both napin and cruciferin in canola and mustard seed have been identified as allergenic, napin exhibits a stronger reaction than cruciferin (Campbell et al. 2016). These structural differences between napin and cruciferin lead to different functional properties in food systems.

1.6.2 Protein Quality

Protein quality is determined by two factors: the amino acid composition of a protein and the digestibility of the protein (Campbell et al. 2016). The protein quality is thus often evaluated by determining the protein digestibility corrected amino acid score

(PDCAAS), which is a measure of percent digestibility multiplied by the first limiting amino acid score (Schaafsma 2000). The first limiting amino acid score is the ratio of the first limiting amino acid (mg/g test protein) to the same amino acid in a reference protein (mg/g reference protein) (Schaafsma 2000). Casein and egg white are two reference proteins customarily used, as both have a PDCAAS value of 1.0 (Singh et al. 2008). A high-quality protein contains all essential amino acids in sufficient amounts and is highly digestible, resulting in a maximum PDCAAS of 1.0. Protein quality is an important factor when determining feasibility of a novel protein ingredient. A protein ingredient with a low PDCAAS value would lose desirability for food applications, as additional processing and ingredient blending would be required to make a quality protein that would allow for health claims to be made. Although camelina protein quality has never been reported, a similar PDCAAS value to canola (0.86) could be assumed, given their similar amino acid composition and digestibility (Campbell et al. 2016).

1.6.3 Protein Functionality

In addition to nutritional requirements of protein consumption, protein ingredients play important functional roles in food products, acting as gelling agents, emulsifiers, stabilizers, foaming agents, and water-holding substances to name a few (Singh et al. 2008; Foegeding and Davis 2011). Soy protein is added to a variety of products such as sausages, frozen desserts, simulated meats, breads, and sauces because it possesses a wide range of these functional properties (Singh et al. 2008). Being the most prominent plant protein on the market, it is helpful to compare soy protein functionality to that of camelina protein. However, camelina protein has yet to be evaluated for its functional abilities in food systems.

Protein functionality is governed by structure, and protein structure is influenced by intrinsic and extrinsic factors (Panyam and Kilara 1996). Inherent to the protein, the amino acid composition and sequence (the primary structure) is the chief determinant of the protein's secondary structure (Berg et al. 2002). Classes of secondary structure include α-helix, β-sheet, β-turn, and random coil. All of these classes can be found in different domains of a single protein (Nature Education 2014a). Protein chains fold upon themselves into globular, fibrous, or open tertiary configurations, which are held together by a variety

of interactions: hydrogen bonds, electrostatic, van der Waals, disulfide bonds, and hydrophobic interactions (Wanasundara 2011). These different structures lend different functional properties to proteins.

Globular proteins, known for their storage and functional roles, are "globe"-like in structure (Li et al. 2015). The majority of plant proteins, including cruciferin and napin from camelina and fellow Brassicaceae crops, are globular and have low molecular flexibility (Wanasundara 2011). This rigidity is due to the stabilization of their folded tertiary structure by disulfide bonds and hydrophobic interactions (Panyam and Kilara 1996; Wanasundara et al. 2016). Another factor influencing molecular flexibility is the presence of free sulfhydryl groups, which result from cysteine residues that are not involved in disulfide bonds. Free sulfhydryl groups allow for sulfhydryl-disulfide interchanges, which contribute to increased flexibility (Damodaran and Paraf 1997). This phenomenon has been reported in canola cruciferin between the α- and β-subunits (Wanasundara et al. 2016). In general, the rigid structure of globular proteins allows them to orient most of their hydrophobic amino acids to the core of the protein, while most hydrophilic residues remain on the surface—a conformation that is the most energetically favorable (Adjonu et al. 2014). In order to act functionally in a food matrix, the protein must be able to interact with the surrounding system (Singh et al. 2008). For globular proteins, this necessitates enhancing molecular flexibility by partial denaturation or unfolding of the stabilized tertiary structure, so that interior residues in the protein are exposed and have access to the system (Panyam and Kilara 1996; Foegeding and Davis 2011). To a certain extent, this transition between native protein structures and partially denatured intermediates is reversible (Foegeding and Davis 2011). Proteins can become irreversibly denatured as extrinsic factors (heat, pH, pressure, ionic strength) become more extreme (Foegeding and Davis 2011). Accompanying the flexibility and partial unfolding, solubility and surface properties of the protein are foundational to many functional properties (Singh et al. 2008). Different functional properties require different structural characteristics, but generally more functional proteins have a balance of hydrophobic/hydrophilic and charged/neutral amino acids on their surface, which allows for interaction with both aqueous and oil phases (Panyam and Kilara 1996). This is

essential for emulsification, foaming, gelation, and rheological properties (Singh et al. 2008).

Intact and partially denatured globular protein structures have been examined for their functionality. Native rapeseed cruciferin has shown poor emulsification properties because of its rigid globular structure, which prevents it from spreading out over a large surface area, adsorbing to oil droplets as a stabilizing film, and solubilizing in aqueous phases (Wanasundara et al. 2016). Similarly, without any structural disruption, globular whey proteins demonstrate poor functionality in their intact structure, as they have a slower rate of diffusion to oil/water interfaces and cover less surface area in emulsion systems (Adjonu et al. 2014). However, once whey proteins are subjected to emulsion formation via homogenization, emulsification capacity increases significantly because the mechanical energy supplied by homogenization partially denatures the proteins, allowing interior hydrophobic amino acids to be exposed, resulting in faster adsorption to the oil/water interface (Adjonu et al. 2014). This is just one example of how functionality of globular proteins can be improved by transitioning to a partially denatured intermediate state.

In addition to input of mechanical energy, pH adjustments or mild heat treatments can achieve flexible, functional protein structures (Panyam and Kilara 1996; Barac et al. 2006). These techniques, when used in mild and controlled degrees, retain the secondary protein structure while disrupting the rigidity of the tertiary structure by breaking hydrogen bonds, disulfide bonds, and hydrophobic interactions (Raikos 2010). Enzymatic modifications also increase molecular flexibility by breaking peptide bonds (Barac et al. 2006). Glycinin showed improved functional properties (emulsification and foaming) at acidic pH (pH 3.0), as this resulted in a higher charge density on the protein, increasing repulsion between similarly-charged residues (Foegeding and Davis 2011; Cui et al. 2013). This caused the naturally globular glycinin to partially denature, allowing it to form stabilizing interfacial films (Foegeding and Davis 2011; Cui et al. 2013). However, decreased functionality was observed at more extreme pH values (pH < 2.0) , as there is greater repulsion, so much so that glycinin was irreversibly denatured and the excessive repulsion prevented necessary interactions to form a film (Cui et al. 2013). This demonstrates that achieving optimal functionality requires balancing extrinsic factors to

best suit the protein and the food application. Thermal treatments are also used to alter globular protein tertiary structure, often for the purpose of forming gels. Gelation depends on partially unfolded proteins aggregating to engage in protein-protein interactions via hydrophobic interactions and disulfide linkages, forming a three dimensional network that traps water (Panyam and Kilara 1996; Foegeding and Davis 2011). In general, this is more successful with larger molecular weight proteins, as their size allows them to have a stronger gel network due to more hydrophobic interactions and disulfide linkages (Tan et al. 2011; Wanasundara 2011). This is observed in canola protein gels. Napin proteins are lower in molecular weight than cruciferin proteins, and accordingly, napin gels are much weaker than cruciferin gels (Campbell et al. 2016). An additional factor influencing gelation is thermal stability. Napin proteins are highly heat stable (thermal denaturation temperature 110°C), making them less susceptible to denaturation by heat, resulting in fewer protein-protein interactions and weak gels (Wu and Muir 2008; Campbell et al. 2016). In contrast, cruciferin is less heat stable (thermal denaturation temperature 91°C), thus denatures more readily and forms more cohesive gels (Wu and Muir 2008; Campbell et al. 2016). Despite functionality challenges inherent to globular proteins, with proper processing techniques such as thermal treatments, pH adjustments, and mechanical energy input, they can contribute significantly as functional ingredients in food products.

Another type of protein structure is fibrous proteins, which are known for their structural and strengthening functions. They form helical-structured, filament-like threads that intertwine together, forming complexes much larger than globular proteins (Xiong 2004). They are not commonly found in plant sources, including Brassicaceae crops, but are mainly found in animal sources (Xiong 2004). Accordingly, their functional uses are largely isolated to meat products, as they exhibit water-holding capacity, swelling capabilities, gelation properties, and emulsification properties in various processed meat products (Xiong 2004). Confectionary products require similar functional properties, and therefore are another application of fibrous proteins, namely gelatin. Gelatin is the most common example of a fibrous protein ingredient added for functionality. Its rope-like structure and ability to form covalent crosslinks between molecules makes it excellent for forming gel networks and trapping water (Gomez-Guillen et al. 2011).

A third type of protein structure is open or random coil proteins. These proteins have little secondary or tertiary structure, due to the abundance of proline residues (Fox and Kelly 2004). Because they lack higher orders of structure, open structure proteins are heat-stable and resist denaturation because they are naturally unfolded (Raikos 2010). The most notable example is casein. Casein's open structure leaves its amino acids exposed and available to interact with food matrices. Due to their open structure and highly surface active amino acids, caseins have gained the reputation of natural emulsifiers, as they are innately able to stabilize immiscible phases (Raikos 2010). Open structure proteins are highly susceptible to proteolytic activity (Fox and Kelly 2004). If this enzymatic action is not controlled, functional properties, like emulsification stability and gelation, can be hindered (Panyam and Kilara 1996).

Protein structure of camelina has been elucidated, but its consequent functional properties have yet to be investigated. Although drawing comparisons to the functional capacities of similarly structured proteins is useful, there is a need for more information on camelina protein's functional abilities and how they can be altered for uses in food applications.

1.6.4 Enzymatic Modification of Proteins

Functionality of plant proteins can be inferior to dairy proteins (whey and casein), egg protein, and to meat protein (gelatin), depending on the final application. Apart from environmental factors (such as pH, ionic strength, and other constituents in the system) and processing conditions, the unique structural characteristics of plant proteins govern their functionality. Being globular with high molecular weights (MW) and low molecular flexibility, most plant proteins may have low functional performance, as previously discussed (Section 1.6.3) (Wanasundara 2011). Targeted protein modification such as enzymatic hydrolysis, however, may lead to improved functionality (Martínez et al. 2007). Enzymatic hydrolysis cleaves peptides bonds, resulting in peptides with lower MW and unique physiochemical characteristics. Hydrolysis can also be achieved through heating or chemical means (acid or alkali hydrolysis), however enzymatic hydrolysis is preferred for its specificity, as different enzymes can be applied to achieve uniquely functional peptides (Cui et al. 2013). Additionally, enzymatic hydrolysis can be more easily controlled than

chemical processes and eliminates any side reactions that may reduce nutritional quality, including loss of tryptophan or creation of carcinogenic byproducts (Nair et al. 1976). It is important that enzymatic modification of proteins is properly controlled to avoid a high degree of hydrolysis (DH) (Wanasundara 2011). High DH values (> 8%) are associated with sensory problems due to the excessive release of bitter peptides and low MW peptides that have reduced functionality (gelation, emulsification stability, foaming stability) because of their small size (Panyam and Kilara 1996; Martínez et al. 2007). However, controlled enzymatic hydrolysis is a common method of protein modification that has been shown to improve functionality.

There are numerous examples of improved protein functionality upon enzymatic hydrolysis. As previously mentioned, the globular structure of native whey protein can hinder its ability to act as an emulsifier, however controlled enzymatic hydrolysis with trypsin resulted in whey protein hydrolysate with exceptional emulsification properties (Adjonu et al. 2014). Trypsin hydrolysates resulted in greater emulsification capabilities than chymotrypsin, alcalase (protease from *Bacillus licheniformis*), and neutrase (endo-protease from *Bacillus amyloliquefaciens*), demonstrating the importance of selecting the enzyme with the specificity needed to release peptides that contribute to the desired functionality enhancement (Panyam and Kilara 1996; Adjonu et al. 2014). Food products have benefited from these modifications, as hydrolyzed whey protein is commonly used in ice cream, sauces, and protein beverages for its emulsification abilities (Euston and Hirst 2000).

In addition to improvements observed in whey protein, hydrolysis of soy protein concentrate with trypsin and pepsin were both shown to significantly improve the emulsification capacity and stability over traditional soy protein concentrate (Barac et al. 2006). Emulsion stability time nearly doubled for soy proteins hydrolyzed with trypsin (60 min, 37°C) compared to untreated soy protein concentrate (34.4 min vs 18.7 min, respectively) (Barac et al. 2006). One contributing factor is that smaller peptides have higher molecular mobility, allowing them to have a faster rate of adsorption to phase interfaces in emulsions and foams, thus exhibiting greater emulsification and foaming capacity (Martínez et al. 2007). A similar increase in foaming capacity was also reported

in rapeseed protein isolate when limited hydrolysis (DH ≤ 7.7%) with alcalase was applied (Wanasundara 2011).

Additionally, soy protein exhibited improved solubility upon enzymatic hydrolysis. Soy protein isolate hydrolyzed with pepsin up to a degree of hydrolysis (DH) of 7.5% exhibited improved solubility around the isoelectric point (pH 4 – 5) (Cui et al. 2013). The peptides released were lower in MW and higher in charge density due to freed carboxyl and amino groups, resulting in improved solubility (Panyam and Kilara 1996; Cui et al. 2013). In a similar study investigating the enzyme modification of canola meal protein isolate, a variety of enzymes were used to hydrolyze the protein up to 5% DH (Alashi et al. 2011). Hydrolysis did not have an effect on solubility; all hydrolysates and the control showed approximately 12% solubility at pH 7. The proposed reason for this lack of improvement was that canola meal protein was extracted by a pH solubilization method where the protein was solubilized at pH 12 for 1 hour and precipitated at pH 4.0. These experimental conditions were harsh, resulting in complete denaturation of the protein, lending to limited solubility. It is possible that solubility could have been improved through the use of a unique enzyme that may break peptide bonds at unique sites releasing hydrophilic peptides.

In contrast to the mostly positive effects reported for emulsification, foaming, and solubility, enzymatic hydrolysis does not always enhance gelation (Panyam and Kilara 1996). Soy protein hydrolyzed with pepsin (DH 15%) showed greatly reduced gel strength compared to SPI gels (Tsumura et al. 2005). When enzymatic hydrolysis is not controlled gelation can be negatively impacted. Smaller peptides have fewer protein-protein interaction sites and create less cohesive, weaker gel networks than unhydrolyzed proteins (Wanasundara 2011). Additionally, nonspecific hydrolysis may release peptides with greater charge densities, resulting in greater repulsion and reduced protein-protein interactions (Panyam and Kilara 1996). This phenomenon was observed in trypsin-hydrolyzed whey proteins with DH values between 2.3% and 6.7%, which failed to produce gels at both acidic (pH 3.0) and neutral pH (Panyam and Kilara 1996). The consequence of enzymatic hydrolysis is not only dependent on the resulting DH, but it is also highly dependent on the specificity of the enzyme, the molecular size and structure of the protein, and on the extrinsic factors. It is important to consider potential drawbacks of

enzymatic hydrolysis on gelation and other functionality properties when working towards improving protein. Evaluating functional properties of camelina protein and the effects of enzymatic hydrolysis can determine appropriate food applications.

1.7 Camelina Dietary Fiber

Camelina seeds contain about 30% dietary fiber by weight, of which there are insoluble and soluble fractions, however the distribution is largely unknown due to the lack of study on dietary fiber from camelina. Comparisons can be drawn to related Brassicaceae crops, such as various canola cultivars. *B. napus, B. rapa, B. juncea,* and *B. carinata* contained between 27 and 35% dietary fiber (Slominski et al. 1994). Of the total dietary fiber present in rapeseed meal, 15.5% was soluble fiber, while in soybean meal 27.6% of the total dietary fiber was soluble fiber (Knudsen 2014). Little is known about the distribution of insoluble and soluble dietary fiber in Brassicaceae crops.

1.7.1 Dietary Fiber Definitions

Dietary fiber is a particular classification of carbohydrates and lignin that corresponds to their functional and nutritional role (Dhingra et al. 2012). Apart from lignin, these polymers are made up of monosaccharides joined by a variety of glycosidic linkages (McCleary and Prosky 2001). Examples of monosaccharides that are commonly found in dietary fiber include glucose, galactose, fructose, arabinose, rhamnose, xylose, mannose, fucose (Blakeney et al. 1983). Starch ($\alpha(1\rightarrow4)$ linked glucose units, interspersed $\alpha(1\rightarrow6)$ branching) is not included in this definition (Zubr 2010). Dietary fiber is strictly defined by the FDA as being: *"non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health"* (US Food & Drug Administration 2018). Examples of dietary fiber include those "intact and intrinsic" fibers from fruits, vegetables, and grains, in addition to the seven FDA approved dietary fiber types: β-glucan soluble fiber, psyllium husk, cellulose, guar gum, pectin, locust bean gum, and hydroxypropylmethylcellulose (US Food & Drug Administration 2018).

A further classification is made for prebiotic fibers. They must resist digestion in the stomach and absorption in the small intestine, be fermentable by gastrointestinal bacteria, and allow changes in the gut microflora that confers positive health effects on the host (Slavin 2013). Inulin, oligofructose, and fructooligosaccharides (FOS) have been classified as prebiotics (Slavin 2013). It is important to note that although all prebiotics are classified as dietary fiber, not all dietary fiber ingredients are prebiotics.

1.7.2 Health Benefits Associated with Dietary Fiber Consumption

The recommendation for adequate intake (AI) of dietary fiber in a 2000 calorie diet is 28 g per day (Anderson et al. 2009). Despite the numerous health benefits that have been associated with dietary fiber consumption, over 90% of Americans still do not meet this recommendation (Clemens et al. 2012). In conjunction with this dietary fiber deficit, coronary heart disease is currently the leading cause of death in the US (National Center for Health Statistics 2017). One of the main benefits associated with dietary fiber intake, particularly soluble fiber, is its ability to lower the risk of developing coronary heart disease (Anderson et al. 2009). This is achieved largely by reducing the risk factors associated with cardiovascular diseases: high cholesterol, hypertension, diabetes, and obesity (American Heart Association 2018).

Soluble fiber consumption showed significant reductions in low density lipoprotein (LDL) cholesterol levels. Numerous studies demonstrated that guar gum (9 – 30 g/day), pectin (12 – 24 g/day), and β-glucans from oats and barley (5 g/day) produced average reductions in LDL values of 10.6%, 13%, and 11.1%, respectively (Anderson et al. 2009). The proposed mechanism for this action involves increasing fecal excretion of bile acids, as well as short chain fatty acid (SCFA) production by fiber fermentation, which plays a role in diminishing the synthesis of cholesterol (Anderson et al. 2009). An additional benefit of fiber fermentation and the production of SCFA is their proposed positive influence on the immune system (Slavin 2013). In animal models, increased SCFA production was linked to increased immune cells, including T cells and macrophages (Slavin 2013). Furthermore, fiber fermentation leads to increased mucus in the gut, which acts as a protective barrier against pathogenic bacteria (Slavin 2013). The beneficial effects

on the immune system demonstrate that the impact of fiber consumption goes beyond gastrointestinal and heart health.

The evidence for high fiber diets (30 – 40 g/day) and the reduced incidence of type II diabetes is robust (Kaline et al. 2007; Anderson et al. 2009; Clemens et al. 2012). In particular, greater levels of dietary fiber intake were shown to decrease insulin resistance and decrease fasting blood glucose up to 39% (McKeown et al. 2002; Kaline et al. 2007). These effects were associated more closely with insoluble fiber, particularly from whole grains, than soluble fiber, however the reason for this has not been deduced (McKeown et al. 2002). Other effects tied to the action of insoluble dietary fiber is decreased constipation by improving laxation and increased fecal bulk (Anderson et al. 2009).

Similarly, fiber plays a critical role in combatting obesity and managing a healthy body weight. Those with high fiber diets were reported to have a 30% reduced risk of weight gain and obesity development over those with marginal to low fiber consumption (Anderson et al. 2009). High-fiber foods lead to greater satiety, as they slow the digestive processes. Subsequently, lower calorie consumption is observed, resulting in overall lower body mass (Clemens et al. 2012).

1.7.3 Functional Roles of Dietary Fiber in Food Applications

The role of fiber in nature is largely structural; found in cell walls, it provides support and strength for plants. Fiber is abundant in fruits, vegetables, and grains, contributing to their protection by maintaining their firm structure (Nature Education 2014b). Fiber can be isolated from plant sources and added to processed foods, which impacts texture, rheological behavior, sensory properties, and enhances the product's nutrition (Dhingra et al. 2012). Addition of fiber to different food products is mostly for the purpose of making health claims, but fiber can also contribute functionally to food products as a gelling agent, thickening agent, stabilizer, and water holding ingredient. Isolated soluble fiber from cereal grains can be used to fortify breads, pasta, and baked goods, while ice cream and frozen desserts can be stabilized with various gums, like locust bean gum or guar gum. Other applications include jams and jellies; pectin provides the gelling power to give a thick, spreadable consistency. Given camelina's rich dietary fiber content, it is possible that soluble and insoluble fiber fractions could be isolated from

camelina meal in order to use them as functional, nutritional ingredients. There is sparse data on camelina fiber, so further research is needed to characterize its fiber types and to match it with appropriate food matrices.

1.8 Limitations Associated with Camelina Use

1.8.1 Antinutritional Factors in Camelina

Antinutritional factors are chemical compounds naturally occurring in plants that have developed as protective or defensive mechanisms for the plant's survival (Gemede and Ratta 2014). Tannins, lectins, saponins, oxalates, protease inhibitors, phytates, and alkaloids are all examples of antinutritional factors. When plant foods are consumed, these compounds prevent mineral and protein absorption, in addition to contributing bitter flavors and pungent odors (Tyagi 2002; Gemede and Ratta 2014). Camelina contains antinutritional factors that could stymie its feasibility in food products. Phytic acid, tannins, sinapine, and erucic acid are all present in camelina, which hinder its overall nutritional quality (Waraich et al. 2013; Sarv et al. 2017). These compounds can render other nutrients and minerals biologically unavailable, such as phytic acid and tannins binding iron, or saponins and tannins binding protein, preventing their digestion and increasing fecal nitrogen (Gemede and Ratta 2014). Erucic acid has been limited in food products because of its role in myocardial lipidosis and necrosis of heart muscles in rats (Zubr 2003a). This effect has not been observed in humans, but based on what is known about erucic acid metabolism, myocardial lipidosis could occur in humans when consumed at high enough levels (Zealand 2003). For this reason, Food Standards Australia New Zealand (Zealand 2003) established a tolerable level for humans at 500 mg erucic acid/day, which incorporates a 120-fold safety margin below the no observed adverse effect level (NOAEL) for pigs. The US FDA (2017) established equivalent regulations, limiting erucic acid to less than 2% of total fatty acids in canola oil, which is the main source of erucic acid in diets (Zealand 2003). This decrease in nutrient absorption, coupled with detrimental organ effects observed in animal models, hinder the use levels of camelina oil and meal allowed by federal regulations (Berti et al. 2016).

Camelina also contains glucosinolates. There is incongruity in the literature as to whether glucosinolates have positive or negative effects on human health (Mithen et al.

2000). Camelina contains lower levels (12 –36 μmol/g seeds) than canola and other Brassicaceae crops (Gugel and Falk 2006). Glucosinolates have been shown to negatively affect ruminants. Improper liver and kidney function due to excessive glucosinolate concentrations hinders livestock productivity (Bischoff 2016). Additionally, glucosinolates have detrimental effects on the thyroid gland of ruminants and resulted in reduced thyroid hormone levels (Bischoff 2016). These drawbacks limit camelina's applicability in animal feed. However, these effects have not been observed in humans (Mithen et al. 2000). On the contrary, glucosinolates from the Brassicaceae family demonstrated positive effects in humans. Glucosinolate breakdown products had anticarcinogenic properties, as they inhibited the induction of tumors, decreased cell proliferation, and increased the rate of programmed cell death (apoptosis) (Mithen et al. 2000). Both the potential advantages of glucosinolates and the drawbacks of reduced nutrient absorption due to other antinutritional factors must be evaluated when considering camelina for food applications.

Methods have been developed to minimize antinutritional factors in plants to reduce their undesirable effects. Soybeans are an example of a widely commercialized crop that necessitate processing to minimize antinutritional factors prior to use. Soy ingredients are required to show a 90% reduction in trypsin inhibitor concentrations (one of the main antinutritional proteins in soy) before incorporation into food products (Yuan et al. 2008). This is typically done through heat treatment, but high-pressure processing has also been shown to denature trypsin inhibitors and lectins (Friedman and Brandon 2001; Yuan et al. 2008). Reduction of antinutritional factors by heat treatment has also been shown effective in canola meal: 43% of phytic acid, 67% of tannic acid, and 94% of glucosinolates were degraded (Mansour et al. 1993). Glucosinolates in canola meal have also been reduced by solvent extraction with ethanol and acetone, but milder water extraction of glucosinolates has been successful as well (Tyagi 2002; Tan et al. 2011). In mustard cake processing, soaking the press cake in water (37°C, 8 h) reduced glucosinolate levels by nearly 80%, thus improving feed intake and cattle productivity (Tyagi 2002). Protein isolation is an additional processing method that has effectively reduced or eliminated tannic acid, phytic acid, and glucosinolates in canola meal, which could likely be successful in camelina protein processing as well (Mansour et al. 1993; Tan et al. 2011).

Current research is underway to explore methods for producing camelina protein isolates free of glucosinolates, phytates, and certain phenolics by ultra- and diafiltration (Sarv et al. 2017). The principle of this approach is that the undesirable compounds are much smaller than the proteins and will filter through, while the protein isolate can be retained (Sarv et al. 2017). Erucic acid, another compound of concern in camelina, is being addressed by breeding efforts (Ehrensing and Guy 2008). Agronomists are developing camelina lines that contain reduced erucic acid levels within regulation (<2%) (Ehrensing and Guy 2008). Given the proven effective methods to reduce antinutritional factors in similar crops and current research efforts in the area of antinutrient factor reduction, camelina, with further processing, is still a viable option for food use.

1.8.2 Knowledge Gaps: Protein and Fiber Functionality

Little is known in regard to camelina's protein and fiber functionality. Although camelina protein has been successfully isolated and characterized to varying degrees, its functional properties (solubility, emulsification, foaming, water holding, and gelling) have yet to be evaluated. Similarly, the structure of camelina fiber has not been elucidated, so its functional and nutritional applications cannot be confirmed. Camelina is a new and upcoming ingredient source for human food products, which leaves a vast space for exploration and discovery.

2 Extraction, Modification and Structural/Functional Characterization of Camelina Protein

2.1 Overview

Camelina sativa, a sustainable oilseed crop, is a novel source of plant protein. The aim of this work was to evaluate two protein extraction approaches, alkaline and salt extraction, and determine their impact on the structural and functional properties of camelina protein. Protein yield, content, structural characteristics, and functional properties of the produced camelina protein concentrates (CPC, 70-80% protein) and hydrolysates (CPH) were assessed and compared to reference proteins, whey protein isolate (WPI) and soy protein isolate (SPI). Compared to alkaline pH extraction, data showed that salt extraction produces less denatured and more functional CPC, composed mainly of cruciferin and napin proteins. The functionality of the salt extracted CPC was comparable and sometimes better than that of SPI. Specifically, the solubility (~70%) of the salt extracted CPC at pH 3.4 was significantly ($P < 0.05$) higher than that (~50%) of SPI. Additionally, salt extracted CPC had significantly higher emulsification capacity and foaming capacity than SPI. On the other hand, the gelation property of CPC was inferior to that SPI, an observation attributed to the molecular size of camelina protein compared to soy protein. Upon hydrolysis of CPC with *Aspergillus oryzae* protease, a limited benefit to solubility was noted at pH 7 post thermal treatment. Overall, results revealed the potential of camelina as a novel source of functional plant protein that might gain a position in the protein market place, and possibly compete with soy protein for several applications targeting the use of plant proteins.

2.2 Introduction

The global demand for protein ingredients is expected to reach 6.8 million tons (~$50 billion) by 2025 (Grand View Research 2018). Specifically, there is a growing interest in novel plant-based protein ingredients that may replace the market sector or at least a portion of it that has been largely dominated by traditional protein ingredients such as dairy and soy proteins. Several reasons have led to this interest. Among the reasons is the cost of the traditional protein ingredients. Plant proteins can offset market share from

animal proteins (dairy, egg, and meat) because they can be produced at competitive prices. Other reasons for the interest in novel plant proteins include the rising incidences of allergenicity (dairy and soy proteins are among the "big eight" allergens) and several functionality limitations. Increases in vegan and health conscientious consumers are another driver for plant protein popularity. As consumers avoid meat products and seek high protein intake, a corresponding rise in products advertised as "high in protein" has been observed. Other opportunistic reasons include utilizing current processing streams to increase value and revenue, finding a unique and a competitive place in the market, replacing chemical ingredients with functional proteins (clean labeling), and utilizing all possible resources to expand the ingredients supply. Some may also argue that the food industry is aging, and there is a pressing need for innovation. Thus, demonstration of equivalent or superior/new functions of novel plant proteins compared to existing alternatives is essential to their market success. However, there is limited consumer and producer knowledge of plant proteins other than soy; nevertheless, novel plant proteins such as proteins from pulses, potato, rice, corn, oats, canola, algae, and ancient grains are gaining traction (Grand View Research 2018).

Among the novel plant proteins that have high potential to become commercially available is camelina protein. Camelina (*Camelina sativa,* a Crucifer seed and a member of the Brassicaceae family) is a sustainable oilseed crop that is high in both fat (30-38%) and protein (25-30%) (Gesch and Archer 2013; Berti et al. 2016). Thus, it is an attractive choice for the production of both oil and protein ingredients. The environmental benefits of this crop include reduced soil and water erosion, reduced soil nitrate leaching, increased carbon sequestration, and reduced inputs of energy and pesticides (Gesch and Archer 2013; Berti et al. 2016). From a consumer perspective, purchasing habits that can improve the environment is gaining prominence. Studies have shown that consumers are seeking transparency and sustainability in their food supply, and companies of all sizes are being receptive (Adams 2014). The use of sustainable crops in food products will allow consumers to feel good about their purchase and their role in supporting a sustainable agricultural system. Accordingly, food industries are interested in commercializing products formulated with ingredients derived from sustainable crops such as camelina. Research, however, on camelina protein is scarce (Li et al. 2014).

Camelina protein is not produced commercially today. Given that it is a relatively new crop being considered for food use, there are few reports on its oil and protein composition (Liu et al. 2014), structure and functionality. Camelina meal would be a by-product from oil production. The meal is rich in protein (40-45%), thus exploring extraction techniques to produce a functional protein ingredient for food applications is warranted. Oilseeds are often pressed to extract the oil, followed by hexane extraction to maximize oil yield. This process is harsh, and most likely leads to protein denaturation, aggregation, and hence loss in functionality. It is, therefore, beneficial to explore the impact of different oil pressing conditions, cold and hot press, and various subsequent protein extraction methods on changes in the protein structure and function.

The amino acid composition and hence the nutritional quality of camelina protein is similar to that of canola protein (Li et al. 2015), which in turn is comparable to that of soy protein, the gold standard among plant proteins (Sarwar et al. 1984). Research on the isolation and characterization of camelina protein fractions is limited (Li et al. 2014, 2015). In particular, no research has been done to determine the functional properties of extracted camelina protein for food applications.

Functionality of plant proteins can be inferior to dairy proteins (whey and casein), and to meat protein (gelatin), depending on the final application. Apart from environmental factors (such as pH, ionic strength, and other constituents in the system) and processing conditions, unique structural characteristics govern protein functionality. Plant proteins are globular, high in molecular weight, and have low molecular flexibility. These characteristics contribute to reduced functional performance. Targeted protein modification such as enzymatic hydrolysis (Martínez et al. 2007), however, may lead to improved functionality. It is, therefore, crucial to not only characterize novel plant proteins, but also investigate protein modifications targeted to enhance their functionality in various applications.

The objectives of this study were to: 1) determine the impact of oil pressing conditions and protein extraction protocol on protein yield and content; 2) characterize structural differences in proteins extracted following salt precipitation and pH solubilization; and 3) determine the impact of structure and enzymatic modification on the functionality of the different protein extracts.

2.3 Materials and Methods

2.3.1 Materials

Camelina seeds and hot pressed (extruded) camelina pellets were kindly provided by General Mills (Minneapolis, MN, USA). The camelina seeds were planted in Morris, MN, and harvested in summer of 2016. The hot press camelina meal was produced by POS Bio Sciences (Saskatoon, SK, Canada) by pressing the seeds using a lab Komet CA 59 G screw press at 50°C through a 6 mm die. The extruded meal had 14.3% fat and 29.2% protein. Whey protein isolate (WPI, 93% protein), BiPro, was kindly provided by Agropur (Eden Prairie, MN, USA). Soy protein isolate (SPI, 92% protein) was prepared in the lab following the method out lined by Margatan et al. (2013). Protease M was obtained from Amano Enzyme (Inc, Elign, IL, USA). SnakeSkin™ dialysis tubing (3.5K MWCO), Falcon™ solid opaque white 96-well plates, electrophoresis grade Dithiothreitol (DTT), and Imperial Protein™ stain were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Criterion™ TGX™ 4-20% precast gels, Laemmli sample buffer, and 10x Tris/Glycine/SDS running buffer were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). 8-anilino-1-napthalenesulfonic acid ammonium salt (ANS) was purchased from Sigma Aldrich (St. Louis, MO, USA). Additionally, immobilized pH gradient (IPG) Strips (ReadyStrip 11 cm, pH 3 -10, linear), Biolyte 3 – 10 pH ampholyte, mineral oil, 4-20% (acrylamide) Criterion TGX gels with 11cm IPG + 1 well, ReadyPrep equilibration buffer II, and Precision Plus molecular weight marker were also purchased from Bio-Rad. UltraPure agarose (16500-1000) was purchased from Invitrogen (Carlsbad, CA, USA). Stainless steel sample pans for DSC were purchased from Perkin Elmer (Waltham, MA, USA). All other chemical grade reagents were purchased from Fisher Scientific or Sigma-Aldrich.

2.3.2 Production of Defatted Camelina Meal (DCM)

For the production of cold press DCM, camelina seeds were pressed at ambient temperature (21°C) for 24 h using a hydraulic press with a starting maximum pressure of 5,000 psi (Carver Model C 12 ton manual bench top laboratory press with 2094 cage equipment, Carver, Inc, Wabash, IN, USA). The pressed cake samples had ~ 20% fat, as verified by the Mojonnier AOAC method 922.06, a gravimetric procedure involving acid

digestion followed by solvent extraction by diethyl ether and petroleum ether (AOAC International 2016). Both the cold pressed cake and the hot pressed camelina pellets provided by General Mills were milled to 50 mesh using a cyclone sample mill (Udy Corp, Fort Collins, CO, USA). Milled camelina cake samples were defatted by batch extraction with hexane in a 3:1 ratio in three 1 h cycles. Residual hexane was allowed to evaporate overnight. Fat content was verified to be below 3% following the Mojonnier method.

2.3.3 Protein Extraction from DCM by pH Solubilization

Cold press and hot press DCM samples were used to produce camelina protein concentrates (CPC) following the method outlined by Li et al (2014), with modifications. A dispersion (2.5% w/v) of DCM and distilled deionized water (DDW) was stirred at room temperature for 1 h and centrifuged at 15,000 x g for 15 min to remove soluble gums and polysaccharides. The pellet was redispersed (5% w/v) in DDW, adjusted to pH 12.0 with 2 N NaOH, stirred at room temperature for 1 h, and centrifuged at 15,000 x g for 15 min. The choice of solubilization at pH 12 was based on the pH solubility curve reported by Li et al. (2014). The supernatant was filtered under vacuum, adjusted to pH 5.0 with 2 N HCl, and centrifuged at 15,000 x g for 10 min to precipitate the protein. The pellet was redispersed (1:4 w/w) in DDW, neutralized with 2 N NaOH, dialyzed to remove salts, and lyophilized. The protein content of the lyophilized sample was determined following the Dumas AOAC method 990.03 using a nitrogen analyzer (LECO, St. Joseph, MI, USA) and a protein conversion factor of 5.3. The camelina protein extracts were kept at -20°C until further analysis. Mass balance was monitored to determine protein yield.

2.3.4 Protein Extraction from DCM by Salt Precipitation

Cold press and hot press DCM samples were used to produce CPC following the method outlined by Wu and Muir (2008), with modifications. A dispersion (5% w/v) of DCM and potassium phosphate buffer (0.05 M, pH 8, 1 M NaCl) was stirred at 50°C (preliminary trials confirmed higher protein yield when stirring at 50°C compared to room temperature) for 1 h and centrifuged at 15,000 x g for 20 min. The supernatant was collected, and ammonium sulfate was added to reach 85% saturation. The solution was stirred for 3 h and centrifuged at 15,000 x g for 20 min to collect the precipitated protein.

The pellet was redissolved in DDW, dialyzed, and lyophilized. The protein content of the sample was determined following the Dumas method. The prepared CPC samples were kept at -20°C until further analysis. Mass balance was monitored to determine protein yield.

2.3.5 Protein Hydrolysis by pH-Stat

Cold press pH extracted CPC and hot press salt extracted CPC were subjected to enzymatic hydrolysis by Protease M following the pH-stat methodology optimized by Walter et al. (2016). A DL22 titrator (Mettler Toldeo, Columbus, OH, USA) was used to conduct a series of end-point titrations to a pH of 6.0 for the duration of the experiment. Dispersions of CPC in DDW (2.5% protein, w/v) were prepared. The dispersions were heated to 40ºC prior to the addition of Protease M. Protease M was added to obtain an enzyme to substrate ratio (E:S) of 0.5 g enzyme : 100 g of protein. The dispersion was then allowed to incubate, while stirring for 5-10 min maintaining a pH of 6.0 throughout by titration with 0.2 N NaOH. Hydrolysis conditions were chosen and optimized based on temperature and pH working range of the enzyme, and a target degree of hydrolysis (DH) less than 8%. After incubation, the hydrolyzed CPC samples were heated at 65ºC for 12 min (inactivation temperature and time were selected based on the thermal stability range for the enzyme) to inactivate the enzyme, then lyophilized. The protein content of the lyophilized camelina protein hydrolysates (CPH) was an average of 67% for cold press pH extracted CPH and 83% for hot press salt extracted CPH as determined following the Dumas method. Lyophilized samples were kept at -20°C until further analysis. The DH was calculated using the formula reported by Adler-Nissen (1984) as follows:

$$\%DH = (h/h_{tot}) \times 100 = (B \times N_b)/(MP \times \alpha \times h_{tot})$$

B is the volume of NaOH that is added in mL; N_b is the normality of the base; MP is the mass of the protein; h_{tot} is the total number of peptide bonds in the protein; and α is the degree of dissociation of the α-NH_2 as determined by the incubation temperature ($\alpha = 1/(1 + 10^{pK-pH})$). Sample calculations can be found in Appendix A.

2.3.6 Protein Structural Characterization

2.3.6.1 Protein Profiling by Gel Electrophoresis

Protein profiling of DCM samples, pH extracted CPC samples, and salt extracted CPC samples was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) based on the method outlined by Laemmli (1970) and reported by Walter et al. (Walter et al. 2016). Precision Plus MW standard, soy protein isolate (SPI), DCM samples, and CPC samples (5 μL; containing ~5 μg protein), prepared in Laemmli buffer, were loaded onto 4 - 20% gradient gel. The gel was electrophoresed, stained and destained as reported previously (Walter et al. 2016). Molecular Imager Gel Doc XR system (Bio-Rad Laboratories) was used to scan the gels. Protein profiling of CPC samples was also performed following 2D gel electrophoresis based on the method outlined by Panda (Panda et al. 2015). Samples were dissolved (0.25 μg protein/ mL) in isoelectric focusing (IEF) sample buffer (8 M urea, 50 mM DTT, 2% CHAPS, and 0.5% ampholyte) and an aliquot (200 μL) was added to individual troughs of the IEF focusing tray. An individual pI 3 – 10 linear IPG strip was submerged in each sample trough and was covered with 4 mL of mineral oil. Active rehydration was performed in a Bio-Rad PROTEAN IEF cell at 50 V for 12 h. Separation was carried out at 250 V for 15 min followed by linear ramping at 8,000 V for 2 h and then rapid ramping at 8,000 V until a total of 40,000 V hours was reached. Proteins were then fixed in position overnight by application of 500 V per hour. The IPG strips were then reduced for 40 min in 130 mM DTT equilibration buffer (ReadyPrep Equilibration Buffer II, 20% glycerol). Each focused strip was placed in the 11 cm well of a 4 – 20% TGX Criterion IPG + 1 well gel, and 7 μL of molecular weight marker was loaded into the small well. An aliquot (1 mL) of molten 0.5% agarose was used to seal the wells. The second dimension electrophoresis was carried out at 150 V for 1h 15 min. The gels were stained using Imperial Protein™ stain and then imaged with the Molecular Imager Gel Doc XR system.

2.3.6.2 Differential Scanning Calorimetry (DSC)

Thermal denaturation of CPC samples was monitored using a DSC 8500 instrument (Perkin-Elmer, Waltham, MA, USA) according to the method of Tang et al. (2007) with slight modification. Samples were solubilized to (10% protein, w/v) in phosphate buffer

(0.05 M, pH 7) overnight to equilibrate and transferred to stainless steel solid pans, which were hermetically sealed. The pans were held in the sample chamber at 25°C for 5 min and then heated from 25 to 120°C at 5°C/min increments. Indium was used to test the calibration of the instrument. A sealed empty pan was used as reference.

2.3.6.3 Surface Hydrophobicity

The surface hydrophobicity of CPC samples was determined spectrofluorometrically as described by Kato and Nakai (1980) and Sava and Plancken (2005), with modifications. Samples were prepared (5 mL) by diluting to concentrations ranging from 0.005 - 0.050% protein (w/v) using 0.017 M: 0.165 M citric acid/sodium phosphate buffer (pH 7). 8-anilino-1-napthalenesulfonic acid ammonium salt (ANS) probe solution (20 μL, 12.6 μg/mL) was added to 200 μL of the diluted samples in a white opaque 96 well plate. The relative fluorescence intensity (RFI) was measured at excitation and emission wavelengths of 400/30 (wavelength/bandwidth) and 460/40 nm and 25 gain after 15 min holding in the dark at room temperature using a microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The calculation of RFI was done as described by Sava and Plancken (Sava et al. 2005). The RFI of each protein dilution containing no ANS probe was subtracted from that of corresponding protein solution with ANS to obtain net RFI. The slope of the net RFIs at each heat treatment time point plotted against % protein concentration was used as an index of the protein surface hydrophobicity. Sample calculations for RFI and surface hydrophobicity index plot can be found in Appendix B.

2.3.7 Protein Functional Characterization

2.3.7.1 Protein Solubility

Protein solubility was measured as outlined by Wang & Ismail (2012), with some modifications. Solutions of CPC, SPI and WPI (5 mL, 1% protein, w/v) were prepared in 10 mL beakers, in triplicate, and adjusted to either pH 3.4 or 7.0 using 2 M HCl and 2 M NaOH and an Orion™ ROSS Ultra™ pH Electrode (Thermo Scientific, Waltham, MA, USA). Solutions were stirred on a magnetic stir plate for a minimum of 1 h. The protein content of each solution (100 µL aliquot) was initially determined by the Dumas method

(AOAC 990.03) using the LECO® TruSpecN™ nitrogen analyzer (St. Joseph, MI, USA). Aliquots (1 mL) at each pH were either left at room temperature or subjected to heating at 80°C for 30 min in a water bath. Samples were then centrifuged at 15,682 x g for 10 min at 23°C, and protein content of the supernatants of the heated and non-heated samples were again determined by the Dumas method. The % protein solubility was determined using the following equation:

$$\%\ protein\ solubility = \frac{(original\ protein\ \%\text{-}supernatant\ protein\ \%)}{original\ protein\ \%\ in\ solution}$$

2.3.7.2 Least Gelation Concentration & Gel Strength

Least gelation concentration (LGC) was measured as the lowest protein concentration at which a protein solution formed a heat-induced gel and held its shape while inverted without slipping. CPC solutions were prepared at 2.5%, 5%, 7.5%, 8%, and 12% protein (w/v, 6 mL) and pH was adjusted to 7.0. Solutions were stirred for 1 h then aliquots were transferred to 15 mL tubes and heated in a 95°C water bath for 10 min. Samples were cooled to room temperature, refrigerated overnight, and inverted to observe slippage. To measure gel strength, heat-induced gels at 15% protein concentration (w/v, 1 mL aliquots in microcentrifuge tubes) were formed under the same conditions listed above. Gel strength was measured using a TA-TX Plus Texture Analyzer (Stable Micro Systems LTD, Surrey, UK) with a 100 mm diameter probe, a 1 mm/s test speed, and a target distance of 0.5 mm from the plate. The force required to rupture the gel (in Newtons) was considered as the gel strength.

2.3.7.3 Water Holding Capacity

Water holding capacity (WHC) was measured according to the procedure outlined by Kocher and Foegeding (1993), with modification. Solutions were prepared in DDW at the LGC for each protein extract, and the pH was adjusted to 7.0. After stirring for 2 h, 1 mL, in triplicate, of each protein solutions was transferred to a microcentrifuge tube, and the weight of the solution was recorded as (T_1). Samples were then heated in a 95°C water bath for 10 min. Samples were cooled to room temperature, and the weight was recorded

as (T_2). Samples were then centrifuged for 5 min at 1,000 x g. Centrifuged samples were inverted for 10 min to drain water expelled from the gel, and the weight was recorded as (T_3). The WHC was determined as the percentage of water that remained trapped in the gel matrix, following the equation below:

$$Water\ Holding\ Capacity = 100\ \times \left(\frac{T_3 - T_1}{T_2 - T_1}\right)$$

Where,

T_1 = weight of protein solution before heating

T_2 = weight of protein solution + microcentrifuge tube after cooling

T_3 = weight of protein solution + microcentrifuge tube after draining excess water

Sample calculations can be found in Appendix C.

2.3.7.4 Emulsification Capacity

Emulsification capacity (EC) was measured following the method outlined by Rickert et al. (2004), with modification. Solutions of CPC and SPI, in triplicate, were prepared in DDW (10 mL, 1% protein concentration, w/v) and stirred for 2 h after adjusting the pH to 7.0. Corn oil dyed with 4 μg/mL of Sudan Red 7B was titrated at a steady flow rate of 4 mL/min into each protein solution while blending using a homogenizer (IKA® RW 20 Digital, IKA Works Inc., Wilmington, NC, US) with a 4 blade, 50 mm diameter shaft (IKA® R 1342) rotating at 860 - 870 rpm. Samples were homogenized while titrating with oil until a phase inversion was observed. Emulsification capacity was expressed as g of oil emulsified by one g of protein as follows:

$$Emulsification\ Capacity = \frac{(Volume\ of\ oil\ titrated\ \times\ Density\ of\ oil\ )}{Grams\ of\ protein}$$

Sample calculations can be found in Appendix D.

2.3.7.5 Emulsion Stability (ES) and Emulsification Activity Index (EAI)

The emulsion stability (ES) was measured according to the method outlined by Rickert et al. (Rickert et al. 2004), with modifications. Solutions of CPC and SPI, in triplicate, were prepared in DDW (0.1% protein concentration, w/v), and the pH was adjusted to 7.0. After stirring the solutions for 2 h, an aliquot (6 mL) of each sample was added to 2 mL of corn oil and homogenized for 1 min using a Scilogex D500 homogenizer (Rocky Hill, CT, USA), with a 20 mm shaft, set at 10,000 rpm. Immediately after homogenization, 50 μL of the emulsion was diluted in 5 mL of 0.1% SDS to prevent the emulsion from flocculating. The sample was then vortexed for 5 sec, and the absorbance at t = 0 min was measured at 500 nm using a spectophotometer (Beckman 12V – 20, Chaska, MN, USA). A second absorbance measurement of the same sample was taken 10 min later (t = 10 min). The ES, in min, was determined using the equation reported by Rickert et al. (Rickert et al. 2004). For each emulsion, the initial absorbance and the turbidity of the oil at 500 nm were used to calculate emulsification activity index (EAI) as reported by Cameron et al. (1991) using the following equation:

$$EAI\ (m^2/\mathrm{g}) = \frac{2T}{(1-\phi)C}$$

$$Turbidity\ of\ Oil\ (T) = \frac{2.303A_0}{l}$$

Where:

C = weight of protein per volume of aqueous phase

Φ = volume fraction of oil

T = turbidity of the oil at 500nm

A_0 is the absorbance at t = 0 min

l = path length of the cuvette

Sample calculations can be found in Appendix E.

2.3.7.6 Foaming Capacity and Stability

Foaming capacity and stability were determined using the method outlined by Bera & Mukherjee (1989), with modification. Solutions (50 mL) of CPC and SPI, in triplicate,

were prepared in DDW (0.5% protein concentration, w/v), and the pH was adjusted to 7.0. After stirring the solutions for 1 h, each sample was blended at 800 rpm using a Sun Beam hand mixer for 2 min. Solutions were poured into a 250 mL short form graduated cylinder, and initial liquid and foam volumes were recorded. Foaming capacity, in mL foam/g protein, was determined as follows:

$$Foaming\ Capacity = \frac{(Total\ solution\ volume - \ Liquid\ volume\ )}{Grams\ of\ protein}$$

Solutions were allowed to rest for 30 min, and foam and liquid levels were recorded. Foaming stability, as a % of initial foam volume, was determined as follows:

$$Foaming\ Stability = \frac{(Total\ solution\ volume_{30\ min} - \ Liquid\ volume_{30\ min}\ )}{(Total\ solution\ volume_{0\ min} - \ Liquid\ volume_{0\ min}\ )} \times 100$$

Sample calculations can be found in Appendix F.

2.4 Statistical Analysis

Analysis of variance (ANOVA) (Appendix G, Tables 10 – 23) was carried out using IBM SPSS Statistics software version 22.0 for Windows (SPSS, Inc., Chicago, IL, USA). Significant differences ($P \leq 0.05$) among the means (n=3) of different samples were determined using the Tukey-Kramer multiple means comparison test.

2.5 Results and Discussion

2.5.1 Effect of Oil Pressing Conditions and Protein Extraction Protocol on Protein Yield and Content

Highest protein extraction yield was obtained from cold press DCM following salt extraction (***Table 2***). However, no statistical differences were noted among the samples, implying that oil pressing conditions (cold vs. hot press) had no direct bearing on the protein yield. Overall, the protein extraction yield was not high, yet it was similar to the protein recovery, i.e yield, reported for canola meal (33-42%) (Tzeng et al. 1988; Pedroche

et al. 2004) and camelina meal (36-48%) (Li et al. 2014), confirming that oil extraction may impair protein solubilization in the extraction solvent in general (Pedroche et al. 2004).

Previous reports on extraction and purification of camelina protein followed a stepwise approach to obtain separate protein fractions, albumins, glutelins, and globulins (Li et al. 2014, 2015). The researchers used different solvents (water, alkaline, and salt solutions) to maximize solubilization of each fraction, respectively. In this study, either alkaline (pH 12) or salt solubilization was used to extract the protein from the DCM samples, targeting a composite protein extract rather than a specific protein fraction. As a result, the constituents of the protein extracts obtained may not represent the actual distribution in the seed, since each constituent would have a varying solubility in the extraction solvents used. Following one isolation protocol, however, rather than fractionation into separate components is more industry feasible for the production of a functional protein ingredient.

Table 2. Protein extraction yields (%) and purity (% protein) for camelina protein concentrate (CPC) samples extracted from cold and hot press camelina meal by pH solubilization and salt precipitation.

CPC	Yield (%)□	Purity (% protein)
Cold press pH extracted	38.4[a^]	71.4[bc]
Cold press salt extracted	42.4[a]	82.2[a]
Hot press pH extracted	36.8[a]	68.8[c]
Hot press salt extracted	35.1[a]	79.5[ab]

□ Yield (%) represent the amount of protein extracted relative to the total amount of protein in the defatted camelina meal (DCM); ^ Means in each column with different lowercase letters indicate significant differences among the different extracts according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

The extraction protocols resulted in a protein concentration of ~ 70%-80% (***Table 2***), with higher concentrations observed following salt extraction compared to alkaline extraction, regardless of oil pressing conditions. Soluble gums and polysaccharides content in camelina seeds is high, and water washing may not have been sufficient to remove them

completely from the meal resulting in a protein concentrate rather than an isolate. The difference in protein content and accordingly difference in residual gums and polysaccharides may have an impact on the functionality of the final product.

2.5.2 Structural Characteristics of CPC

2.5.2.1 Protein Profile

Under non-reducing conditions, two major protein bands, just under 50 kD and 15 kD, were apparent in all DCM and CPC samples (***Figure 1***). These two bands correspond to cruciferin and napin, respectively (Tan et al. 2011; Wanasundara 2011). Legumin-type globulins, cruciferin (11S), and napin-type albumins, napin (2S), are the major storage proteins (~80-85% of total seed proteins) of Brassicaceae seeds, including the Crucifer seed, camelina (Camelina sativa) (Wanasundara 2011). A cruciferin polypeptide consists of a heavy α- (acidic, ~ 30 kD) and a light β- (basic, ~ 20 kD) subunit linked with one disulfide bond (Wanasundara 2011). The α- and β- subunits are observed upon running the SDS-PAGE gel under reducing conditions (***Figure 1***, lanes 2-9). Similarly, a napin polypeptide consists of two basic subunits, a long chain subunit (~ 10 kD) and a short chain subunit (~ 4.5 kD) linked with 1:1 disulfide linkages, which are seen when the gel was run under reducing conditions. There are a number of genetic variants for the cruciferin and napin subunits that vary slightly in amino acid composition and length (Wanasundara 2011); hence the bands seen with slightly different molecular weights (***Figure 1***, lanes 2-9). Glutelin-type polypeptide (~15-20 kD) was also observed, but only in DCM and pH extracted CPC samples (***Figure 1***, lanes, 2, 3, 6, 7, 10, 11, 14 and 15). Glutelins are soluble in high alkaline and not in salt solutions (Li et al. 2014, 2015), which explain why all salt extracted samples lack this band around 20 kD (***Figure 1***, lanes 4, 8, 9, 12, 16 and 17). Globulins (cruciferin), on the other hand, while mostly salt-soluble, were present in both pH and salt extracted CPC (***Figure 1***, all lanes). Aluko and McIntosh (2001) similarly showed that canola globulins are present in alkaline extracts, demonstrating that globulins, which are soluble in salt solutions, can also be extracted by a strong alkaline solution. There was no apparent difference in protein bands, albumins, globulins and glutelins, between hot press and cold press DCM and corresponding CPC samples. Similar to yield and total protein content, oil extraction conditions did not seem to influence the protein profile.

Given the similarity in the protein profile between hot and cold press samples, 2D gel electrophoresis was only run on one set of samples (cold pH and cold salt extracted CPC samples). Running 2 D gel electrophoresis with isoelectric focusing as first dimension and PAGE (under reducing conditions) as second dimension revealed that napin subunits at or less than 10 kD hardly moved along the IEF strip for both salt and pH extracted CPC (***Figure 2***, a & b), indicating an isoelectric point of 10 or higher. This finding aligns with the reported pI (10.25 – 12.16) of napin subunits based on the amino acid composition (Crouch et al. 1983). A clear distinction between cruciferin α- (acidic, ~ 30 kD) and β- (basic, ~ 20 kD) subunits was also apparent (***Figure 2***). Finally, two faint spots, in the acidic region of the gel between 15 and 20 kD were apparent in the pH extracted CPC (***Figure 2*** a) and absent in the salt extracted CPC (***Figure 2*** b). The molecular weight location of these spots is consistent with the glutenin-type polypeptide bands observed for DCM and the pH extracted CPC (***Figure 1***), further confirming that glutelin-type polypeptides are not extracted using a salt solution.

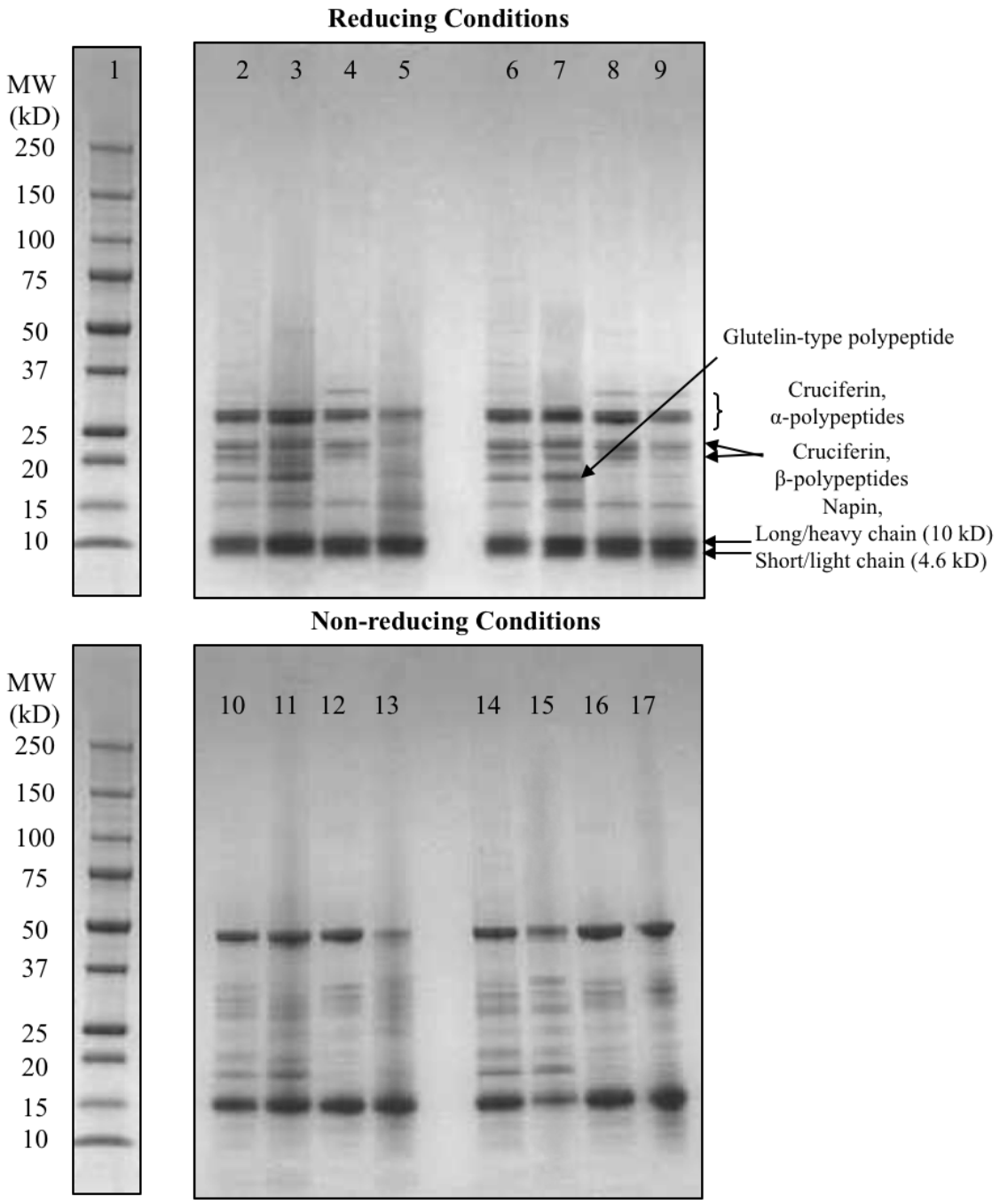

Figure 1. SDS-PAGE gel visualization of the protein profiles of camelina samples under reducing (lanes 2 – 9) and non-reducing (lanes10 – 17) conditions. Lane 1: Molecular weight marker (MW); Lanes 2, 10: Cold press DCM; Lanes 3, 11: Cold press pH extracted CPC; Lanes 4, 12: Cold press salt extracted CPC; Lanes 5, 13: Cold press pH extracted CPH; Lanes 6, 14: Hot press DCM; Lanes 7, 15: Hot press pH extracted CPC; Lanes 8, 16: Hot salt extracted CPC; Lanes 9, 17: Hot salt extracted CPH.

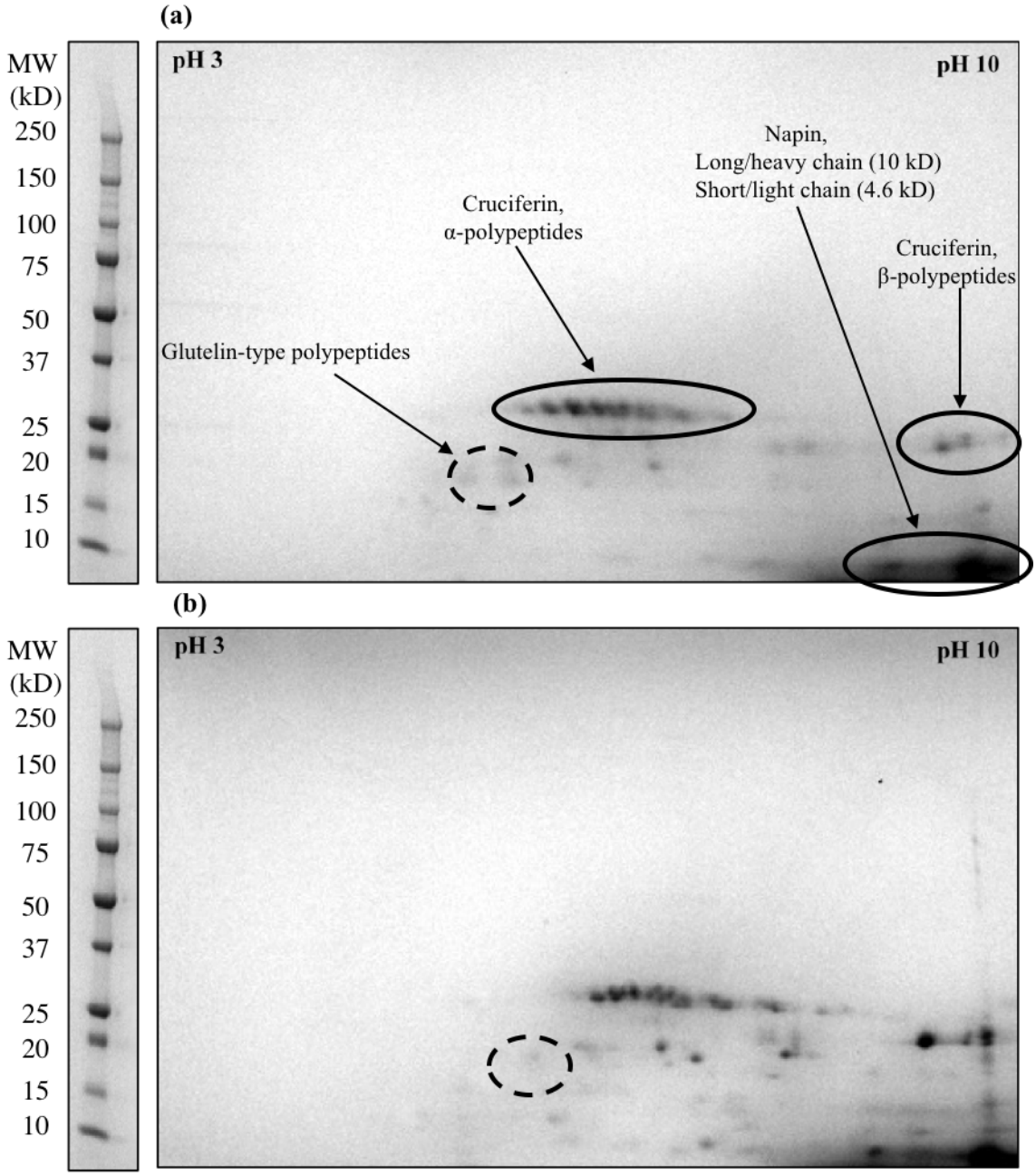

Figure 2. Two dimensional gel electrophoresis (linear pH 3 – pH 10 range) visualization of Cold pH extracted CPC **(a)** and Cold salt extracted CPC **(b)**.

2.5.2.2 Denaturation State

Severe alkaline conditions during oilseed protein extraction may result in protein denaturation (Tan et al. 2011). Accordingly, DSC was performed to determine the impact of high pH extraction conditions, as compared to salt extraction, on the denaturation state

of the protein in CPC. An endothermic peak with denaturation temperature (T_d) of ~ 95 and 93ºC, and ΔH (enthalpy) of ~ 14 and 9 J g^{-1} was apparent in the cold and hot press salt extracted CPC, respectively, and was absent in both cold and hot press pH extracted CPC (Table 2). This endothermic peak corresponds to cruciferin, which was reported to have a T_d ~ 91ºC and ΔH of 12.5 J g^{-1} (Wu and Muir 2008), very close to our findings. Another endothermic peak with denaturation temperature (T_d) of ~ 104-107ºC, and ΔH (enthalpy) of ~ 0.2 - 3 J g^{-1} was apparent in all CPC samples regardless of extraction method. This endothermic peak corresponds to napin, which was reported to have a T_d ~ 110ºC and ΔH that ranged between 1-16 J g^{-1} (Wu and Muir 2008), depending on extraction conditions. This observation confirms that pH 12 extraction resulted in complete denaturation of cruciferin as compared to salt extraction. The denaturation of cruciferin may have a detrimental effect on functionality of the pH extracted CPC. On the contrary, there did not seem to be a clear impact of extraction solvent on napin protein. However, it was apparent that both cruciferin and napin proteins were partially denatured (partially unfolded), as denoted by a reduced ΔH, in hot press compared to cold press samples (***Table 3***). Heating during oil extraction, even at a temperature (50ºC) below the measured onset of denaturation for extended time, may result in minor structural change, causing a decrease in enthalpy. This structural difference may influence functionality.

2.5.2.3 Surface Hydrophobicity

Surface hydrophobicity was determined to assess the impact of oil and protein extraction conditions on unfolding of the protein and the revealing of hydrophobic residues, originally buried within the interior moiety of the protein. Complementary to the DSC results, surface hydrophobicity index was significantly ($P < 0.05$) higher for pH extracted CPC compared to salt extracted CPC (***Figure 3***), confirming that denaturation, and hence unfolding, resulted in the exposure of hydrophobic residues other than the ones already on the surface of the native protein. There was no statistical difference at $P \leq 0.05$ between cold and hot press samples. Yet the trend was toward higher surface hydrophobicity index in hot compared to cold press samples (532 vs. 430 S_o for salt extracts and 1300 vs 1100 S_o for pH extracts, respectively), again complementing DSC results. Increase in surface hydrophobicity will have a direct bearing on the functionality of the protein (Tan et al.

2011), such as solubility. Specifically, increase in surface hydrophobicity may result in a decrease in solubility.

Table 3. Denaturation temperatures and enthalpy values for camelina protein concentrates (CPC) as determined by differential scanning calorimetry.

CPC	Denaturation Temperature (T_d, °C)		Enthalpy (ΔH, J g^{-1})	
	Cruciferin	Napin	Cruciferin	Napin
Cold press pH extracted	NA^	104.0	NA	2.905
Cold press salt extracted	95.50	106.9	13.86	0.228
Hot press pH extracted	NA	106.5	NA	0.425
Hot press salt extracted	93.32	106.9	9.01	0.208

^Endothermic peak not apparent

All CPC samples had lower surface hydrophobicity than SPI (reference oilseed protein). This SPI sample was produced from minimally heated and defatted soy flour under mild extraction conditions (pH 7.5), and was not denatured (Margatan et al. 2013). Soy protein is known to have high surface hydrophobicity coupled with relatively high polar and charged residues on the surface of the protein (Lampart-Szczapa 2001). Thus, higher surface hydrophobicity of SPI may not necessarily imply lower solubility compared to CPC. However, changes in surface hydrophobicity among CPC samples as impacted by denaturation (unfolding) is predicted to have a causative change in the protein solubility (i.e. the protein with higher surface hydrophobicity will likely have lower solubility).

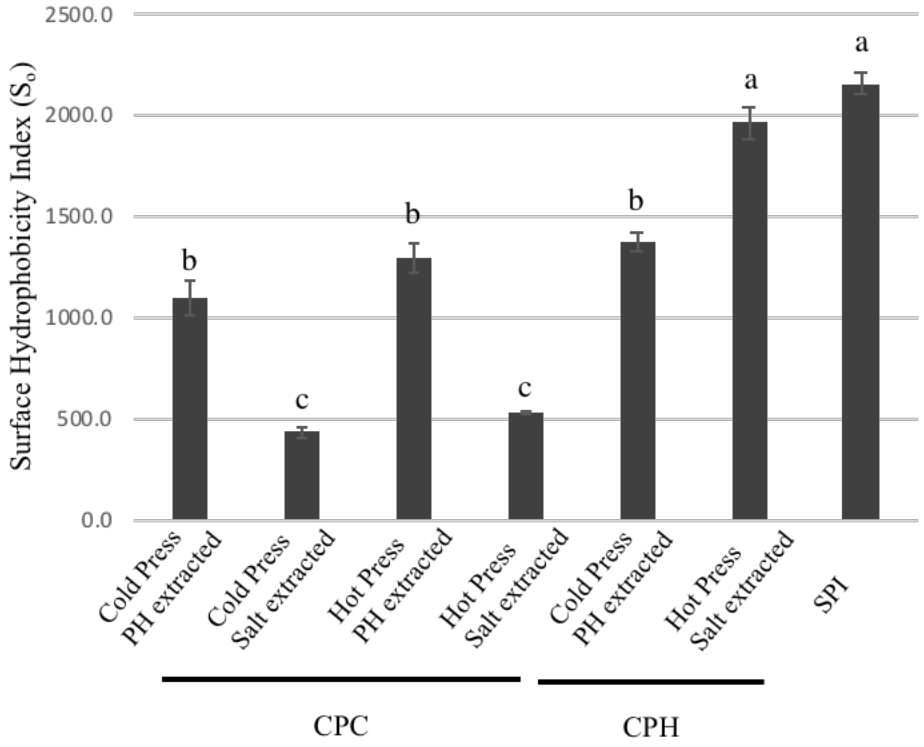

Figure 3. Surface hydrophobicity index of camelina protein concentrates (CPC) and hydrolydates (CPH), as well as soy protein isolate (SPI). Error bars represent standard error; n=3. Different lowercase letters above the bars indicate significant differences among samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

2.5.3 Functional Properties of CPC

2.5.3.1 Solubility

Protein solubility of all CPC samples was significantly ($P < 0.05$) higher at pH 3.4 than pH 7 (***Table 4***). Heating at pH 7 resulted in reduced solubility only for the salt extracted CPC, while heating at pH 3.4 did not. This observation could be attributed to the protein carrying higher net positive charge at pH 3.4. Given that a number of camelina protein subunits are basic with high pI (***Figure 2***), it is expected that as the pH drops below the pI, the protein will have higher net positive charge. At pH 3.4 even the acidic cruciferin α-polypeptides (its pI is greater than 3.4, ***Figure 2***) will be protonated, thus will carry a positive charge. Having higher net positive charge at pH 3.4 not only contributes to higher solubility but also to higher thermal stability, as demonstrated by the solubility results of the heated samples.

Among the CPC samples, salt extracted samples had significantly ($P < 0.05$) higher solubility (up to ~ 70%) under all conditions tested (***Table 4***). This observation is related to the salt extracted CPC being less denatured (***Table 3***) with lower surface hydrophobicity (***Figure 3***) compared to pH extracted CPC. There was no statistical difference in protein solubility between cold and hot press samples (***Table 4***) in accordance with the non-significant difference in surface hydrophobicity between cold and hot press pH extracted or salt extracted CPC (***Figure 3***).

The solubility of reference proteins, WPI and SPI, was also determined. SPI was selected as reference given that it is a common oilseed protein ingredient, while WPI was selected as a reference given that it is the gold standard protein ingredient for beverage applications. All CPC samples had significantly ($P < 0.05$) lower solubility than WPI under all conditions. The solubility results for WPI is consistent with previously published data (Wang and Ismail 2012). While all CPC samples had significantly ($P < 0.05$) lower solubility than SPI at pH 7, salt extracted CPC had higher solubility than SPI at pH 3.4 (***Table 4***). The solubility results of SPI are in the range of reported solubility of SPI at acidic and neutral pH (Lee et al. 2003; Jiang et al. 2010). These results imply that a camelina protein ingredient extracted using salt, may be a better choice than soy protein for acidic beverage applications targeting the use of plant proteins.

To the best of our knowledge, there is no published work on the solubility of camelina protein concentrate or isolate. However, there are few protein solubility reports on canola protein, which is a related Brassica protein. The solubility of canola protein isolate was between 30-40% at acidic pH and ~ 50 % at pH 8 (Pedroche et al. 2004). The solubility of salt extracted CPC samples is higher than the reported solubility of canola protein at acidic pH, partially due to the alkaline extraction used to isolate canola protein, and to possible differences in the protein subunits between the two species. It is speculated that the higher solubility of canola protein at pH 8 than that of pH extracted CPC samples at pH 7, is attributed to higher solubility at higher pH. Varietal differences could be another factor that may result in different protein solubility (Tan et al. 2011).

Relative to other plant proteins and with a protein solubility between 50-70%, salt extracted camelina protein is considered to have acceptable solubility at both acidic and neutral pH. However, having lower solubility than WPI, camelina protein may require

structural modification (such as hydrolysis) in order for it to be a competitive ingredient in the protein market place. Protein solubility may need to be enhanced further not only for beverage applications, but also to improve other functional properties including emulsification, foaming, and gelation needed for various applications.

Table 4. Percent solubility of camelina protein concentrates (CPC) and hydrolysates (CPH), soy protein isolate (SPI), and whey protein isolate (WPI) at 1% protein concentration, pH 3.4 and 7, non-heated and heated at 80°C for 30 min.

	% Protein Solubility			
	pH 3.4		pH 7	
	Non-heated	Heated at 80°C	Non-heated	Heated at 80°C
Cold press pH extracted CPC	40.3[d^]	45.0[d]	17.6[c]	16.7[d]
Cold press salt extracted CPC	69.9[b]	68.8[b]	48.6[b*]	27.5[cd]
Hot press pH extracted CPC	45.8[d]	44.9[d]	17.6[c]	23.9[cd]
Hot press salt extracted CPC	62.4[bc]	65.7[bc]	49.7[b*]	29.1[c]
Cold press pH extracted CPH	26.6[e]	24.0[e]	16.0[c*]	23.7[cd]
Hot press salt extracted CPH	69.3[b]	69.2[b]	43.1[b]	41.6[b]
SPI	51.1[cd]	51.2[cd]	100[a]	100[a]
WPI	100[a]	100[a]	99.6[a*]	94.4[a]

[^] Means in each column with different lowercase letters indicate significant differences among the different extracts according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$). [*] Represent significant ($P \leq 0.05$) difference between a heated and a non-heated sample.

2.5.3.2 Emulsification capacity (EC), Stability (ES) and Activity Index (EAI)

Similar to solubility results, among the CPC samples, salt extracted samples had significantly ($P < 0.05$) higher EC and EAI than pH extracted samples (***Figure 4***), and slightly higher ES. This observation is in agreement with previously reported data showing that higher protein solubility is correlated with better emulsification properties (Khattab

and Arntfield 2009). Others have reported that as the extraction pH of canola protein increases (up to pH 12), emulsification properties decrease (Pedroche et al. 2004). Adequate emulsification requires a balance between hydrophobic and hydrophilic residues on the surface of the protein. While surface hydrophobicity is needed for interaction with the oil phase, poor protein solubility in the aqueous phase will result in low surface activity, more protein/protein interactions and hence lower EC and EAI.

While there are no reports on the emulsification properties of camelina, there are several on those of canola protein (Tan et al. 2011). However, given that different research groups use a variety of different analytical methods, protein sources, concentrations, and assay conditions, it is impossible to compare published emulsification properties of canola protein extracts to our CPC samples (Tan et al. 2011). While several of the reported results show variation due to variety and extraction conditions, some are contradictory (Tan et al. 2011). Accordingly, emulsification properties of SPI were determined alongside the CPC samples. Comparison to SPI is reasonable given that it is a common oilseed protein ingredient, with acceptable and well-documented emulsification properties (Chen et al. 2014).

The EC and EAI of salt extracted CPC were not significantly different from those of SPI (***Figure 4*** a & c). However, ES of CPC was significantly ($P < 0.05$) lower than that of SPI, which could be due to lower solubility of camelina protein at pH 7 compared to that of SPI (emulsification test was done at pH 7). Lower protein solubility may result in more protein/protein interactions and less protein/water interactions, leading to quicker coalescence. While SPI has significantly higher surface hydrophobicity than CPC (***Figure 3***), it has higher solubility implying an equivalently higher surface hydrophilicity, thus attributing to a good balance and better ES. However, ES of camelina protein can be improved upon enhancing solubility following modification such as enzymatic hydrolysis, as demonstrated for canola protein (Pedroche et al. 2004). Accordingly, camelina protein may have the potential to replace SPI in the protein market place for applications (at neutral pH) requiring good emulsification properties.

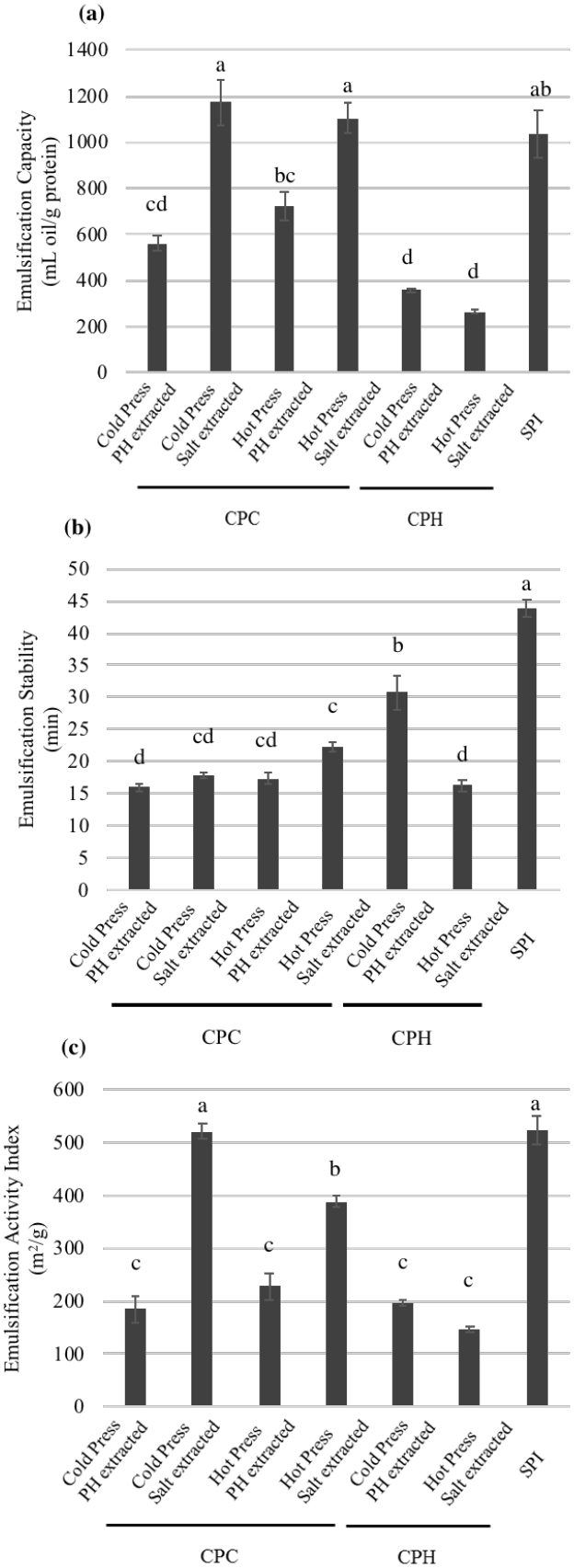

Figure 4. Emulsification capacity **(a)**, emulsion stability **(b)**, and emulsification activity index **(c)** of camelina protein concentrates (CPC) and hydrolydates (CPH), as well as soy protein isolate (SPI). Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

2.5.3.3 Foaming Capacity and Stability

Among the CPC samples, salt extracted samples had a much higher ($P < 0.05$) FC than pH extracted samples (***Figure 5*** a), and similar FS values (***Figure 5*** b). Similar to the findings of EC and EAI, this observation is in agreement with previously reported data on canola protein showing that higher protein solubility is correlated with better foaming properties (Aluko and McIntosh 2001). Protein denaturation during extraction at high pH (10-12) has also been found to reduce FC of canola protein (Pedroche et al. 2004). Once again, the oil extraction conditions (hot vs. cold press) had no major impact on foaming properties. On the other hand, compared to SPI, salt extracted CPC had significantly ($P < 0.05$) higher FC and similar FS values. This result suggests that camelina protein may have the potential to replace SPI in the protein market place for applications (at neutral pH) requiring good foaming properties.

2.5.3.4 Gel Strength and Water Holding Capacity

Given that oil extraction conditions (hot vs. cold press) had no impact of consequence on any of the thus far presented structural and functional properties of CPC, WHC and gel strength data are shown for one set of CPC samples (***Figure 6***). The WHC, which did not statistically vary among the samples (***Figure 6*** a), was run at the LGC of the different samples, 8% protein for pH extracted samples and 12% for the salt extracted samples and SPI (LGC data not shown). Ability to form a gel at lower concentration could be attributed to the denaturation state of cruciferin and the overall higher surface hydrophobicity of the pH extracted CPC compared to salt extracted CPC, facilitating better protein/protein interactions upon thermally inducing gel formation. However, the gels formed were too soft to get accurate rupture force data. Accordingly, the strength of the gels (rupture force, ***Figure 6*** b) was determined at 15% protein for all samples including SPI. While there was no statistical difference between pH and salt extracted CPC samples, both had significantly ($P < 0.05$) lower gel strength (almost by half) than SPI. These results are not surprising given the much lower molecular weight of camelina protein compared to soy protein (up to 50 kD for camelina and 300 kD for soy protein, both under non-reducing conditions). Proteins with large molecular size were found to form better networks, thus stronger gels (Oakenfull et al. 1997). Modification of the protein structure

may result in enhancement of gelation properties. For example, polymerization using trans-glutaminase with or without protein hydrolysis resulted in enhanced gelation properties of canola protein (Hyun and Kang 1999; Pinterits and Arntfield 2007). Thus, it would be interesting to determine if modification of camelina protein could enhance its gelation properties.

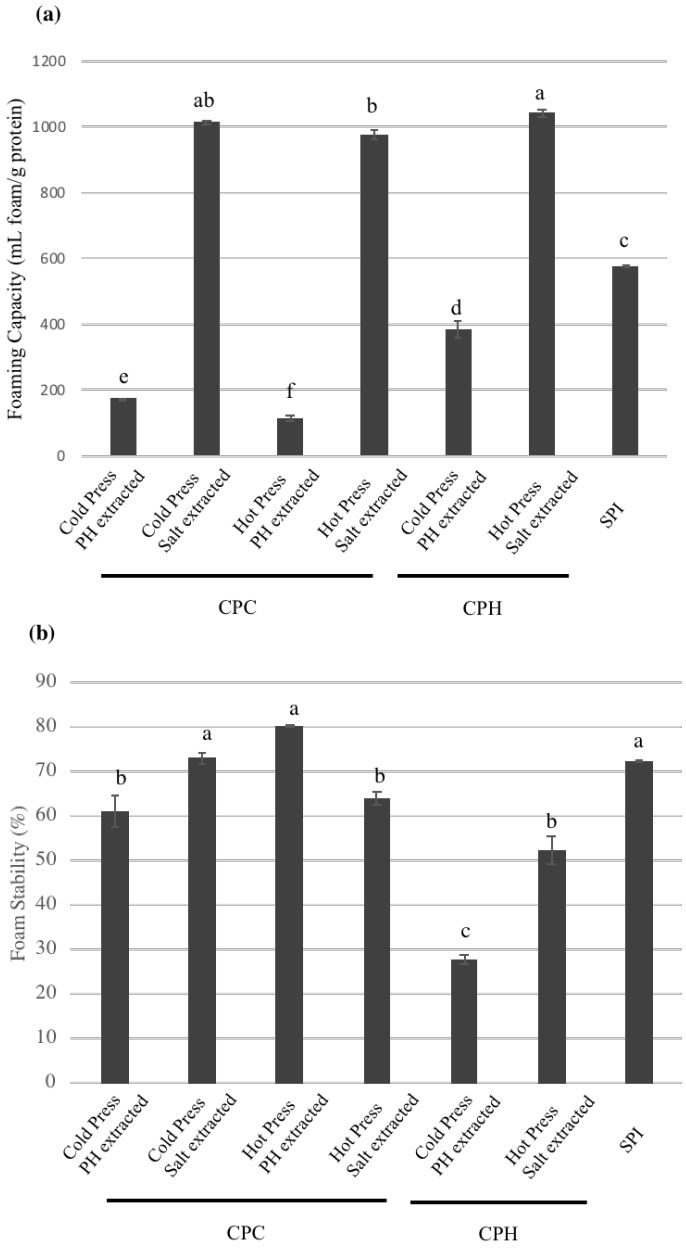

Figure 5. Foaming capacity **(a)** and foam stability **(b)** of camelina protein concentrates (CPC) and hydrolydates (CPH), as well as soy protein isolate (SPI). Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

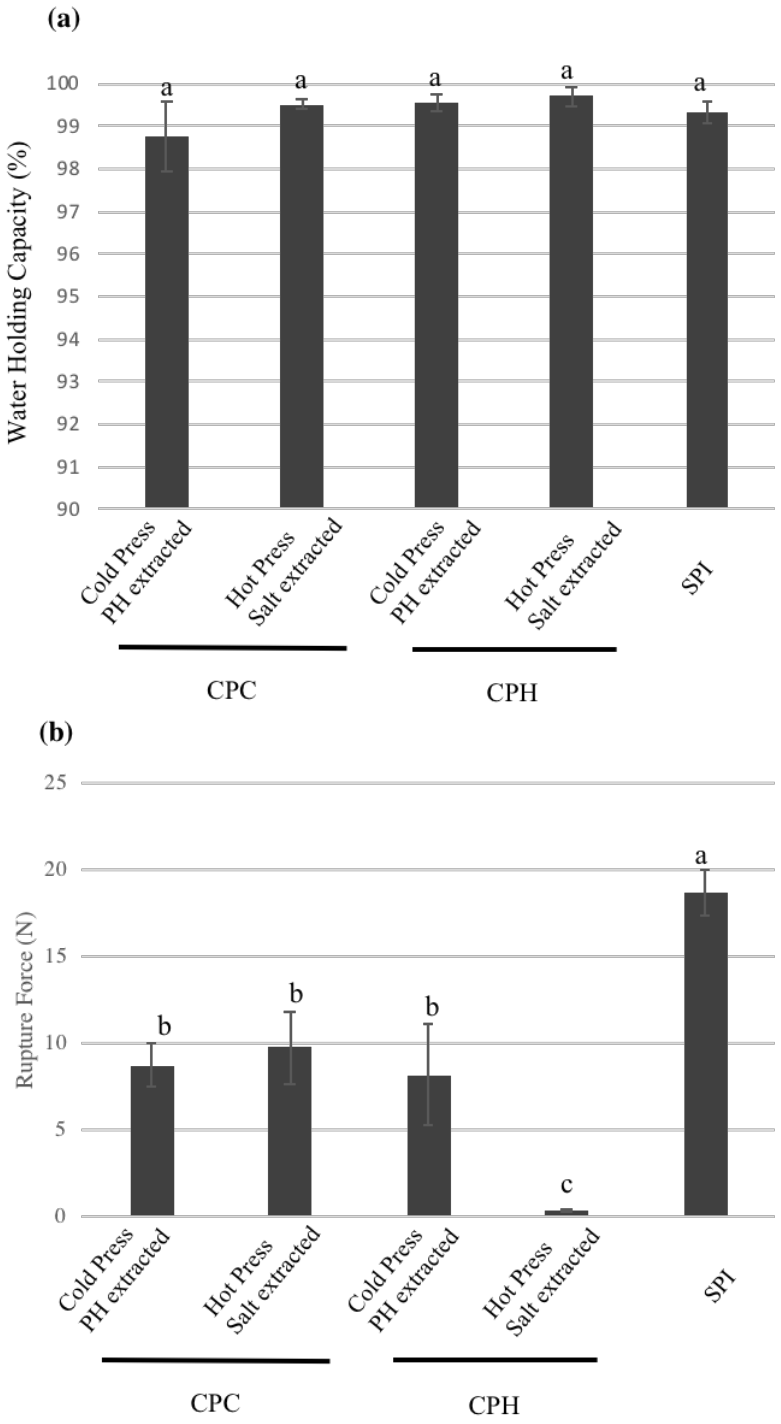

Figure 6. Water holding capacity (WHC) **(a)** and gel strength determined by rupture force (all gels were prepared at 15% protein w/v) **(b)** of camelina protein concentrates (CPC) and hydrolydates (CPH), as well as soy protein isolate (SPI). Gels for WHC were prepared at 8% protein concentration, w/v, for all pH extracted samples, and at 12% protein concentration, w/v, for all salt extracted samples and SPI. Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

2.5.4 Impact of Hydrolysis on Structural and Functional Properties of CPC

Enzymatic hydrolysis of CPC was attempted to determine potential enhancement of the protein functionality. Since there was no impact of oil extraction conditions (cold vs. hot press) on structural and functional characteristics of the produced CPC, only two out of the four CPC samples, cold press pH extracted and hot press salt extracted CPC, were subjected to enzymatic hydrolysis. Enhancing solubility of CPC was the target in an attempt to enhance the overall functionality of the protein. Amano Protease M, a fungal enzyme produced from *Aspergillus oryzae* was chosen as it was advertised to produce hydrophilic peptides. Theoretically, production of hydrophilic peptides contributes to enhanced solubility of the hydrolysate. Limited hydrolysis (DH ≤ 8%) was targeted to avoid excessively reducing the molecular weight of camelina protein. Post several preliminary trials, two hydrolysates, pH extracted CPH (DH = 7.3%) and salt extracted CPH (DH = 8.6%), were produced.

Protease M targeted the globulin-like, cruciferin, both acidic and basic polypeptides (***Figure 1***, lanes 5 and 9), as noted by a reduction in the corresponding band intensities. On the other hand, albumin-like, napin polypeptides resisted hydrolysis, which is consistent with the compact, enzyme resistant structural characteristics of albumins (Wanasundara 2011). Previous research has shown that Alcalase®, which is also a fungal enzyme, targeted the hydrolysis of canola cruciferin (Chabanon et al. 2007). The surface hydrophobicity of salt extracted CPC increased significantly upon hydrolysis (***Figure 3***), while that of pH extracted CPC did not. Protein hydrolysis results in opening up of the structure and revealing hydrophobic residues originally buried within the protein moiety. Compared to salt extracted CPC, pH extracted CPC was already denatured (unfolded) (***Table 3***), prior to enzymatic hydrolysis, thus explaining the different impact of hydrolysis on the surface hydrophobicity of the two samples.

The impact of hydrolysis with Protease M on camelina protein functionality was not favorable in most instances. There was a limited benefit to solubility with enhancement only noted at pH 7 post thermal treatment (***Table 4***). Hydrolyzing the protein potentially increases the charge load, by releasing carboxyl and amine groups. As mentioned earlier, a higher charge load contributes to thermal stability of the protein, as seen specifically at pH 7, where the charge on camelina protein is low compared to that at pH 3.4. Hydrolysis

had either no effect or a detrimental effect on the other functional properties, emulsification, foaming, and gelling (***Figure 4***, ***Figure 5***, ***Figure 6***). Since solubility at pH 7 with no heat was not improved, it is not surprising that the other functional properties measured also at pH 7 did not improve either.

There is no published work on the impact of hydrolysis on the functionality of camelina protein, but there are several on canola protein. Hydrolysis of canola protein resulted in mixed effects on functionality. Hydrolysis of canola protein with Alcalase® resulted in enhanced solubility (up to 67%) at pH 9 (Pedroche et al. 2004), while its hydrolysis with pepsin caused an increase in solubility, foaming capacity, and foaming stability between pH 4 and 7. On the other hand, hydrolysis with trypsin, ficin and bromelin resulted in reduced gel strength (Pinterits and Arntfield 2007), similar to our observation for salt extracted CPH (***Figure 6*** b). The authors contributed their observation to a reduction in the average molecular weight of the protein. The impact of hydrolysis on protein functionality is dependent on many factors including the enzyme specificity, chain length of the peptide, and test conditions. Further work is needed to investigate the effect of hydrolysis, using different enzymes and hydrolysis conditions, on the functionality of camelina protein tested under a number of testing conditions (e.g. pH, temperature, and ionic strength).

2.6 Conclusions

Results confirmed that oil-pressing conditions (cold vs. hot press) had no major impact on protein yield, content, structural characteristics and functional properties presented in this work. The data generated provided basic information, for the first time, on the structural and functional properties of CPC as affected by alkaline pH extraction and salt extraction. Compared to alkaline pH extraction, data showed that salt extraction produces less denatured and more functional protein concentrate, composed mainly of cruciferin and napin proteins. Results also suggested that a camelina protein ingredient, extracted using salt, may be comparable and in some cases a better choice than soy protein for different applications targeting the use of plant proteins. Accordingly, further optimization of protein yield and content of the extracted protein to produce an isolate (>90% protein) is warranted.

Nevertheless, this research revealed the potential of camelina as a novel source of functional plant protein that might gain a position in the protein market place.

3 Camelina Dietary Fiber Characterization

3.1 Overview

Dietary fiber isolated from defatted camelina meal (DCM) prepared by two oil pressing conditions was quantified and characterized. Total dietary fiber (TDF) from DCM was determined, and three fractions —insoluble dietary fiber (IDF), soluble dietary fiber that precipitates in 78% ethanol (SDFP), and soluble dietary fiber that is soluble in 78% ethanol (SDFS) — were isolated preparatively. Saccharides in the SDFS fraction were identified by liquid chromatography coupled with mass spectrometry (LC-MS) and quantified by high performance anion exchange chromatography coupled with a pulsed amperometric detector (HPAEC-PAD). Disaccharides in DCM were differentiated and quantified spectrophotometrically following a standard enzymatic assay. IDF and SDFP were analyzed spectrophotometrically for pectin content by measuring galacturonic acid. Degree of methylation (DM) of pectin was analyzed by proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Monomer composition of IDF and SDFP fractions was determined by alditol acetate derivatization followed by analysis with gas chromatography coupled with a flame ionization detector (GC-FID). TDF from DCM averaged 51.2% (45.3 – 49.1% IDF, 2.00 – 5.98% SDFP, 1.1 – 1.2% SDFS). Of the pectic polysaccharides measured in SDFP, low methoxyl pectin represented the major constituent, with a DM of 12.5 – 14.5%. Pectin was also present in the IDF fraction (2.61 – 2.85% of DCM). The SDFS fraction was comprised mainly of stachyose and raffinose, which is in line with other Brassicaceae crops. The chief disaccharide present in DCM was verified to be sucrose (2.43 – 3.36%). Free glucose and fructose were also present in the SDFS fraction. Glucose was the main monosaccharide in the IDF fraction. Other monosaccharides detected in the IDF fraction were xylose, arabinose, mannose, and galactose. The monosaccharide composition indicated the presence of cellulose, xyloglucans, galactomannans, and arabinoxylans in the IDF fraction. In SDFP, the monosaccharides rhamnose, arabinose, galactose, and mannose were evenly distributed. Monomer composition of the SDFP fraction indicated the presence of pectin and galactomannans. Results show that camelina meal contains a significant amount of dietary fiber that can be isolated into potentially functional ingredients. Camelina presents a sustainably sourced alternative to traditional fiber. Further

investigation will be conducted to understand how isolated camelina fiber interacts in various food matrices and to identify potential applications.

3.2 Introduction

Dietary fiber is a classification of carbohydrates and lignin that are non-digestible, made of three or more monomeric units, and confers a physiological benefit to human health (US Food & Drug Administration 2018). Fiber is ubiquitous in nature, as it comprises the structure in cell walls of fruits, vegetables, and cereal grains, yet less than 3% of Americans consume the recommended daily intake of dietary fiber (30 g/day) (Anderson et al. 2009; Clemens et al. 2012). Consumption of dietary fiber has been linked to many positive health effects, so considering the vast lack of fiber in American diets, it warrants the title "an under-consumed nutrient of public health concern," which was assigned by the Dietary Guidelines Advisory Committee in 2010 (Anderson et al. 2009; Clemens et al. 2012).

Dietary fiber is associated with numerous health benefits: lowering the risk of developing coronary heart disease, lowering cholesterol, reducing hypertension, preventing development of diabetes, and combating obesity by contributing to satiety (McKeown et al. 2002; Kaline et al. 2007; Anderson et al. 2009; Slavin 2013; American Heart Association 2018). Dietary fiber can be either soluble or insoluble. Soluble dietary fiber is linked to lowered cholesterol levels and slowed digestion rate leading to satiety. Insoluble dietary fiber is more closely associated with diabetes prevention and healthy laxation (McKeown et al. 2002; Anderson et al. 2009). Considering these numerous ways fiber can improve health, there is a need for more dietary fiber in American diets. This can be achieved by fortifying foods with fiber ingredients.

Not only does added fiber contribute nutritional benefits to food products, but fiber ingredients also play a key functional role in foods. Fiber has the ability to greatly impact the textural and rheological properties of a food matrix. Soluble fibers such as pectin have excellent water binding ability, making them effective as fat mimetics or thickening agents in low-calorie products (McCleary and Prosky 2001). Pectin also acts as a stabilizer for frozen desserts or baked goods. Furthermore, hygroscopic properties of soluble fiber allow

it to maintain texture and prevent cooking losses in meat analogues. Accordingly, dietary fiber plays an essential role in a wide variety of food products.

In the food industry, there is a drive to develop more plant-based ingredients from crops that require fewer inputs (irrigation, fertilizers, pesticides) than traditional crops in order to create food products in an environmentally responsible manner (González et al. 2011). Therefore, it is desirable to investigate novel crops that have not been previously cultivated for their potential as a source of fiber ingredients.

Camelina sativa is an oilseed gaining interest as a novel source of dietary fiber. Camelina was traditionally grown for its high quality nutritional oil and for its use as a feedstock for biodiesel. However, the press cake following oil extraction is rich in protein and fiber (Fleenor 2011). While the compositional constituents are highly attractive to those in the ingredient industry and consumers, camelina also boasts many environmental benefits. It is a fast-growing cover crop, useful in relay-cropping systems. Camelina has been shown to boost the productivity of soybean harvests by as much as 11.6% (Clark 2015; Berti et al. 2016). Additionally, camelina is cold weather hardy, retains moisture in the soil, requires fewer fertilizer inputs, and is resistant to common pests that jeopardize canola crops (Obour et al. 2015). These agricultural benefits coupled with its nutritional potential as a food ingredient make camelina a good candidate for investigation into its use as a source of fiber ingredients.

Similar crops to camelina, such as canola and rapeseed, have been shown to be rich in dietary fiber. Canola is a genetically modified variety of rapeseed that has been bred to have less than 2% erucic acid in the seed oil and less than 30 mmol/g aliphatic glucosinolates in the meal (Raymer 2002). Rapeseed and canola contain as high as 38% dietary fiber in defatted meal, including fermentable fibers such as pectin and raffinose family oligosaccharides (RFO) (Wanasundara 2011). Given the genetic similarity of camelina and these fellow Brassicaceae members, we hypothesize that defatted camelina meal (DCM) will exhibit similarly high levels of dietary fiber, some of which will be soluble dietary fiber including pectin and RFO. We also hypothesize that the oil pressing conditions the seeds are subjected to will not have a significant impact on the fiber composition of DCM. Analyzing the chemical composition of camelina fiber will help elucidate the anticipated functional attributes it could contribute to a food system.

Therefore, the objective of this work was to quantify total dietary fiber in camelina, as well as characterize the three fiber fractions: insoluble dietary fiber (IDF), water soluble dietary fiber that precipitates in 78% aqueous ethanol (SDFP), and water soluble dietary fiber that remains soluble in 78% aqueous ethanol (SDFS). An additional objective of this work was to determine the effect of oil pressing temperature on dietary fiber composition.

3.3 Materials and Methods

3.3.1 Materials

Camelina seeds and hot pressed (extruded) camelina pellets were kindly provided by General Mills, Inc. (Minneapolis, MN, USA). The camelina seeds were planted in Morris, MN, and harvested in the summer of 2016. The hot press camelina meal was produced by pressing the seeds using a lab Komet CA 59 G screw press at 50°C through a 6 mm die (POS Bio Sciences, Saskatoon, SK, Canada). The extruded, pelletized meal had 14.3% fat and 29.2% protein. Enzyme kits for isolating and quantifying dietary fiber and for measuring maltose, sucrose, and glucose were purchased from Megazyme International (K-INTDF, K-MASUG, Wicklow, Ireland). Mixed-bed ion exchange resins Amberlite® FPA53 (OH^-) and Ambersep® 200 (H^+) were purchased from Rohm and Haas France S.A.S. (Semoy, France). Celatom® (C8656), D-mannose (63580), D-glucose (G5767), D-galactose (G-0625), L-xylose (851590), L-rhamnose (R38750), and D-galacturonic acid (48280-5G-F) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stachyose (453142500) and 0.45 μm nylon membrane filters were purchased from Thermo Fisher Scientific (Waltham, MA, USA). D-arabinose (A0513) was obtained from TCI America (Portland, OR, USA), meso-erythritol (A15813) was obtained from Alfa Aesar (Tewksbury, MA, USA), and D-fucose (21940) and D-raffinose (02022) were obtained from Chem Impex (Wood Dale, IL, USA). All other chemical grade reagents were purchased from Thermo Fisher Scientific, Sigma-Aldrich, or VWR International (Randor, PA, USA).

3.3.2 Production of Defatted Camelina Meal (DCM)

For the production of cold press defatted camelina meal (DCM), camelina seeds were pressed at room temperature for 24 h using a hydraulic press (Carver, Inc, Wabash,

IN, USA). The press cake samples had ~ 20% fat, as verified by the Mojonnier AOAC method 922.06 (AOAC International 2016). Both the cold press cake and the hot press camelina pellets were milled to 50 mesh using a cyclone sample mill (Udy Corp, Fort Collins, CO, USA). Milled camelina cake samples were defatted by batch extraction with hexane in a 3:1 ratio in three 1 h cycles. Residual hexane was allowed to evaporate overnight. Fat content was verified to be under 3% following the Mojonnier method. The protein content (34.8%) of the DCM samples was determined following the Dumas AOAC method 990.03 using a nitrogen analyzer (LECO, St. Joseph, MI, USA) and a protein conversion factor of 5.3 (AOAC International 2016). DCM samples were kept at -20°C until further analysis.

3.3.3 Total Dietary Fiber Quantification

Total dietary fiber in DCM was determined according to integrated total dietary fiber AOAC method 2011.25 (Mccleary et al. 2012; AOAC International 2016), with minor modifications in sample amounts and the addition of centrifugation steps. In duplicate, 0.5 g DCM samples were dispersed in ethanol (1 mL) and digested with pancreatic α-amylase and amyloglucosidase in 20 mL sodium maleate buffer (50 mM, pH 6.0, 2 mM $CaCl_2$) for 16 h at 37°C while shaking in a water bath. Before filtration, samples were centrifuged at 2500 x *g* for 5 min. IDF was collected onto Celatom® as a filtering aid and washed with water, 78% ethanol, 95% ethanol, and acetone sequentially. The residue was dried and weighed. The filtrate containing SDFP and SDFS was suspended with 78% ethanol (160 mL) for 1 h. The soluble fiber was filtered through Celatom®, and the remaining SDFP residue was washed with 78% ethanol, 95% ethanol, and acetone, then dried and weighed. Both IDF and SDFP residues were corrected for protein and ash by the Kjeldahl (AOAC 981.10) and dry ashing methods (AOAC 942.05), respectively (AOAC International 2016). The remaining filtrate constituting SDFS in ethanol was desolventinized by evaporation using a rotovap (Rotavapor R114, BUCHI Corporation, New Castle, DE, USA), redissolved in DDW, deionized by percolating through two mixed-bed ion exchange resins in a polypropylene column, and filtered through a 0.45μm nylon syringe filter before being analyzed by liquid chromatography-mass spectrometry (LC-MS) and quantified by high

performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

3.3.4 Identification of Saccharides in SDFS by Liquid Chromatography Coupled with Mass Spectrometry (LC-MS)

Based on the method outlined by Hernández-Hernández et al. (2012), lyophilized SDFS samples were dissolved in 2 mL deionized distilled water (DDW), filtered through a 0.45 μm nylon membrane, and analyzed by a Shimadzu LC-10AD liquid chromatography system coupled with quadrupole mass spectrometry detection (Waters Micromass ZQ, Waters Corporation, Milford, MA, USA), and electrospray ionization (ESI) under positive polarity. A CarboSep CHO-411 ligand exchange column (Transgenomic, Inc., Omaha, NE, USA) was used with DDW as the mobile phase. LC parameters were as follows: flow rate of 0.4 mL/min, sample injection volume of 40 μL, oven temperature of 80°C. Optimized MS instrument tune parameters were as follows: capillary voltage 4.00 kV, cone voltage 50 V, and nebulizing gas (N_2) at a flow rate of 600 L/h and 400°C. Oligosaccharides were detected as sodium adducts. The intensity of the lactose adduct at 365 m/z was monitored and adjusted to the maximum using the automatic tuning procedure built into the Mass Lynx software in order to maximize the response to specific oligosaccharides in the camelina SDFS sample (Waters Corporation). SDFS samples were anticipated to contain hexose saccharides up to a degree of polymerization (DP) of 7, so lactose was chosen as the tuning compound because its molecular weight would be in range with saccharides in camelina samples. Raffinose and stachyose standards were prepared and analyzed in the same manner to identify the sample constituents. Sample and standard chromatograms can be found in Appendix H.

3.3.5 Quantification of Saccharides by High Performance Anion Exchange Chromatography Coupled with Pulsed Amperometric Detection (HPAEC-PAD)

HPAEC-PAD analysis was carried out as described by Gangola et al (2014). In duplicate, lyophilized SDFS samples (~ 25mg) were dissolved in 1 mL DDW and filtered through 0.2 μm syringe filters prior to analysis on a Dionex ICS 5000+SP system (Thermo Scientific, Stevens Point, WI, USA) with a CarboPac PA100 (4 x 250 mm) column

(Thermo Fisher Scientific, Stevens Point, WI, USA) held at 30°C. Sample injection volume was 10 μL. Two solvents were used, degassed DDW (solvent A) and 200 mM NaOH (solvent B). The eluent had a flow rate of 1.0 mL/min, and the gradient (% solvent A/% solvent B) was as follows: 0 – 25 min 90/10, 25 – 26 min 0/100, 26 – 35 min 90/10, followed by column equilibration. Samples were detected by an electrochemical detection cell with a gold electrode and silver chloride reference electrode. A standard PAD waveform at four different potentials was used for 500 milliseconds. The potentials (*E*) were as follows: +0.1 V for 40 s (*E1*), -2.0 V for 1 s (*E2*), +0.6 V for 1 s (*E3*) and -0.1 V for 6 s (*E4*). The detection and integration of samples were controlled by Chromeleon v. 7.2.6 software (Thermo Scientific, Stevens Point, WI, USA). Standards (stachyose, raffinose, sucrose, fructose, and glucose at 0.1 mg/mL) were also run under the same conditions. Sample and standard chromatograms can be found in Appendix I.

3.3.6 Maltose, Sucrose, and Glucose Quantification

Quantification of maltose, sucrose, and glucose in DCM was carried out in triplicate using a Megazyme kit. DCM samples were prepared such that glucose concentrations were within the linear limits of the assay (4 – 80 μg/mL glucose) and clarified to eliminate interfering protein by treatment with Carrez reagents (Mitchell 1990). Each DCM sample (0.20 g) was solubilized with 6 mL DDW in a 10 mL volumetric flask. Carrez I solution (0.5 mL, 15% $K_4[Fe(CN)_6]$ x $3H_2O$), Carrez II solution (0.5 mL, 30% $ZnSO_4$ x $7H_2O$), and NaOH (1 mL, 100 mM) were added to the flask before bringing to volume with DDW. The solution was filtered with a 0.45 μm nylon syringe filter. For the analysis of each saccharide of interest, one sample cuvette and one sample blank cuvette were prepared. For measuring sucrose, 100 μL β-fructosidase in sodium citrate buffer (pH 4.6) and 50 μL filtered, clarified sample were added to polymethyl methacrylate (PMMA) cuvettes. For measuring maltose, 100 μL α-glucosidase in sodium citrate buffer (pH 6.6) and 50 μL filtered, clarified sample were added to PMMA cuvettes. For measuring glucose, only 50 μL filtered, clarified sample were added to PMMA cuvettes. Cuvettes were covered and gently inverted to mix before incubating at ambient temperature for 20 minutes. To each cuvette the following were added: DDW (1 mL to sucrose and maltose samples, 1.1 mL to glucose samples), sodium citrate buffer (100 μL, pH 7.6), and aqueous nicotinamide-adenine

dinucleotide phosphate ($NADP^+$) with adenosine triphosphate (ATP) (50 μL). After 3 min hold time, absorbance (A_1) at 340 nm was read using a Beckman DU 800 spectrophotometer (Beckman Coulter, Brea, CA, USA). The enzymatic reaction was initiated by adding 10 μL hexokinase with glucose-6-phosphate (G6P) dehydrogenase suspension. After 5 min hold time, absorbance (A_2) at 340 nm was read. The amount of nicotinamide-adenine dinucleotide phosphate (NADPH) that is formed and measured spectrophotometrically is in stoichiometric relationship with the amount of sucrose, glucose, and half the amount of maltose. Sucrose, maltose, and glucose concentrations were calculated based on measured absorbances as shown in Appendix J.

3.3.7 Pectin Analysis by Galacturonic Acid Quantification

Pectin content of camelina dietary fiber was analyzed spectrophotometrically by measuring galacturonic acid content. Dietary fiber fractions were isolated preparatively by enzymatic digestion and filtration as described by the integrated total dietary fiber method with the modification of omitting Celatom® and not using any filtering aids. IDF and SDFP fractions were dried in an oven (103°C) overnight and ground to a powder using a mortar and pestle. The method outlined by Robertson (1979) was followed, with modifications. Dried sample (0.160 g) was weighed, in triplicate, into a 50 mL centrifuge tubes, dispersed in 3.5 mL DDW, vortexed for 10 min, and centrifuged at 1000 x *g* for 15 min. The supernatant was poured into a 10 mL volumetric flask and brought to volume with DDW after the addition of 500 µL 1 N sodium hydroxide (NaOH). The remaining residue was vortexed for 10 min with 3 mL 0.75% ammonium oxalate, centrifuged, and the supernatant was poured into a 10 mL volumetric flask. 1 N NaOH (500 µL) was added, and the extract was brought to volume with 0.75% ammonium oxalate. To the remaining residue, 1 N NaOH (500 µL) was added and brought to 10 mL with DDW. All three extracts were centrifuged to separate insoluble particles. In triplicate, aliquots from the supernatants were taken and diluted to fit the standard curve (0 – 240 µg galacturonic acid). Sodium tetraborate (0.0125 M) in concentrated sulfuric acid (1 mL) was added to each sample tube, heated in a boiling water bath for 6 min, and cooled in an ice bath. The chromagen *m*-hydroxydiphenyl (0.15% in 0.5% NaOH) was added to each tube (20 µL), samples were

vortexed for 20 s, and absorption was read at 520 nm by a Beckman DU800 spectrophotometer. Absorbance readings were taken after 15 min, allowing for the colorimetric reaction products to stabilize. Sample blanks were analyzed alongside all samples to correct for color present in samples and colors produced by neutral sugars with sulfuric acid. A standard curve was constructed by preparing and analyzing galacturonic acid standard solutions under the same analysis conditions and over a range of concentrations (0 – 240 μg galacturonic acid/mL). Sample calculations can be found in Appendix K.

3.3.8 Determination of Degree of Methylation by ^{1}H NMR Spectroscopy

Degree of methylation was measured by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) as described by Rosenbohm et al. (2003). SDFP samples were incubated with stirring (2 h at ambient temperature) with 0.7 mL deuterium oxide (D_2O), being exchanged with D_2O three times before centrifugation at 3200 x *g* for 20 min. Supernatants were filtered through a 0.45 μm nylon syringe filter. Samples were analyzed by an AVANCE III HD ^{1}H NMR spectrometer (Bruker, Milton, ON, Canada), at 400 MHz. with Bruker Topspin v 3.5 software (Milton, ON, Canada). Sample NMR spectra can be found in Appendix L. Degree of methylation was determined using the following equation:

$$Degree\ of\ Methylation = \frac{I_{COOMe}}{I_{COOMe} + I_{COO^-}}$$

Where:

I_{COOMe} = the integrals of H-5 adjacent to esters

I_{COO^-} = the integrals of H-5 adjacent to carboxylates

3.3.9 Alditol Acetate Derivatization for Monosaccharide Identification of IDF and SDFP

Samples were prepared and analyzed as described by Blakeney et al. (1983). IDF (30 mg) was hydrolyzed, in triplicate, with sulfuric acid (H_2SO_4) and allowed to stand 30 min in an ice bath plus 2 h at room temp, while SDFP samples (30 mg) were dispersed in 3 mL 2 M H_2SO_4 without any holding time. The IDF samples were diluted to 2 M H_2SO_4 before

both sample types were heated in a boiling water bath for 1 h. Ammonium hydroxide (NH_4OH, 25%) was added to cool samples until alkaline (at least 8 – 9) pH was achieved, as indicated with litmus paper. Samples were transferred to 10 mL volumetric flasks, brought to volume with DDW, and reduced by adding 1 mL 2% sodium borohydride ($NaBH_4$) in dimethyl sulfoxide (DMSO) to filtered (0.45 μm nylon syringe filters) 100 μL aliquots. Samples were incubated at 60°C in a water bath for 1 h while shaking. To the cooled samples, 100 μL 80% acetic acid with 0.8 mg/ml erythritol was added, which acted as the internal standard. Samples underwent acetylation with the addition of 2 mL acetic anhydride and 200 μL 1-methylimidazole to act as a catalyst. The reaction proceeded for 10 min at ambient temperature before samples were cooled in an ice bath and 5 mL DDW was added. Alditol acetates were extracted by adding 2 mL chloroform, and the aqueous phase was separated and discarded. The chloroform phase was washed twice with water before freezing at -20°C to freeze out residual water. Aliquots (1 μL) were analyzed without dilution using a Hewlett Packard 5890A gas chromatograph system (Palo Alto, CA, USA) with flame ionization detection. A DB1701 column was used and samples were analyzed with an isothermal profile at 220°C and hydrogen as the carrier gas. A mixture of seven monosaccharides (0.5 mg/mL each of rhamnose, arabinose, xylose, fucose, mannose, galactose, glucose) were prepared as standards. Standard solutions were dried under nitrogen, dissolved in 100 μL 0.1 M NH_4OH, and reduced and acetylated in the same manner as the samples. Concentrations were calculated from chromatograms by generating response factors using the following equations:

$$Internal\ Response\ Factor = \frac{area_{IS} \times concentration_{CI}}{concentration_{IS} \times area_{CI}}$$

$$Concentration_{CI} = \frac{area_{CI} \times concentration_{IS} \times IRF_{CI}}{area_{IS}}$$

Where:

IS = internal standard

CI = compound of interest

IRF = internal response factor

Chromatograms for standards and samples can be found in Appendix M. Example calculations can be found in Appendix N.

3.4 Statistical Analysis

Analysis of variance (Appendix O, Tables 24 – 42) was carried out using IBM SPSS Statistics software version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences ($P \leq 0.05$) among the means (n = 3) of different samples were determined using the Tukey–Kramer multiple means comparison test.

3.5 Results & Discussion

3.5.1 Total Dietary Fiber in Cold and Hot Press DCM

TDF amounted to over 50% in the DCM samples (***Table 5***). There was no significant difference in TDF between cold and hot press samples. In comparison, defatted canola meal contained 27.3 and 30.1% for yellow- and brown-seed canola, respectively (Slominski et al. 1994). Rapeseed has been reported to have 30.6 – 38.2% TDF (Wanasundara 2011). Camelina has a smaller seed size (1 mg per seed) than canola and rapeseed (3 – 5 mg per seed) (***Figure 7***) (Iskandarov et al. 2014). The smaller seed size of camelina accounts for its higher TDF content compared to canola and rapeseed.

Although the cold and hot press samples do not differ in TDF, there is a significant ($P < 0.05$) difference in the distribution of SDFP and a complementary difference in IDF. Hot press DCM contained significantly higher SDFP and lower IDF than cold press DCM (***Table 5***). A potential explanation for this difference can be attributed to the mild heat treatment applied to the seeds before oil pressing. Seeds naturally contain enzymes that act on oligo- and polysaccharides in order to make available monosaccharides necessary for growth during germination (Martínez-Villaluenga et al. 2005). The seeds were heated to 50°C, which is less than the traditional blanching temperature range (70 - 95°C), meaning it was likely not high enough to inactivate native enzymes present but resulted in enhanced enzyme activity (Jaiswal et al. 2012). Any pectinases or galactosidases naturally present in camelina would have increased their activity upon heat treatment, potentially cleaving

portions of the IDF into smaller, more soluble polysaccharides, thus shifting the distribution of IDF and SDFP in hot press DCM.

Table 5. Percent insoluble dietary fiber (IDF), soluble dietary fiber that precipitates in 78% ethanol (SDFP), and soluble dietary fiber that remains soluble in 78% ethanol (SDFS) found in defatted camelina meal (DCM) samples.

Sample	IDF (%)	SDFP (%)	SDFS (%)	Total Dietary Fiber (%)
Cold Press DCM	49.1[a^]	2.00[b]	1.20[a]	52.3[a]
Hot Press DCM	45.3[a]	5.98[a]	1.11[a]	52.4[a]

[^] Means (n=2) in each column with different lowercase letters indicate significant differences between two DCM samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

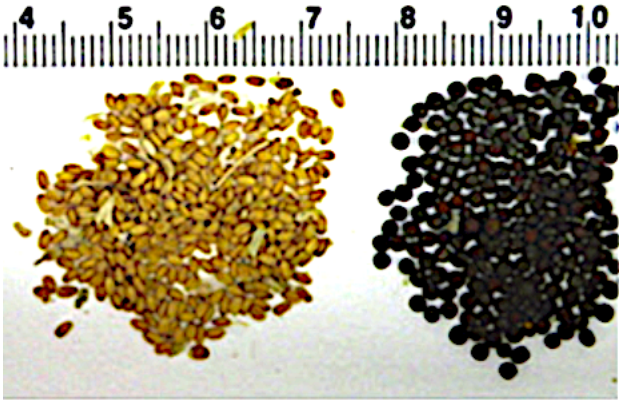

Figure 7. Camelina seeds (left), approximately 1 mg per seed. Rapeseeds (right), approximately 3 – 5 mg per seed (Iskandarov et al. 2014, with permission).

SDFS fraction is thought to be a mixture of mono-, di-, and oligosaccharides. Raffinose family oligosaccharides (RFO), namely raffinose and stachyose, were found in camelina (Zubr 2010) in roughly similar concentrations, on average totaling 1.00%.

Differences are likely due to growing locations and conditions (Zubr 2003b). SDFS data are also similar to reported values for combined raffinose and stachyose in rapeseed (1.57% on average) (Knudsen and Li 1991). RFO are commonly associated with beans and legumes and have been shown to have positive effects on human health by promoting the growth of beneficial bacteria such as *Bifidobacteria* (Martínez-Villaluenga et al. 2005). The presence of RFO in camelina indicates its potential for use as a functional food ingredient.

3.5.2 Saccharides in SDFS

The SDFS fraction contained mono- and disaccharides as confirmed by LC-MS identification (***Figure 8***) and HPAEC-PAD quantification (***Table 6***). These mono- and disaccharides are utilized by the plant for energy during maturation, however most of its energy stores are bound in larger polysaccharides. Glucose and fructose were observed at greater concentrations than what was reported previously for camelina (0.42% and 0.04%, respectively) (Zubr 2003b, 2010). This variability can be attributed to growing conditions. On the other hand, canola seeds have as much as 5% free glucose and fructose (Wanasundara et al. 2016). Therefore, values observed for camelina are within the reported range for the Brassicaceae family (Wanasundara et al. 2016).

Sucrose concentrations were greater than glucose and fructose, which is in accordance with findings from Zubr (2010) and Wanasundara (2011), yet the reported values were relatively higher, 5.5% and 6.9%, respectively. A reason for this discrepancy is proposed to be genetic, as observed in lupin seeds. Sucrose and verbascose are more influenced by seed genotype than raffinose and stachyose, which were shown to fluctuate more with varying environmental conditions (Trugo et al. 1988; Macquet et al. 2007). Another explanation for the lower sucrose levels could be attributed to the maturity of the plant. Sucrose acts as a substrate for deriving stachyose and raffinose and thus its concentration decreases as seeds develop. Additionally, overtime galactose units are linked to sucrose to form more complex oligosaccharides (Macquet et al. 2007). This phenomenon has been shown in soybeans, lupin, peas, and faba beans (Lowell and Kuo 1989). Considering the influence of plant maturity, the camelina samples used in this study could have been more mature than those used in previously referenced works. A further

explanation for these differences is the method of analysis used. Zubr and Wanasundara referred to analytical methods for sucrose in animal feed that were less specific than the HPAEC and enzymatic methods used for camelina samples, which would result in higher sucrose content than those found in the SDFS fractions.

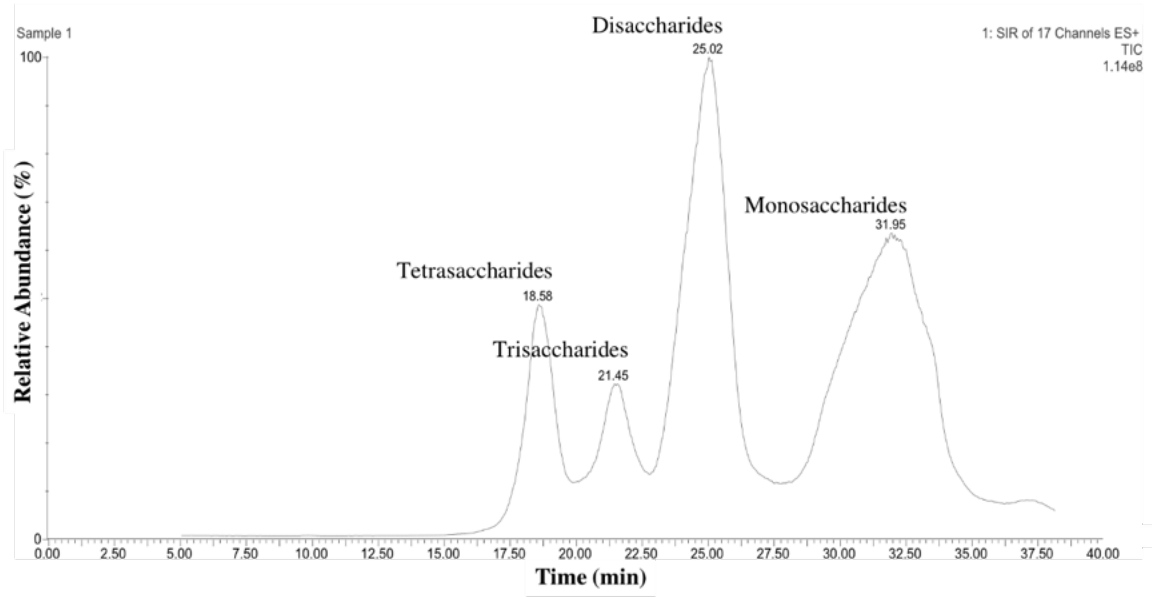

Figure 8. LC-MS total ion chromatogram of a SDFS sample.

Raffinose and stachyose were the dietary fiber components found in the SDFS fraction, as they have DPs of three and four, respectively (the minimum DP for classification as dietary fiber). Both raffinose and stachyose, the main dietary fiber constituents of the SDFS fraction (1.1 – 1.2%), were found in camelina in previous work by Zubr (2010) at similar concentrations: 0.64% raffinose and 0.36% stachyose. Both cold and hot press samples had significantly ($P < 0.05$) higher stachyose concentrations than raffinose. It has been shown that the content of these oligosaccharides in seeds is dependent upon a variety of environmental and intrinsic conditions, two of which being maturation temperature and seed development stage (Trugo et al. 1988; Martínez-Villaluenga et al. 2005). Raffinose and stachyose are synthesized in the seed as the plant matures, with raffinose (α-D-galactopyranose-(1,6)-α-D-glucopyranose-(1,2)-β-D-fructofuranose) forming first, after which another galactose unit is added to the structure, forming stachyose. The results, therefore, indicate that the plants had a longer time to mature. The

significantly more raffinose and less stachyose present in the hot press sample in comparison to the cold press sample, could be due to the activating effect of the heat treatment on native enzymes present, as discussed earlier. As stachyose was acted upon by enzymes, a galactose unit could have been cleaved off resulting in raffinose.

Table 6. Saccharides in soluble dietary fiber that remains soluble in 78% ethanol (SDFS) fraction as percent of defatted camelina meal (DCM) determined by HPAEC-PAD.

Sample	Glucose (%)	Fructose (%)	Sucrose (%)	Raffinose (%)	Stachyose (%)
Cold Press DCM	1.83[a^]	1.02[a]	2.02[a]	0.27[b]	0.93[a]
Hot Press DCM	1.45[b]	0.74[b]	1.88[a]	0.38[a]	0.73[b]

[^] Means (n = 2) in each column with different lowercase letters indicate significant differences between the two DCM samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

3.5.3 Disaccharide Differentiation in DCM

Data gathered from LC-MS analysis of the SDFS fraction showed a sizeable abundance of disaccharides (**Figure 8**). Having the same mass, disaccharides in camelina could not be differentiated by the LC-MS analysis performed. In order to accurately identify disaccharides that could be present, an enzymatic assay was used to quantify sucrose and maltose, as these are the two likely disaccharides in camelina (Zubr 2010). Sucrose is expected, given that it is commonly found in Brassicaceae crops, and camelina has a low starch content (1.21%) as reported by Zubr (2010), that could be partially hydrolyzed during processing, contributing to maltose.

To differentiate and quantitate sucrose and maltose, an enzymatic assay was performed, which also allowed for the quantification of free glucose. Minimal free glucose (***Table 7***) was detected in DCM by this assay compared to the glucose measured in the SDFS fraction by HPAEC (***Table 6***), while this assay showed greater sucrose levels than the HPAEC data. The discrepancy could be attributed to differences in methods; measuring

saccharides in SDFS by HPAEC was the last stage of a multi-step assay, in which samples were treated with heat, digestive enzymes, ethanol precipitations, and concentrated by thermal evaporation and lyophilization. This lengthy process most likely resulted in sucrose hydrolysis into its monomers, glucose and fructose, thus contributing to higher glucose and lower sucrose levels. The enzymatic assay conducted to quantify sucrose, maltose, and glucose had fewer and less disruptive steps, thus contributing to the higher sucrose and lower glucose levels observed. The sucrose values from the enzymatic assay are closer to the values reported by Zubr (2010) and Wanasundara (2011), 5.5% and 6.9%, respectively, however they are still about 50% lower than the literature values. A reason for this difference is the use of different analytical methods. Previously reported data for sucrose in camelina used a method for measuring sucrose in animal feed that was less specific than the enzymatic assay, potentially resulting in an overestimation of sucrose. Glucose levels were within the range of published values: 0.02% reported by Berhow et al. (2014) and 0.42% reported by Zubr (2010). Maltose was not detected in DCM, which was not surprising due to camelina's scarce starch content. This finding is in agreement with literature reports for camelina (Berhow et al. 2014) and canola (Tan et al. 2011).

Table 7. Percent glucose, sucrose, and maltose present in defatted camelina meal (DCM) as determined by an enzymatic assay.

Sample	Glucose (%)	Sucrose (%)	Maltose (%)
Cold Press DCM	0.35[a^]	3.42[a]	< 0.1
Hot Press DCM	0.17[b]	3.33[a]	< 0.1

[^] Means (n = 3) in each column with different lowercase letters indicate significant differences between the two DCM samples according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

3.5.4 Pectin Content and Degree of Methylation

Camelina contains mucilage on its seed coat that allows it to form a gel when soaked in water. Camelina seeds can bind water up to ten times their weight. Accordingly, pectin content was analyzed, as it is a known gelling substance commonly found in plant cell walls. Pectin was quantified by measuring galacturonic acid, the primary monomer of

pectic substances. Camelina fiber contained measurable quantities of pectin (***Table 8***), which is consistent with reports on similar crops like rapeseed and *Arabidopsis thaliana*, a genetically close relative of camelina (Macquet et al. 2007; Tan et al. 2011; Wanasundara 2011). IDF contained significantly ($P < 0.05$) more pectin than the SDFP fractions (***Table 8***). Although pectin is largely considered a soluble fiber, portions of the molecule can be insoluble or bound to other insoluble fiber types like cellulose as reported in *Arabidopsis*, thus contributing to the presence of pectin in IDF (Macquet et al. 2007). When considering the ratio of pectin contributing to each dietary fiber fraction, SDFP contains a much higher percentage (24 – 27%) of pectin than IDF (5.8%).

Hot press DCM contained significantly more pectin than cold press DCM, which is attributed mostly to the significantly higher pectin content in the SDFP fraction. It is not clear why the hot press process would result in a greater concentration of pectin. One possible explanation for this difference could be attributed to the method and the homogenization of the sample. Isolated, dried dietary fiber fractions from DCM samples were extraordinarily difficult to grind to a powder, thus sample homogenization and distribution may have caused the noted variation in pectin content, potentially leading to an underestimation in the cold press DCM.

The degree of methylation (DM) of pectin in the SDFP fraction was also measured, which allows for classification as low or high methoxyl pectin and indicates gelling behavior. Low methoxyl (LM) pectin is classified as having 50% or less of the carboxyl groups on galacturonic acid units esterified with methyl groups, while high methoxyl (HM) pectin has greater than 50% methylated carboxyl groups (***Figure 9***). LM pectin in the presence of divalent cations, often calcium ions in food systems, forms stronger gels than HM pectin (McCleary and Prosky 2001). HM pectin requires acid and sugar in order to form gels (Rosenbohm et al. 2003).

Table 8. Pectin in insoluble dietary fiber (IDF) fraction and soluble dietary fiber that precipitates in 78% ethanol (SDFP) fraction, and total pectin in defatted camelina meal (DCM), as percent of DCM.

Sample	Pectin by Fiber Fraction (% of DCM)	Total Pectin in DCM (% of DCM)
Cold Press IDF	2.85[a^]	
		3.33[b]
Cold Press SDFP	0.48[c]	
Hot Press IDF	2.61[a]	
		4.22[a]
Hot Press SDFP	1.61[b]	

^ Means (n = 3) in each column with different lowercase letters indicate significant differences among the different dietary fiber fractions according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

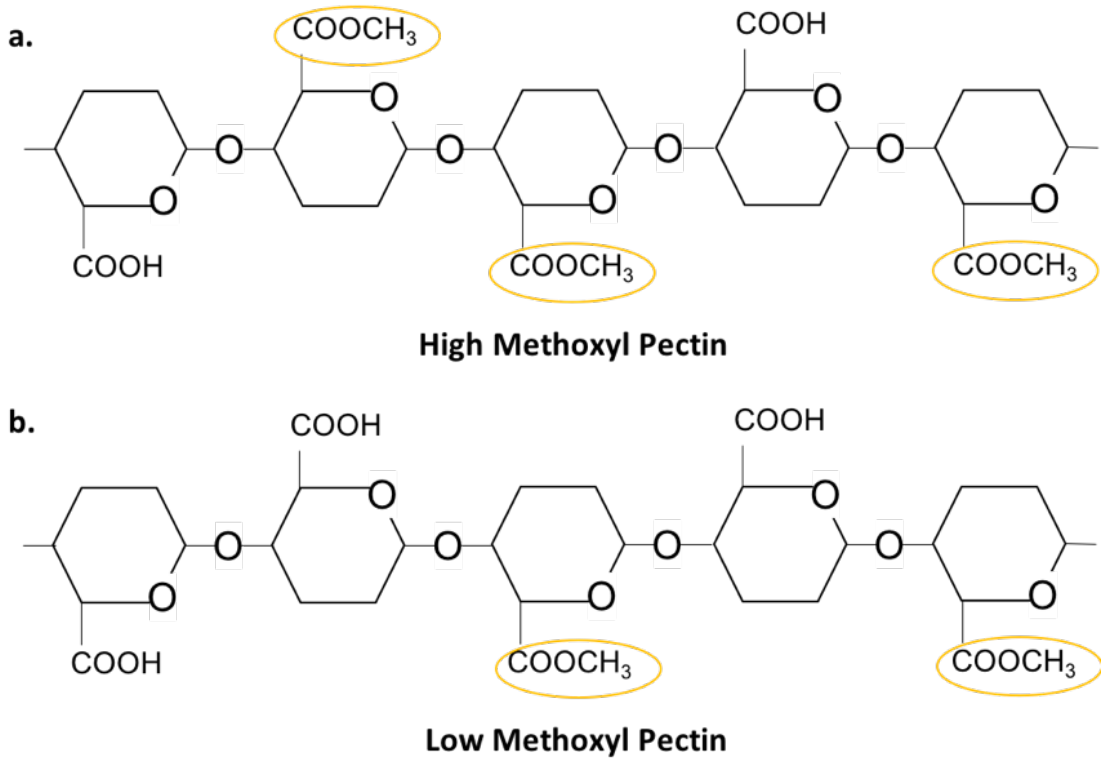

Figure 9. Chemical structures of high **(a.)** and low **(b.)** methoxyl pectin, with methylated carboxyl groups circled in yellow.

Camelina pectin showed low DM, 12.5% in cold press and 14.5% in hot press SDFP, demonstrating that it is LM pectin. It is not clear why hot press DCM had a significantly ($P < 0.05$) higher DM than cold press DCM. There are no reported values on the DM for camelina, however it has been reported that underesterified or LM pectin predominates in the endosperm cell walls of *Arabidopsis thaliana* (Lee et al. 2012). Additionally, rapeseed press cake had a DM of 25% (Müller-Maatsch et al. 2016). This is the first determination of DM in camelina, and the results are in agreement with the LM pectin reported for similar crops. Knowing the pectin DM can help predict the functional properties of pectin from camelina and determine what food products it would best be applied to. Because it can form strong gels without the presence of sugar, it can be used in low-calorie products to impart thickness, or it can act as a fat mimetic in sauces (McCleary and Prosky 2001).

3.5.5 Monosaccharide Composition of IDF and SDFP

Alditol acetate derivatization allowed for the elucidation of monosaccharide composition of IDF and SDFP. Seven of the most common monosaccharides present in plant fibers (fucose, glucose, galactose, mannose, xylose, arabinose, and rhamnose) were chosen as standards. Knowing the monomers of polysaccharides can provide insight into possible types of dietary fiber that are present in each fraction.

In the SDFP fractions, an approximately even distribution of galactose, mannose, arabinose, and rhamnose was found (***Table 9***). The only significant difference observed between the cold and hot press SDFP samples was in the galactose content, however this difference is minor. The presence of galactose and mannose indicates the potential presence of galactomannans, which is supported by their reported presence in rapeseed (Wanasundara 2011). Furthermore, galactomannans are particularly abundant in the cell walls of seeds, making this a logical conclusion for camelina (McCleary and Prosky 2001). Galactose can also participate in pectic structures, which is true for arabinose and rhamnose as well, the two other monosaccharides accounted for in SDFP. Given that pectin is present in SDFP (***Table 8***), galactose, arabinose, and rhamnose likely contributed to pectin branching (McCleary and Prosky 2001).

Table 9. Monosaccharides present in soluble dietary fiber that precipitates in 78% ethanol (SDFP) and insoluble dietary fiber (IDF) fractions (% of fiber fraction) as determined by alditol acetate derivatization method.

	Percent (%) Monosaccharide in Fiber Fraction					
	Rhamnose	**Arabinose**	**Xylose**	**Mannose**	**Galactose**	**Glucose**
Cold Press SDFP	23.2[a^]	20.4[a]	–	28.8[a]	27.7[b]	–
Hot Press SDFP	25.5[a]	21.2[a]	–	25.0[a]	28.3[a]	–
Cold Press IDF	–	13.2[a]	15.7[a]	18.7[a]	11.8[a]	40.7[a]
Hot Press IDF	–	12.4[a]	15.4[a]	19.4[a]	12.1[a]	40.8[a]

[^] Means (n = 3) in each column with different lowercase letters indicate significant differences between cold and hot press samples within each fiber fraction according to the Tukey-Kramer multiple means comparison test ($P \leq 0.05$).

IDF differed from SDFP in its monomer composition most notably in the presence of glucose. This observation can be attributed to the presence of insoluble cellulose and hemicelluloses, two of the most common polysaccharides in nature. Cellulose is strictly comprised of β-(1,4)-linked glucose units, while hemicellulose is comprised of xylose, galactose, mannose, rhamnose, and arabinose in addition to glucose. Given the more diverse monosaccharide profile of IDF, it is likely that the presence of these sugars can be attributed in part to hemicellulose. Other specific polysaccharides that may be present are galactomannans, given the presence of galactose and mannose, and arabinoxylans, due to the presence of arabinose and xylose. Similar to SDFP, there were no significant differences in monomer composition between cold and hot press IDF samples, demonstrating that processing temperature had minimal, if any, effect on fiber composition and distribution.

In addition to the presence of glucose and xylose in IDF, another major difference between SDFP and IDF was the presence of rhamnose in the soluble fraction. Rhamnose is frequently a constituent of pectin, either in a branching structure on a homogalacturonan chain or interspersed as part of the backbone, in which case the polysaccharide is defined as rhamnogalacturonan type I (RGI) (α-(1,2)-linked L-rhamnosyl and α-(1,4)-linked D-

galacturonosyluronic acid residues) (McCleary and Prosky 2001). *Arabidopsis thaliana* has water soluble mucilage on its seed coat (Macquet et al. 2007; Berti et al. 2016). Macquet et al. (2007) analyzed the pectin in the seed coat to be RGI. Given the genetic relationship between camelina and *Arabidopsis*, the rhamnose measured in SDFP is likely part of the RGI structure on the seed coat, which contributes to its water binding properties. Since rhamnose was not detected in the IDF fraction and the same conclusions regarding RGI cannot be made, the pectin present in IDF is likely branched with predominantly galactose and arabinose.

Although the monomeric analysis by alditol acetate derivatization was helpful in gaining insight into potential polysaccharides present in camelina fiber, confirmation of these fiber types is still necessary. Future directions of this project will include the analysis of glycosidic linkages in these fiber fractions to obtain an accurate profiling of camelina dietary fiber.

3.6 Conclusions

Dietary fiber isolated from DCM was quantified and found to be present in higher concentrations than previously studied Brassicaceae relatives (canola and rapeseed). The majority of the dietary fiber was insoluble dietary fiber (~90%). Monosaccharide analysis allowed the prediction of fiber types in different fiber fractions. Camelina contains RFO and pectin, which are fiber types that have been shown to have health benefits. Oil pressing temperature did not have an effect on camelina fiber composition and distribution. Although previous studies have reported individual characteristics of camelina fiber, this is the first comprehensive chemical characterization of camelina dietary fiber. Understanding the composition and structure of camelina fiber can help predict functional properties and applicability in food systems.

4 Overall Conclusions, Implications, Recommendations

Isolation, evaluation, and characterization of protein and dietary fiber from camelina was successfully carried out. Salt precipitation of protein from DCM resulted in CPC with higher purity and more favorable functionality than CPC produced by pH solubilization. In some cases, salt extracted CPC had comparable or better functional properties than SPI, namely solubility at pH 3.4, emulsification capacity and activity index, foaming capacity and stability, and water holding capacity. Structural characterization revealed that CPC have overall low molecular weights and high denaturation temperatures, which is in agreement with the cruciferin and napin proteins of related Brassicaceae crops. These specific traits hindered the gelation properties of CPC. Controlled enzymatic hydrolysis was investigated in an attempt to improve functional properties. Aside from increasing solubility at pH 7.0 post thermal treatment, hydrolysis demonstrated few beneficial effects. Oil pressing conditions had minimal effect if any on protein extraction yield, protein structure, and functionality of CPC.

Dietary fiber was isolated from DCM. The majority of TDF was insoluble fiber. The SDFS fraction consisted mainly of raffinose and stachyose, as the dietary fiber portion, and free mono- and disaccharides (glucose, fructose, sucrose). Pectin was found in both the IDF and the SDFP fractions. When analyzed for degree of methylation, camelina pectin was found to be mostly low methoxyl pectin. Glucose was the main component of IDF, in addition to galactose, mannose, xylose, and arabinose. This composition indicated the presence of cellulose, galactomannans, and arabinoxylans in IDF. SDFP had an even distribution of galactose, mannose, rhamnose, and arabinose, indicating the presence of galactomannans and pectin. Little if any effect of oil pressing temperature on fiber composition and distribution was observed.

Understanding the chemical structure and function of camelina protein provide the basis for potential food applications. Some functional properties of CPC are comparable or better than SPI, indicating that camelina protein may be a viable and novel plant protein option in different applications, such as high protein acidic beverages, given their relatively higher solubility at acidic pH. The small molecular weight of camelina proteins renders CPC not suitable for gelling applications. However, camelina's small protein size may

hinder protein-protein interactions and polymerization in systems such as protein bars, which often harden over storage due to protein polymerization. Therefore CPC might be a better option than SPI in protein bars. Additionally, as of yet CPC is a non-allergenic and non-GM ingredient, which are favorable and marketable traits over SPI.

Camelina fiber also has potential to act as a functional and nutritional ingredient in food products. Camelina is high in dietary fiber, which can help address the vast under-consumption of dietary fiber in westernized diets. Additionally, camelina fiber can contribute viscosity and rheological properties to foods, especially due to the low methoxyl pectin that predominates in DCM.

In light of these potential applications for camelina ingredients, further work must be done to increase camelina's marketability. Research on extraction or masking of flavor compounds in camelina would be useful to address any unpleasant, characteristic aromas or tastes that are imparted to final products. Additionally, antinutritional factors limit camelina's feasibility for inclusion in feed and food products at higher rates. More work regarding processing treatments needs to be done in order to reduce levels of antinutritional factors, such as erucic acid and phytates, by thermal, solvent extraction, or filtration techniques, while managing glucosinolate levels to balance positive and negative nutritional effects. Furthermore, this study provides a springboard for continued investigation into the structure of camelina dietary fiber. Glycosidic linkage analysis would complement the alditol acetate analysis well, allowing for more conclusive results to be drawn regarding the fiber types present. Finally, investigating different enzymes for enzymatic hydrolysis of camelina protein may result in directed functionality enhancement, as different enzymes produce different peptides with different functionality.

Overall, camelina, a crop with several benefits, has the potential to be a source of nutritional and functional, plant-based ingredients. Although previous studies have reported individual characteristics of camelina protein and fiber, this is the first comprehensive chemical characterization of these constituents. This work provides a foundation for future exploration of camelina as a novel ingredient source for food applications.

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Appendix A. Sample Calculation for Determining % Degree of Hydrolysis (DH) by pH Stat Method

$$\% DH = \left(\frac{h}{h_{tot}}\right) x\ 100 = \left(\frac{B\ \times N_b}{MP \times \alpha \times h_{tot}}\right) \times 100$$

Where:

B = the volume of the NaOH necessary to keep the pH constant = 0.43 mL

N_b = Normality of the NaOH = 0.2 N

MP is the mass of the protein = 4 g

h_{tot} = the total number of peptide bonds for soy protein expressed in equivalents of free amines = 7.75 meq/g (soy was used as a reference protein)

α = the degree of dissociation of the α-NH_2 groups and is expressed as:

$\alpha = 1/(1 + 10^{pK-pH})$

The pK value varies significantly with temperature and can be estimated as follows:

pK = 7.8 + (298-T)/(298 x T) x 2400

Where T is the absolute temperature (K)

At 40°C, pK = 7.8 + [(298-(40 + 273))/(298*(40 + 273))] = 7.80

Therefore, at pH 6.0, $\alpha = 1/(1 + 10^{7.8-6.0}) = 0.016$

$$\%DH = \left(\frac{0.43\ mL\ x\ 0.2\frac{moles}{L}}{4\ g \times 0.016 \times 7.75\frac{meq}{g}}\right) \times 100 = 7.34\%$$

Appendix B. Sample Calculation for Determining Surface Hydrophobicity Index

Determining Net Relative Fluorescence Intensity (RFI) at a single protein concentration

$RFI_{Initial} = Sample_{NoANS} - Blank_{NoANS}$

$RFI_{Final} = Sample_{ANS} - Blank_{ANS}$

$Net\ RFI = RFI_{Final} - RFI_{Initial}$

Where:

$Sample_{NoANS}$ = fluorescence of protein sample before ANS probe is added
$Blank_{NoANS}$ = fluorescence of buffer blank before ANS probe is added
$Sample_{ANS}$ = fluorescence of protein sample after ANS probe is added and 15 min hold time
$Blank_{ANS}$ = fluorescence of buffer blank after ANS probe is added and 15 min hold time

Example calculation for cold press pH extracted protein (0.005%)

$RFI_{Initial} = 13 - 12 = 1$

$RFI_{Final} = 33 - 10 = 23$

$Net\ RFI = 23 - 1 = 22$

Net RFI values for all protein solutions (0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.05%) are plotted against protein concentration as shown below.

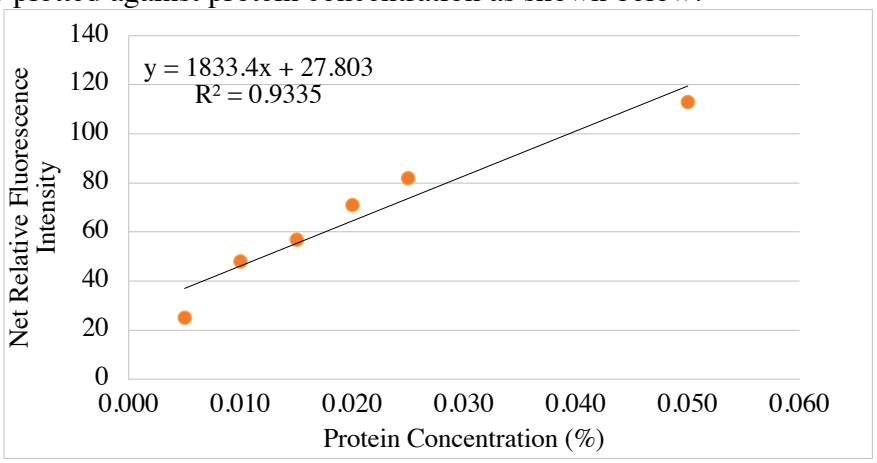

Figure 10. Net Relative Fluorescence Intensity (RFI) plotted against protein concentration (%) for cold press pH extracted protein to determine surface hydrophobicity index.

The slope of the trendline in **Figure 10** is the surface hydrophobicity index (1833.4). Three replicates are averaged to determine final surface hydrophobicity index.

Appendix C. Sample Calculation for Water Holding Capacity (WHC)

$$Water\ Holding\ Capacity = 100\ \times \left(\frac{T_3 - T_1}{T_2 - T_1}\right)$$

$$Water\ Holding\ Capacity = 100\ \times \left(\frac{1.9787 - 1.2048}{1.9833 - 1.2048}\right) = 99.41\%$$

Where:

T_1 = Mass of protein solution before heating

T_2 = Mass of protein solution + microcentrifuge tube after cooling

T_3 = Mass of protein solution + microcentrifuge tube after draining excess water

Appendix D. Sample Calculation for Emulsification Capacity (EC)

$$Emulsification\ Capacity = \frac{(\ Volume_{oil\ titrated}\ x\ Density_{oil})}{Grams\ of\ Protein}$$

$$Emulsification\ Capacity\ (EC) = \frac{\left(63\ mL\ x\ 0.868\frac{g}{mL}\right)}{0.05\ g}$$
$$= 1093.7\ g\ oil/g\ protein$$

Where:

0.868 g/mL = density of corn oil

0.05 g = grams protein in 5 mL of a 1% protein solution

Appendix E. Sample Calculation for Emulsification Stability (ES) and Emulsification Activity Index (EAI)

Emulsification Stability:

$$ES = \frac{Abs_0}{Abs_0 - Abs_{10}} \times 10\ min$$

$$ES = \frac{0.333}{0.333 - 0.115} \times 10\ min = \mathbf{15.23\ min}$$

Where:

A_0 = the absorbance at t = 0 min

A_{10} = the absorbance at t = 10 min.

Emulsification Activity Index:

$$EAI\left(\frac{m^2}{\mathrm{g}}\right) = \frac{2T}{(1-\phi)C} = \frac{2(2.303)A_0}{l(1-\phi)C} = \frac{2(2.303 \times A_0)}{l(1-\phi)C} =$$

$$= \frac{2(2.303 \times 0.333)}{(0.01)(1-0.25)(1)} = \mathbf{204.5\ m^2/g}$$

Where:

C = weight of protein per volume of aqueous phase

= 0.1% protein solution = 0.1g/100mL = 1g/m^3

Φ = volume fraction of oil = 2 mL of oil in 6 mL of 0.1% protein solution

= (2 mL)/(2 mL + 6 mL) = 0.25

T = turbidity of the oil at 500 nm

$$Turbidity\ of\ Oil\ (T) = \frac{2.303A_0}{l}$$

l = path length of the cuvette = 10 mm = 0.01 m

Appendix F. Sample Calculation for Foaming Capacity (FC) and Foaming Stability (FS)

Foaming Capacity:

$$FC = \frac{(Total\ solution\ volume - \ Liquid\ volume\ )}{Grams\ of\ protein}$$

$$FC = \frac{(260\ mL - 5\ mL\ )}{0.25\ g} = 1020\ {}^{mL\ foam}/_{g\ protein}$$

Foaming Stability:

$$FS = \frac{(Total\ solution\ volume_{30\ min} - \ Liquid\ volume_{30\ min}\ )}{(Total\ solution\ volume_{0\ min} - \ Liquid\ volume_{0\ min}\ )} \times 100$$

$$FS = \frac{(240\ mL - \ 47\ mL\ )}{(260\ mL - \ 5\ mL\ )} \times 100 = 75.7\%$$

Appendix G. Analysis of Variance (ANOVA) Summary Tables for Chapter 2 Statistics

Table 10. Analysis of variance of the effect of extraction protocol on protein yield.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs	Extraction protocol	3	19.477	4.821	0.081
	Error	4	4.040		

Table 11. Analysis of variance of the effect of extraction protocol on protein purity.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs	Extraction protocol	3	81.975	16.255	0.010
	Error	4	5.043		

Table 12. Analysis of variance of the effect of extraction protocol on surface hydrophobicity.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	1150212	119.795	0.000
	Error	12	9602		

Table 13. Analysis of variance of the effect of extraction protocol on gel strength.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	2563718	69.659	0.000
	Error	12	36803		

Table 14. Analysis of variance of the effect of extraction protocol on water holding capacity.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	4.904	6.760	0.002
	Error	14	0.725		

Table 15. Analysis of variance of the effect of extraction protocol on emulsification capacity.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	405796	31.808	0.000
	Error	12	12757		

Table 16. Analysis of variance of the effect of extraction protocol on emulsification stability.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	315.868	85.910	0.000
	Error	13	3.677		

Table 17. Analysis of variance of the effect of extraction protocol on emulsification activity index.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	77077	80.864	0.000
	Error	13	953.170		

Table 18. Analysis of variance of the effect of extraction protocol on foaming capacity.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	485412	1079.837	0.000
	Error	14	449.524		

Table 19. Analysis of variance of the effect of extraction protocol on foaming stability.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI	Extraction protocol	6	61167	184.532	0.000
	Error	14	1980.482		

Table 20. Analysis of variance of the effect of extraction protocol on the pH 7.0 solubility of unheated CPC, CPH, SPI, and WPI

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI, WPI	Extraction protocol	7	3837.985	129.682	0.000
	Error	16	29.595		

Table 21. Analysis of variance of the effect of extraction protocol on the pH 7.0 solubility of heated CPC, CPH, SPI, and WPI.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI, WPI	Extraction protocol	7	3502.403	190.815	0.000
	Error	16	18.355		

Table 22. Analysis of variance of the effect of extraction protocol on the pH 3.4 solubility of unheated CPC, CPH, SPI, and WPI.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI, WPI	Extraction protocol	7	1665.418	63.675	0.000
	Error	15	26.155		

Table 23. Analysis of variance of the effect of extraction protocol on the pH 3.4 solubility of heated CPC, CPH, SPI, and WPI.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press, pH and salt extracted CPCs and CPHs, SPI, WPI	Extraction protocol	7	1705.233	67.538	0.000
	Error	15	25.248		

Appendix H. Sample Chromatograms from LC-MS Analysis of SDFS Fractions

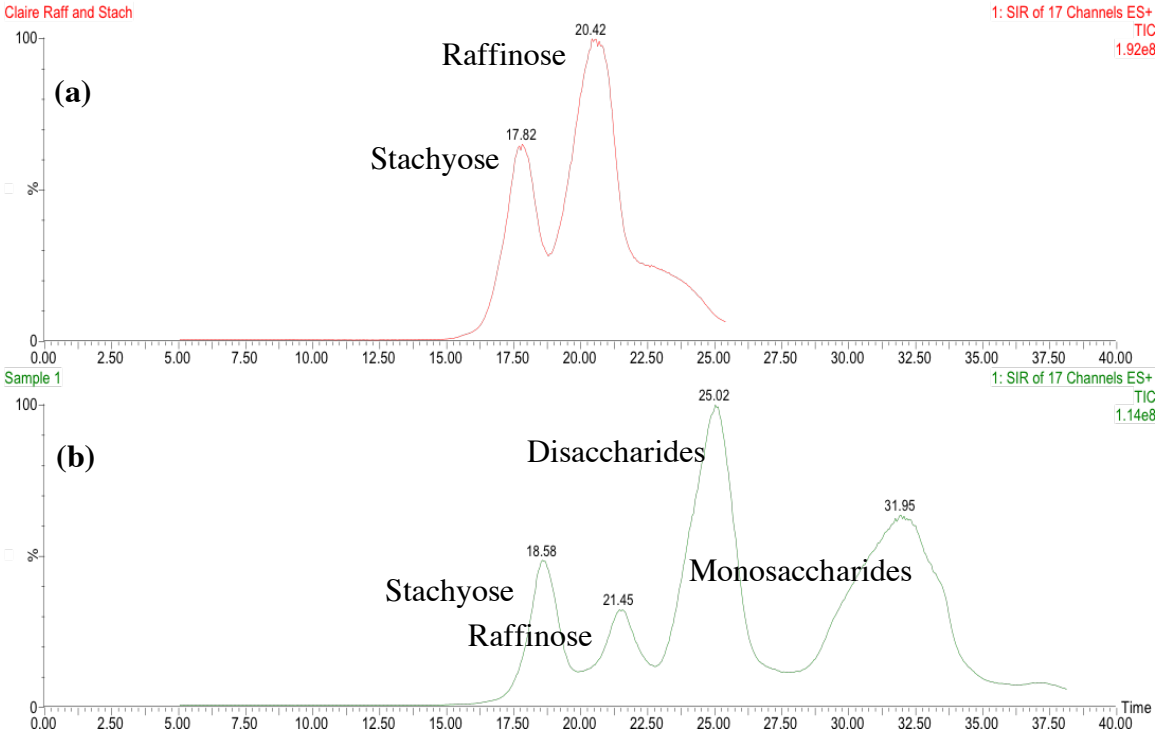

Figure 11. Chromatograms from LC-MS analysis of raffinose and stachyose standards **(a)** and SDFS fraction of cold press DCM **(b)**.

Appendix I. Chromatograms from HPAEC-PAD Analysis for Standards and Samples

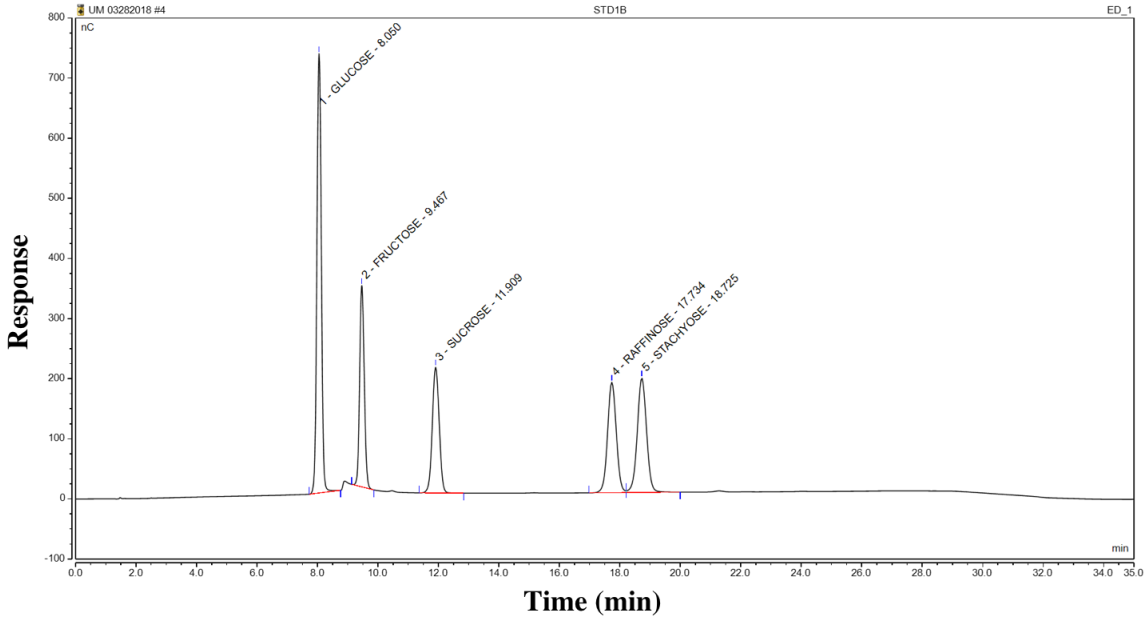

Figure 12. HPAEC-PAD chromatogram for saccharide standard mixture (0.1 mg/mL).

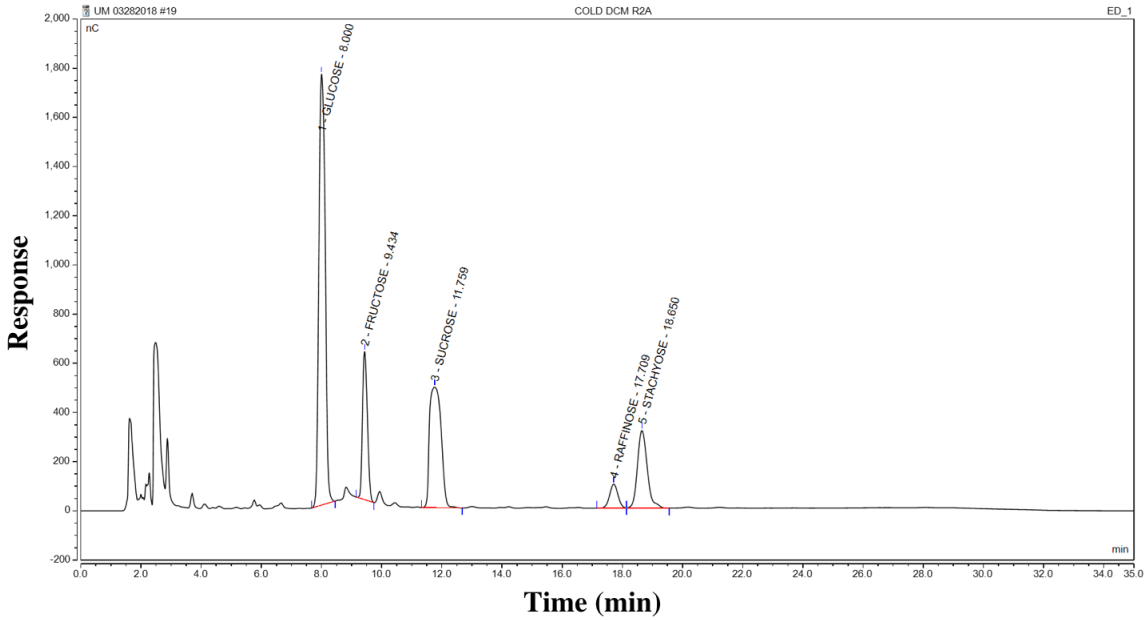

Figure 13. HPAEC-PAD chromatogram for saccharide quantification of cold press SDFS fraction.

Appendix J. Sample Calculation for Sucrose, Maltose, and Glucose Concentrations in DCM

A_2 = Final absorbance at 340 nm
A_1 = Initial absorbance at 340 nm
$\Delta A = (A_2 - A_1)_{sample} - (A_2 - A_1)_{sample\ blank}$

Determination of sucrose:

$$\Delta A_{sucrose} = \Delta A_{sucrose\ +\ free\ glucose} - \Delta A_{free\ glucose}$$

Determination of maltose:

$$\Delta A_{maltose} = \Delta A_{maltose\ +\ sucrose} - \Delta A_{sucrose}$$

Determination of maltose, sucrose, and glucose concentrations:

$$\text{Concentration } [{}^{g}/_{L}] = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A$$

Where:
V = final volume (mL)
MW = molecular weight of the substance assayed (g/mol)
ε = extinction coefficient of NADPH at 340 nm = 6300 ($l \times mol^{-1} \times cm^{-1}$)
d = light path (cm)
v = sample volume (mL)
2 = 2 molecules of glucose released from each molecule of maltose hydrolyzed

For sucrose:

$$\text{Concentration } [{}^{g}/_{L}] = \frac{1.31 \times 342.3}{6300 \times 1.0 \times 0.05} \times \Delta A_{sucrose}$$

$$\text{Concentration } [{}^{g}/_{L}] = 1.4235 \times \Delta A_{sucrose}$$

For maltose:

$$\text{Concentration } [{}^{g}/_{L}] = \frac{1.31 \times 342.3}{6300 \times 1.0 \times 0.05 \times 2} \times \Delta A_{maltose}$$

$$\text{Concentration } [{}^{g}/_{L}] = 0.7118 \times \Delta A_{maltose}$$

For glucose:

$$\text{Concentration } [{}^{g}/_{L}] = \frac{1.31 \times 180.16}{6300 \times 1.0 \times 0.05} \times \Delta A_{glucose}$$

$$\text{Concentration } [{}^{g}/_{L}] = 0.7492 \times \Delta A_{glucose}$$

Example calculation for sucrose concentration:

Determining corrected $\Delta A_{sucrose + free\ glucose}$

Sucrose sample: $A_1 = 0.1590$
$A_2 = 0.7431$
$\Delta A = 0.7431 - 0.1590 = 0.5841$
Sucrose sample blank: $A_1 = 0.1572$
$A_2 = 0.1523$
$\Delta A = 0.1523 - 0.1572 = -0.0049$
Corrected $\Delta A_{sucrose + free\ glucose} = 0.5841 - (-0.0049) = 0.5890$

Determining corrected $\Delta A_{free\ glucose}$

Glucose sample: $A_1 = 0.1683$
$A_2 = 0.2549$
$\Delta A = 0.2549 - 0.1683 = 0.0866$
Glucose sample blank: A1 = 0.1716
$A_2 = 0.1679$
$\Delta A = 0.1679 - 0.1716 = -0.0037$
Corrected $\Delta A_{free\ glucose} = 0.0866 - (-0.0037) = 0.0903$

Determining corrected $\Delta A_{sucrose}$

$\Delta A_{sucrose} = \Delta A_{sucrose\ +\ free\ glucose} - \Delta A_{free\ glucose}$
$= 0.5890 - 0.0903 = 0.4987$

Calculating sucrose concentration based on corrected absorbances

$$\text{Concentration } [{}^{g}/_{L}] = \frac{1.31 \times 342.3}{6300 \times 1.0 \times 0.05} \times 0.4987$$

$$\text{Concentration } [{}^{g}/_{L}] = 1.4235 \times 0.4987 = 0.710$$

Converting sucrose concentration (g/L) to % sucrose in DCM

$$\%\ \text{sucrose in DCM} = \frac{0.710}{20.05\ {}^{g}/_{L}\ \text{DCM}} \times 100 = 3.541\%$$

Where 20.05 g/L DCM is the converted mass of sample weighed (0.2005 g DCM into 10 mL)

Appendix K. Sample Calculation for Pectin Concentration in DCM

Blanks		Standards				
μg GalA/mL	Abs	μg GalA/mL	Rep	Abs	Corrected Abs	Average
0	0.0004	0	1	0.0096	0.0092	0.0067
			2	0.0080	0.0076	
			3	0.0037	0.0033	
20	0.0050	20	1	0.1048	0.0998	0.1035
			2	0.1075	0.1025	
			3	0.1132	0.1082	
40	0.0085	40	1	0.2309	0.2224	0.2208
			2	0.2351	0.2266	
			3	0.2220	0.2135	
60	0.0131	60	1	0.3929	0.3798	0.3784
			2	0.3934	0.3803	
			3	0.3881	0.3750	
80	0.0143	80	1	0.5714	0.5571	0.5376
			2	0.5373	0.5230	
			3	0.5470	0.5327	
100	0.0178	100	1	0.7275	0.7097	0.7310
			2	0.7711	0.7533	
			3	0.7477	0.7299	
120	0.0204	120	1	0.8654	0.8450	0.8864
			2	0.8979	0.8775	
			3	0.9571	0.9367	
160	0.0228	160	1	1.3632	1.3404	1.3610
			2	1.3718	1.3490	
			3	1.4163	1.3935	
200	0.0276	200	1	1.9228	1.7490	1.7570
			2	1.9357	1.7572	
			3	1.9527	1.7647	
240	0.0310	240	1	2.3265	2.2955	2.2722
			2	2.2689	2.2379	
			3	2.3143	2.2833	

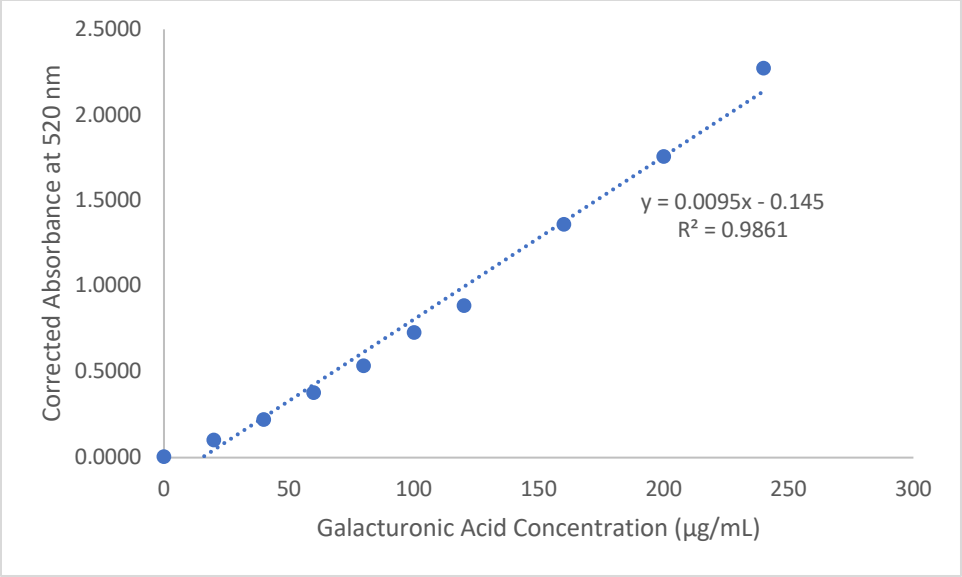

Figure 14. Absorbance values at 520 nm for galacturonic acid standards (µg/mL) in order to quantify pectin concentration. R^2 values indicate goodness of fit for the trend-line.

Determining galacturonic acid concentration

$$\text{Galacturonic acid concentration } \left({}^{\mu g}/_{mL}\right) = \frac{A + 0.145}{0.0095} \times 100$$

Where:
A = Corrected Absorbance = $A_{sample} - A_{sample\ blank}$
0.145 = standard curve y-intercept
0.0095 = standard curve slope
100 = accounting for sample dilution factor

Determining % pectin in each of the three extracts

$$\%\ \text{Pectin}_E \left({}^{\text{mg pectin}}/_{\text{mg fiber sample}}\right) = \frac{\frac{G}{1000}}{161.9} \times 100$$

Where:
% Pectin_E = % pectin in each extract (water, ammonium oxalate, or sodium hydroxide)
G = Galacturonic acid concentration (μg/mL)
1000 = conversion from μg to mg
161.9 = mass of fiber sample (mg)
100 = conversion to %

Determining % pectin in each fiber fraction (IDF or SDFP)

$$\%\ \text{Pectin}_{FF} = \%\ \text{Pectin}_{\text{water extract}} + \%\ \text{Pectin}_{\text{ammonium oxalate}} + \%\ \text{Pectin}_{\text{sodium hydroxide}}$$

Where:

% $Pectin_{FF}$ = % Pectin in each fiber fraction (IDF or SDFP)

Determining % pectin in DCM contributed by each fiber fraction (IDF or SDFP)

$$\% \text{ pectin in DCM by fiber fraction} = [\% \text{ Pectin}_{FF} \times \left(\frac{100}{F}\right) \times 100]$$

Where:
% $Pectin_{FF}$ = % pectin in fiber fraction
F = % fiber fraction in DCM

Determining total % pectin in DCM contributed by both IDF and SDFP

$$\text{Total \% pectin in DCM from all fiber fractions} = \% \text{ pectin in DCM}_{IDF} + \% \text{ pectin in DCM}_{SDFP}$$

Example calculation for pectin concentration in cold press DCM:

Determining galacturonic acid concentration in cold press IDF water extract

$$\text{Galacturonic acid concentration } \left({}^{\mu g}/_{mL}\right) = \frac{(0.1700 - 0.0333) + 0.145}{0.0095} \times 100 = 29.65$$

Determining % pectin in cold press IDF water extract

$$\% \text{ Pectin}_{E} \left({}^{\text{mg pectin}}/_{\text{mg fiber sample}}\right) = \frac{\frac{29.65}{1000}}{161.9} \times 100 = 1.83\%$$

Determining % pectin in IDF fraction

% PectinFF = 1.83% + 1.71% + 2.23% = 5.77%

Determining % pectin in DCM contributed by IDF

$$\% \text{ pectin in DCM by fiber fraction} = [5.77\% \times \left(\frac{100}{0.4907}\right) \times 100] = 2.846\%$$

Where 0.4907 is the percent of IDF in DCM (49.07%)

Determining total % pectin in DCM contributed by both IDF and SDFP

Total % pectin in DCM from all fiber fractions = 2.846% + 0.483% = 3.329%
Where 0.483% is the pectin contributed by SDFP (calculations not shown)

Appendix L. ^{1}H NMR Spectra for Determination of Pectin Degree of Methylation

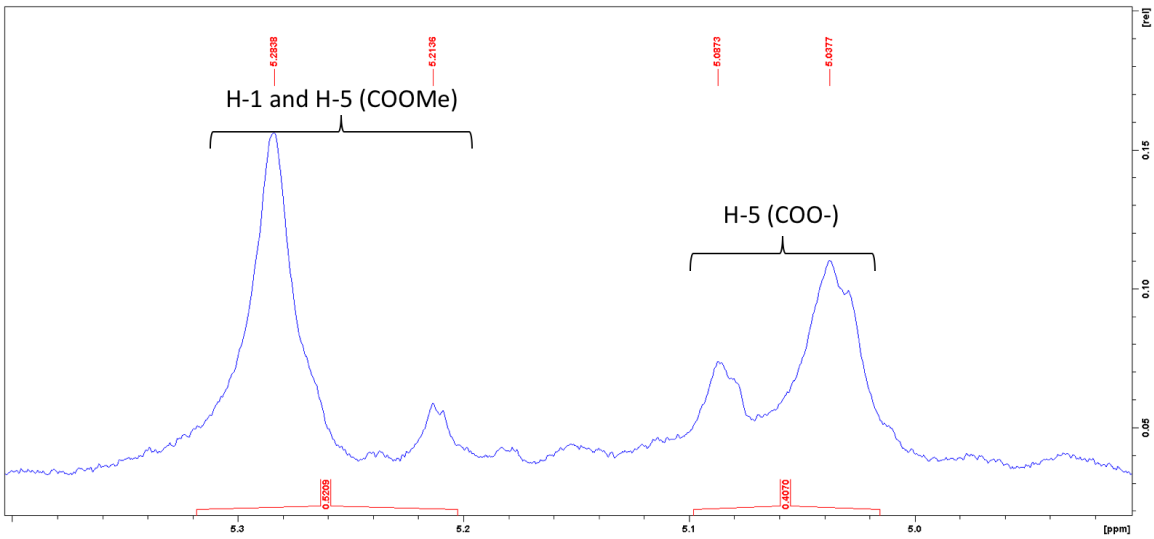

Figure 15. ^{1}H NMR spectrum for cold press SDFP to determine pectin degree of methylation.

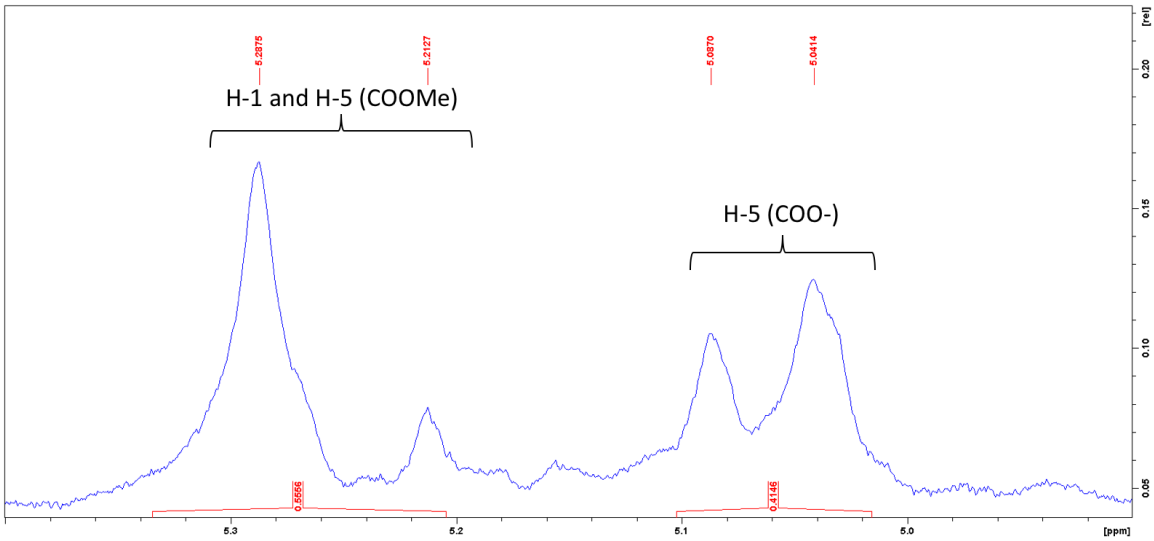

Figure 16. ^{1}H NMR spectrum for hot press SDFP to determine pectin degree of methylation.

Appendix M. Chromatogram of Standard Compounds in Alditol Acetate Analysis

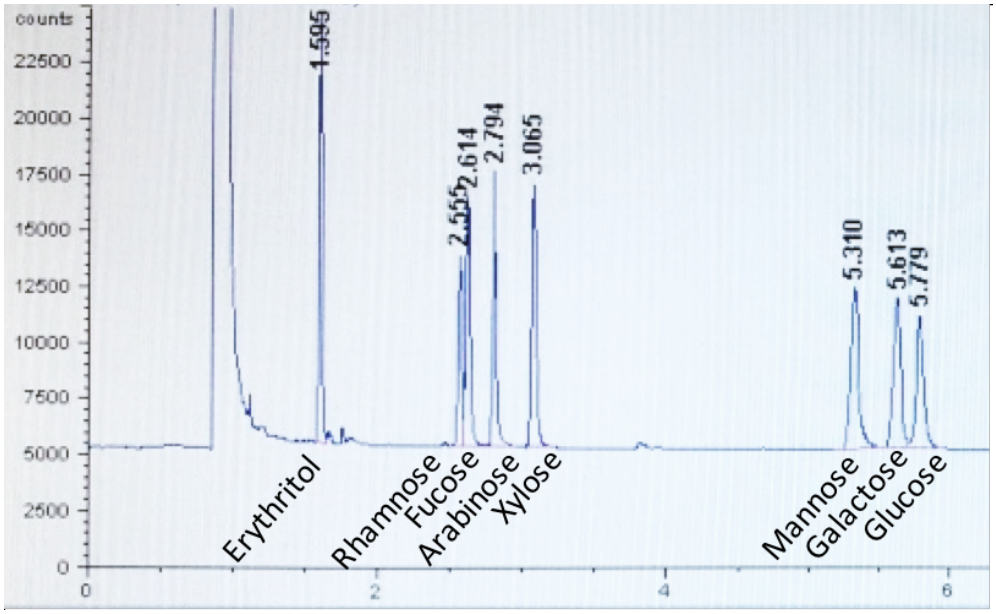

Figure 17. Gas chromatogram of standard compounds used in alditol acetate analysis.

Appendix N. Sample Calculations for Determining Response Factors and Monosaccharide Concentrations

Determining internal response factors

$$\text{Internal Response Factor} = \frac{\text{Area}_{IS} \times \text{Concentration}_{CI}}{\text{Concentration}_{IS} \times \text{Area}_{CI}}$$

Where:
IS = Internal Standard (erythritol)
CI = Compound of Interest
IRF = Internal Response Factor

Determining monosaccharide concentrations using IRF

$$\text{Concentration}_{CI} = \frac{\text{Area}_{CI} \times \text{Concentration}_{IS} \times \text{IRF}_{CI}}{\text{Area}_{IS}}$$

Converting monosaccharide concentration (mg/mL) to mg/g fiber fraction

$$\text{Concentration}\left(\frac{\text{mg}}{\text{g fiber fraction}}\right) = \frac{C \times 10}{30} \times 1000$$

Where:
C = $\text{Concentration}_{CI}$ (mg/mL)
10 = dilution volume (mL)
30 = mg fiber sample
1000 = conversion from mg to g

Converting monosaccharide concentrations (mg/g fiber fraction) to % of total neutral monosaccharide composition

$$\%\ \text{monosaccharide} = \frac{C_M}{S} \times 100$$

Where:
C_M = Concentration of monosaccharide of interest (mg/g fiber fraction)
S = Sum of all monosaccharide concentrations (mg/g fiber fraction)
100 = Conversion to %

Example calculation for rhamnose concentration in cold press SDFP:

Determining internal response factor for rhamnose

$$\text{Internal Response Factor} = \frac{25248.1 \times 0.5\ \text{mg/mL}}{0.8\ \text{mg/mL} \times 14835.6} = 1.0637$$

Determining concentration of rhamnose in cold press SDFP

$$\text{Concentration of Rhamnose} = \frac{2279.6 \times 0.8 \text{ mg/mL} \times 1.0637}{27959.9} = 0.069 \text{ mg/mL}$$

Converting rhamnose concentration (mg/mL) to mg/g SDFP

$$\text{Concentration} \left(\frac{\text{mg}}{\text{g fiber fraction}}\right) = \frac{0.069 \text{ mg/mL} \times 10}{30} \times 1000 = 23.13 \text{ mg/g SDFP}$$

Converting rhamnose concentration (mg/g SDFP) to % of total neutral monosaccharide composition

$$\%\ \text{Rhamnose} = \frac{23.13}{23.13 + 20.33 + 28.72 + 27.60} \times 100 = 23.2\%$$

Where:

20.33 = Arabinose (mg/g SDFP) (calculations not shown)
28.72 = Mannose (mg/g SDFP) (calculations not shown)
27.60 = Galactose (mg/g SDFP) (calculations not shown)

Appendix O. Analysis of Variance (ANOVA) Summary Tables for Chapter 3 Statistics

Table 24. Analysis of variance on the effect of oil pressing temperature on IDF, SDFP, and SDFS.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF	Oil pressing temperature	1	14.512	1.424	0.355
	Error	2	10.190		
Cold and hot press SDFP	Oil pressing temperature	1	15.858	66.685	0.015
	Error	2	0.238		
Cold and hot press SDFS	Oil pressing temperature	1	0.009	1.700	0.262
	Error	4	0.006		

Table 25. Analysis of variance on the effect of oil pressing temperature on glucose content of SDFS fractions as determined by HPAEC-PAD.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFS	Oil pressing temperature	1	19.253	85.775	0.001
	Error	4	0.224		

Table 26. Analysis of variance on the effect of oil pressing temperature on fructose content of SDFS fractions as determined by HPAEC-PAD.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFS	Oil pressing temperature	1	10.157	54.689	0.002
	Error	4	0.186		

Table 27. Analysis of variance on the effect of oil pressing temperature on sucrose content of SDFS fractions as determined by HPAEC-PAD.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFS	Oil pressing temperature	1	2.807	2.373	0.198
	Error	4	1.183		

Table 28. Analysis of variance on the effect of oil pressing temperature on raffinose content of SDFS fractions as determined by HPAEC-PAD.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFS	Oil pressing temperature	1	1.658	24.047	0.008
	Error	4	0.069		

Table 29. Analysis of variance on the effect of oil pressing temperature on stachyose content of SDFS fractions as determined by HPAEC-PAD.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFS	Oil pressing temperature	1	5.086	21.884	0.009
	Error	4	0.232		

Table 30. Analysis of variance on the effect of oil pressing temperature on sucrose content of SDFS fractions as determined enzymatically.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press DCM	Oil pressing temperature	1	0.008	0.202	0.697
	Error	2	0.039		

Table 31. Analysis of variance on the effect of oil pressing temperature on maltose content of SDFS fractions as determined enzymatically.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press DCM	Oil pressing temperature	1	0.030	12.126	0.073
	Error	2	0.002		

Table 32. Analysis of variance on the effect of oil pressing temperature on glucose content of SDFS fractions as determined enzymatically.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press DCM	Oil pressing temperature	1	0.033	71.800	0.014
	Error	2	0.000		

Table 33. Analysis of variance on the effect of oil pressing temperature on glucose content of SDFS fractions as determined enzymatically.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press DCM	Oil pressing temperature	1	0.033	71.800	0.014
	Error	2	0.000		

Table 34. Analysis of variance on the effect of oil pressing temperature on pectin content within fiber fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFP and IDF	Oil pressing temperature	3	3.495	187.082	0.000
	Error	8	0.019		

Table 35. Analysis of variance on the effect of oil pressing temperature on total pectin content in DCM.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press DCM	Oil pressing temperature	1	1.188	32.928	0.005
	Error	4	0.036		

Table 36. Analysis of variance on the effect of oil pressing temperature on pectin degree of methylation.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFP	Oil pressing temperature	1	4.060	87.836	0.011
	Error	2	0.046		

Table 37. Analysis of variance on the effect of oil pressing temperature on rhamnose content in SDFP fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press SDFP	Oil pressing temperature	1	3.069	0.169	0.690
	Error	10	18.181		

Table 38. Analysis of variance on the effect of oil pressing temperature on arabinose content in IDF and SDFP fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF and SDFP	Oil pressing temperature	3	26.631	3.187	0.046
	Error	20	8.357		

Table 39. Analysis of variance on the effect of oil pressing temperature on xylose content in IDF fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF	Oil pressing temperature	1	3.898	0.899	0.365
	Error	10	4.337		

Table 40. Analysis of variance on the effect of oil pressing temperature on mannose content in IDF and SDFP fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF and SDFP	Oil pressing temperature	3	10.718	13.918	0.000
	Error	20	0.770		

Table 41. Analysis of variance on the effect of oil pressing temperature on galactose content in IDF and SDFP fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF and SDFP	Oil pressing temperature	3	302.758	73.180	0.000
	Error	20	4.137		

Table 42. Analysis of variance on the effect of oil pressing temperature on glucose content in IDF fractions.

Sample Analysis	Source of Variation	Degrees of Freedom	Mean Square	F	Sig.
Cold and hot press IDF	Oil pressing temperature	1	20.300	0.716	0.417
	Error	10	28.371		