Effects of isolation condition and spray drying on camelina gum yield and properties

by

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Abstract

Camelina sativa (L). Crantz that belongs to Brassicaceae family has been grown as a dicotyledonous oilseed crop in the cold places like America and Canada. Camelina seeds are widely used for the extraction of oil and protein. Recently, research found that camelina gum is an excellent candidate for food and industrial uses as thickener or stabilizer. The objectives of this research were 1) to increase camelina gum isolation efficiency using spray drying technology, and 2) to develop an innovative method to remove gum from seed bran to increase protein and oil extraction efficiency and quality.

The camelina gums isolated using ethanol precipitation and spray drying method from the whole camelina seeds were compared. Effects of spray drying temperature on yield, gum morphology, and gum rheological and thermal properties were studied. The representative sample dried at 165°C was chosen to study the effects of concentration, temperature, pH and additives (NaCl, $CaCl_2$, sucrose, and ethanol) on viscosity and viscoelastic properties of the isolated gum. The gum showed a shear thinning behavior when shear rate increased gradually, higher concentrations of additives only slightly affect the rheological properties. Results showed that spray drying is an effective method in terms of saving time and energy, and provided positive rheology benefits on camelina gum isolation.

Pre-removal of gum from camelina seeds can increase protein and oil yield and their quality. Decortication can separate 10-17% of the total camelina seed as bran. A wind tunnel was used to separate lighter bran particles from heavier endosperm and unbroken seeds. Camelina gum isolation from the separated seed bran using the traditional ethanol precipitation method was optimized using response surface methodology where the simultaneous effect of the three independent variables (seed bran to water ratio, isolation temperature, and isolation time) were

investigated for gum yield, purity and optimum rheological properties. Three independent quadratic modules were developed and the original data fitted the models fitted (R^2 = 0.995, 0.877, and 0.804). The optimal isolation conditions were seed bran to water ratio of 1:39, isolation temperature of 35 ºC, and isolation time of 1.5 h and 0.839 desirability was obtained by the rigorous statistics analysis. The protein yield and quality extracted from decorticated endosperm were improved significantly compared with that extracted from whole seeds meal without decortication. In addition, the degumming step can be eliminated before protein and oil extraction that increase protein and oil extraction efficiency.

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Dedication

I dedicate my thesis work to my loving parents, Hongyi Cao and Jian Zhang for their support, encouragement and helping me develop my life skills.

Chapter 1 - Introduction

1.1 Introduction

Camelina sativa (L.) Crantz as healthy supplier and clean sources of energy has widely planted in Canada and America as an oilseed crop (Berti et al., 2016; Pilgeram, 2007). The plant can growth under cold and low rainfall environment and soil conditions (Sintim et al., 2016; Zubr, 2003). It have strong cold and pesticides resistant ability with a shorter growth period (85 to 100 days) and can germinate and emerge in the early spring (Sintim et al., 2016). However, this crop has a lower seeding rate at 4-6 kg/ha compare to other oilseed, like rapeseed (Christensen and Drabble, 1984). The camelina seed is very small and light, around 0.7 mm x1.5 mm and 1,000-seed weight ranging between 0.8-1.8 g (Berti et al., 2016). The small seed size compared to canola oilseeds may hinder its application in modern agriculture, because the separation procedure is can be complicated and difficult for pre-cleaning or large farm equipment to process (Li et al., 2016). Camelina products have been used in cooking and fuel oil, many previous researches are focus on the utilization like cosmetics and animal feeds (Bernardo et al., 2003; Berti et al., 2016; Taasevigen, 2010; Vollmann et al., 1996; Zubr, 1997).

As a by-product of camelina oil and protein production, camelina gum surrounds the camelina seed may be a source of carbohydrates source (Li et al., 2014). When camelina meal is cold pressed, three steps of degumming procedures are used to obtain pure oil and protein, which is expensive and has to use environmental unfriendly organic reagents. In order to get more pure oil and reduce the refining steps, a pre-degumming process has been introduced (Li et al., 2014; Zhu et al., 2016).

Camelina seed bran is a potential source of soluble polysaccharides which is used as thickener and suspending agent in food and non-food products (Li et al., 2016). Camelina gum has

a unique fibrillary structure linked to each other which can provide unique rheological properties (Li et al., 2016). Camelina gum can be used as a thickener in aqueous solution because of its high viscosity in the low concentration range. It also exhibits a high viscosity at low shear rates, and unique shear thinning properties which can be widely applied to industrial products (Sanchez Gil, 2014). Temperature and additives are not known to affect the gum properties, similar to Xanthan gum (Urlacher and Noble, 1997).

The gum isolation methods have a significant effect on gum yield and quality and on the protein and oil extraction process. In this research, two different drying methods, spray drying and freeze drying, were used to isolate gum from whole camelina seed and seed bran. Previous methods for gum isolation were achieved by using freeze drying after ethanol extraction, no determined the effect of the drying method on gum yield and properties. The first part of this study, attempted to systematically study the effect of spray drying on gum yield and rheological properties, thermal properties and morphology properties of the isolated gums at different drying temperature. The effect of additives on rheological properties was also studied.

In order to convenient use of the various components separately, decortication process was used because the separated components can produce high production yield and quality individually. Effect of the decortication on the gum property and rheological properties and the effect on protein and oil extraction was investigated. Camelina gum isolation from the separated seed bran using the traditional ethanol precipitation method was optimized using response surface methodology with three variables (seed bran to water ratio, isolation temperature and time). The protein extraction quality from the decorticated endosperm was evaluated and compared with the traditional extraction method (Li et al., 2014; Zhu et al., 2016).

1.2 Polysaccharides

Natural gums or hydrocolloids are polysaccharides commonly used as stabilizing, gelling, emulsifier, adhesive, and thickening agent in pharmaceutical, food, and non-food applications. Nowadays, the carbohydrates biosynthesized by plants and photosynthesizing bacteria in the world is around 400 billion tons (Robyt, 2012). The earliest gum is harvest by hand from the specific tree species, the procedure is cumbersome and unsanitary, quality issue cannot be effectively controlled. The early studies only focused on gum arabic and tragacanth gum characteristics as the representatives of exudates gum (Al-Assaf et al., 2008; Montenegro et al., 2012; Whistler, 2012). Later, the studies found that some plant components can be used to make a thick soup and achieve a better food taste through the practice. These products are subjected to further extrusion, extraction, drying and filtering to produce extract gum, such as agar, pectin, gelatin, etc. After the ripe of technology, some hydrocolloids are obtained from the separated plant's seed hull or endosperm (Whistler, 2012; Yapo et al., 2007). Extracting gum from plant seeds is the most convenient method, variety of gum are extracted from plant seeds. Seeds often contain large amounts of oil, protein, and polysaccharides to provide structural support. The polysaccharides obtained from seeds such as guar gum, locust bean gum, camelina gum, durian gum, and flaxseed gum are important source of food additives (Barak and Mudgil, 2014; 2002; Li et al., 2016; Qian et al., 2012; Srichamroen, 2013; Whistler, 2012). Fermentation techniques are also used to biosynthesizing gum such as xanthan, which can obtain pure polysaccharides by chosen the optimal bacterial strains and substrates (Maurer et al., 2012; Urlacher and Noble, 1997). Another way to get hydrocolloids is chemical modification and synthesis. Organic chemistry technology has been used to develop the unique hydrocolloid polymers that have the same or better characteristics compared to ordinary gums. However, the biosynthetic products such as

methylcellulose and carboxyvinyl polymers need more time to approval by customer (Wang and Cui, 2005; Yang and Zhu, 2007).

Gums are dried sticky colloids carbohydrate with uncrystallized and brittle mass that can dissolve or swell in aqueous solution. The internal structure of polysaccharides affects the nature of its application and is a big challenge to understand the structure and function of the polysaccharides. Monosaccharide composition, linkage patterns, furanosidic and pyranosdic ring size, anomeric configuration, sequences of monosaccharide residues and repeating units and substitutions as well as molecular weight and distribution of polysaccharides have significant effects on the functional properties of gum (Cui, 2005). For example, linear and branched polysaccharides exhibit different solubility and affect rheology properties. Branched polymer may disrupt intermolecular association and dissolve in aqueous solution readily compared with linear molecules at the same molecular weight range and shows lower viscosity, which are really hard to retrograde or precipitate (Cui, 2005; Glicksman, 1982; Phillips and Williams, 2009). The research of gum has been relatively popular recently and there is a great market for pure modified gums.

1.3 *Camelina sativa*

1.3.1 Camelina

Camelina sativa (L.) as a member of the Brassicaceae family (mustard family) has distant relationship with canola, the oil, protein and polysaccharide contents in seed are all potential treasure to study. Camelina is also known as ‘gold of pleasure’, ‘wild flax’, ‘linseed dodder’, ‘German sesame’, ‘leindotter’ and ‘flax seed’ (Berti et al., 2016; Pilgeram, 2007). The earliest planted history can be found for camelina is in central Europe since 4000 BCE and originated in Southern Europe and South-West of Asia since the Bronze Age (1800BCE). Evidence indicated camelina seeds were cultivated in Eastern Tukey from 700-900 BC. During the medieval age,

cultivation of camelina was decreased. In the early of 20th century, camelina was occasionally found to be planted in some areas of Europe. Recently, camelina as potential oilseed crop widely spray to the Canada and Northern America, especially Montana. Fig 1.1 shows the *camelina sativa* plant distribution

1.3.2 Camelina plant

Camelina is a fast-growing annual plant with 85 to 100 growth period and harsh climate resistant which can cultivated in both spring and winter season. Research showed that camelina plant is frost tolerant, no seedling damage occurs at the temperature as low as 12 ºF around Montana, and seeds can be germinated as low as 38 ºF (Ehrensing and Guy, 2008). Camelina can be planted in rotation following wheat, barley, peas, or lentils (Grady and Nleya, 2010). Compared to the crop from the same family species, camelina has stronger insect resistant capability, which benefits from the synthetizing of phytoalexins characteristics (Vollmann et al., 2001). There is no serious disease found in camelina plants, such as no damage from cutworms, aphids, bird damage and *Phoma* spp (Berti et al., 2011).

Camelina plant steam are usually smooth or hairy at the bottom, the height at maturity period is 60 to 110 cm. Camelina flowers are autogamous with pear-shaped siliques or pods (5-14mm) which contains 8 to 15 golden, oblong and rough seeds (Berti et al., 2016; Berti et al., 2011). Camelina seed (0.7 mm × 1.5 mm) obtained from the pod are very small, 1,000 seeds weight is only 0.8-1.8 g depends on the variety species and growth environment (Ehrensing and Guy, 2008; Sintim et al., 2016). Special harvest settings need to be adjusted for camelina harvest rate, including combine speed, wind flow power, and screen size for sifter (Sintim et al., 2016). In Montana, camelina can yield 1,300 to 2,000 lb/acre under the normal rainfall rate. Inappropriate harvesting work may cause seed shattering. Dark brown color present may cause by the ripening

and long storage period. Fig 1.2 shows a camelina plant. Camelina seed contains 25-45% crude protein, 30-49% oil, 10% crude fiber and 2-3% gum in dry basis.

1.3.3 Camelina oil and protein

Camelina is famous about its 35-39% of omega-3 fatty acid (C 18:3) content in the oil. The crude oil contains 64% polyunsaturated, 30% monounsaturated, and 6% saturated fatty acids. Except the linolenic acid, the same proportion of oleic acid (18:1), linoleic acid (18:2), eicosenoic acid (20:1), and erucic acids (22:1) were reported by multiple studies (Bernardo et al., 2003; Berti et al., 2016; Berti et al., 2011; Katar, 2013; Zubr, 1997). Warm or cold mechanical extraction, solvent extraction, and enzymatic extraction are the major methods used for camelina oil production. Berti et al. (2016) reported the highest oil extraction yield (35.9%) can be obtained with Soxhlet extraction with hexane for 6 h. Crude camelina oil exhibited pure yellow color, accompanied by aroma flavor. The unrefined oil has gamma tocopherol which is vitamin E, have antioxidant ability and can have longer shelf life up to two years. The product remaining after oil extraction, camelina meal contains 10-14% of residual oil, 40% of protein, and 2-3% gum.

Fig 1.3 shows the potential markets for *camelina sativa* (Pilgeram, 2007). Most of the usage are depends on the fatty acid content which can be used for edible and industrial products, such as salad dressing, medicinal use, lamp oil, biodiesel, cattle feed, soaps, skin care products (Berti et al., 2016; Ehrensing and Guy, 2008; Pilgeram, 2007). Camelina oil are mainly used as biodiesel for jet fuel, which can provide lower soot and carbon monoxide emission (Bernardo et al., 2003). Camelina oil used as biodiesel has the similar properties compared with canola as a low cost feedstock (Berti et al., 2011). Camelina oil are used in cosmetics as skin conditioning agents-emollient regulated according to EU regulation. As the personal care products additive, it can use as emollient and pass the skin irritation, allergens and phototoxicity tests (POPA et al., 2017).

The product remaining after oil extraction, camelina meal contains 10-14% of residual oil, 30-40% of protein, 10-12% crude fiber, and 2-3% gum (Li et al., 2015; Russo and Reggiani, 2015; Zhu et al., 2016). The exploitation of the by-product can increase camelina's revenue and utilization (Russo and Reggiani, 2015). The defatted step mixing hexane was introduced before extraction the protein from grounded camelina meal with particle size <0.5 mm. Legumin-type globulins (12S) mixture with napin-type albumins (2S) represents the storage protein predominate in camelina seeds (Russo, 2013). Four fractions of camelina protein can be isolated from the defatted meal, such as albumins, globulins, prolamins, and glutelins (Li et al., 2014). Albumins extracted from camelina meal are water-soluble proteins. The method of isolating glutelin using acid-sedimentation is also mentioned (Zhu et al., 2016), which may form more covalent bonds through oxidation than globulins due to tyrosine contained (Li et al., 2015). The purity of extracted protein was between 55-85%, scanning electron microscopy picture showed some holes and filament substances on the surface of extracted protein which indicates the participation of gum (Li et al., 2015). The folding degree of camelina protein greatly affected its application characteristics. Camelina meal are rich in valuable oil and protein, used as animal feed, pet food, cosmetic, dairy products have been widely studied. 38% of the remaining oil in camelina meal are composed of omega-3 fatty acids which can bring an increase in health, reduces the stress use as the livestock and poultry feeds. Camelina proteins exhibited comparable biological value and appropriate amino acid profile to rapeseeds or other species (Russo, 2013). Camelina meal can be used for producing thermoplastics by grafting various vinyl monomers. The camelina proteins have melting peaks without plasticizers and use as the thermoplastic films exhibited good wet tensile properties (Reddy et al., 2012). Petroleum-based adhesives application is one major

research direction of camelina protein, which showed comparable water resistance to sorghum protein and soy protein (Li et al., 2015).

1.3.4 Camelina gum

Camelina gum are present in the seed bran, which accounts for about one-fourth of the seed bran weight. This gum layer allows the seeds to absorb enough water quickly to supply the seed germination and growth. Hydrolyzed camelina gum showed linear fibrillary structure (4-6 nm diameter), which can interact to each other and form a strong framework to provide viscosity in aqueous solution (Li et al., 2016; Sanchez Gil, 2014). Camelina gum is a mixture of neutral and acidic sugars consisting primarily of arabinose, rhamnose, galactose, glucose, mannose, xylose, glucuronic acid and galacturonic acid (Sanchez Gil, 2014). The research found that camelina gum is similar to gum isolates from locust beans. It can be isolated in small quantity compared with guar gum but are easily discernible. As a plant derivative polysaccharide, it can disperse in both cold and hot water to produce viscous solutions. The colloidal solvent showed shear-thickening properties at low shear rate and shear-thinning properties at high shear rates (Li et al., 2016; Sanchez Gil, 2014). The effects of concentration, pH, temperature, and additives on the rheological properties of camelina gum isolated from the whole seed using freeze drying method was investigated as the control group (Li et al., 2016; Sanchez Gil, 2014).

1.4 Drying methods for gum dehydration

1.4.1 Drying

Drying is a dehydration process, mass and heat transfers take place between the dry air and wet solid or semi-solid, or liquid with low solid content. Temperature, humidity, time and pressure all have significant effects on the drying rate and final product quality. Drying methods also have significant effect on the final products characteristics, such as molecule weight, physical and

chemical properties, appearance and color (Salehi and Kashaninejad, 2015). Air drying, oven drying, spray drying, freeze drying and dehumidification drying are the common methods used in the research and industry. The selection of appropriate drying technology and conditions is important to the desired products characteristics. Freeze drying and spray drying are two major methods used for gum dehydration.

1.4.2 Freeze drying

Freeze drying (or lyophilisation, cryodesiccation) uses low or vacuum pressure surrounding the materials to allow the water molecules sublime directly from solid state into a gaseous state. Steam is then collected by a water vapor condenser to reach the objective of drying. This process may take several weeks to fully remove the moisture. Freeze drying temperature can avoid heat damage and produce pure and colorless products. Low temperature environment is particularly suitable for heat-sensitive substances, as well as inhibited the growth of microorganisms and enzyme. Oxidizable material such as grease, can be protected in the vacuum conditions and get a long-term shelf life without deterioration. Freeze dried material usually have longer preservation period without spoilage, because the moisture content is extremely low, which inhibits the growth of microorganisms and enzymes. The product can quickly be rehydrated to the original moisture content and retain the nutrients after adding moisture, this feature is widely used in the gum extraction and food preservation process to maintain the flavors and nutrition. This drying method is extensively applied in food and agricultural industries, chemical synthesis, pharmaceutical and biotechnology industries.

1.4.3 Spray drying

Spray drying is widely used in industry to produce consistent dry powder from a liquid solution, suspensions, pastes and slurries (Kudra and Mujumdar, 2009). It can be quickly reduced

the transport weight of foods and other materials and ensure the high product quality. The liquid feed is introduced at very low pressure, and the pulse dryer can handle very viscous liquids even those containing solid particles without clogging of the atomizer. All homogenous and pumpable suspensions can be dried using spray drying technology. The application range is wide, it might work as hot air drying or cool air consolidation according to the property of material (Dutta, 2007). The high velocity air instantly atomized the liquid and breaks the liquid into tiny droplets and disperses the slurry droplets into the heating chamber to drives off the water. Atomization is a critical process that increases liquid surface area greatly and makes the water evaporated instantly in the hot air. This procedure can control the drying rate and particle size. The turbulence in the atomization zone can fast evaporation and nearly instantaneous dry (Desobry et al., 1997). Spray drying can also be used for heat sensitive products because only the atomizing head outlet have the desired temperature level. The temperature will drop rapidly as the moisture is evaporated in the chamber.

Not all sample transit to the cyclone follows the air flow and discharges the excess moisture into the atmosphere through the hot outlet air to complete the drying step. The designed particles size can be easily obtained by spray drying through adjusting the nozzle speed, the ground step can be omitted in shortcut. Short residence time is required because rapid spray can disperse the liquid into droplets. Spray drying can reduce the cost by about 30 times compared to freeze drying. Minimum overall flavor loss can be achieved with the appropriate drying conditions (Desobry et al., 1997). Simultaneously, spray drying have a lot benefits for industrial product, such that spray dried gum Arabic had improved emulsion properties and lower bacterial influence (Islam et al., 1997). The downside for spray drying method is costly to assemble a device and the equipment is bulky and difficult for cleaning and sanitizing process.

1.5 Rheology

Rheology is the study of the deformation and flow of matter. Rheological property studies have been focused on two parts, viscosity for fluid state material and viscoelastic for solid or semi-solid state material (Rao, 2010). Liquid state rheology is to study the relationship between flow and force of a material as a function of shear, time, and temperature. The study with solid or semi-solid state is the deformation responding manner to applied stress (σ) and strain (ε) (Barnes et al., 1989; Steffe, 1996). Measured material is categorized into elastic material or viscous material as shown in the Fig 1.4 (Steffe, 1996).

Rheology is a primary behavior of polysaccharides in aqueous solution. Interaction between the high molecular weight polymer and polymer chains when dissolved may influence the solution viscosity. Many of the functionality of hydrocolloids are integrated into the food and non-food materials to obtain the desired mouthfeel, texture, flow characteristics and color (Yaseen et al., 2005). Furthermore, the impact of concentration, temperature, pH and additives on rheological properties of hydrocolloids was also studied by many literatures (Chen et al., 2015; Marcotte et al., 2001; Sanchez Gil, 2014; Wu et al., 2009; Wu et al., 2015; Yaseen et al., 2005).

1.5.1 Viscosity

Some gums exhibit low viscosity behavior at fairly high concentrations but most of the gums are thickener with high viscosity even at low concentration. The rheological properties of semi-dilute solutions are divided into Newtonian and non-Newtonian fluids, and the non-Newtonian contains shear thinning, shear thickening, time dependent, and time independent fluids. Most of the gums exhibit shear thinning properties, which viscosity decreased with the increased shear rate, such as xanthan gum, guar gum and wild sage seed gum. The number of chain entanglements decreased due to the high shear rates occupied in shear thinning fluids. (Cui, 2005;

Salehi and Kashaninejad, 2015; Srichamroen, 2013; Urlacher and Noble, 1997). For the shear thickening fluids, the viscosity of the fluids increases as shear rate increases. For some non-Newtonian fluids also have a yield stress appears at the beginning of measurement. For a given hydrocolloids, the rheological properties can be changed by interrupting with extra cosolutes to change the chain segments (Higiro et al., 2007; Yaseen et al., 2005).

Viscosity measurement can be conducted using concentric cylinder, plate and cone plate, parallel plate, and capillary viscometer, the suitable instrument can be selected according to the different experimental design (Rao, 2010). The rheological properties of gums influenced by the extraction methods, additives, and physical properties, such as particle size, linkage pattern and molecular weight were also important to their applications (Salehi and Kashaninejad, 2015).

1.5.2 Viscoelasticity

Viscoelastic properties can be studied in the linear viscoelastic region use the following three measurement types: frequency sweep, temperature sweep, and time sweep (Rao, 2010). Frequency sweep is used for measuring strong gel and week gel, crossover between elastic or storage modulus G' and a viscous or loss modulus G''. Elastic modulus is a stress divided by the corresponding elastic strain and viscous modulus is the imaginary part of the complex modulus. Temperature sweep is used to study the gel formation preference during cooling of a heated dispersion (e.g., starch gelatinization). Time sweep is also called gel cure test that is used to determine the modulus as a function of time at fixed frequency and temperature (Rao, 2010).

1.6 Response surface methodology

Response surface methodology (RSM) is an integrated consists of mathematical and statistical tool used for developing, improving, and optimizing processes (Myers et al., 2016). The RSM optimization method uses a special experimental design to find the optimal response by

changing several variables at the same time (Cai et al., 2008). RSM has a function to find the optimal in response patterns within the design space and regressed points. The reason for abandoning a single factor experiment is due to resources and consuming the time. RSM is a reliable method used to find the optimal experimental conditions if several conditions interact with one another to give an object characteristic. (Karazhiyan et al., 2011; Koocheki et al., 2009; Psomas et al., 2007; Salah et al., 2010; Toufeili et al., 1994). It guarantees that the polynomial function can converge to the failure probability of the real implicit limit state function by selecting the test points and the iterative strategy. The linear response surface has higher accuracy when the true limit state function is nonlinear. Independent variables with major effects, also called factors, choose by screening experiment before the experimental design. All post-analysis and modeling are worked on the credibility of the variables data, RSM is to generate a suitable approximation model. In general, the first-order model and second-order model can be obtained at the same time when the data is analyzed using software, the higher-order model is more powerful to find the optimal conditions from the curvature in the mound-shape graph (Karazhiyan et al., 2011). RSM can use the three-dimensional graphical perspective of the convenient to intuitively express the data information, to find the optimal conditions for pre-defined response, it can be a performance measures or quality characteristics (Myers et al., 2016). Contour of constant response and three-dimensional graphics with an obviously stationary point describing the estimated relationship between variables and response (Cui et al., 1994; Myers et al., 2016).

RSM is a pellucid modeling system that can be easily established to study the interaction effects of variables that act on a targeted response. Similarly, this model also can infer the levels of the independent variables according to a desired response level (Cai et al., 2008). This model can shorten the time for data collection and reduce the random experimental error. First order

polynomial model, second order polynomial model, even third order model can be used to simulate the response surface model.

The first-order model has the form which only reflects the planer response surface:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \epsilon \qquad (1-1)$$

To evaluate curvature and find the critical point, the second-order model fitted to the following equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ij} X_i^2 + \epsilon \qquad (1-2)$$

where X_i and X_j are represent the coded independent variables, β_0 is a constant, β_i, β_{ij} (i, j=1, 2, 3) are the estimated regression coefficients of variables and Y is the calculated response function. For establishment of the second order model, at least two independent variables with three levels need be contained (Myers et al., 2016). In this experiment, the quadratic term is already sufficient for the simulation equation. In general, the higher degree of the model has more terms, which can fit the data better and reflected in the coefficient of determination more closely to 1(Myers et al., 2016).

RSM is also widely used to find the optimal experiment conditions (Cui et al., 1994). Previously, there was a lot experiments to optimization the best gum extraction conditions using RSM (Cai et al., 2008; Cui et al., 1994; Karazhiyan et al., 2011; Oomah and Mazza, 2001; Psomas et al., 2007; Razavi et al., 2009).

1.7 Objectives

The goal of this research was to improve the utility value of camelina gum, obtain gum with higher quality and isolation yield, and establish the rheological understanding of camelina

gum in dilute solutions thorough spray drying and freeze drying. The goal of this research was achieved through the following four objectives:

1. To study the effect of spray drying technology on the yield, purity, and rheological properties of camelina gum.
2. To study the effects of additives (e.g., NaCl, $CaCl_2$, sugar and ethanol) and pH on the rheological properties of camelina gum isolated from whole seed using spray drying technology.
3. To develop an innovative method for high efficient gum isolation from seeds bran using decortication technology.
4. To optimize the gum isolation from seed bran using response surface methodology.

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Figure 1.1 *Camelina sativa* distribution in US (EDDMapS, 2017)

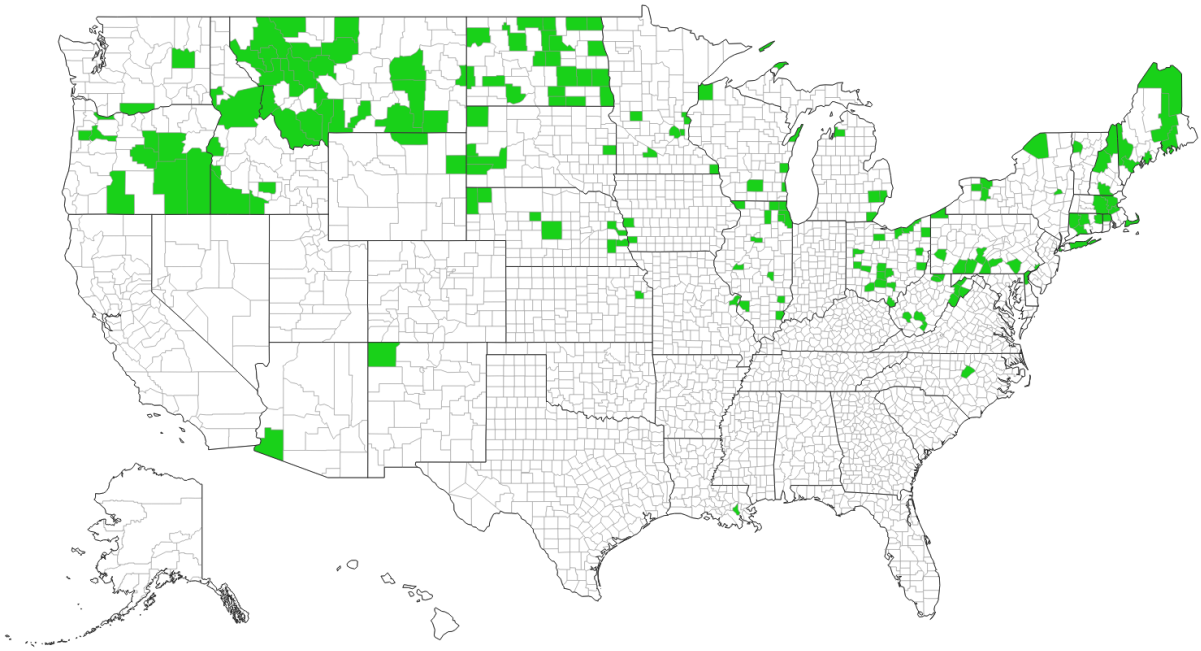


Figure 1.2 *Camelina sativa* plan (Masclef, 1891)

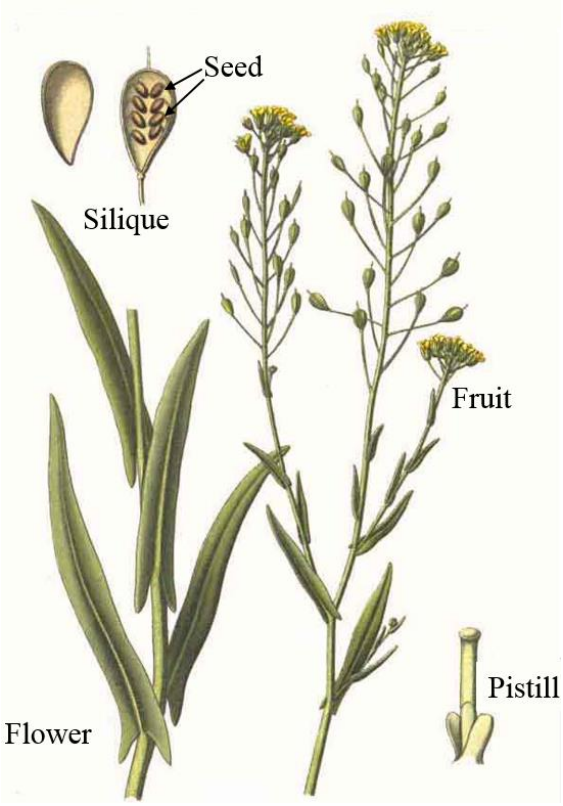


Figure 1.3 Potential markets for *camelina sativa* (Pilgeram, 2007)

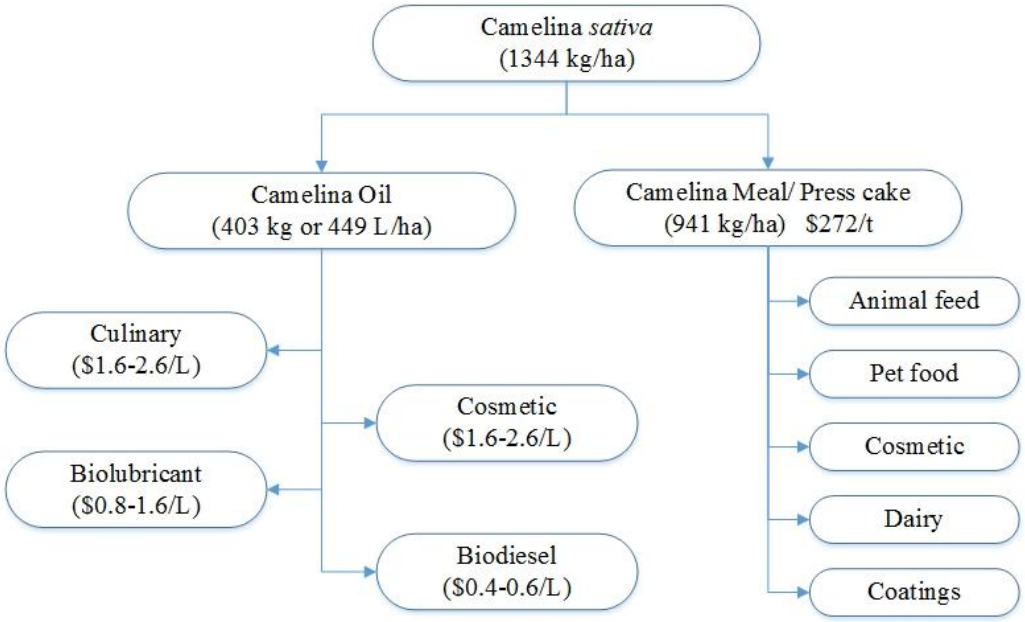
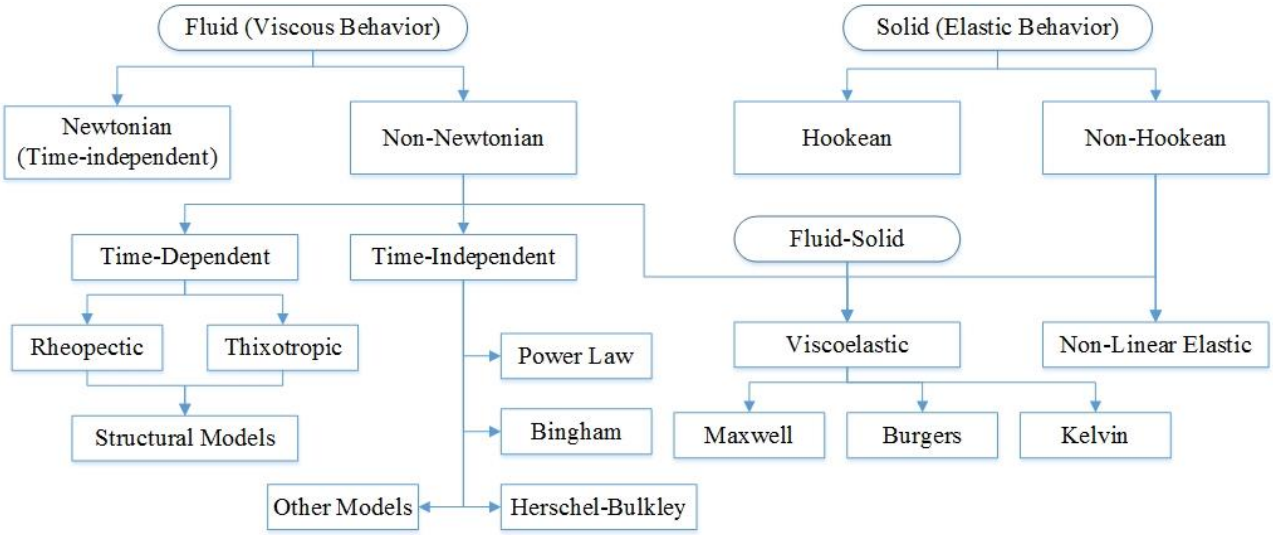


Figure 1.4 Classification of rheological behavior (Steffe, 1996)



Chapter 2 - Properties of Camelina Gum Isolated from Camelina Seeds with Spray Drying Method

Abstract

Camelina seed as an oilseed widely use in biofuels, jet fuel, and feed product. Its functional characteristics have been studied extensively over the last five years. Camelina gum from camelina seed was solubilized with water and isolated use spray dryer. Ethanol precipitation method was used as control. The effect of drying temperature (140 to 180 °C) on the properties of camelina gum was studied. The camelina gum yield from spray drying isolation is up to 1.89% of camelina seed, which is slightly less than that from ethanol isolation method (2.04%). In addition, the camelina gum isolated from spray drying has lower polysaccharides (62 vs 72%) and higher protein (7 vs 6%). In general, the viscosity of camelina gum isolated by spray drying is less than that isolated by ethanol precipitation. Results showed that relative high temperature (165 °C) had a positive effect on the gum yield, purity, and viscosity. High drying temperature (180 °C) led to decreased gum yield and viscosity due to chemical structure decomposition of gum. Camelina gum solution exhibited superior stability in both acidic and weakly basic ranges. At pH 12, its' viscosity was greatly increased, attributed to the promotion of crosslinking between polysaccharides and protein in camelina gum. The effect of varied additives on the rheological properties of camelina gum was also characterized. Results revealed that camelina gum has a good compatibility with those additives studied, indicating that camelina has great potential for being used as the stabilizer.

2.1 Introduction

Camelina falls into the Brassicacease family and oilseed crop in North America, has been native to Europe and Asia, and its growing history can be traced back to 600 BC in Germany (Li et al., 2016). Camelina was recognized as "Gold of pleasure" and spread to the United States because of it has the merits of a short growth cycle (85 to 100 days) and strong cold tolerance (Budin et al., 1995). Camelina's chemical composition varies with species, growing location, and environment, and it roughly contains 30 to 49% oil, 23 to 30% protein, 10% carbohydrates and 6.6% ash (Berti et al., 2016; Budin et al., 1995). Camelina oil is known for rich alpha-linolenic acid (ALA) and omega-3 fatty acid, which greatly promotes camelina research value for food and non-food usage such as biodiesel and biolubricant (Budin et al., 1995; Li et al., 2014).

Camelina gum extracted from camelina seed has shown excellent viscoelastic property and has the potential for use as a thickener, suspending agent, film, and stabilizer (Li et al., 2016; Qi et al., 2016). Previous studies indicated that around 2% of camelina gum was extractable from camelina seeds, and camelina gum is the heterogeneous material that consists of polysaccharide (70%) and protein (12.3%) (Li et al., 2016; Li et al., 2010). The extracted polysaccharide from camelina seed has been composed of galactose (58.1%), glucose (25.0%), rhamonse (11.6%), and xylose (5.2%) (Li et al., 2016). Camelina gum extracted from seeds showed superior viscosity and elastic modulus compared with commercial gums of k-carrageenan and hydroxyethlcellulose (HEC) (Li et al., 2016). Similar to other gums, camelina gum showed the potential of being used as emulsifier, stabilizer, gelling agent and adhesive in food and non-food manufacturing (Glicksman, 1982; Cui, 2005). Based on previous studies, to isolate camelina gum, the gum needs to be solubilized with water and separated from the insoluble part with a process called filtration

or centrifugation, then, the gum is precipitated with organic solvent such as ethanol, followed by a freeze-drying process (Li et al., 2010; Li et al., 2016).

The drying method is paramount in gum production and plays an important role to the properties of gum (York, 1983). Freeze dry, spray dry, vacuum dry, and air-dry methods are the major drying methods that have been widely used in gum isolation. Previous studies have revealed that spray drying could be used to harvest pure, white, uniform gum powder with improved product properties and eliminated harmful bacteria (Glicksman, 1982; Aponte et al., 2016). In addition, spray drying is more cost-effective; it can be 30-50 times less expensive than freeze drying for industrial scale production of pharmaceutical and food additives (Oomah and Mazza, 2001; Silva et al., 2011).

The previously described method for camelina gum isolation requires a mass of organic solvent and energy consumption. There is no study regarding isolating camelina gum with the spray drying method that has been reported so far. The objective of this study was to investigate the effect of spray drying technology at varied temperatures on the yield, purity, and rheological properties of camelina gum. The effect of different additives on the properties of camelina gum was also evaluated.

2.2 Materials and Methods

2.2.1 Materials

Camelina seed was supplied by Montata Gluten Free Processors LLC and was manually cleaned with 48 meshes sieve (W. S. Tyler Company, Belgrade, MT, USA) before use. Phenol 5.0% (w/v), sucrose, glucose, NaCl and HCl were purchased from Fisher Scientific Co., (Fair Lawn, NJ, USA). Experimental data were commonly obtained in duplicate with the mean values reported.

2.2.2 Camelina Gum Isolation

Cleaned camelina seeds were mixed with distilled water at a seed bran/water ratio of 1:20 (w/w), and the mixture was stirred for 2 h at room temperature. The mixed solution was then passed through a sifter with 40-mesh screen (420 microns) to separate the extracts solution and seeds residues. Centrifugation of the camelina gum suspension at 7000 rpm for 10 minutes was performed to remove the precipitate (Sorvall RC 6+ Centrifuge, Thermo Scientific Asheville, NC, USA). The supernatant was collected, filtered and dried in a spray dryer (LPG-5 Centrifugal spray dryer, Jiangsu, China) operated at an inlet temperature of 140 °C to 180 °C and outlet temperature of 100 °C, respectively. The sample feeding rate was controlled at 4 to 5 kg/h by adjusting the pump speed. The collected gum powder was then ground using an Udy cyclone sample mill with 0.25 mm screen (Udy, Ft. Collins, CO, USA), packed and stored at room temperature before further analysis.

The control sample was extracted using ethanol precipitation and freeze drying for comparison. The camelina seeds mixed with distilled water at seed bran/water ratio of 1:20 (w/w) were stirred for 2 h at 25 °C. The extracted solution was filtered with 40 mesh sieves followed by centrifugation at 7000 rpm for 10 min. The supernatant was treated with absolute ethyl alcohol to precipitate camelina gum out of solution. The freeze drying technique was then applied to remove moisture in camelina gum suspensions. The precipitate was collected and ground using an Udy cyclone sample mill with 0.25 mm screen (Udy, Ft. Collins, CO, USA).

2.2.3 Chemical analysis

The total sugar content of camelina gum was measured by the phenol-sulphuric acid method (Dubois et al., 1956). First, Standard curve ($y=268.33x-1.1401$ with R^2 of 0.997, which x represents the colorimetric measurement value and y represents the total sugar content) was

established using glucose. Next, 20 mg of the sample was placed in a 500 mL volumetric flask, dilutes with distilled water to the full-scale volume. One mL of the dissolved sample solution was mixed with 1 mL of the pre-configured phenol (5%, w/w) solution and 5 mL of sulfuric acid, and the mixture was shaken using Maxi Mix Ⅱ mixer (Barnstead International, Dubuque, IA, USA). After the mixture was cooled to room temperature, the absorbance was measured at 490 nm by using Colorimetric (BioMate^{TM} 3 Spectrophotometer, Thermo Electron Co. Madison, WI, USA). Nitrogen (N) was measured via PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA). Protein content was converted from Nitrogen (N) with a coefficient of 6.25.

2.2.4 Rheological Properties

The Bohlin Model CVOR 150 rheometer (Bohlin Instruments, Southborough, MA, USA) was used to measure apparent viscosity, elastic modulus (G') and viscous modulus (G'') as a function of shear rate. The cone and plate spindle with 40 mm diameter head and an angle of 4° were used for all rheological measurements with a small amount of mineral oil which has low viscosity compared to sample to ensure that the solution will not volatilize.

2.2.4.1 Apparent Viscosity Measurement

To prepare the gum solutions, spray-dried camelina gum was mixed with distilled water to reach the desired concentration gradient (0.1%, 0.5%, 1.0%, and 2.0%), and the solutions were then stirred with a magnetic stirrer for 2 h until the solutions became homogenous. The apparent viscosity test with exponentially increasing in shear rate between 0.001-10 (1/s) was measured at 25 °C. The effect of temperature on the viscosity was determined by measuring the change in camelina gum viscosity at continuously changed temperature from 4 to 85 °C with a constant shear rate at 0.1 (1/s). To investigate the effect of pH, the pH of camelina gum solution (2%) was adjusted by HCl or NaOH. Apparent viscosity was measured at the pH range from pH 1.0 to 12.0 with 1.0

pH unit gradient (only results at pH 3.0, 7.0, 10.0, and 12.0 were reported) at room temperature at a shear rate range of 0.001-10.0 (1/s). Effects of salts and sugar additive (0.5-10%) on apparent viscosity of camelina gum were studied at a shear rate range of 0.001-10 (1/s). To investigate the effect of ethanol on apparent viscosity, camelina gum solution with 10, 25, and 50% ethanol concentration was measured at a shear rate range of 0.001-10 (1/s).

2.2.4.2 Frequency Sweep

Oscillatory evaluations were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA). Samples were covered with low viscosity mineral oil to prevent evaporation of water during measurement. Frequency sweeps tests were performed from 0.01 Hz to 10Hz at 25 ℃ with at least three in duplicates in order to be in the linear viscoelastic region. The elastic modulus (G') and viscous modulus (G'') were continuously registered. The viscoelastic properties of camelina gum at different pH were also exhibited including pH 3, 7, 10, and 12. The viscoelastic properties of the interaction of camelina gum with other substances by adding NaCl, $CaCl_2$, sucrose, and ethanol was also studied.

2.2.5 Scanning Electron Microscopy

For scanning electron microscopy (SEM) image, the thin layers of the camelina gum isolated at different drying conditions were prepared, and the gum was coated with an alloy of 60% gold and 40% palladium with a sputter cotter (Desk II Sputter/Etch Unit, Moorestown, NJ, USA). The coated samples were viewed and photographed using a Hitachi S-3500N SEM (Hitachi Science system, Ibaraki, Japan) at an accelerating voltage of 5kV, 10kV, and 20kV using magnification from 3kV, and 18kV to obtain the morphological properties.

2.2.6 Transmission Electron Microscopy

Transmission electron microscopy (TEM) measurement of camelina gum was performed with the CM 100 (FEI Company, Hillsboro, OR, USA) at an accelerating voltage of 130kV. The gum sample was prepared to a seed bran to water ration at 0.05% (w/w). The sample was coated with formvar and carbon copper grids (Electron Microscopy Sciences, Fort Washington, PA, USA) with 200 mesh grids. The coated sample was stained with 2% (w/v) uranyl acetate (Ladd Research Industries Company, Burlington, VT, USA) for 60 seconds before viewing.

2.2.7 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurement of camelina gum was studied with the TA instrument Q200 V24.4 (New Castle, DE, USA), which was calibrated with indium and zinc as recommended by TA instruments. Camelina gum dried powder was used for the measurement with sample size around 7-15 mg in the sealed aluminum plate. The temperature for camelina gum sample was increase stepwise from 25 °C to 275 °C corresponding a heating rate 10 °C/min under a flowing nitrogen atmosphere environment. The enthalpy calculation was used the peak between the onset temperature and the end temperature.

2.2.8 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) for camelina gum stability behavior was performed using a Perkin-Elmer TGA 7 (Norwalk, CT, USA) in a nitrogen atmosphere. The requirement of sample were approximately 5 mg. The temperature range was from 25 °C to 700 °C at a rising rate of 10 °C/min under N_2 atmosphere.

2.3 Results and Discussion

2.3.1 Comparison of Analytical Data of Camelina Gum Extracted at Different Temperature

Gum yield was obtained by calculating the ratio of dried gum powder to seed weight. The gum yield from 1.28-1.89% was obtained from camelina seed with varied spray drying temperatures of 140-180 °C (Table 2.1). Inlet air temperature of spray dryer affects the gum yield. Generally, the gum extracted at 165 °C has the highest yield of 1.89%. When the inlet temperature of the spray dryer reaches the lower limit of 140 °C, it is necessary to reduce the feeding rate to ensure that gum was completely dried. As inlet temperature increased, the drying time decreased. However, the production rate at 180 °C was lower than that of 165 °C because higher drying temperature could volatilize the water faster and result in some sample stick on the wall of the spray dryer, thereby reducing gum yield. Compared to spray drying with freeze drying method, no significant difference to camelina gum yield was observed (Table 2.1). Camelina gums isolated with spray dryer at different temperatures consist of 59 to 62% polysaccharide, which is lower than the gum isolated with ethanol precipitation and freeze dryer method (75%), attributing the fact that ethanol can wash some impurities including fats, proteins, etc., away during the precipitation process. The protein content of spray dryer extracted gum was 7.06 to 7.56% (Table 2.1).

2.3.2 Morphological Properties

Fig 2.1 shows the SEM and TEM images of camelina gum. The morphological property of the camelina gum was affected by the isolation method. There was no difference among the four samples isolated with spray drying in term of particle dimensions (Fig 2.1 A and B). Only gum isolated at 165 °C was listed due to the higher gum yield and higher viscosity. The particle size

ranged from 10 to 150 μm. All of the camelina gum samples isolated with spray drying were hollow, light and fluffy sphere particles. The spray dried sample had smaller particle structure as well as more uniform particle size distribution compared to the freeze dried gum (Fig 2.1 C) which is in agreement with previous study (Nep and Conway, 2011). The turbulence during the spray drying process could cause erosion on the surface of particles, thereby, resulting in smoother surface. The particle size and the structure of surface play an important role in the hydration behavior of gum. The dissolution rate of camelina gum increased with increasing surface area as well as decreasing particle size (Cui, 2005). As wall thickness is an important factor to determine the stabilization of gum, larger particles size will provide gum better stability and prohibit the wall collapses. Freeze drying causes large pores within the sample extending in the interior structure, where will cause the sample to be more easily dissolved (Desobry et al., 1997). The compact microstructure was observed with TEM (Fig 2.1 D), and the result indicted that camelina gums are fibrils with average diameters of 5 nm and greater than micron length level in solution. The spray dried sample had similar microstructure compared to the control sample (Fig 2.1 E). However, more impurities were observed in the spray dried sample.

2.3.3 Thermal Analysis

The DSC curves of four samples are shown in Fig 2.2. In general, there were two exothermic peaks. The first peak appears at temperature ranging from 121 to 205 °C. This peak is due to the dehydration reaction during recrystallization period in the exothermic process. At a specific temperature, the gum polymers gained energy to form intermolecular bonds and move into ordered arrangements, and loses random chain arrangement. The thermogram for gum with low spray drying temperature, the exothermic peak was larger than that with high temperature, which was associated with the large amount of intermolecular bond forms. The second significant

exothermic peak is considered as gum decomposition, which is in agreement with previous studies (Maurer, 1969; Li et al., 2016). The onset of decomposition was at 210 ℃ and an exothermic peak appeared at approximately 225 ℃ with transformation enthalpy being 253.15 J/g.

For TGA and DTG curves (Fig 2.3), there are three distinguished stages of mass loss. The first stage with onset temperature near 30 ℃ lasted to 150 ℃ in the vicinity was considered the loss of moisture or structural water as hydrogen water bonded to gum closely (Bothara and Singh, 2012; Nep and Conway, 2011). The second peak, around 290-304 ℃, was considered to be gum decomposition, which started at 265 ℃ (Qi et al., 2016). Xanthan gum, tragacanth gum, and guar gum had the similar result and decomposition process resulted in the formation of H_2O, CO, and CH_4 (Bothara and Singh, 2012; Zohuriaan and Shokrolahi, 2004). The decomposition temperature decreased with the increasing of spray drying temperature. The gum isolated at 180 ℃ had the lowest decomposition temperature as the higher isolation temperature may break the molecular bond and result in loss of some cross-linking structure (Daoub et al., 2016). The third peak appeared around 350 ℃, which was partially overlapped with gum decomposition peak, was attributed to protein decomposition. As shown in Fig 2.3, camelina gum contains around 7% of protein which can be degraded, involves breakage of intermolecular and intramolecular hydrogen bonds and electrostatic bonds (Li et al., 2014).

2.3.4 Rheological Properties

2.3.4.1 Flow Behavior

2.3.4.1.1 Effects of Concentration and Spray-drying Temperature

Camelina gum at different extraction temperatures of 140, 150, 165, and 180 ℃ were evaluated for their apparent viscosity at concentrations of 0.1, 0.5, 1.0, and 2.0% and the results are summarized in Fig 2.4. Overall, the drying temperature did not have a significant effect on

viscosity characteristics and all gums behave as typical non-Newtonian materials. At low shear rate (less than 0.01 1/s), the viscosity increased as shear rate increased and reached the peak value at 0.01 1/s. The rheological properties tended to become shear-thinning as the viscosity of camelina gum solutions decreased and shear rate increased. The viscosity of camelina seed gums isolated using spray drying was 5-10 times lower than that of control sample isolated using ethanol precipitation and freeze drying (2,000 Pa.s) and locust bean gum (500-1,000 Pa.s) (Phillips and Williams, 2009). The significant change in the apparent viscosity may be due to the fact that the spray drying method affects the chemical composition, microstructure, and molecular weight of the camelina gum which may affect the viscosity (Nep and Conway, 2011; Salehi and Kashaninejad, 2015). Viscosity increased at a low shear rate due to an increasing number of chain entanglements (Nep and Conway, 2011). Camelina gum molecules in solution become aligned and transformed to uniform sequences when shear rate increased, this causes the internal friction to decrease and show a decrease in viscosity at higher shear rates (Glicksman, 1982). The sample become easy to extrude, pour, and mix due to this behavior. The viscosity of camelina gum increased with increasing gum concentration as expected (Fig 2.4). With the increase of concentrations, the number of macromolecules per unit volume has increased and enhance the binding capacity (Barnes et al., 1989). The samples with 165 °C extraction temperature was selected for subsequent analysis because of higher production yield and rheological performance.

The control sample at 2% concentration had the same viscosity trend compared with spray dried samples, however, the viscosity value was significantly higher than the spray-dried samples at the same concentration. The control sample had higher polysaccharide purity, thus providing a higher viscosity (Li et al., 2016).

2.3.4.1.2 Effects of Temperature

The viscosity of camelina gum solutions at different concentrations (0.1 - 2%) exhibited different trends when the temperature increased from 4 to 85 °C (Fig 2.5). For high concentration solutions (0.5, 1.0, and 2.0%), viscosity decreased with increasing temperature in the range of 25 to 70 °C. At low temperatures, the apparent viscosity decreased slowly with increasing temperatures. Increasing temperatures promote chain disentanglement; the intermolecular thermal motion increases lead enlarged the intermolecular distance and weaken the interaction between each other (Lapasin and Pricl, 1995; Wang and Cui, 2005; Wu et al., 2015), resulting in decreased viscosity. Conformational changes of gum in solution from an ordered helix to a disordered coil was influenced by the measuring temperature (Wang and Cui, 2005). The similar finding was found in other commercial grade gums; for instance, xanthan gum retains the same viscosity property for a wide temperature range before reaching the melting point (Urlacher and Noble, 1997). Although low viscosity mineral oil was used to prevent volatilization, some viscosity still increased at high temperature ranges due to the fact that moisture was decreased for all of the rheological measurements.

2.3.4.1.3 Additives

The effects of four additives (ethanol, NaCl, $CaCl_2$, and glucose) on rheological properties of camelina gum were studied with 2% gum concentration (Fig 2.6 A, B, C, and D). The addition of ethanol up to 10 and 25% to camelina gum decreased the apparent viscosity, but significant increase the viscosity when sustaining add ethanol to 50% (Fig 2.6 A). The reduction may be interrelated with a decrease in the camelina gum polymer level in sample association at 10 and 25% ethanol. After an addition of 50% of ethanol in the camelina gum solution, the excess alcohol result of the molecular repulsions, the aggregation between the molecular species may occur,

resulting in increased viscosity of camelina gum. When a small quantity of salt was added, the solution essentially retained the same rheological properties as the absence of salt, just showed a minor decrease in viscosity due to strong attraction between molecules. However, when 10% divalent ions from $CaCl_2$ were added, the result showed a more reduction in the viscosity comparing to monovalent ions from NaCl (Fig 2.6 A, B, and C). It was possibly caused by the crosslinking between camelina gum and bivalent ions which leaded to more extension of molecular contraction. Salt is a necessary additive for food industry, the effects on rheological properties of gum solution depend on the amount of salt added (Marcotte et al., 2001). Salt additives do not affect some gums, such as xanthan gum. However, in some gums, for instance flaxseed gum, rheological properties decrease as the salt concentration increases (Higiro et al., 2007). By the same theory, the sugar content affects the rheological properties of the gum. In camelina gum solution, no significant changes were observed when the sucrose content increased to 10% (Fig 2.6 D).

2.3.4.1.4 Effects of pH

Fig 2.6 E shows the effect of pH on the viscosity of 2% camelina gum solution at a constant shear rate. Camelina gum solution exhibited superior stability in both acidic and weakly basic pH ranges. In a strongly alkaline environment at pH 12, the viscosity of the camelina gum solution was greatly increased. The huge increase in apparent viscosity at pH 12 may be related to the conformational change in the molecules of the mucilage. The effect of pH on camelina gum viscosity cannot be well explained by the incompletely structural analysis of camelina gum, such as linkage and arrangement of the different types of carbohydrates in the camelina gum. Camelina gum showed stability of the viscosity at pH 3 to 10 which is similar to tragacanth gum (Yokoyama et al., 1988). The stability of gum under wide range of pH is a desire property for its application

in food industry. Xanthan gum and methyl cellulose showed stable in a wide range of pH in viscosity (Williams and Phillips, 2009).

2.3.4.2 Viscoelastic Properties

Frequency sweep was used to study the differences between viscous behavior and elastic behavior due to the changes in the rate of strain. Material functions need to be determined over a wide range of frequencies to study the effectively rheological behavior and evaluate material specification and compare viscoelastic behavior. Viscoelastic behavior of camelina gum have been conducted by using oscillatory experimental measurements and was determined over the frequency range from 0.1 to 10 (ω) at 25 ℃. Fig 2.7 illustrated the differences in storage modulus (G') and loss modulus (G'') as a function of frequency for camelina gum isolated at 165 ℃. Modulus G' indicates the temporarily stored elastic energy in measured material, and G" reflects the energy the capacity required to activate the flow, which is applied, or converted to heat energy and lost (Saha and Bhattacharya, 2010). Dilute solution (Newtonian behavior) always reveal G'' being larger than G' for the entire frequency range, but the moduli become closer at the end. Concentrated solutions (viscoelastic behavior) have an overlapping at the middle of the frequency range which is between plateau and rubber-like regions. Gel always exhibited G' greater than G" during the entire experiment, which classified in elastic behavior (Ptaszek et al., 2009; Steffe, 1996).

2.3.4.2.1 Effects of Concentration

As shown in Fig 2.7 A, the G' and G'' of camelina gum at 0.1% concentration showed an overlapping at high frequencies. Elastic modulus (G') was predominant at high frequency after the crossover, but was less than the viscous modulus (G'') before the intersect point, which indicated the samples have a solid-like behavior at higher frequency ranges after the crossover with a clearly tendency structure. This crossover appears at lower frequencies, meaning the system is more

structured (Piermaría et al., 2016). The material developed gradually more liquid-like at the concentration of 0.1%; in turn, it became progressively more gel-like at the concentration levels of 0.5, 1.0, and 2.0%. Some studies have discussed that the overlapping frequency values shifted to lower frequency range with the increasing gum concentration (Wu et al., 2009; Wu et al., 2015); however, in this study, the measurement range was determined in order to achieve the linear viscoelastic region in which the overlapping could occur at lower frequency (i.e. outside the linear viscoelastic region). The higher concentration samples include more camelina gum molecules which will cause stronger intermolecular entanglement. It showed solid-like behavior for the high concentration sample which G' increased exponentially and faster than G" at higher frequency with concentration 0.5%. At concentration 1%, G' and G" increased constantly regardless oscillation frequency. The reaction difference between G' and G" to the concentration of 2% was larger than that of 1% as the higher concentration has a firmer gel structure (Li et al., 2016).

2.3.4.2.2 Effects of pH

Fig 2.7 B shows the effect of pH (pH 3, pH 7, pH 10, and pH 12) on viscoelastic properties of camelina gum solution (2% w/v). Modulus G' was significantly higher than G'' over the entire frequency range that occurred without any crossover at lower frequency range. With the increase of pH level from pH 7 to 12, the G' and G'' increased gradually, promoting a more stable gel. Compared with the locust bean gum and guar gum extracted from seeds, the solution had a similar pH value, which is slightly lower than the neutral level (Yaseen et al., 2005). The lowest protein solubility at pH 2.5 to 3.0 caused phase separation and opacity in the camelina gum solution at pH 3, resulting in decreased viscoelastic modulus at acidic condition (Li et al., 2016)

2.3.4.2.3 Additives

Fig 2.7 C, D, E, and F shows the addition of ethanol, sugar, and salts (NaCl and $CaCl_2$) on the viscoelastic properties of camelina gum. Approximately 50% of ethanol had a significant effect on the viscoelastic behavior of camelina gum. As observed, the moduli (G' and G'') of all samples increased as sweep frequency increased. The gain of viscoelasticity is related to several factors, such as high ethanol content, which have the ability to extract gum out of the solution or the addition of 50% ethanol providing the sample to have strong ability of bonding water and forming stronger gel structure (Maurer et al., 2012). The effect of salts and sugar on the viscoelastic properties showed similar trends as to the viscosity of camelina gum. Results revealed that camelina gum has good compatibility with those additives studied, indicating that camelina has a great potential for being used as stabilizer.

2.4 Conclusions

The spray drying temperature showed great effect on the yield, purity, and rheological properties of camelina gum. Results showed that the modest temperature of 165 °C can be chosen to achieve high efficiency in camelina gum isolation with better composition and properties compared with other drying temperatures. Camelina gum, a heterogeneous material consisting of polysaccharides and protein, exhibited superior stability in both acidic and weakly basic environment and good compatibility with additives of salts, sugar, and ethanol, enabling it to be a great candidate as stabilizer in food and non-food usage. Spray drying method is simple and cost-effective compared with freeze drying in isolating camelina gums and can be considered as an alternative method in camelina gum production for further applications.

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Table 2.1 Spray drying time and yield of camelina gum.

Treatment	Gum yield (%)	Total carbohydrate (%)	Protein content (%)
140 ℃	1.28	59.15	7.56
150 ℃	1.81	60.85	7.31
165 ℃	1.89	62.08	7.13
180 ℃	1.72	61.74	7.06
Control	2.04	71.57	5.88

Figure 2.1 SEM and TEM images of camelina gum samples with different drying temperature treatment at different magnifications: A (3,000X); B (18,000X); C (3,000X); D (130,000X); and E (130,000X).

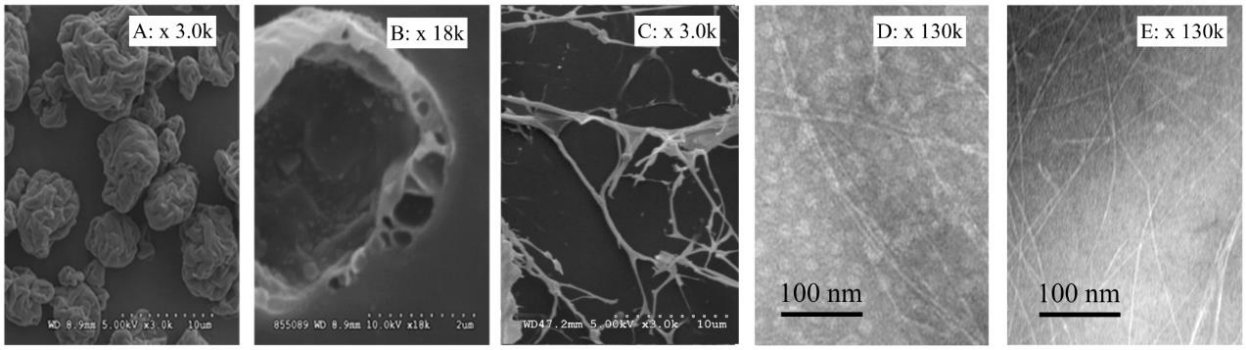


Figure 2.2 DSC thermograms of camelina gum solutions with different extraction temperature.

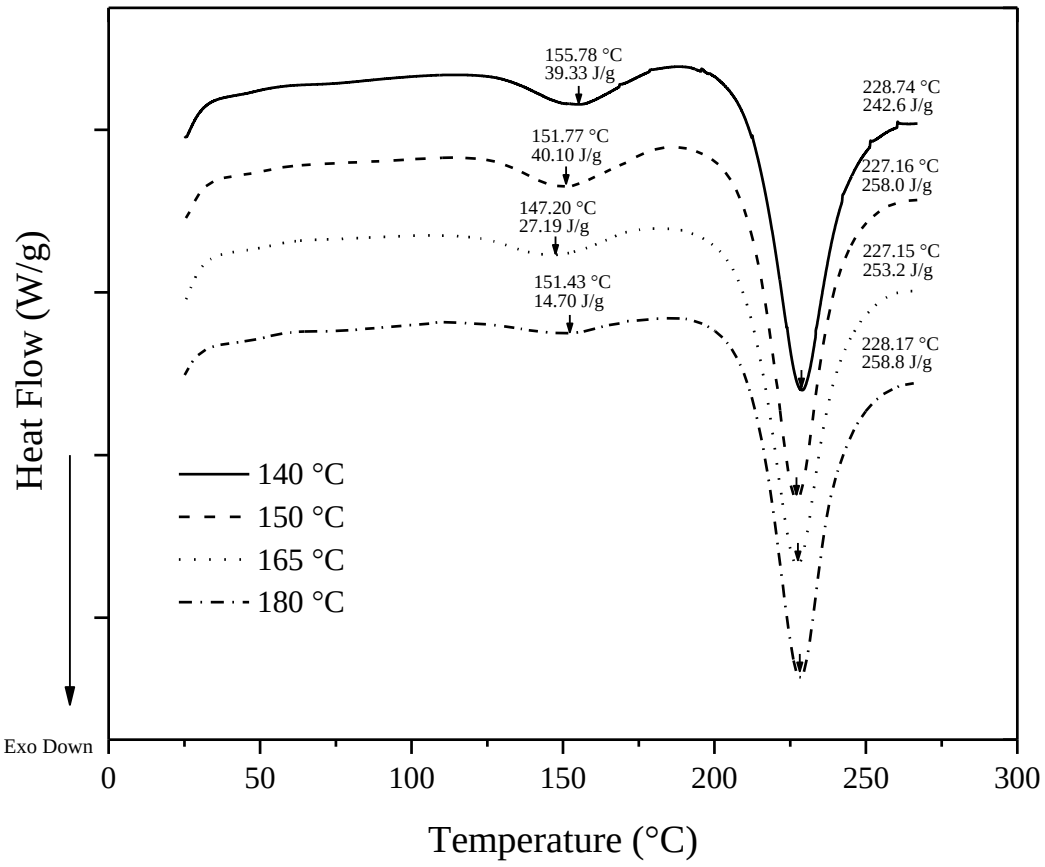


Figure 2.3 TGA curves of camelina gum solutions with different extraction temperature.

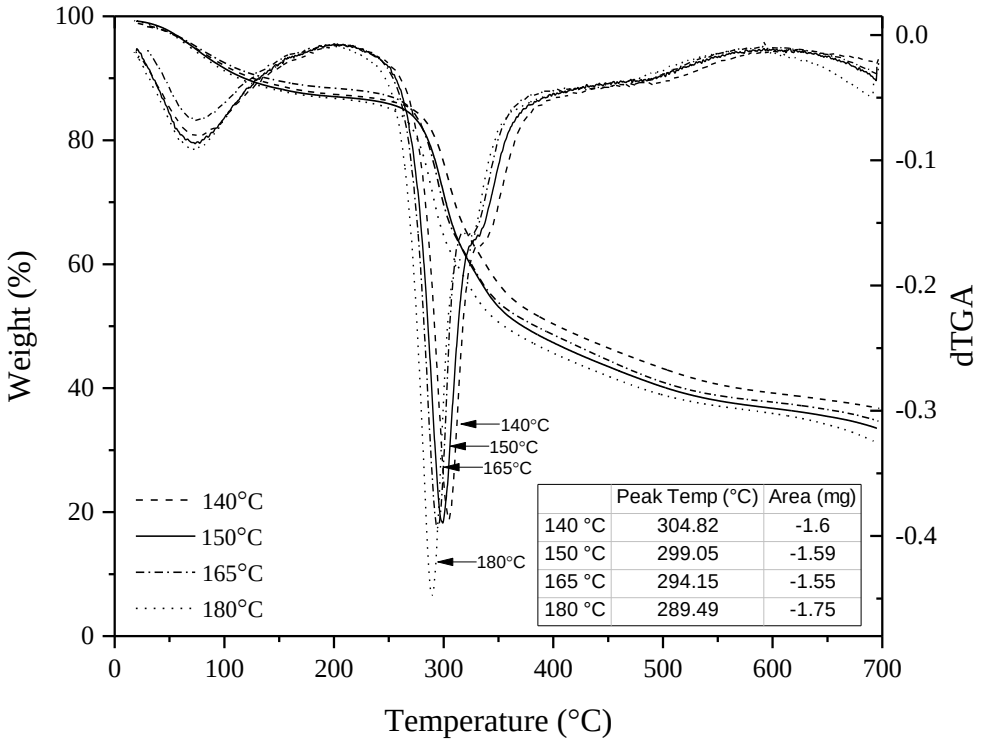


Figure 2.4 Apparent viscosity of camelina gum solutions with different extraction temperature.

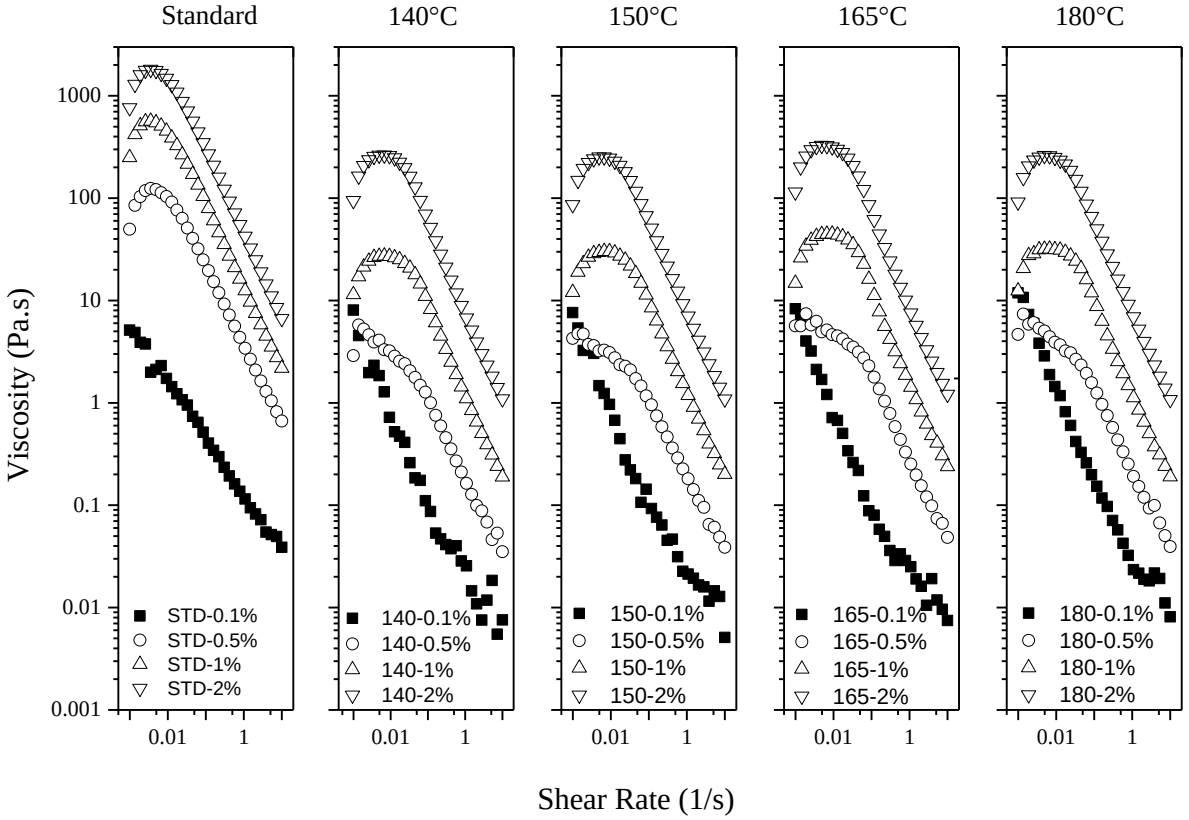


Figure 2.5 Apparent viscosities of camelina gum solutions at different temperature at constant shear rate of 0.1 (1/s) with varying concentrations (0.1-2.0%).

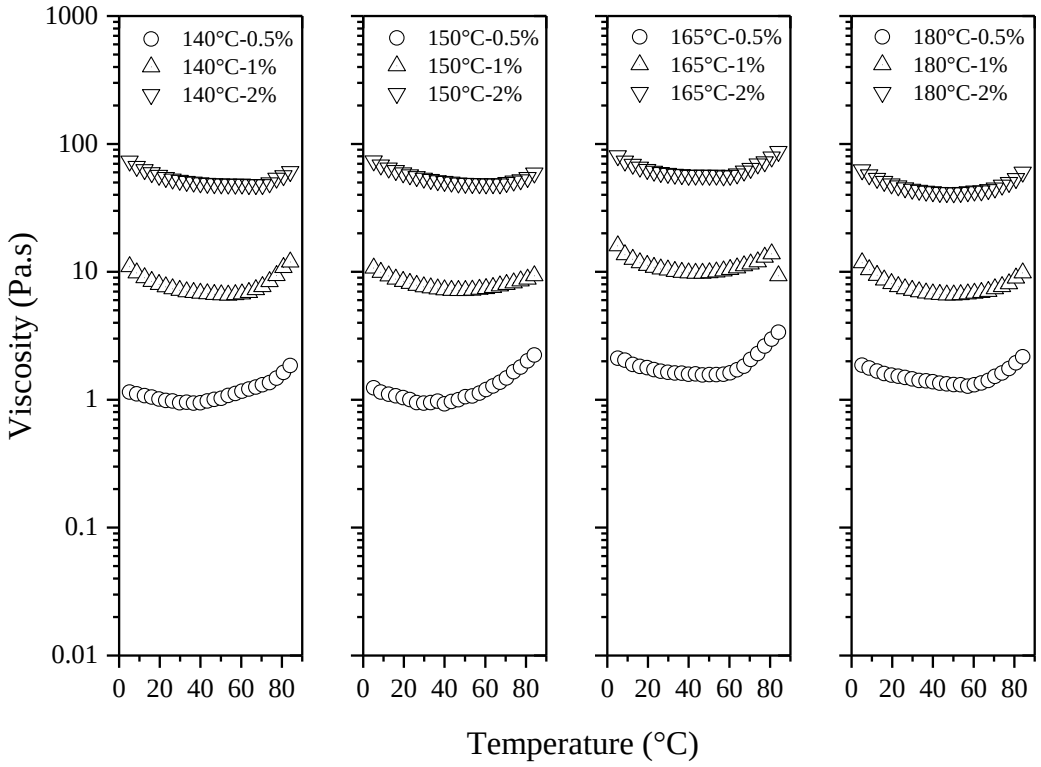


Figure 2.6 Effect of ethanol, NaCl, $CaCl_2$, sucrose, and pH on a pparent viscosity of camelina gum (A: ethanol, B: NaCl, C: $CaCl_2$, D: Sucrose, and E:pH).

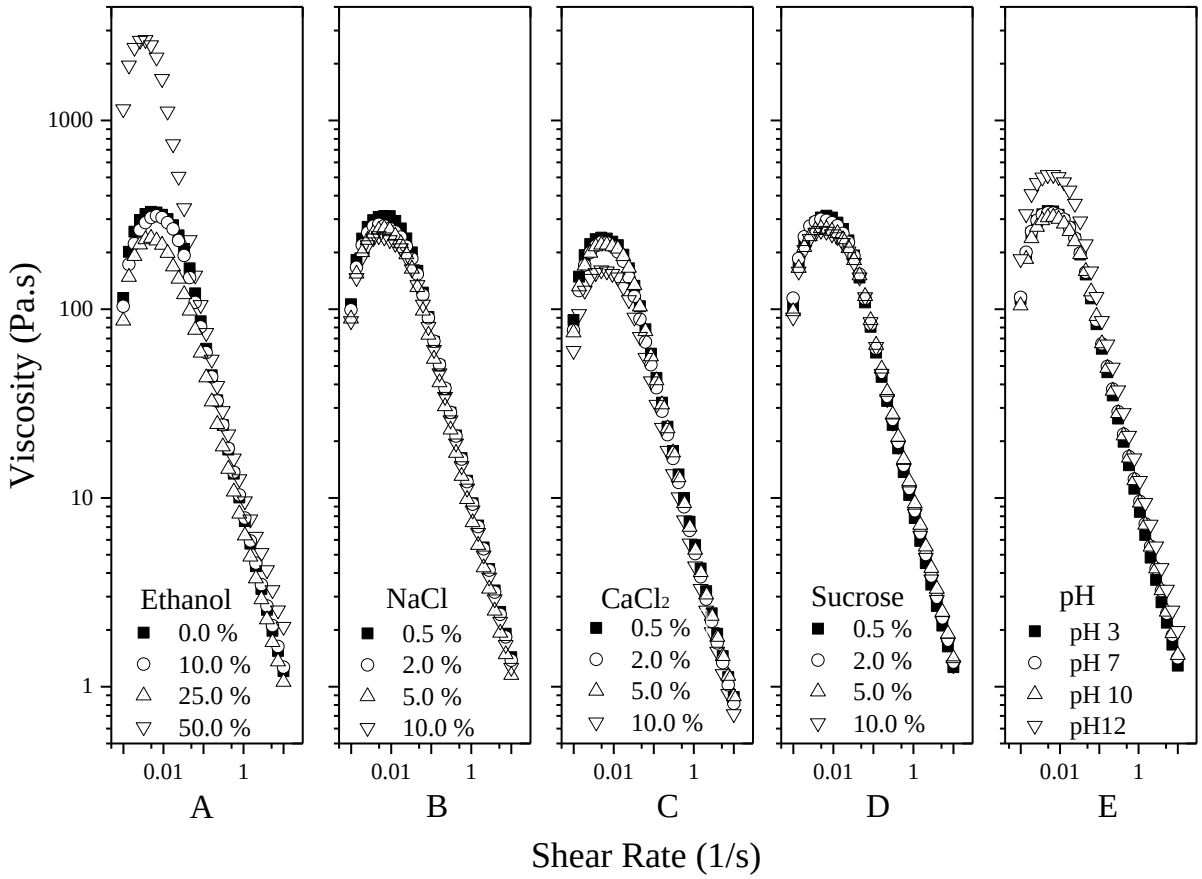
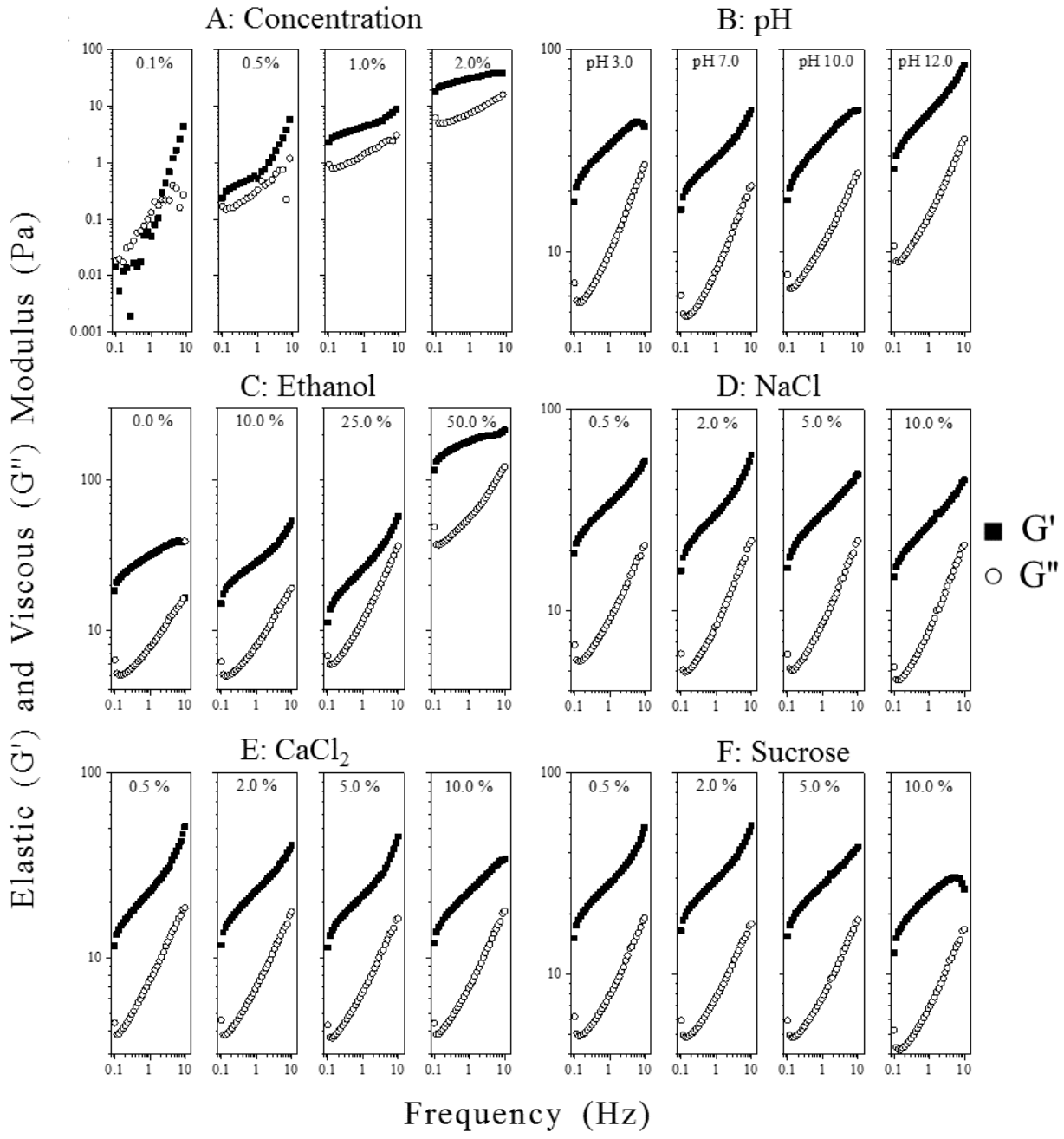


Figure 2.7 Effect of A: gum concentration at 165 °C, B: pH, C: Ethanol, D: NaCl, E: $CaCl_2$, and F: Sucrose on the viscoelastic properties of camelina gum (2%) dried at 165 °C.



Chapter 3 - Optimization of gum isolation from camelina (*Camelina sativa* L. Crantz) seed bran using response surface methodology

Abstract

Camelina, as other oil seeds, has a significant amount of gum; the camelina bran itself contains the most of the polysaccharides (gums). Decortication procedure was used to remove the seed bran for increased gum isolation and increased camelina protein isolation efficiency and protein quality. The effects of seed bran to water ratio (1:30, 1:40, and 1:50 g/mL); isolation temperature (25, 50, and 75 ºC); isolation time (0.5, 1.5, and 2.5 h); and their interactions on the gum yield, purity, and rheological property were studied. Response surface methodology was used to determine the optimal conditions for isolating the gum from camelina seed bran. The gum yield, gum purity, and gum viscosity data fit the full quadratic models (R^2 = 0.995, 0.877, and 0.804) better than the first-order models (R^2 =0.813, 0.568, and 0.636), respectively. Regression results from the second-order models indicated that seed bran to water ratio, isolation temperature, and isolation time all had significant effect on gum yield and gum viscosity, in contrast, isolation temperature had no significant effect on gum purity. According to the data analysis, the optimum isolation conditions were found as follows: seed bran to water ratio, 1:39 g/mL; isolation temperature, 35 ºC; and isolation time, 1.5 h. Under this condition, gum yield, gum purity, and viscosity were 19.08% (w/w), 56.24% (w/w), and 62.82 Pa.s, respectively. This optimal condition provided a probability of 0.839 for the numerical optimization function of the responses simultaneously. The protein yield and quality extracted from decorticated endosperm were improved significantly compared with that extracted from whole seeds meal without decortication.

3.1 Introduction

Camelina seed contains 25-45% crude protein, 30-49% oil, 10% crude fiber, and 2-3% gum (Berti et al., 2016; Li et al., 2016; Zubr, 2003). As a co-product, camelina gum has showed high apparent viscosity, and strong temperature and pH tolerance (Li et al., 2016). Camelina gum also showed higher viscosities, storage (G'), and viscous modulus (G") than commercial gums such as κ-carrageenan and hydroxyethylcellulose (HEC) gums, and showed great potential for food and industrial uses. Currently, camelina gum are mainly isolated from the whole seeds (Li et al., 2016; Sanchez Gil, 2014). The seeds are soaked in water and the gum is solubilized with water and harvested. This wet process makes camelina seed with high moisture content and significantly affects the subsequent oil extraction and protein isolation. Our research showed that the majority of the camelina gum are presented in bran and about 10-20% of the bran are monosaccharides and polysaccharides (camelina gum). In general, the degumming process is needed before oil extraction and protein isolation because gum content affects oil extraction and protein isolation. If seed bran can be removed before oil extraction and protein isolation, product quality will be increased and processing procedure can be simplified. Therefore, we proposed to remove the camelina bran through decortication process and the only bran is used for gum isolation, then the oil and protein will be extracted from decorticated camelina endosperm, this not only saves the camelina seeds for oil and protein production, but also makes the oil extraction and protein isolation more efficient and may increase the oil and protein yield and purity.

There are many studies focused on finding the effects of processing conditions on the gum extraction performance using factorial designs, or using the response surface methodology (RSM) to figure out the best conditions (Cai et al., 2008; Myers et al., 2016; Razavi et al., 2009; Salah et al., 2010). The RSM combines the optimization theory of mathematics and scientific test

arrangement method, with the minimum human and material resources, to achieve the best scientific results in the shortest time (Li et al., 2011; Myers et al., 2016). The methodology can also reveal the continuous analysis to each level of the experiment when combined with other analysis method, such as orthogonal methodology (Salah et al., 2010). The RSM refers to use polynomial functions to approximate the implicit limit state function through a series of deterministic experiments. By successfully selecting the test points and iterative strategies, RSM can insure that the failure probability on polynomial function can be more accurate compared to the real implicit limit state function (Myers et al., 2016).

To date, the existing research on camelina gum seems to focus on characteristics the morphology, physical and chemical properties as a by-product from camelina oil extraction. To our knowledge, there is no published study for optimizing the gum isolation from camelina seed bran due to that a small seed size increases the difficulty of separation procedure. In this research, we successfully separated the seed bran from seed endosperm and investigated the effects of seed bran to water ratio, isolation temperature, and isolation time; as well as interaction of these factor on the gum yield, gum purity, and rheological behavior of camelina gum. In addition, rheological properties were compared with standard camelina gum isolated from whole seed, and the effect of decortication on protein and oil quality were also studied.

3.2 Materials and methods

3.2.1 Camelina seeds

The camelina seeds used in this study were purchased from Field Brothers, LLC (Pendroy, MT, USA). The seeds were manually pre-cleaned with 48 meshes sieve (W. S. Tyler Company, Belgrade, MT, USA) to remove all foreign materials such as stones, broken seeds, and branches.

The moisture, ash, fat, and protein content of the seeds were previously analyzed in recent studies by the authors (Li et al., 2014).

3.2.2 Separation of camelina seed bran

Fig 3.1 shows the processing flowchart of separation of camelina bran from endosperm using decortication and air classification procedures.

Decortication. The pre-cleaned whole camelina seeds were decortication by the impact dehuller "Dry Granular Separations" (Forsberg's Inc. Thief River Falls, MN, USA) using impact force to separate the bran from seed endosperm. The separation procedure was carried out at a low feed rate using the automatic feeder and repeated three times (Fig 3.2A). Further impact could cause small endosperm fragmenting to influence the gum purity.

Bran separation. A seed blower with wind tunnel (Seedburo Equipment Company, Des Plaines, IL, USA) was used to separate the seed bran from the endosperm and un-decorticated seeds by different suspending ability for 5 minutes (Fig 3.2B). Seed endosperm and other heavier components remain at the bottom of the blower. The seed bran, fine endosperm, and light germ were blowing with the wind in the blower and collected for further separation. Forty mesh sieving was used to separate the pure seed bran component from the fine materials. Fig 3.3 shows the whole seed before decortication (A), camelina seed bran (B), and endosperm (C). With this procedure, the seed bran yield is about 10-17% of total whole seeds.

3.2.3 Gum isolation

After decortication process, the optimal gum isolation conditions were determined by the following three factors: seed bran to water ratio (1:30 – 1:50 g/mL), isolation temperature (25 – 75 ºC), and isolation time (0.5 to 2.5 h) using MSR method. The bran was separated from the gum slurry using a Sorvall RC 6+ Centrifuge (Thermo Scientific, Asheville, NC, USA) at 7,000 rpm

for 10 min. Alcoholic precipitation process was introduced to precipitate gum from the supernatant. Three times of 95% ethanol was used to precipitate the filtered colloids overnight for at least 24 h at 4 °C. Then centrifuge was used to recover the precipitate at 7,000 rpm for 5 min. The precipitates were collected and dried with freeze dryer to remove the moisture, providing a white product. The dried isolated gums were grounded, packed, and stored in room condition for further analysis.

3.2.4 Experimental methodology

Based on the previous single factor experiment results, the feasible independent variable range of the seed bran to water ratio, isolation temperature, and isolation time for high camelina gum production yield was obtained. Box-Behnken factorial design (BBD) was selected for optimization of the camelina gum isolation conditions. The BBD is able to determine the optimum of response functions based on incomplete factorial design. The individual and interactive effects of the seed bran to water ratio (*X1*: 1:30 – 1:50 g/mL), isolation temperature (*X2*: 25 – 75 °C), and isolation time (*X3*: 0.5 – 2.5 h) at three levels on response variables including camelina gum yield (Y_1), purity (Y_2), and rheological property (Y_3). Table 3.1 presents the coded and encoded form of independent variable and each one with three levels, namely, -1, 0, and 1. Each experiment was done by at least duplicated and the average results were taken as the response.

Response surface methodology (RSM) was selected for the full experimental analysis. The RSM was widely used for developing, improving, and optimizing the production process conditions (Myers et al., 2016). The “three level full fractional” design with four center points was used in this experiment. Multiple center points can make the measurement process more robust and reliable, providing estimates of pure error and checking curvature (Myers et al., 2016). The professional statistical software released by Design-Expert (version 10) by Statease, Inc.,

(Minneapolis, MN, USA) was used to analyze and optimized the response surface methodology model. The fitted model with 2-D contour plot and 3-D response surface graph was expressed from the software, easier to analyze the correlative between the various factors, the optimal isolation conditions were deduced.

3.2.5 Evaluation methods

3.2.5.1 Determination of camelina gum yield

The camelina gum yield from various isolation conditions was measured in dry basis use equation 3-1, expressed as percentage:

$$Y_{(yield)} = \frac{weight\ of\ isolated\ camelina\ gum\ (g)}{weight\ of\ camelina\ seed\ bran\ \text{sample}(g)} \times 100\% \qquad (3\text{-}1)$$

3.2.5.2 Determination of purity

The analyses for content of nitrogen in camelina gum use freeze drying process were carried out on instrument PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA) after weight the camelina gum samples between 2 to 2.5 mg and wrapped use foil paper. The protein content in the sample was obtained by multiplying the nitrogen content with a factor of 6.25. The purity of the sample was obtained by subtracting the protein content and the various impurities, like oil and fiber. Some further verified the exact test by determining the total sugar content with the phenol-sulphuric acid method (Dubois et al., 1956). The standard curve ($y = 268.33x - 1.1401$ with R^2=0.997 which x represents the colorimetric measurement value and y represents the total sugar content) has been established before the 17 trails sample measurement.

3.2.5.3 Determination of rheological property

Sample was prepared by hydrolyzing the camelina gum powder in distilled water at room temperature and stirring for 2 h to dissolve completely. The sample was well equilibrated before measuring at a fixed shear rate 20 s^{-1} for 3 min. The shear rate was chosen based on the chewing

method, to study the performance as a food additives (Glicksman, 1982). A Bohlin CVOR 150 rheometer, Malvern Instruments, (Southborough, MA, USA), was used during the apparent viscosity measurement. Depending on the rheological properties of the sample, an 8 mm parallel plate measuring head and 0.5 mm gap was selected for all the measurements. All measurements were done in duplicate and the average was reported.

3.2.6 Camelina protein isolation and characterization

Camelina proteins were isolated from undecorticated defatted meal and decorticated defatted meal separately. For undcorticated meal, samples were mixed with distilled water at a seed bran/water ratio of 1:30 (g/mL), stirred for 2 h, and then centrifuged; and the residues were collected for further camelina protein fraction isolation following the previous method (Li et al., 2014). In this procedure, degumming is needed from supernatant for albumin isolation. For decorticated meal, without the effect of gum (mainly exists in the bran), the meal and water ratio of 1:15 was used for protein isolation which is much simpler than meal with bran. Protein content and yield rate was determined using PerkinElmer Elemental Analyzer. The color of protein was obtained by color contrast. Apparent viscosities of extracted proteins were performed using the Bohlin CVOR150 rheometer with 20mm parallel plate head and 500 um gap. The viscosity measurements were tested at a steady shear rate of 25 s^{-1}.

3.3 Results and discussion

3.3.1 Optimization of isolation condition by RSM

Box-Behnken factorial design was selected in this experimental, this method has a central point as well as one high and one low, a total of three levels of the experimental design. The central point of the experiment is determined by the preliminary experiments to estimate a pure error sum of squares, and the significant factor with the highest corresponding value in the test that was

selected. The 17 trials, experimental designs exported from Design Expert, were generated by the software in a random sequence that can avoid the model affect by the extraneous factors as much as possible (Razavi et al., 2009). Experiments were performed in the order of disruption, and each experiment was completed at least in duplicate. Seventeen experiments were carried out according to the conditions which showed in Table 3.1.

The first-order and quadratic models were developed to study the effect of seed bran to water ratio, isolation temperature, isolation time, and their interactions on gum yield, purity, and viscosity. Because the gum yield, gum purity, and viscosity data fit the quadratic models (R^2 = 0.995, 0.877, and 0.804) better than the first-order models ((R^2 =0.813, 0.568, and 0.636), respectively. Higher determination coefficient (R^2) was expected, but the value of R^2 always increased as we add terms to the model. In contrast, R^2 (adj) will decrease when nonsignificant terms are added into the model. Thus, the closer of R^2 and R^2 (adj) are preferred which will make the regression model fits the data better (Montgomery, 2017; Myers et al., 2016). The first-order regression results were not reported. The ANOVA table for the three reduced models was shown in Table 3.2. The significance of each terms in the fitted model was analyzed by statistical methods and check the probability (*p*-value), generally at a *p*-value of 0.05, some of which use 0.1 (Myers et al., 2016). The predicted reduced and modified equations for gum yield, gum purity, and rheological property was given as following equations:

For gum yield (*Y1*):

$$Y_1 = +18.71 + 3.12 \times A - 2.58 \times B + 2.30 \times C + 1.81 \times A \times B - 0.36 \times A \times C - 0.41 \times A^2 - 2.94 \times B^2 - 0.80 \times C^2 \quad (3\text{-}2)$$

For gum purity (*Y2*):

$$Y_2 = +55.86 - 1.71 \times A - 0.47 \times B - 1.17 \times C + 1.68 \times A \times C - 1.53 \times A^2 - 1.93 \times C^2 \quad (3\text{-}3)$$

For rheological property (*Y3*):

$$Y_3 = +60.44 - 14.08 \times A + 2.18 \times B - 9.44 \times C - 16.00 \times A \times B + 11.07 \times B \times C + 10.63 \times B^2 + 10.65 \times C^2 \quad (3\text{-}4)$$

where A represents seed bran to water ratio (g/mL), B represents isolation temperature (°C), and C represents isolation time (h).

3.3.2 Gum yield

The decortication process yield approximately 16% of seed bran fraction (Fig 3.3. B) for gum isolation and 84% of endosperm fraction (Fig 3.3. C) for subsequent use to extract camelina oil and protein. The yield of camelina gum isolated with the alcohol extraction and freeze drying method was between 7.5 to 22.3% based on the seed bran weight or 1.21 to 3.61% of the whole seed which is close to previous study (Li et al., 2016). The results indicate that the process of gum isolated from seed bran is very comparable with the method from the whole seed.

The second-order response function (Eq. 2) for gum yield (*Y1*; Eq. 3-2) was obtained based on the data showed in Table 3.1. The β_{23} term was removed from the full model based on statistical analysis that the *p*-value is not significant (Table 3.2). The other terms except β_{13}, which *p*-value (0.10) is at an acceptable range, and others were all at significant level. The *p*-value (<0.0001) for the reduced model indicates the model for gum yield (*Y1*) response is highly significant (Table 3.2 *Y1*). R-squared for the reduced model was 0.995, which means the regression model has perfect prediction. The lack-of-fit value can be indicative of a model's failure to represent data in the experimental domain at points, which are not included in the regression model. Lack-of-fit was not significant, which means the model fit the experiment data. Seed bran to water ratio is the most

important factor that affects the camelina gum yield based on the positive magnitudes of coefficients which are larger than isolation time, whereas temperature had a negative value and effect.

In order to identify the optimum conditions for gum yield, the 3-D response surface image and the interaction information of response *Y1* were developed (Fig. 3.4). Fig 3.4A shows the effect of seed bran to water ratio and isolation temperature on gum yield. The gum yield increased as the seed bran to water ratio increased at a constant temperature. The gum yield reached the proximity maximum value close to the center of isolation temperature at the fixed high seed bran to water ratio range. Gum yield did not change significantly in the temperature range between room temperature to 50 °C. However, it decreased significantly when the temperature exceeds 50 °C. This is probably because the higher extraction temperature resulted in reduced viscosity and increased hydrolysis of polysaccharides at higher extraction temperatures (Karazhiyan et al., 2011; Mirhosseini and Amid, 2012). In addition, higher extraction temperature causes liquid evaporation which may also affect gum yield. Fig 3.4B shows that the gum yield increased significantly as the seed bran to water ratio and isolation time increased. The more solvent used in the extraction process, the greater yield can be obtained due to the driving force for the mass transfer increased which makes a less sticky slurry and lead more efficient isolation (Karazhiyan et al., 2011; Koocheki et al., 2009; Mirhosseini and Amid, 2012). At constant seed bran to water ratio and temperature, gum yield increased significantly as the isolation time increased. As the stirring time increased, more substances in the seed bran were dissolved in the solution due to the mass transfer from bran to the solution increased as time increased (Karazhiyan et al., 2011; Mirhosseini and Amid, 2012; Yapo et al., 2007)

3.3.3 Gum purity

The reduced polynomial equation on gum purity (*Y2;* Eq. 3-3) has been shown previously on (Eq. 3). The reduced model of gum purity shows that isolation temperature (term β_2) did not affect the gum purity significantly, and all the other terms are at significant levels (Table 3.2). The reduced model has a large *F*-value of 9.34 which indicates the reduced model is significant. The lack of fit implies the model is not significant relative to the pure error. The determination coefficient R^2, as a measure of the model's goodness-of-fit of this model is 0.847 and R^2 (adj) is 0.758 (Table 3.2). Indicating that the model can explain 85% of the variability of the response data around its mean values.

Fig 3.5 shows the 3-D response surface graph for the polynomial model with a regular pattern in relation to seed bran to water ratio and isolation time. The 3-D response surface image suggested that the seed bran to water ratio and time had significant effect on gum purity. The gum purity increased as the seed bran to water ratio and isolation time decreased. With low seed bran to water ratio and low isolation time the gum purity could increase up to 57.08%, compared with high seed bran to water ratio and long isolation time. The excess liquid used for the isolation process may cause the mass transfer ability increase and consequently cause more water-soluble material dissolved into the aqueous (Mirhosseini and Amid, 2012). This results is in agreement with the results reported by Karazhiyan et al. (2011). Shorter agitation time causes the polysaccharide of the epidermis to dissolve but other material may maintain in the seed bran. The protein content and other components in the isolated camelina gum were considered as impurity materials. Camelina gum purity also influenced by the protein content and other components, because shattering may happened in the separation process, which can cause some slightly shatter fall off from the endosperm, such as seed germ (Karazhiyan et al., 2011; Koocheki et al., 2009).

The feed speed for granular separation machine, wind tunnel speed, and other factors can affect the purity of seed bran separation (Berti et al., 2016). This leads directly to the purity of the gum isolated from the seed bran, structural proteins, and enzymes contamination in natural present can be the main reason (Koocheki et al., 2009). Although the interactions of protein and polysaccharide may improve the application characteristics of some gums, such as locust bean gum (Glicksman, 1982); higher protein content was observed with higher isolation temperature and also reflect browning in gum color (Fedeniuk and Biliaderis, 1994; Koocheki et al., 2009). Browning may affect the application prospect of polysaccharides, using a lower isolation temperature should be considered (Qian et al., 2012). Temperature was not an important factor relevant to the gum purity analysis.

3.3.4 Rheological property

A reduced quadratic model was selected to study the third response variable, viscosity property (*Y3;* Eq. 3-4) due to the first-order model does not fit the original data. The reduced quadratic model fits the original data well with *p*-value of 0.019 and R^2 of 0.781. This indicates that only about 21.87% of the total variations was not explained by the reduced model (Table 3.2). In addition, the lack of fit is not significant.

The effect of isolation temperature and seed bran to water ratio on rheological properties are presented in Fig 3.6A. At the lower temperature range, seed bran to water ratio did not have much effect on the rheological properties while at higher temperature range, the gum viscosity increased as the seed bran to water ratio decreased. This result is in agreement with the results reported by Razavi et al. (2009). Fig 3.6B shows the effect of isolation time and temperature on gum viscosity. The inverted umbrella shape is presented, which indicates that the position of the center point is the least responsive point. *Lepidium perfoliatum* seed gum has the same trend at the

low temperature and time range due to stronger molecules interaction at lower temperature (Koocheki et al., 2009). However, camelina gum viscosity rebounded at high temperature and long isolation time. This is probably because polysaccharide and protein interaction play significant roles in some polysaccharides which affect the rheological property (Montenegro et al., 2012).

3.3.5 Statistical analysis and validation of the model

The purpose of the experiment is to find the optimum conditions for the maximize gum yield and high purity with acceptable viscosity range. The following optimal encoded results were calculate by Design-Expert software: X1= - 0.096, X2= - 0.593, and X3= 0.033, which corresponding to the optimum condition of seed bran to water ratio, 1:39 g/mL; isolation temperature, 35 ºC; and isolation time, 1.5h. Under this optimal condition, the gum yield and gum purity are 19.08 and 56.24%, respectively. The gum yield from decorticate meal is similar to the yield isolated from whole camelina seed (Li et al., 2016). In contrast, the gum purity is low compared with that from whole seed due to more soluble impurities on the seed bran. However, the rheological properties of gum achieve the acceptable level (62.80 Pa.s). The predicted value of all three responses has the maximum value of 0.839. Considering the influencing factors of the three independent variables, seed bran to water ratio is the most important factor affecting the gum yield, purity and rheological property.

3.3.6 Effect of decortication on protein extraction and quality

Decortication process has advantages not only on camelina gum isolation, but also protein yield, and protein quality (Table 3-3). First, the degumming procedure in camelina protein isolation can be omitted when the camelina protein was extracted from pure endosperm. The aqueous protein extract can be prepared by mixing defatted endosperm with water at ratio of 1:10 - 1:15 instead of 1:30 for defatted camelina meal without decortication, which is able to be stirred.

Therefore, decortication makes the camelina protein extraction process easier and more cost effective. Second, the defatted camelina endosperm contains high protein (56.3%) compared to 39.2% protein in defatted camelina meal without decortication. The camelina protein extraction yield and extraction rate from defatted endosperm were improved to 42 and 67.45%, respectively, compared with 28.31 and 65.25% from defatted meal without decortication. Third, camelina protein extracted from whole seed meal had dark brown color, which may be caused by the pigment existed in the bran, while camelina protein from pure endosperm is a yellow white color.

3.4 Conclusion

Decortication was used to remove the seed bran for increased gum isolation and increased camelina protein isolation efficiency and protein quality. The RSM was used to determine the optimal gum isolation conditions. Three reduced quadratic models were developed for gum yield, gum purity and gum viscosity. With the optimal isolation condition of seed bran to water ratio of 1:39 g/mL, isolation temperature of 35 °C, and isolation time of 1.5 h, 19.08% gum yield from bran and 56.24% gum purity were achieved. The viscosity of the gum isolated from bran is lower than the gum isolated from whole seeds but it is at an acceptable level (62.80 Pa.s). Decortication process has advantages on protein isolation, such as improve the protein yield and quality. Decortication increased endosperm protein contents to 56.3% compared to 39.2% protein in camelina meal without decortication. The protein yield and extraction rate after decortication were improved to 42 and 67.45%, respectively, compared with 28.31 and 65.25% from undecorticated meal.

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Table 3.1 Experimental design with response surface methodology

Trials	Seed bran/Water (w/w)	Temperature (°C)	Time (h)	Yield (%, w/w)	Purity (%)	Rheological (Pa.s)
1	1:50 (1)	50 (0)	0.5 (-1)	18.59	48.77	49.4
2	1:40 (0)	25 (-1)	0.5 (-1)	15.14	56.55	102.6
3	1:40 (0)	50 (0)	1.5 (0)	18.98	55.48	51.1
4	1:30 (-1)	50 (0)	2.5 (1)	17.10	52.53	85.8
5	1:50 (1)	25 (-1)	1.5 (0)	19.62	53.87	81.8
6	1:40 (0)	25 (-1)	1.5 (0)	18.29	54.67	77.1
7	1:30 (-1)	75 (1)	1.5 (0)	7.47	54.14	95.6
8	1:40 (0)	75 (1)	0.5 (-1)	10.03	54.94	86.5
9	1:50 (1)	75 (1)	1.5 (0)	17.64	53.06	52.4
10	1:40 (0)	50 (0)	1.5 (0)	18.47	56.01	53.7
11	1:50 (1)	50 (0)	2.5 (1)	22.30	50.92	50.8
12	1:40 (0)	75 (1)	2.5 (1)	15.17	52.79	79.8
13	1:40 (0)	50 (0)	1.5 (0)	19.00	56.01	55.6
14	1:40 (0)	25 (-1)	2.5 (1)	19.52	51.72	51.6
15	1:40 (0)	50 (0)	1.5 (0)	18.80	56.82	58.3
16	1:30 (-1)	50 (0)	0.5 (-1)	11.97	57.09	104.8
17	1:30 (-1)	25 (-1)	1.5 (0)	16.68	56.55	60.9

Table 3.2 Significance of the fitted quadratic regression model parameters for all three responses

	Gum Yield (*Y1*)		Gum Purity (*Y2*)		Gum Viscosity (*Y3*)	
Regression	F Value	Pr>F	F Value	Pr>F	F Value	Pr>F
Model						
Quadratic	193.94	0.0001	9.34	0.0013	4.59	0.0190
Residual						
Lack of fit	1.96	0.2658	2.87	0.1633	1.73	0.3081
Variables[a]						
A		0.0001		0.0018		0.0102
B		0.0001		0.2762		0.6280
C		0.0001		0.0164		0.0580
A×B		0.0001				0.0287
A×C		0.1009		0.0156		
B×C						0.1054
A^2		0.0575		0.0214		
B^2		0.0001				0.1096
C^2		0.0026		0.0063		0.1090

[a] A=Seed bran to water ratio (w:w); B=Isolation temperature (ºC); C=Isolation time (h)

Table 3.3 Effect of decortication process on protein yield, purity.

	Whole seed meal	Endosperm
Protein content in defatted meal (%, db)	39.2	56.3
Protein extraction yield (%, w/w)[1]	28.31	42.0
Protein purity (%)	90.35	90.42
Protein extraction rate (w/w)[2]	65.25	67.45
Color	Dark brown	Yellow white

[1] Weight of isolates compared to defatted camelina meal.

[2] Weight of isolated protein compared to the total protein in defatted camelina meal.

Figure 3.1 Bran separation processing flowchart using impact decortication method.

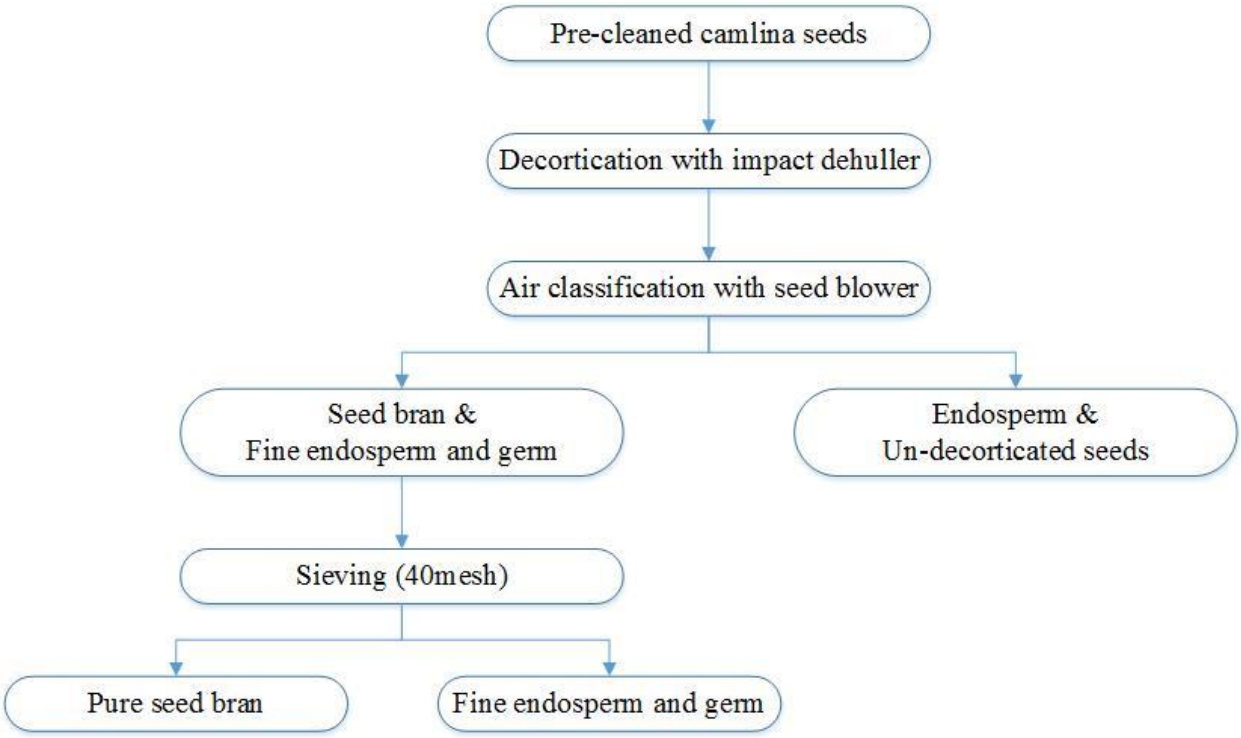
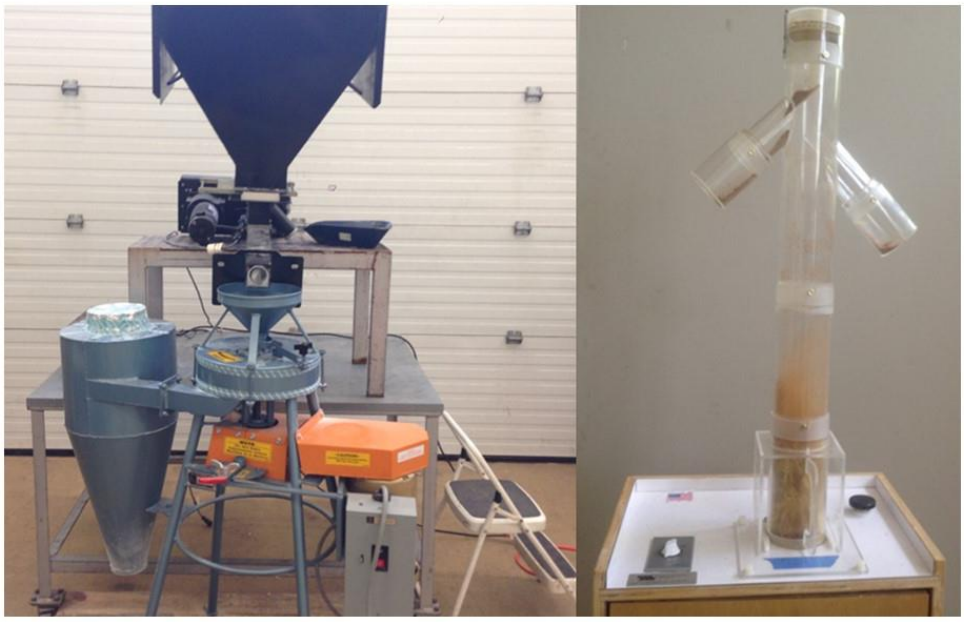


Figure 3.2 (A) impact dehuller with automatic feeder, and (B) wind tunnel seed blower.



(A) Impact dehuller with automatic feeder

(B) Seed blower

Figure 3.3 Morphological properties of (A) whole camelina seed, (B) decorticated seed bran, and (C) seed endosperm.

(A) Camelina seed (B) Seed bran (C) Seed endosperm

Figure 3.4 3-D response surface of gum yield (*Y1*) in relation to (A) seed bran to water ratio and isolation temperature, and (B) seed bran to water ratio and isolation time.

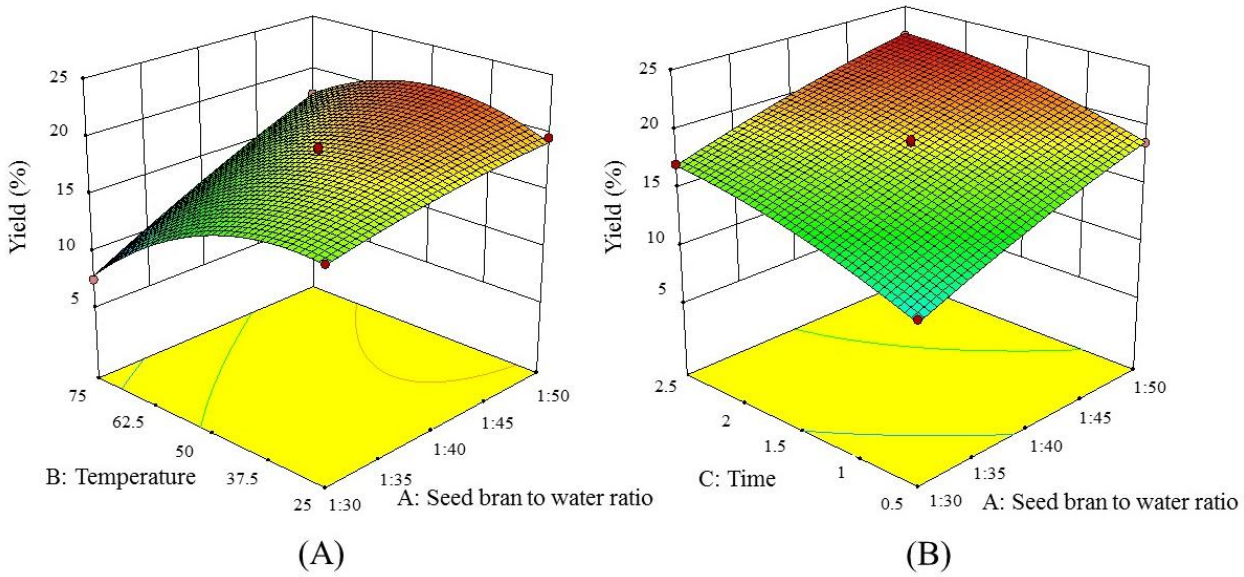


Figure 3.5 Response surface and contour plot of the effect of the seed bran to water ratio and isolation time on the gum purity (*Y2*) at 50 °C.

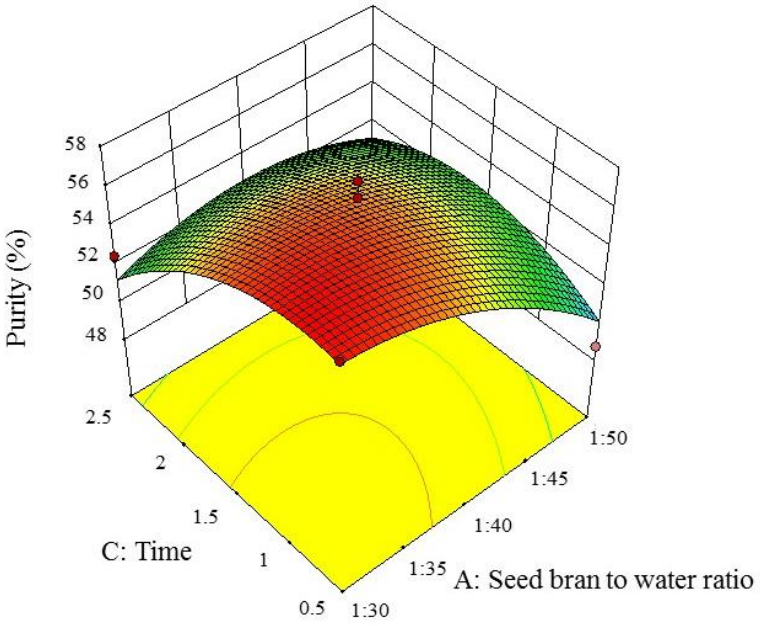
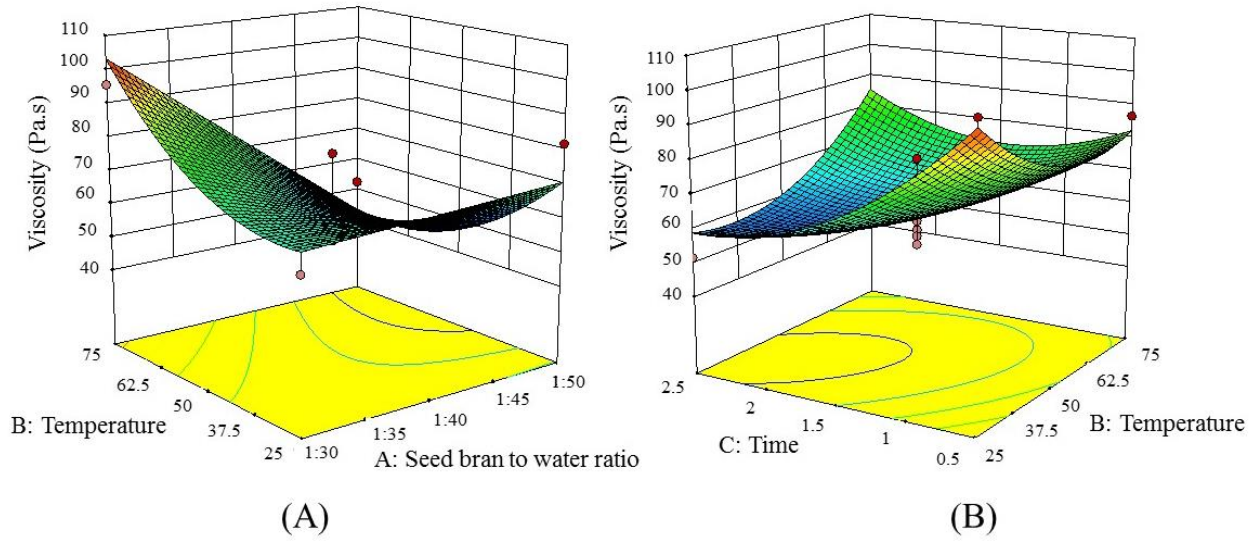


Figure 3.6 3-D response surface and contour plots of viscosity (*Y3*) in relation to (A) seed bran to water ratio and isolation temperature, and (B) isolation temperature and isolation time.



Chapter 4 - Conclusion and future work

4.1 Conclusion

The camelina gum isolated using spray drying methods consumes less time and energy compared with freeze drying method. The yield of camelina gum from spray drying isolation can be reached up to 1.89% of camelina seed, which is slightly less than that from ethanol isolation method (2.04%). The camelina gum obtained by using spray drying method showed a hollow, light and fluffy sphere structure compared to the fibrillary structure obtained from freeze drying method. Camelina gum exhibits shear thinning properties and modest drying temperature at 165 ℃ was considered as optimum drying temperature with better gum yield, purity, and viscosity. The influence of salts, sugar and ethanol on camelina gum influence the rheological properties but in a compatible situation.

The pure seed bran from camelina seeds obtained from decortication process was 17% of the whole seed, and camelina gum obtained from bran was between 7-23% of the seed bran on the dry basis depending the isolation conditions. The optimal conditions for isolating camelina gum from seed bran were found using the response surface methodology (seed bran to water ratio: 1:39, isolation temperature: 35 ºC, and isolation time: 1.5 h). The decortication procedure produced similar quantity of camelina gum compared to that from the whole seed. In addition, the protein yield and quality extracted from the remaining endosperm components were improved significantly, and the degumming step can be eliminated during the protein and oil extraction process.

4.2 Future work

The continuous study of decortication methods of camelina seed, such as the frequency of impact, wind speed and sifter screen size, is important to continue increase the gum yield and

quality. Compared to previous camelina gum isolation articles, the gum purity appears in a low range which also affects the applicate ability (Li et al., 2016). The following ideas may improve the purity of gum in later experiments. According to some previous studies, known that camelina protein has the lowest dissolution capability at pH 4.5 and camelina gum had great tolerance ability with wide pH range (Li et al., 2014; Zhu et al., 2016). Isolation gum at low pH range also helpfully to get product with whiter color (Koocheki et al., 2009). According to this information, we can reverie to remove the protein with extra centrifugal process before isolate camelina gum with ethanol, by adjust the pH to reach the lowest protein solubility range and optimal pH for enzyme activity (Burkus and Temelli, 1998; Zhu et al., 2016). These methods are often used for removing proteins from carbohydrates products in the literature such as Sevag method (Staub, 1965), Pronase agent (Brummer et al., 2003), and dithiothreitol agent (Clayton et al., 1996). Reduce the protein content in the sample can increase the polysaccharide content in the sample thereby, increase viscosity (Burkus and Temelli, 1998). Excluding gum, other polysaccharides such as starch, fibers may appear on the outer layer of the camelina seed. The purification process with thermostable α-amylase can be introduced to improve the camelina gum purity. Similar question were discussed on barley gum (Burkus and Temelli, 1998). According to the response surface methodology data, it can be easily found out that the yield of gum and purity at low isolation temperature is higher. So low temperature has a broader prospect for gum isolate without the risk of thermal degradation.

Research of camelina gum need be more focus on the microscopic level. The analysis of the sugar component needs to be carried out using the high performance anion exchange chromatograph with pulsed amperometric detection that can be used to detect the monosaccharide composition. Structural studies of camelina gum after purification need to be studied used NMR and varied methods. Similarly, such as linkage-pattern, methylation analysis, molecular weight

and surface and interfacial tension need be studied in depth, the impact of different extraction methods and conditions on the structure is also an important project.

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