# IMPROVEMENT OF ORGANOLEPTIC ATTRIBUTES OF YELLOW PEA FLOUR THROUGH AN EMERGING GREEN TECHNOLOGY: SUPERCRITICAL CARBON DIOXIDE + ETHANOL EXTRACTION

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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## ABSTRACT

An increasing demand for following healthier eating pattern has created a rapidly growing food market, including more nutrient dense and healthier plant-based foods. Nutrient-dense yellow pea flour is ideal for addressing these new-generation foods. However, its utilization in foods is limited due to its unpleasant flavor. Therefore, an eco-friendly deflavoring method has recently been found effective to improve sensory quality of pulse ingredients. Supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction was applied as deflavoring method. The goals of this research were to evaluate (1) the applicability of this extraction at optimized conditions to reduce off-flavor compounds of pea flour, and (2) interaction effect of extraction and particle size on flavor profile, physicochemical properties, particle size distribution, moisture sorption isotherms of deflavored pea flours. Findings of this study showed that operating conditions of SC-$CO_2$+EtOH extraction significantly ($p$<0.05) optimized using a central composite rotatable design under response surface methodology to ethanol (22%), temperature (86 °C), and pressure (42.71 MPa). Extraction at optimum conditions reduced total volatile (TV) content (0.55 µg/g) and improved sensory attributes of pea flour. TV contents of non-deflavored and deflavored whole pea flour and its fractions ranged from 7.1 to 18.1 µg/g and 0.4 to 2.7 µg/g, respectively. Similarly, the total volatile intensity of deflavored pea flours were significantly lower than non-deflavored flours as detected by the GC-Olfactory system. The extraction decreased moisture, resistant starch, damage starch, and lipid content of pea flours. Flours with coarse particles had lower protein, total starch, and starch damage than other flours. Medium and fine fractions had greater protein and total starch, respectively. Deflavored pea flours had lower viscosity parameters and water solubility index depending on particle size. Water sorption capacity of deflavored pea flours decreased with

increased water activity. SC-$CO_2$+EtOH extraction and particle size had a significant interaction effect for most response variables.

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## DEDICATION

I dedicate this work to my mom, husband, and older sister:

To my mom, Serife Ozel, without your positive attitude for all my decision, and endless love and support, I would not be an optimistic, smiling, strong, patient, and hard-working person for a long term-study and overall for the life, including good days and bad days.

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## LIST OF ABBREVIATIONS

AACC .....American Association of Cereal Chemists

ANOVA .....Analysis of Variance

C.....Celsius

CCRD.....Central Composite Rotatable Design

D.....Deflavored

FAO .....Food and Agriculture Organization

FDA .....Food and Drug Administration

FID .....Flame Ionization Detector

g.....Gram

GC .....Gas Chromatography

GC-O.....Gas Chromatography-Olfactometry

GI .....Glycemic Index

HCA .....Hierarchical Cluster Analysis

HS-SPME.....Headspace-Solid-Phase Microextraction

kg.....Kilogram

LOX .....Lipoxygenase

LPI.....Lentil Protein Isolate

LSD .....Least Significance Difference

mg .....Milligram

min .....Minute

mL .....Milliliter

mm .....Millimeter

MPa .....Megapascal

MSI .....Moisture Sorption Isotherm

µg .....Microgram

µl .....Microliter

NCI.....Northern Crops Institute

N.....Non-deflavored

OAC .....Oil Absorption Capacity

PPC .....Pea Protein Concentrate

PPEF .....Pea Protein Enriched Flour

PPI.....Pea Protein Isolate

PUFA .....Polyunsaturated Fatty Acids

RSM .....Response Surface Methodology

rpm .....Revolutions per Minute

RVA .....Rapid Visco Analyzer

PCA.....Principal Component Analysis

SCCA .....Sulphur-Containing Amino Acid

SC-$CO_2$+EtOH.....Supercritical Carbon Dioxide + Ethanol

TV .....Total Volatile

TVI.....Total Volatile Intensity

QDA.....Quantitative Descriptive Analysis

v/v .....Volume to Volume

VOC .....Volatile Organic Compounds

VSA .....Vapor Sorption Analyzer

USDA.....United State Department of Agriculture

WAI.....Water Absorption Index

WSI .....Water Solubility Index

# CHAPTER 1. GENERAL INTRODUCTION

Dry peas, one of the most important pulses, are an excellent source of protein and complex carbohydrates (e.g., dietary fiber and starch) and exhibit many health benefits (e.g., lowering the glycemic index, reducing the risk of diabetes) (Boye, Zare, & Pletch, 2010a; Hall, Hillen, & Garden-Robinson, 2017; Tulbek, Lam, Wang, Asavajaru, & Lam, 2017). Recently, researchers have focused on how to improve nutritional value and functional characteristics of pulses, create new foods as a functional product to increase customer consumption, and develop unique sensory properties of foods (Boye et al., 2010b; Hall et al., 2017; Li & Ganjyal, 2017; Kaiser, Barber, Manthey, & Hall, 2019). Therefore, whole dry pea flour might be a potential food ingredient to produce nutrient-dense and healthy plant-based foods and gluten-free foodstuffs. This flour also complements cereal flours resulting in improved protein quality in the combined flours (Kaiser et al., 2019; Maskus, Bourre, Fraser, Sarkar, & Malcolmson, 2016; Vatansever & Hall, 2020). However, dry pea flour, as with other pulse flours, has not been widely used in the food applications due to its unpleasant flavor or pea off-flavor (Murat, Bard, Dhalleine, & Cayot, 2013; Nosworthy, Tulbek, & House, 2017; Vatansever & Hall, 2020). Thus, undesirable pea off-flavor restricts the use of pea ingredients in the global food system (Malcolmson et al., 2014; Murat et al., 2013; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). In addition to this, impacts of different particle size on the physicochemical and functional properties of whole pea and split pea flour have been previously reported by Kaiser et al. (2019) and Maskus et al. (2016). But, effects of different particle size on the volatile profile and sensory properties of dry pea flours have not been extensively investigated.

The objective of this study was to better understand pea off-flavor compounds and deflavoring method to mitigate the intense pea flavor through deflavoring protocol. Also, research

on flours with different particle sizes was completed to illustrate particle size impacts on the flavor profile and deflavoring method. Therefore, the first chapter of the research completed documented significant ($p < 0.05$) removal of unpleasant flavor compounds from whole yellow pea flour using Supercritical Carbon Dioxide + Ethanol (SC-$CO_2$+EtOH) extraction. The efficacy of this removal was determined through the application of gas chromatographic (GC) analyses, sensory evaluation, and mathematical and statistical modeling for process optimization.

For the second and third chapters of the study, the influences of different particle sizes along with SC-$CO_2$+EtOH extraction at optimum conditions were investigated for various pea flour samples. Proximate composition, functional and pasting characteristics, moisture sorption isotherms (i.e., for predictive critical water activity value via mathematical modeling), and particle size distribution were determined using the AACC International approved methods, rapid visco analyzer (RVA), vapor sorption analyzer (VSA), and particle size analyzer, respectively. The changes in volatile profile and sensory attributes of pea flours were determined through the application of GC analyses, including GC-Olfactory, sensory evaluation, and chemometrics. The unique findings related to particle size, the interaction effect between two factors, significant changes for proximate composition, functional properties, moisture sorption isotherms, volatile profile and organoleptic properties of pea flours were obtained.

### 1.1. General Research Methodology

The general research methodology is presented in Figure 1.1. Whole yellow pea provided by three different sources were blended at equal ratio of each source and hammer milled. Then, milled dry pea was subjected to SC-$CO_2$+EtOH extraction. The operating conditions of SC-$CO_2$+EtOH extraction was optimized using response surface methodology (RSM) with central composite rotatable design (CCRD). After the system optimization, pea flours of different particle

size were subjected to the optimized SC-$CO_2$+EtOH extraction and evaluated through gas chromatographic analyses and sensory evaluation. Thereafter, obtained deflavored pea flours and non-deflavored (raw) pea flours were tested to determine their chemical compositions, pasting, functional, and morphological properties, particle size, and moisture sorption isotherms.

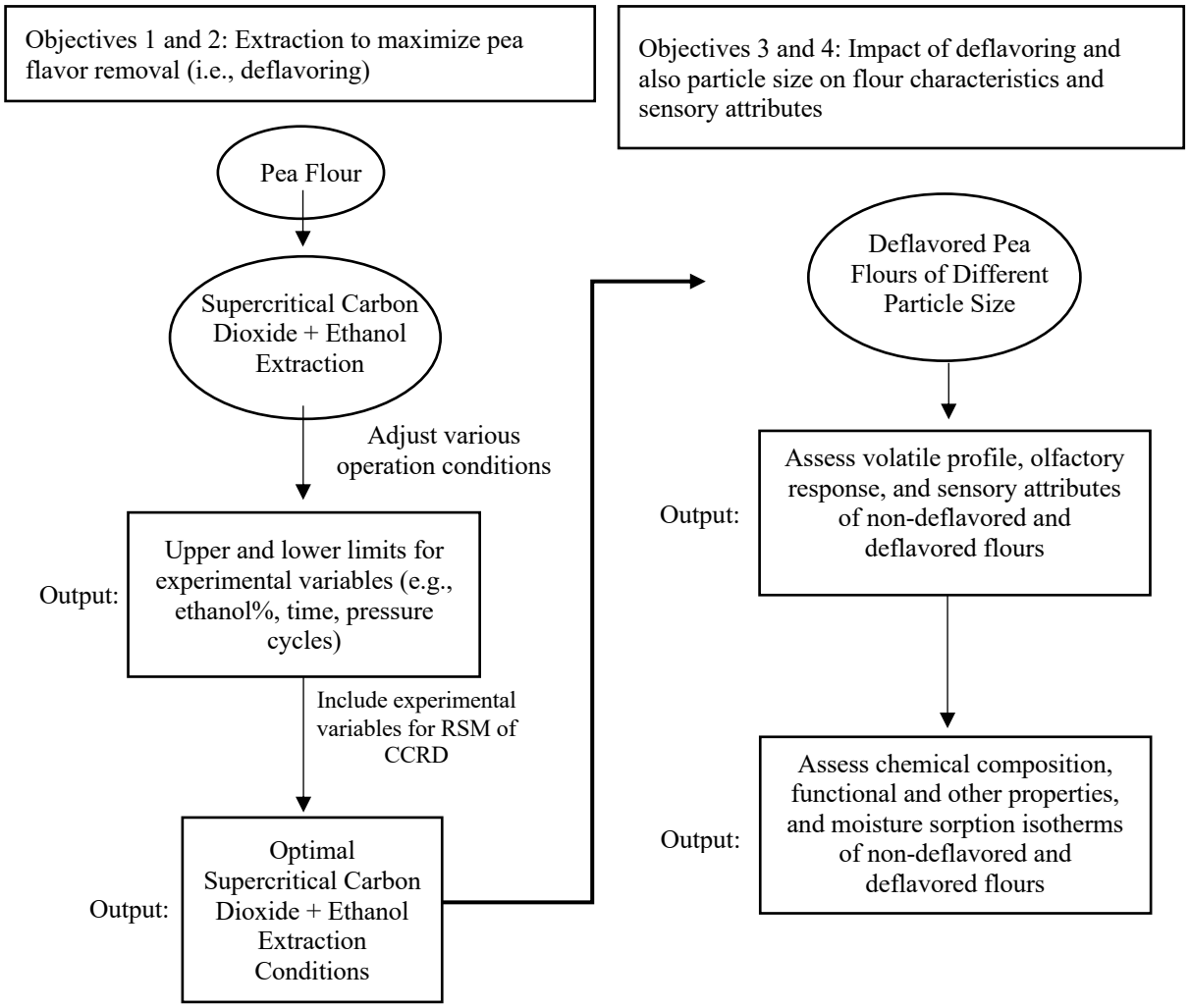


Figure 1.1. The design of optimally deflavored whole dry pea flours and their evaluation.

## 1.2. Objectives and Hypotheses

### 1.2.1. Objectives

Objective 1: To optimize the experimental factors of SC-$CO_2$+EtOH extraction using CCRD under RSM to reduce off-flavor in whole yellow pea flour.

Objective 2: To investigate changes in the physicochemical properties, particle size distribution, and moisture sorption isotherms of pea flours deflavored using SC-$CO_2$+EtOH extraction.

Objective 3: To determine the interaction effect between the two factors (i.e., SC-$CO_2$+EtOH and particle size) on the physicochemical properties, particle size distribution, and moisture sorption isotherms of pea flours.

Objective 4: To assess the applicability of SC-$CO_2$+EtOH extraction for deflavoring pea flour with different particle sizes.

Objective 5: To determine the interaction effect between the two factors (i.e., SC-$CO_2$+EtOH and particles size) on the volatile profile and sensory quality of yellow pea flours.

**1.2.2. Hypotheses**

Objective 1: SC-$CO_2$+EtOH extraction will be optimized using RSM with CCRD to reduce off-flavor compounds of whole yellow pea flour.

Objective 2: Deflavored pea flours will have different physicochemical properties and moisture sorption isotherms, but particle size distribution will be same.

Objective 3: SC-$CO_2$+EtOH and particle sizes will have an interaction effect for physicochemical properties and particle size distribution.

Objective 4: SC-$CO_2$+EtOH extraction will be an efficient deflavoring method for different particle size flours to reduce off-flavor.

Objective 5: SC-$CO_2$+EtOH and particles size will have an interaction effect on the volatile profile and sensory quality of yellow pea flours.

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# CHAPTER 2. LITERATURE REVIEW

## 2.1. Classification and Production of Dry Peas Among Pulses

Pulses are the edible seeds and a member of the Leguminosae family, where the Food and Agricultural Organization (FAO) of the United Nations defines them as "Leguminosae crops harvested exclusively for their grains, containing dry beans, peas, and lentils." The FAO fundamentally divides pulse grains into 11 groups. These are dry beans (kidney, haricot, lima, butter, adzuki, mungo, golden, gram, scarlet runner, moth, tepary, and rice bean), dry broad beans (horse, faba, broad and field beans), dry peas, pigeon peas, chickpeas, dry cow peas, lentils, bambara beans, vetches, pulses nes (sword, yam, velvet, guar, and winged beans), and lupins (FAO, 1994). According to the FAO definition, dry beans, peas, and lentils are referred to as pulse grains while soybeans, fresh beans, and fresh peas are not.

Dry pea (*Pisum sativum* L.) has been produced in the United States as a high-value and versatile crop to address the demands for human and feed processing (Hall et al., 2017; Simsek, Tulbek, Yao, & Schatz, 2009). This crop is a cool climate crop with the optimum growing temperatures of 13 to 18 $^{o}$C. Cultivated peas can be classified into garden pea (*Pisum sativum ssp. hortense* L.) with wrinkled seeds and field pea (*Pisum sativum ssp. arvense* L.), also known as dry pea, with a smooth seed surface, including green and yellow cotyledon varieties (U.S. Dry Pea & Lentil Council, 2017).

The production of dry peas among total world production of pulse crops accounts for approximately 8 to14.6% (Joshi & Rao, 2017). The top dry pea producers in the global market are Canada, Russia, United States (US), China, and India. In 2018, the total U.S. dry pea production was approximately 722,530 metric tons (FAO, 2018) and mostly exported as feed and human products (Li & Ganjyal, 2017). The production of dry peas within the U.S. is predominantly in the

Midwest, including North Dakota, Montana, Minnesota, and South Dakota, and also in the Pacific Northwest, including Washington, Idaho, and Oregon. North Dakota has become a major producer of dry pea since 2009; accounting for over 40% of the annual U.S. production (~322,000 metric tons) (Kaiser, 2019). The use of dry pea in the food system has been rising along with other pulse crops due to increased health concerns and demands for nutritious and gluten-free products (Hall et al., 2017; Li & Ganjyal, 2017).

**2.2. Proximate Composition and Nutritional Importance of Dry Peas**

Pulses have been significant food crops throughout human history due to their high nutritional components and health-promoting benefits besides their contributions to agriculture sustainability and global food security (Rochfort & Panozzo, 2007; Vaz Patto et al., 2015). Pulses are particularly notable crops in terms of their high protein contents. Furthermore, they are important starchy stable foods after cereals in the world and are rich sources of dietary fiber, vitamins, minerals, and bioactive compounds. The protein content of pulses is more than twice that of cereals (Hall et al., 2017; Kaiser 2019).

Recently, researchers have focused on nutritional value and functional characteristics of pulse components for use in new food formulation and as a functional food ingredient (Hall et al., 2017; Tulbek et al., 2017). Among pulses, dry pea is nutritionally important grain legume like other pulse crops (bean, chickpea, and lentil); therefore, dry pea is grown and consumed worldwide as a healthy food in different forms (Rempel, Geng, &, Zhang, 2019; Tulbek et al., 2017). The physical seed characteristics of dry pea include a 1000 seed weight of ~206-223 g, an outer seed coat that accounts for ~10% of the seed weight, a pair of cotyledons surrounded by the seed coat accounting for ~89% of seed weight, and an embryo at ~1% of seed weight (Chibbar, Ambigaipalan & Hoover, 2010; Kaiser, 2019). The seed coat, also called as the hull or testa,

composed of ~89% dietary fiber, ~5% protein, ~3% ash, and ~3% starch, whereas the remaining part of seed has ~48% starch, ~28% protein, ~14% cell wall-based dietary fiber, ~3% ash, and ~1% lipid (Dalgetty & Baik, 2003). Overall, dry pea is relatively high in crude protein (14-31%), which is a rich source of lysine, gluten-free, and has a low allergenicity; total carbohydrates (55-72%), including mainly starch (30-49%) and total dietary fibers (3-20%); and vitamins (e.g. folate) and minerals (Hall et al., 2017). Due to the rich nutritional contents of dry pea, it is a good raw material that can be used to fortify food formulations. Folate and lysine fortification from pea are important part of the benefits of using pea flours in food systems (Hall et al., 2017; Tulbek et al., 2017). Furthermore, dry peas exhibit promising health benefits due to the presence of high dietary fiber content, which slow the digestion of starch, lower glycemic index and act as prebiotics that are linked to a lower risk of cardiovascular disease, cancer, diabetes, a reduction of LDL cholesterol, supporting gastrointestinal microflora, and other benefits (Simons, Hall, & Vatansever, 2017). In addition to dietary fibers, dry pea is a good source of vitamins, such as riboflavin, niacin, thiamin, folate, and vitamin A (mainly its precursor beta-carotene) (Hall et al., 2017).

### 2.2.1. Proteins

Proteins are large, complex macromolecules, consisting of amino acid chains. Pulses are an inexpensive source of proteins. Pulse proteins are an alternative plant-based protein that can economically replace animal proteins in poor countries (Boye et al., 2010a). In particular, pulses play a major role in providing protein and calories in African and Asian diets. The crude protein content of pulses falls between 14-44% (Foschia, Horstmann, Arendt, & Zannini, 2017; Hall et al., 2017), where that of North American dry pea is 15.7-28.6% (Boye et al., 2010a; Amber, 2019; Tulbek et al., 2017; U.S. Dry Pea & Lentil Council, 2018). Therefore, pulses have higher protein

content than cereals, which is in the range of 7-14 % (Foschia et al., 2017). Among the pulses, dry pea protein exhibits a similar protein bioavailability and techno-functionality to soy protein. Therefore, dry pea protein is a non-GMO and low allergenic alternative to soy protein in the global food system, including plant-based foods (Boye et al. 2010a; Samard & Ryu, 2019).

The Osborne fractionation classified plant proteins into four groups based on solubility: (i) albumins (water soluble proteins); (ii) globulins (salt soluble); (iii) prolamins (alcohol soluble); and glutelins (soluble in dilute acid or base) (Delcour & Hosoney, 2010). In pulse proteins, globulins are 70-80 % of the total protein (Foschia et al., 2017; Lu, He, Zhang, & Bing, 2019; Shevkani, Singh, Chen, Kaur, & Yu, 2019), whereas pea globulins range from 49 to 70% (Hall et al. 2017; Lu et al., 2019; Tzitzikas, Vincken, de Groot, Gruppen, & Visser, 2006). Pulse globulins, based on sedimentation coefficients, are further classified into legumin (11S) and vicilin (7S) fractions. The ratio of legumin to vicilin of dry pea is 1-3:1, which depends on intrinsic (e.g., amino acid profile, surface charge) and extrinsic factors (e.g., processing, the cultivar, and growing environment) (Lu et al., 2019; Shevkani et al., 2019). Also, pea has the minor fraction convicilin (7S-8S), which contains sulphur-containing amino acids (SCAAs) that are methionine and cysteine (Lu et al., 2019). Legumin (11S) is a hexamer of 300-410kDa, containing six subunits (~ 60-65kDa) that are composed of an acidic, α-chain (~40kDa) and a basic, β-chain (~20kDa) polypeptides. These polypeptides are covalently linked through a disulfide bridge at the presence of SCAAs (Barac et al., 2010; Lam, Karaca,Tyler, & Nickerson, 2018; Shevkani et al., 2019). Also, legumin is high in glutamic acid, alanine, valine, and leucine (Lam, Karaca, Tyler, & Nickerson, 2018). Pea vicilins is a trimer of 150-170kDa, including ~48-52kDa subunits with 70-80% similarity of legumin amino acids except for SCAAs, which are relatively low in this fraction. Therefore, vicilin subunits interact via non-covalent hydrophobic bonding linkage due to the

absence of cysteine residues (Barac et al., 2010; Lam et al., 2018). Convicilin is similar to vicilin with 80% amino acid sequence homology, but it is high in SCAAs (Tzitzikas et al., 2006).

Albumins (2S, 5-80kDa) are the second major storage proteins of pulse seeds. These proteins are responsible for metabolic reactions during seed germination through formation of enzymatic proteins (e.g., proteases, amylases) and are rich in lysine content (Lu et al., 2019; Shevkani et al., 2019). Pea albumins are about 15-25% of total seed proteins (Hall et al., 2017; Lu et al., 2019; Tzitzikas et al., 2006). Pea albumins and globulins are the main fractions, involved in techno-functionality of proteins (e.g., solubility, emulsifiying, gelling, foaming) (Tulbek et al., 2017). Pulse prolamins, containing a high amount of proline and glutamine, and glutelins, containing a high proportion of methionine and cystine, are minor seed proteins. Pea prolamins and glutelins are ranged of 4-5% and 11% for total seed proteins, respectively (Hall et al., 2017; Lu et al., 2019; Tzitzikas et al., 2006).

Pulse crops have a different amino acid profile than cereals, specifically lysine in pulses are higher than cereals at almost three times. However, cereals have relatively higher SCAAs than pulses (Kaiser, 2019). In general, pulse protein has high amounts of lysine, leucine, glutamate, aspartate, and arginine; however, they are deficient in cysteine, methionine, and tryptophan (Boye et al., 2010a). In addition to this, protein quality of dry pea is a promising protein ingredient in the food systems since its protein digestibility corrected amino acid scores (PDCAAS) calculated with amino acid profile and true protein digestibility is about 93%, which is close to soybean protein (100%) (Yang et al., 2012).

### 2.2.2. Carbohydrates

Carbohydrates in pulses are the major components with the range of 42-76% (Hall et al., 2017). Dry pea is comprised of approximately 55-72% carbohydrate, containing the main

components, starch (30-49%) and dietary fibers (15-39%), and the minor constituents that are sugars, such as sucrose (2.2-2.6%) and oligosaccharides, including raffinose (0.2-1.0), stachyose (1.3-3.2%), and verbascose (1.2-4.0%), depending on seed cultivar, growing location, and environment (Hall et al., 2017; Tulbek et al., 2017).

Pea starch is composed of 25-45% amylose and 55-75% amylopectin (Hall et al., 2017; Hoover, Hughes, Chung, & Liu, 2010; Tulbek et al., 2017). The ratio of amylose and amylopectin plays a key role in starch functionality. Dry pea starch has a lower degree of crystallinity (i.e., 7.8-36.8%) than typical cereal (e.g., corn starch), tuber starches (e.g., potato starch), and other pulse crops (e.g., kidney bean, pigeon pea) (Kaiser, 2019; Singh, Nakaura, Inouchi, & Nishinari, 2008; Zhou et al., 2015). In general, the starch granule crystallinity is negatively correlated with the amount of amylose (Zhou et al., 2015) and amylopectin branching (Kaiser, 2019). Thus, dry pea might have a low crystallinity due to its higher amylose content and highly branched amylopectin structure. Dry pea, as with other pulses, has a C-type crystallinity, which is a combination of A-type, exhibiting a greater resistance to swelling and disruption due to denser structure with 4 water molecules than B-type, and B-type crystalline, containing 36 water molecules (Raghunathan, Hoover, Waduge, Liu, & Warkentin, 2017; Simsek et al., 2009; Xu, Ma, Ren, Li, Liu, & Hu, 2019a). Therefore, an increase of A-type crystalline to B-type crystalline results in higher gelatinization temperature of pea starch (Raghunathan et al., 2017).

Furthermore, pea starch can be divided into slowly digestible, rapidly digestible, and resistant starch based on digestibility. Resistant starch, which is indigestible fraction of starch in the gastrointestinal tract, is 2-10% of dry pea seed weight and can be used as a dietary fiber (Tulbek et al., 2017). Dietary fiber of dry pea, including resistant starch, is an important functional ingredient with its greater levels in comparison to cereals. American Association of Cereal

Chemists (AACC, 2001) defines fiber as “the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.”

Total dietary fibers are classified in two groups, which are soluble and insoluble fibers. Soluble fiber helps to slow digestion in the colon, on the other hand, insoluble fiber is fermented in the colon, so it contributes to the growth of the colonic bacteria; therefore, these fibers are considered as prebiotic (Jukanti, Gaur, Gowda, & Chibbar, 2012). Thus, dietary fibers are important in terms of lowering cholesterol, diabetes control, and improving digestive system (Mudgil & Barak, 2013). In general, insoluble dietary fibers in pulses consist of cellulose, hemicellulose and lignin, on the other hand, soluble dietary fibers are composed of oligosaccharides, such as stachyose, raffinose, verbascose, and pectin (Annor, Ma, & Boye, 2014). Particularly, the hulls of pulses are very rich in dietary fiber contents and during processing, this part is removed as a by-product. The use of the pulse hulls as food ingredients might be a good approach to increase dietary fibers of foods (Annor et al., 2014). Besides the hulls of pulses, Dalgetty & Baik (2003) stated their cotyledon fibers also are rich in dietary fibers, such as cotyledon fibers in common beans are reported approximately 55 to 60% of total dietary fibers. These authors stated that cellulosic and noncellulosic polysaccharides differ in cotyledon and hull fibers of pulses. The cotyledon fibers in legume grains are nonstructural polysaccharides (e.g. hemicelluloses, pectin, and gums), while the hull fibers in pulses are cell wall polysaccharides, such as cellulose, hemicellulose, and lignin. Also, the soluble and insoluble dietary fiber contents are found in different amounts in pulses depending on the pulse crops and cultivars. Stoughton-Ens, Hatcher, Wang, & Warkentin (2010) reported the soluble dietary fiber in peas was 0.6-3.7% and insoluble dietary fiber in peas was 8.7-12.0%. The dietary fiber compositions of peas are

composed of cellulose (55%) and hemicellulose (23%), and some pectin-like polysaccharides (8%) (Hall et al., 2017). Also, dry peas are higher in galacturonic acid (10-18.4%) (Brummer, Kaviani, & Tosh, 2015).

### 2.2.3. Lipids

Lipids are minor constituent in pulses and mainly found in a small amount in dry pea seed. Lipid contents of pulses are low around 1-4% (Hall et al., 2017), where dry pea produced in North America contain approximately 1.5-2-0%, depending on the cultivar (Boye et al., 2010a; Tulbek et al., 2017). Dry pea lipids are composed of combination of different lipids: 55-61% phospholipids and 31-40% triacylglycerol, with minor lipid components accounting for 1.3-2.7% free fatty acids, 2.0-4.0% diacylglycerols, 0.8-2.4% steryl esters, and 0.5-0.9% hydrocarbons (Yoshida, Tomiyama, Saiki, & Mizushina, 2007). Free fatty acid profile includes 10-20% saturated fatty acids (e.g., palmitic and stearic acid), 27-58% mono-unsaturated fatty acids (MUFA), such as oleic acid, and 30-57% polyunsaturated fatty acids (PUFA), such as linoleic acid (Solis, Patel, Orsat, Singh, & Lefsrud, 2013). Among free fatty acids of pea lipids, linoleic acids are the major fatty acid (46-54%), follow by oleic acid (15-31%), linolenic acid (9-11%), palmitic acid (7-13%), and stearic acid (2-3%) (El-Saied, Amer, & Gabran, 1981; Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007; Solis et al., 2013; Wang & Daun, 2004).

### 2.2.4. Micronutrients: Minerals, vitamins, and carotenoids

Pulses are a good source of dietary micronutrients, including vitamins and minerals as well as macronutrients, which play a key role in nutritional quality (Hall et al., 2017). Mineral constituents of dry pea produced in North America have been reported in the range of 588-850, 5.8-7.0, 46-60, 1092-1450, 11.7-12.7, 1.6-2.7, 3320-10376, 7478-9832, 25-43 mg/kg for calcium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, and zinc, respectively and

220-469 µg/kg for selenium, respectively (Gawalko, Garrett, Warkentin, Wang, & Richter, 2009; Ray et al., 2014; Tulbek et al., 2017; U.S. Dry Pea & Lentil Council, 2018). Mineral contents of dry pea seeds were greater than wheat and other cereals (Tulbek et al., 2017). Furthermore, Gawalko et al. (2009) showed that heavy metals, including cadmium, arsenic, lead, and mercury in dry pea were lower than the maximum residue levels determined by the Codex Alimentarius. The large range of mineral content in dry peas might be associated with pea variety and most likely the mineral availability of the soil during seed development (Kaiser, 2019).

Dry pea seeds are a rich source of vitamins such as folate, niacin, thiamine, riboflavin, pyridoxamine, pyridoxal, and pyridoxine. Specifically, dry peas contain a high amount of folate, which is about 25.0-64.8 µg/100g for green pea and 23.7-350 µg/100g for yellow pea. Therefore, dry peas are relatively rich in this vitamin, which is a limiting micronutrient in refined cereals such as wheat flour (Han & Tyler, 2003; Tulbek et al., 2017). Folate is an important nutrient for humans, specifically pregnant women (Kaiser, 2019).

In addition, dry peas are a good source of carotenoids, containing predominantly lutein (no provitamin activity) and trace amounts of other carotenoids, such as ß-carotene (precursor of Vitamin A), zeaxanthin (no provitamin activity), and violaxanthin (no provitamin activity). Both green and yellow pea cotyledons have a high amount of lutein, in the range of 6.65-17.47 µg/g, which has several health benefits (e.g., antioxidant activity, skin and eye maintenance). However, green peas (0.47-1.52 µg/g) are relatively richer in ß-carotene than yellow peas (0.01-0.04 µg/g) (Ashokkumar, Tar'an, Diapari, Arganosa, & Warkentin, 2014). Overall, luteins and total carotenoids for green pea cotyledons were greater than yellow pea cotyledons (Ashokkumar et al., 2014).

## 2.3. Dry Pea Ingredients

### 2.3.1. Pea flour

Dry pea flour is produced either from the whole pea seed or split peas in different granulations according to the purpose of the food product, such as snacks, bakery products, pasta, bread, extruded pet foods (Tulbek et al., 2017). The high nutritional profile of dry pea flour (22-28% protein, 40-53% starch, and 6-20% dietary fibers, and rich micronutrient contents) has gained interest by food manufacturers as a way to improve nutritional quality of food products (Hall et al., 2017; Kristiawan et al., 2018). Specifically, fortification of dry pea flour (i.e., high in lysine, but low SCAAs) with low-lysine cereal ingredients is a potential application of pea flour to address nutritionally inadequate protein profile of cereal-based products (Kaiser et al., 2019; Kristiawan et al., 2018). Recent studies showed that incorporation of pulse ingredients at 9-11% into cereal-based products, such as crackers, cookies, and granola bars, were found sensorily acceptable (Fujiwara, Hall, & Jenkins, 2017). Also, fortification of wheat flour with pea flour at 20% to produce nutrition-dense biscuits exhibited an acceptable sensory quality (Qayyum et al., 2017).

Furthermore, there has been rising demands for gluten-free, non-GMO, protein-dense, and high fiber plant-based foods. Therefore, whole dry pea flour or split pea flour might directly be used to address these popular food demands (Kristiawan et al., 2018; Koksel & Masatcioglu, 2018). Non-deflavored whole yellow pea flour was used to produce banana bread, biscotti, and pasta to replace whole wheat flour (100%). Marinangeli, Kassis, & Jones (2009) found that sensory parameters (e.g., taste, smell, texture, appearance, overall acceptance) of banana bread and biscotti made with pea flour were not significantly ($p > 0.05$) different from products made with wholewheat. However, pea pasta was not acceptable due to off-flavor of pea flours. Based on this study, using higher levels of sugar and other ingredients for production of pea banana bread and

pea bisquit might mask off-flavor of pea flour (Marinangeli, Kassis, & Jones, 2009). Also, yellow pea bread produced with non-deflavored pea flour had a significantly lower sensory acceptability due to off-flavor issue of pea flour in comparison with commercial premix bread sample. However, yellow pea bread had a longer shelf life than control sample (Jeradechachai, 2012). New generation gluten-free, protein-rich, plant-based extruded snacks were produced using dry pea flour with low-moisture extrusion, but sensory acceptabilities of extruded pea snacks were not investigated (Kristiawan et al., 2018; Koksel & Masatcioglu, 2018). Consequently, dry pea flour could be used as a potential value-added ingredient for various purposes. However, off-flavor of pea flour has limited the use of pea flour, specifically complete pea flour replacement in gluten-free food products.

### 2.3.2. Pea protein ingredients

Extraction of proteins from dry pea seeds can be carried out through dry fractionation and wet fractionation (Tulbek et al., 2017). Dry fractionation produces a product with 48-58% pea protein and is referred to as pea protein concentrate (PPC). In comparison, wet fractionation results in pea protein isolate (PPI), which has protein contents for 75 to 90% (Boye et al., 2010a; Ettoumi & Chibane, 2015; Lu et al. 2019; Pelgrom et al., 2013; Schutyser, Pelgrom, van der Goot, & Boom, 2015; Tulbek et al., 2017).

#### *2.3.2.1. Pea protein concentrate*

PPC is produced by dry fractionation of finely milled flour combined with air classification. A typical processing of PPC process includes the following: dehulling dry peas; fine milling to liberate starch granules (~20 μm) that is embedded in the protein matrix, which is surrounded by a fiber-rich cell wall (Figure 2.1.), from protein-rich particles (1-3 μm), and fiber-rich particles (Pelgrom et al., 2013; Schutyser et al., 2015; Tyler & Panchuk, 1982); and separation

of the larger starch particles from the lighter particles with air classification (Schutyser et al., 2015).


Figure 2.1. Starch-protein matrix surrounded by cell wall. The figure was adopted from Schutyser et al., 2015).

In recent years, there has been increased interest for dry fractionation (Figure 2.2) because the processing deemed to be a sustainable and clean route to produce protein-dense products from protein crops compared to wet fractionation. Particularly, dry fractionation has become more popular due to reduction in water and energy consumption as a result of less process intensity and enabling the product to carry a clean label since no chemicals were used during processing (Schutyser et al., 2015). PPC, containing high protein content (48-58%) and other components, such as 5-10% starch, 2.5-3.0% fat, and 2.7-3.1% ash, have been used for production of high-protein food products, e.g., extruded snacks (Tulbek et al., 2017).

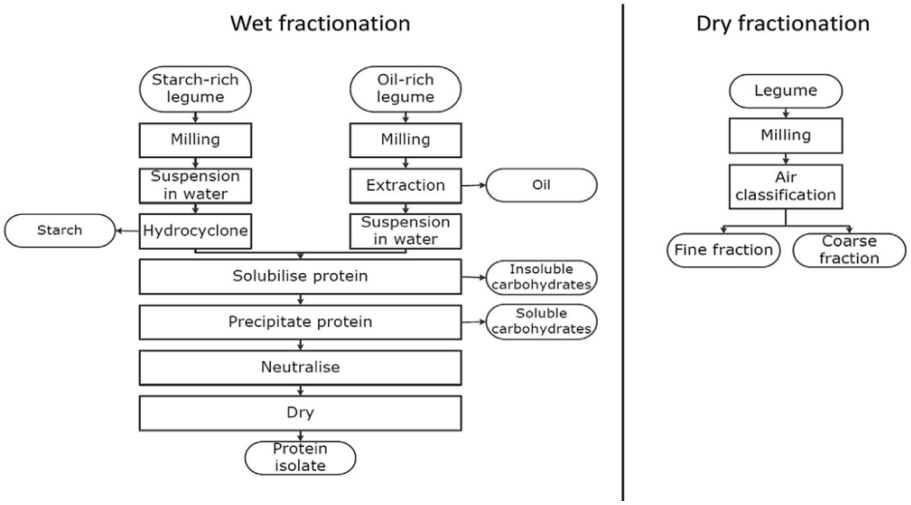


Figure 2.2. The wet and dry fractionation of legumes. The figure was adopted from Schutyser et al. (2015).

#### *2.3.2.2. Pea protein isolate*

PPI has been used as an outstanding gluten-free and non-GMO ingredient to soy protein and replacement for animal proteins in the food market. The wet fractionation (Figure 2.2) has been applied to separate protein and starch of pea flour using various extraction protocols, such as alkali extraction/isoelectric precipitation (AE/IP), ultrafiltration, salt extraction and micellization) (Lam et al., 2018; Tulbek et al., 2017). Amongst these protocols, the AE/IP is the most common way to wet fractionate dry pea ingredients, PPI (main product), pea starch (by-product), and pea cotyledon fiber (by-product). Through the AE/IP, starch granules and protein solution are separated from the slurry, which is a dispersion of pea flour in water, using hydrocyclone. Thereafter, protein solution is precipitated at pH 4.8, which is the isoelectric point (pI) of pea protein. At the same time, solubilized fibers present in protein solution are removed and the resulting precipitated proteins are re-solubilized at pH 7. Later, the PPI (75-90% protein) is obtained after drying (Boye et al., 2010b; Schutyser et al., 2015; Tulbek et al., 2017). The PPI sample may contain trace (0.4%) amount of starch (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014).

### 2.3.3. Pea starch

Pulse starches, as in cereal starches, are the primary carbohydrate in the crop (Hoover et al., 2010; Hoover & Ratnayake, 2002; Raghunathan et al., 2017). Dry pea starch ingredients, pea starch concentrate and pea starch isolate, are obtained using either dry fractionation or wet fractionation methods that result in starch contents in the range of 60-90%. Pea starch concentrate consists of 65-75% starch, 10-15% protein, 0.9-1.3% fat, and 1.2-1.4% ash (Tulbek et al., 2017). On the other hand, pea starch isolate (90-99%) is almost free from protein (0.5-2%), fat (0.07%),

and ash (0.01-0.20%) (Huang, Schols, van Soest, Jin, Sulmann, & Voragen, 2007; Simsek et al., 2009; Tulbek et al., 2017).

Unlike protein ingredients, dry pea starch ingredients have not gained the same level of interest by food manufacturers despite research and development efforts demonstrating functionality (Hoover et al., 2010). Pulse starches have been considered as by-products of protein isolate and concentrate production. However, the renewed interest in pulse starch in food formulation, particularly pea starch, has been rising due to their many functional characteristics (Ambigaipalan et al., 2011; Raghunathan et al., 2017; Tulbek et al., 2017).

Pea starch has been used for production of gluten free foods, noodles, pet foods, and other food formulations due to its superior functional properties, such as resistant to mechanical and thermal shear, high retrogradation rate, strong gelation and texturizing, which correspond to its high amylose content (30-65%) and intrinsic properties (Huang et al., 2007; Li et al., 2019; Raghunathan et al., 2017; Ratnayake, Hoover, & Warkentin, 2002; Simsek et al., 2009; Tulbek et al., 2017). Additionally, pea starch is fairly high in resistant starch (4-20%), which is slowly digestible starch; thus, results in slow release of glucose to the blood, resulting in a lower glycemic index (Raghunathan et al., 2017; Simons et al., 2018; Tulbek et al., 2017).

Moreover, starch, which is often preferable ingredients for meat products (e.g., coarse ground and emulsion-style meat products) due to their desired functionalities, such as high viscosity and high-water binding, that help to improve texture and slice-ability, and extend shelf-life of meat products. Furthermore, starch is a cost-effective ingredient. The most important criterion for starch to be used in meat products is its gelatinization temperature. This temperature needs to meet thermal processing temperature of the products, such as meat proteins denaturation temperature. During the protein denaturation, the water is released, and the starch serves as a water

binding agent (Balestra & Petracci, 2019). Based on functionality properties of starch, pea starch can be added to protein-based foods, such as texturized vegetable proteins or related foodstuffs.

### 2.3.4. Pea fiber

Dry pea fiber is another ingredient produced after fractionation of dry pea seeds into pea protein and pea starch. The total dietary fiber in pea seeds are in the range of 3-27% based on the seed cultivar, growing location and environment (Hall et al., 2017; Tulbek et al., 2017). Dietary fiber of pea seeds includes the fibers found in the hull and cotyledon cell wall, which consist-of cellulose, hemicellulose, and pectin-type polysaccharides, and resistant starch. Pea hull fiber composes of mostly cellulose while pea cotyledon fiber is high in pectin-type polysaccharides. Besides these polysaccharides, other dietary fibers in dry pea seeds are raffinose family oligosaccharides/α-galacto-oligosaccharides (i.e., raffinose, stachyose, and verbascose), which are present at 2-10% depending on cultivar, environment and growing conditions (Tosh & Yada, 2010; Tulbek et al., 2017).

Dry pea fiber is obtained by either dry- or wet-fractionation processing and result in fiber content of 50 to 90% (Tulbek et al., 2017). Pea hull fiber (~90% insoluble and 10% soluble dietary fiber) is produced using dry-fractionation as a fine powder, whereas pea fiber from cotyledon cell wall/inner pea fiber (50% insoluble and 50% soluble) is produced using wet-fractionation (Tosh & Yada, 2010; Tulbek et al., 2017).

The functional properties of pea fiber are water-retention capacity, water absorption capacity, and swelling based on hydration properties of dietary fiber and fat-retention capacity (Tosh & Yada, 2010). Among these properties, water absorption capacity plays an important role since pea hull fiber and inner pea fiber can bind water at approximately 3.5-4.0 and 9 times their weight, respectively. Water absorption is the primary functionality of pea fiber for food

formulations. Commercial dry pea fiber is commonly used in fiber fortification of bread and extruded snacks to provide structure along with enrichment of dietary fiber content (Tulbek et al., 2017). Also, this fiber is applicable for meat sector due to its superior water-binding capacity, texturization through viscosity and gelation ability, fat stabilization in emulsified products and nutrient enhancement (Balestra & Petracci, 2019). Gluten-free products produced with rice, corn and/or potato flour are enriched with pea fiber (Tosh & Yada, 2010). Due to the low viscosity of pea fiber, particularly inner pea fiber, it could be applicable for non-viscous functional beverages (Dalgetty & Baik, 2003).

### 2.4. Techno-Functionality of Pea Flour

#### 2.4.1. Water and oil absorption capacity

The water absorption index (WAI) is the measure of the volume occupied by flour components (e.g., starch, protein) that swells in excess water (Kaur, Sandhu, & Singh, 2007; Sharma, Singh, Hussain, & Sharma, 2017). Water solubility index (WSI) relates to the presence of soluble components (e.g., starch, fibers, sugar, proteins) in excess water (Sharma et al., 2017). These two parameters are useful to provide the knowledge about water absorption capacity of dry pea flour. Kaur et al. (2007) reported a range of WAI from 4.84-5.01 g/g for dry pea flours but was higher (5.17-6.11 g/g) for pigeon pea flours. Furthermore, Kaur & Sing (2005) and Simons (2013) have reported similar results for different pulse flours, 2.39-2.66 g/g WAI for chickpea flours, and 2.92 and 2.69 g/g for whole pinto bean flour and its high starch fraction, respectively. WSI values for different pulse flours have been reported in the range of 19.8-20.6% for dry pea flours, 13.7-14.5% for pigeon pea flours, 20.42-22.89% for chickpea flours, 22.66 % for whole pinto bean flour, and 14.01% for pinto bean high starch fraction (Kaur & Sing, 2005; Kaur et al., 2007; Simons, 2013).

The WAI and WSI values depend on food processing methods. For instance, Simons (2013) reported an increased WAI value (4.39 g/g) for extruded pinto bean high starch fraction and suggested that structural changes of starch and other components likely contributed to the observe increase. Furthermore, particle size might influence WAI and WSI values. Kaiser et al. (2019) and Maskus, Bourre, Fraser, Sarkar, & Malcolmson (2016) reported that as particle size decreased, starch damage increased in yellow dry pea flours. Maskus et al. (2016) reported that whole yellow dry pea flour with decreased particle size had significantly ($p < 0.05$) higher starch damage but lower water absorption capacity. Sharma et al. (2017) indicated that higher starch damage, due to greater damaged polymer chains, could reduce the availability of hydrophilic groups to participate in water binding, resulting in a decreased WAI (Sharma et al., 2017).

Oil absorption capacity (OAC) is a relationship between the surface availability of hydrophobic amino acids and non-polar chains of flour components (e.g., dietary fibers, proteins, starch) found in flour and oil. Maskus et al. (2016) reported OAC of hammer milled whole yellow pea flour and yellow split pea flour as 0.9 g/g and 1.12 g/g. The main difference between flour samples was the dietary fiber content, which was relatively higher in whole pea flour than split pea flour. Furthermore, Kaiser et al. (2019) reported that hammer milled split dry pea flour, containing higher total dietary fiber content, had a lower OAC (0.9 g/g) than roller milled yellow split pea flour (1.1 g/g). Effects of particle size on the OAC of dry pea flours have been reported by Maskus et al. (2016). Maskus et al. (2016) showed that coarse, medium, and fine particles with d (0.9) values of 1,142 µm, 593.6 µm, and 298 µm had non-significant OAC values of 0.8 g/g, 0.9 g/g, and 0.9 g/g, respectively. But, in this study they observed that finer particles had a significantly lower OAC (1.04 g/g) than other medium and coarse particles, which had OAC values of 1.12 g/g. Furthermore, Kaiser (2019) found that roller-milled split pea flour had significantly higher starch

damage than hammer-milled split pea flour. Higher starch damage in roller milled pea flour might cause better oil binding and result in higher OAC.

### 2.4.2. Pasting properties

Pasting properties of dry pea flour is primarily related to its starch content. Pea starch exhibits unique techno-functionalities. These functional properties are as follows: (1) high resistance to shear thinning, indicating more resistance of starch granules to collapse subsequently obtaining low breakdown viscosity that is needed for extensively mixed products (e.g., extrusion processing of gluten-free pasta); high gel elasticity, and rapid retrogradation that is favorable for gluten-free oriental foods (e.g., glass noddle) as well as providing health benefits (e.g., lowering glycemic index) through high resistant starch levels due to high amylose content (Ambigaipalan et al., 2011; Hoover et al., 2010; Huang et al., 2007; Kaiser et al., 2019; Tulbek et al., 2017). Functionality properties of pea starch are dependent to its gelatinization, which is an irreversible process in which starch granules swell in the presence of excessive water at the elevated temperature and its retrogradation, which is a reassociation between starch chains, particularly amylose chains and formation of a gel at the cooling temperature after gelatinization (Simsek et al., 2009; Marta & Tensiska, 2017).

The knowledge about starch gelatinization can be obtained using differential scanning calorimetry (DSC) and rapid visco-analyzer (RVA) for gaining information about the temperature range of gelatinization, and overall pasting properties, respectively (Kaiser 2019). According to the literature, pea starch gelatinization transition temperatures, which are onset ($T_o$), peak ($T_p$), and conclusion($T_c$) temperatures obtained by DSC, have been recorded as 53.6-66.6 $^{\circ}$C, 58.8-75.5 $^{\circ}$C, and 62.8-85.4 $^{\circ}$C, respectively (Gomes, Cordoba, Rosa, Spier, Schnitzler, & Waszczynskyj, 2018;

Hoover et al., 2010; Huang, et al., 2007; Leite, de Jesus, Schmiele, Tribst, & Cristianini, 2017; Simsek et al., 2009; Wang, Sharp, & Copeland, 2011).

The pasting properties of dry pea starch obtained by RVA provide a general pasting behavior of starch, including various parameters. These parameters are as follows (1) pasting time and temperature indicate time and temperature where an increase in viscosity occurs due to granule swelling; (2) peak viscosity of hot slurry measures maximum swelling of starch granules and indicates the water-holding capacity of the starch; (3) breakdown in the viscosity describes the holding strength of starch granules before disruption depending on the temperature, shear rate, and ingredient; (4) final viscosity is the cold paste viscosity obtained after reassociation of starch chains during cooling and is closely related to texture of end-products, such as viscous paste or gel from the material after cooking and cooling process (Perten, 2015). Pasting properties of isolated dry pea starch provided by RVA have been reported in the range of 69.6-75.8 ˚C (pasting temperature) and viscosity values, 303-4663 cP, 10-2397 cP, 190-2665 cP, and 284-6026 cP for peak, breakdown, setback, and final/cold paste viscosities, respectively (Gomes et al., 2018; Hoover et al., 2010; Huang et al., 2007; Leite et al., 2017; Raghunathan et al., 2017; Simsek et al., 2009; Wang et al., 2011). Large differences have been observed in the literature for viscosity values of isolated dry pea starch due to inconsistency of the method applied for RVA testing, particularly associated with amount of starch sample used. Pasting profile of U.S. whole dry pea flours were between 70.2-82.4 ˚C (pasting temperature) and viscosity values, 1104-2100 cP, 10-312 cP, 648-1968 cP, 1800-3756 cP for peak, breakdown, setback, and final/cold paste visocisties, respectively (U.S. Dry Pea & Lentil Council, 2018).

### 2.4.3. Moisture sorption isotherms

Water is a vital component in foods and profoundly impacts the chemistry, nutritional value, sensory attributes (e.g., texture, taste, appearance), shelf-life, microbiological safety of the food ingredients and products (Schmidt, 2004; Syamaladevi et al., 2016). Therefore, monitoring changes and controlling of food moisture during processing and storage are essential due to many roles of water in food reactions and food quality (Nurtama & Lin, 2010). Moisture content and water activity ($a_w$) are the terms used to assess relationship of water with food components (e.g., carbohydrates, protein).

The moisture sorption isotherm (MSI), which is a plot between moisture content and $a_w$ is beneficial to evaluate changes in the food matrix due to chemical reactions and also for food safety. From MSI curve, which provides three regions: region I ($a_w < 0.2$) for tightly bound water molecules in the food matrix at limited mobility; region II ($0.2 < a_w < 0.85$) for higher increase in water activity with smaller increase in moisture and water molecules interacted with food components at some mobility; region III ($a_w > 0.85$) for higher water activity and moisture content and free water molecules interacted with food components at high mobility. Based on the regions, the texture of foods is dry and crispy at region I, chewy and moist at region II, and soft and juicy at region III (Damodaran et al., 2008; Schmidt, 2004).

Gaining knowledge of MSI is crucial for process design, modeling, and optimization (Al-Muhtaseb, McMinn, & Magee, 2002). Therefore, knowledge of MSI is helpful to design dehydration process and also predict shelf-life of dried products; to formulate food mixtures; to develop new products; to investigate moisture barrier characteristics; to estimate microbial growth; and to evaluate chemical and physical changes of food products (e.g., lipid oxidation, non-enzymatic browning), specifically during storage conditions (Damodaran et al., 2008; Schmidt,

2004). Henderson (1987) showed that after mixing dry (7% moisture) and wet (27% moisture) barley grains, the moisture equilibrium of grains was reached in three days. However, equilibration was temperature dependent, where equilibrium occurred faster at 20 °C than 10 °C. Hogan, Chaurin, O'Kennedy, & Kelly (2012) investigated the impacts of milk protein type, formulation, and storage conditions for protein bar samples. They observed that protein bar hardness was dependent to protein type. Hardness development of protein bars was associated with non-equilibrium changes owing to hydration behaviors of each food components and their competition for moisture. From this study, diminishing water activity differences among food ingredients provides better hardness control during storage. Also, the effect of processing on the WSI of food ingredients and food products are important to estimate their behavior for further food applications and their shelf-life stability during the storage (Al-Muhtaseb et al., 2002).

There are five types of MSI curves: type I (Langmuir), II (sigmoidal), III, IV, and V. Sigmoidal shape (type II) occurs most often in foods (Al-Muhtaseb et al., 2002) and is the behavior that is extensively seen for cereal-based foods. This behavior is associated with the occurrence of sorption multilayers, where small pores are saturated at lower water activities; conversely, large pores are saturated at higher water activities (Syamaladevi et al., 2016). The most common mathematical models used to characterize MSI curves of foods are Brunauer-Emmett-Teller (BET) for up to 0.5 $a_w$, and Guggenheim-Anderson-de Boer (GAB) for the entire $a_w$ range and widely used model (Ricardo et al., 2011). Decagon (2010) has developed a better model, Double Log Polynomial (DLP), than GAB to describe complex MSIs of foods.

**2.5. Techno-Functionality of Pea Protein**

Functional properties of pulse proteins have gained increasing interest for novel food applications, particularly replacing animal proteins in food systems as alternative to soy and wheat

proteins, which are highly allergenic proteins, as well as their nutritional characteristics and health-promoting benefits (Balestra & Petracci, 2019; Boye et al., 2010a; Lam et al., 2018). The promising functional properties of pulse proteins, which are commonly applicable in food applications, are water and oil absorption, protein solubility, emulsification, foaming, and gelation properties (Boye et al., 2010a).

Protein solubility is described as the equilibrium between protein-protein (hydrophobic) and protein-solvent (hydrophilic) interactions. Therefore, it is the retention of proteins in protein-solvent suspension, such as protein-water (Lam et al., 2018). The balance between hydrophobicity and hydrophilicity of protein molecules determines protein solubility and depends on molecular surface compositions (i.e., hydrophilic and hydrophobic amino acids) of proteins. Furthermore, protein solubility changes based on cultivars, solvent polarity, pH, ionic strength, interactions with other food components, such as lipids and carbohydrates, and food processing methods, such as extraction protocol, heating, freezing, drying (Nielsen, 2009).

Solubility of pulse proteins is greater at strong acid and high alkaline pH values but low (2-4%) at pH 4 to 6, which is close to the pI (Boye et al. 2010a; Lu et al., 2019; Shevkani, Singh, Kaur, & Rana, 2015). Karaca, Low, & Nickerson (2011) reported that isolated dry pea proteins had 70-95% solubility at pH 9. Depending on extraction protocols, including AE/IP, alkaline extraction with ultrafiltration (AE/UF), salt extractions, PPIs might have different protein solubility (Lam et al., 2018). Stone, Avarmenko, Warkentin, & Nickerson (2015) determined salt extracted PPI had greater protein solubility than PPIs extracted using AE/IP and micellization. Improved protein solubility in the salt extraction may be due to enhanced surface charge and a decrease in surface hydrophobicity of proteins. Compared to other extraction protocols, PPI extracted through micellization had poor protein solubility (Stone et al., 2015).

Water absorption capacity (WAC) of a protein measures the amount of water absorbed per gram of protein (Lam et al., 2018; Shevkani et al., 2015). WAC of proteins have significant impacts on sensory quality of final food products (e.g., mouthfeel, texture, and flavor retention) due to the water retainability of protein after food processing (Lam et al., 2018; Shevkani et al., 2019). Specifically, water retention is important for several food products (e.g., dough, soup, bread, bakeries, meat analogs) for final product quality (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Lam et al., 2018; Shevkani et al., 2019). Amino acid composition of proteins influence WAC, for instance highly charged amino acids tend to interact with water through electrostatic attractions (Stone et al., 2015). Similar to protein solubility, WHC is lowest at the pI of proteins due to greater protein-protein interactions, through hydrophobic forces, that occur at the pI (Lam et al., 2018). The WAC of PPIs extracted through AE/IEP ranged from 1.9 to 4.8 g/g (Shevkani et al., 2015; Stone et al., 2015; Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2011). Additionally, Boye et al. (2010b) reported that PPC (AE/IEP) produced from yellow split peas exhibited greater WHC (~4.4 ml/g) than that of PPC (3.9 ml/g) from AE/UF.

Fat absorption capacity (FAC) of proteins measures the amount of fat absorbed by per gram of protein (Lam et al., 2018). The interaction between protein and lipid depends on nonpolar side chains of proteins and the aliphatic chains of lipids, which are bound through hydrophobic interactions (Lam et al., 2018; Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2011). The FAC influence sensory quality (e.g., texture, flavor) of final food products, such as meat extender, meat replacer for meat-based foods, and baked goods (Shevkani et al., 2019). Based on processing conditions and cultivar types, the FAC of PPIs were different. Shevkani et al. (2015) reported the FAC range (i.e., 5.5-7.2 g/g) of PPIs, whereas commercial PPIs had 1.3-1.7 ml/g FAC (Osen et al., 2014).

Foam occurs when proteins unfold to form an interfacial membrane, which encapsulate the air bubbles in a suspension and preventing their collapse. Foaming property of proteins has been mainly applied in food systems for beverages, mousses, meringues, and whipped topping applications. Foaming properties of food proteins are determined using foam expansion (FE), foam capacity (FC) and foam stability (FS). The change in the volume of foam over a certain time associates with FS; however, FC or FE measures the degree of volume created (Boye et al., 2010a; Ettoumi & Chibane, 2015). Foaming stability of a protein is closely associated with the hydrophobicity and electrostatic repulsion of protein. High hydrophobicity and low electrostatic repulsion are desired for a good foaming stability unlike emulsion property of proteins. Surface charge of proteins can be modified based on pH value of the suspension. The pH value near to pI reduces foam formation due to lower protein solubility and results in lower FC (Kaiser, 2019). However, at the pI surface elasticity of protein films is maximum level thus enhances FS. Additionally, the FC and FS differ based on protein concentration (Hoang, 2012). Foaming capacity and stability of dry pea protein suspensions at 10, 25, 50, and 100 mg/ml were evaluated by Aluko, Mofolasayo, & Watts (2009). The protein suspension at 100 mg/ml had the lowest FC and FS at pH 3, 5, and 7. Protein suspension at 25 mg/ml had a greater FC at pH 3 and 5, but that of 10 mg/ml was higher than 25 mg/ml at pH 7. Compared to FC, protein suspension at 100 mg/ml had a better FS at pH 3, 5, and 7 due to development of a thicker interfacial film, including a finer and more dense foam.

An emulsion is a mixture of two or more immiscible liquids where one of liquids is dispersed as droplets into the other liquid. Pulse proteins can be good emulsifying agents and could be applicable to food emulsions, such as butter, milk, cream, mayonnaise, salad dressing and several meat products (Nielsen, 2009). Emulsifying properties of food proteins are measured by

emulsion capacity (EC) or emulsifying activity index (EAI, $m^2/g$), and emulsifying stability index (ESI, min) (Ettoumi & Chibane, 2015). The EC describes the ability of proteins to form an emulsion (Ettoumi & Chibane, 2015) and is positively correlated to protein surface charge and solubility. The EAI measures an estimation of the interfacial area formed per unit weight of protein stabilized at a defined time period (Karaca et al., 2011). ESI defines protein ability to impart strength to an emulsion for resistance to stress over time (Ettoumi & Chibane, 2015). Emulsifying property of proteins increases as surface hydrophobicity increase with high electrostatic repulsion, which prevents coalescence. Emulsifying properties of pea proteins are impacted by extraction protocols and also the ratio of legumin to vicilin (Kaiser, 2019). Specifically, vicilin content increases emulsion capability of pea proteins (Aluko et al., 2009). Aluko et al. (2009) also showed that PPI had a greater emulsion capability than soy protein isolate (SPI) owing to possibly higher sugar content, which improves solubility, in PPI than SPI.

Gelation property of globular proteins plays a major role in various food products, specifically texturization of plant proteins. Protein gel is a three-dimensional network that in trapped in water and other food components. For instance, texturized pea proteins formed through gelation generates a structured network to provide textural and rheological properties to foodstuffs (Lam et al., 2018; Mession, Chihi, Sok, & Saurel, 2015). The mechanism of protein gelation consists of three stages and can be induced by heat, pH, or salt concentration. Based on heat-induced gelation of globular proteins, these three steps are (1) protein denaturation, which is when native proteins unfold through thermal process, thereby buried residues in protein interior are expose to protein-protein interactions; (2) aggregation of unfolded proteins where buried residues aggregate through disulfide bridge and/or non-covalent bonding; and (3) gel-formation where a continuous three-dimensional network is formed through arrangement of protein aggregates,

which is stabilized by several bonds (i.e., disulfide bridge and/or non-covalent bonding) (Mession et al., 2015; Mession, Sok, Assifaoui, & Saurel, 2013).

Heat-induced gelation of pea globular proteins are associated with the ratio of legumin/vicilin since legumin has a poor gelling ability (Sun & Arntfield, 2010). Mession et al. (2015) compared mixed pea globulins, vicilin-enriched samples, and legumin-enriched samples and found that legumin-enriched samples had lower gelling ability than other samples. But, O' Kane, Vereijken, Gruppen, & van Boekel (2005) reported gelling ability of pea proteins were more dependent on cultivars than pea legumins. They observed that chemical interactions (e.g., disulfide bridge ability of proteins) were more associated with gelling capacity of pea proteins than their legumin contents (O' Kane et al., 2005). Furthermore, protein concentration has been reported a determinative factor for protein gelation. Inadequate protein concentrations result in an insufficient surface for protein-protein interaction. On the other hand, high concentration leads to a bad dispersion of protein suspension unless an additional energy source, such as mixing, shearing forces, is applied for proper protein dispersion. In both cases, protein concentration can impact the formation of sufficient network structure of pea protein gel (Lin et al., 2017). Pea protein concentration to generate proper network structure range from 5.5 to 20% based on extraction protocols of pea protein and other extrinsic factors, including ionic strength and pH values (Adebiyi & Aluko, 2011; Lin et al., 2017; Mession et al., 2013; Mession et al., 2015; Shand, Ya, Pietrasik, & Wanasundara, 2007; Sun et al., 2010).

### 2.6. Off-Flavor Profile of Dry Peas

Flavor plays a crucial role for overall acceptability of food products. Products produced using pulse ingredients have a lower acceptability owing to a pea off-flavor (i.e., green, earthy, mushroom, grassy, bitter). Therefore, off-flavor compounds of pulse ingredients, particularly pea

ingredients, are a significant limiting factor in the utilization of pea ingredients in food applications (Heng, 2005; Malcolmson et al., 2014; Nosworthy et al., 2017). Pea off-flavor compounds are the combination of off-aroma (i.e., volatiles) and off-taste (e.g., saponins) compounds (Heng, 2005; Roland et al., 2017). These compounds are found in dry peas inherently or develop during handling, processing, and storage (Azarnia, Boye, Warkentin, & Malcolmson, 2011a; Murray, Shipton, Whitfield, & Last, 1976; Sessa & Rackis, 1977).

### 2.6.1. Volatile organic compounds in dry peas

All food and food products contain hundreds of volatile compounds, specifically volatile organic compounds (VOCs). Sensory evaluation of food products has been an important criterion for the selection of food (Maarse, 1991). Pea off-flavor compounds contain predominantly various VOCs, which are alcohols, aldehydes, ketones, furans, and alkyl methoxypyrazines (Heng, 2005; Roland et al., 2017). These VOCs are responsible for a distinct pea aroma, including green, grassy, beany, mushroom, earthy, metallic and other aromas (Murat et al., 2013; Azarnia et al., 2011a). Degradation of pea lipids via enzymatic (hydrolytic and oxidative process) and non-enzymatic (autoxidative) reactions and of pea amino acids cause the formation of off-aroma compounds and leads to flavor reversion in dry peas (Azarnia et al., 2011a; Murray et al., 1976; Sessa et al., 1977).

#### *2.6.1.1. Lipid oxidation*

Dry pea lipids (1-4 %), containing various lipids (e.g., triacylglycerol, free fatty acids) can be degraded into secondary products (e.g., alcohols, aldehydes, ketones) through lipid oxidation. Lipase may increase the amount of free fatty acids in dry peas through degradation of pea lipids into free fatty acids. Free fatty acids, particularly PUFA, can be degraded into off-aroma compounds by lipoxygenase (linoleate: oxidoreductase, EC 1.13.11.12, LOX) (Hayward, Cilliers, & Swart, 2017) or the non-enzymatic process autoxidation (Azarnia et al., 2011a). Nevertheless,

LOX-catalyzed degradation of PUFA is considered as a major contributor of unpleasant pea-off aroma development in dry peas (Roland et al., 2017). Dry pea contains predominantly linoleic acid (18:2), accounting for approximately 48% of total free fatty acid content (Hall et al., 2017), thereby it makes dry pea susceptible to LOX enzyme because the natural substrates of plant LOX are the C18-polyunsaturated fatty acids (e.g., linoleic and α-linolenic acid) (Hayward et al., 2017).

LOX has a group of non-heme metal-containing dioxygenases that catalyzes the insertion of molecular oxygen into *cis*, *cis*, 1,4-pentadiene units of PUFAs to form conjugated unsaturated fatty acid hydroperoxides, which are 9S- or 13S-hydroperoxides (Hayward et al., 2017). Wu & Robinson (1995) detected two types of LOX isozymes in pea seeds, LOX-2 and LOX-3, which are similar to soybean LOX-2 and -3. Soybean LOX-2, also known as 9/13 LOX, catalyzes linoleic acid with oxygen to 9S-hydroperoxy-octadecadienoic acid (HPODE) and 13S-HPODE, equally. But, pea seed LOX-2 catalyzes the oxygenation of linoleic acid predominantly to 13S-HPODE (Wu & Robinson,1995). Additionally, LOX-2 has an ability to use the esterified unsaturated fatty acids found in seed membranes (Hayward et al., 2017). On the other hand, soybean LOX-3 catalyzes the oxidation moderately at the 9 position, resulting in 9S-HPODE (Hayward et al., 2017). Likewise, pea seeds LOX-3 produces predominantly 9S-HPODE (Wu & Robinson, 1995).

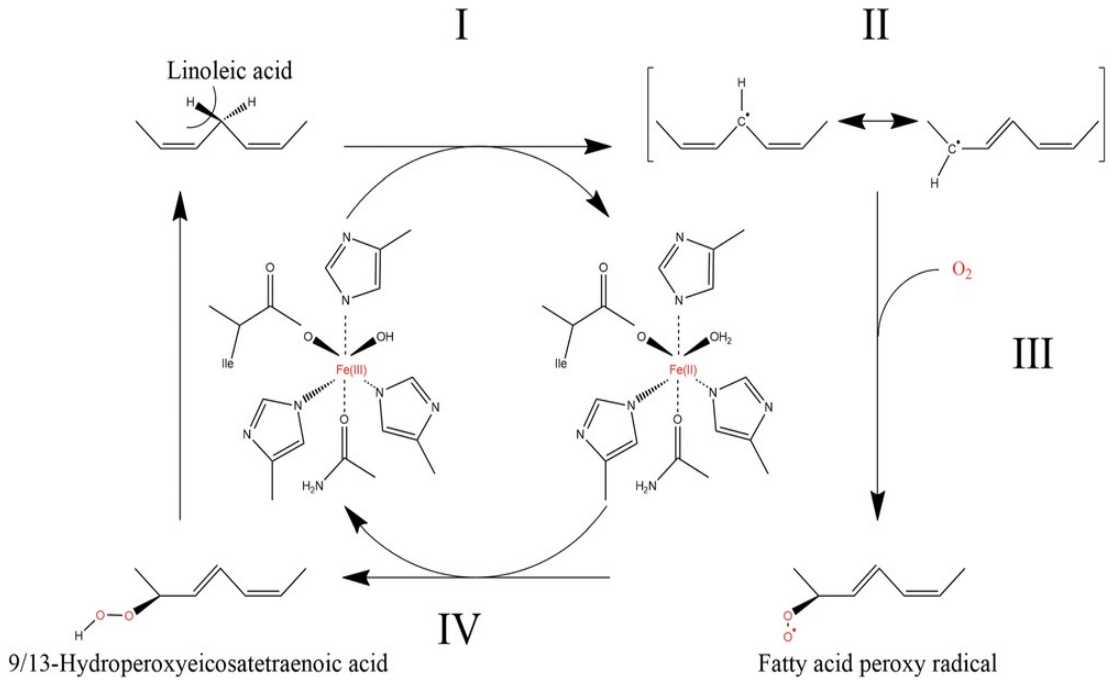


Figure 2.3. The mechanism of LOX-catalyzed fatty acid oxygenation. Numbers represent the following: I: Cycling between active Fe (III) and inactive Fe (II) states for hydrogen abstraction from fatty acid; II: Radical rearrangement; III: Oxygen insertion; and IV: Reduction of fatty acid peroxy radical (Adopted from Hayward et al., 2017).

The mechanism of fatty acid oxidation promoted by LOX include four consecutive stages (Figure 2.3): (i) hydrogen abstraction, (ii) radical rearrangement, (iii) oxygen insertion, and (iv) peroxy radical reduction (Hayward et al., 2017). Hydrogen abstraction and oxygen insertion occur in *cis*, *cis*, 1,4-pentadiene units of PUFAs. After hydrogen abstraction, occurs at the double bonds, via cycling electron between active Fe (III) to Fe (II) of LOX, which creates an alkyl radical, a molecular rearrangement of double bonds takes place to stabilize free alkyl radical, a conjugated diene ($L^{\bullet}$). Molecular oxygen is scavenged by the carbon centered radical to yield lipid peroxy radicals ($LOO^{\bullet}$). This peroxy radical is highly reactive and abstract a hydrogen from another PUFA; which then results in a lipid hydroperoxide (LOOH) and another alkyl radical ($L^{\bullet}$). For instance, after hydrogen abstraction at the carbon 9, the double bond at carbon 9-10 shifts to 10-11 position that gives a rise to the carbon 9 radical and eventually formation of the carbon 9 hydroperoxide. Likewise, after hydrogen abstraction at the carbon 12, the double bond at carbon

12-13 shifts to 11-12 position, giving rise to the carbon 13 radical and eventually creating the carbon 13 hydroperoxide (Hayward et al., 2017).

Furthermore, LOX can function under anaerobic conditions, limiting oxygen concentration, depending on the availability of PUFAs and hydroperoxide products (Figure 2.4). Anaerobic reactions take place in a similar way as the aerobic mechanism, which has available molecular oxygen. In this case, a free alkyl radical is generated from the linoleic acid substrate and subsequently causes the formation of several carbonyl compounds. Under oxygen limiting condition, available hydroperoxides instead of oxygen can oxidize the active site of LOX iron to Fe (III). The homolytic cleavage of hydroperoxide is reduced into a hydroxyl ion and an alkoxy radical.

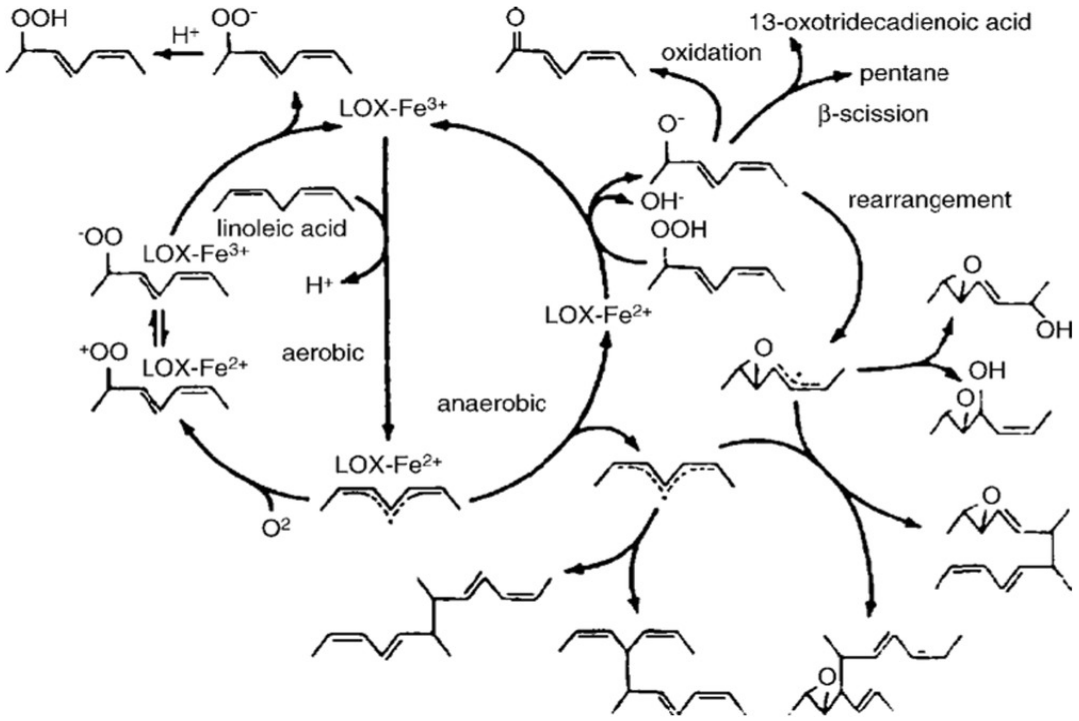


Figure 2.4. LOX-catalyzed fatty acid oxidation during aerobic and anaerobic reactions. The figure was adopted from Gardner (1988).

Autoxidation is non-enzymatic lipid oxidation pathway, which produces lipid hydroperoxides through the reaction of triplet oxygen/ground state oxygen (electrons within a

single orbital) with unsaturated fatty acids depending on extrinsic factors (e.g., temperature, metals, enzymes) in plant tissues. Direct reaction of triplet oxygen and unsaturated fatty acids does not occur since electrons are within a single orbital for triplet oxygen (Shahidi & Abad, 2019). Therefore, autoxidation by triplet oxygen requires additional factors (e.g., temperature, metals, enzymes) to catalyze the oxidation (Kallenbach, 2016). The overall stages of lipid autoxidation (Figure 2.5) are initiation (hydrogen abstraction or homolytic cleavage of hydroperoxides), propagation (oxygen insertion and radical formation through lipid-lipid interactions), and termination (interaction of two free radicals to form a non-radical product) (Gardner, 1988; Kallenbach, 2016).

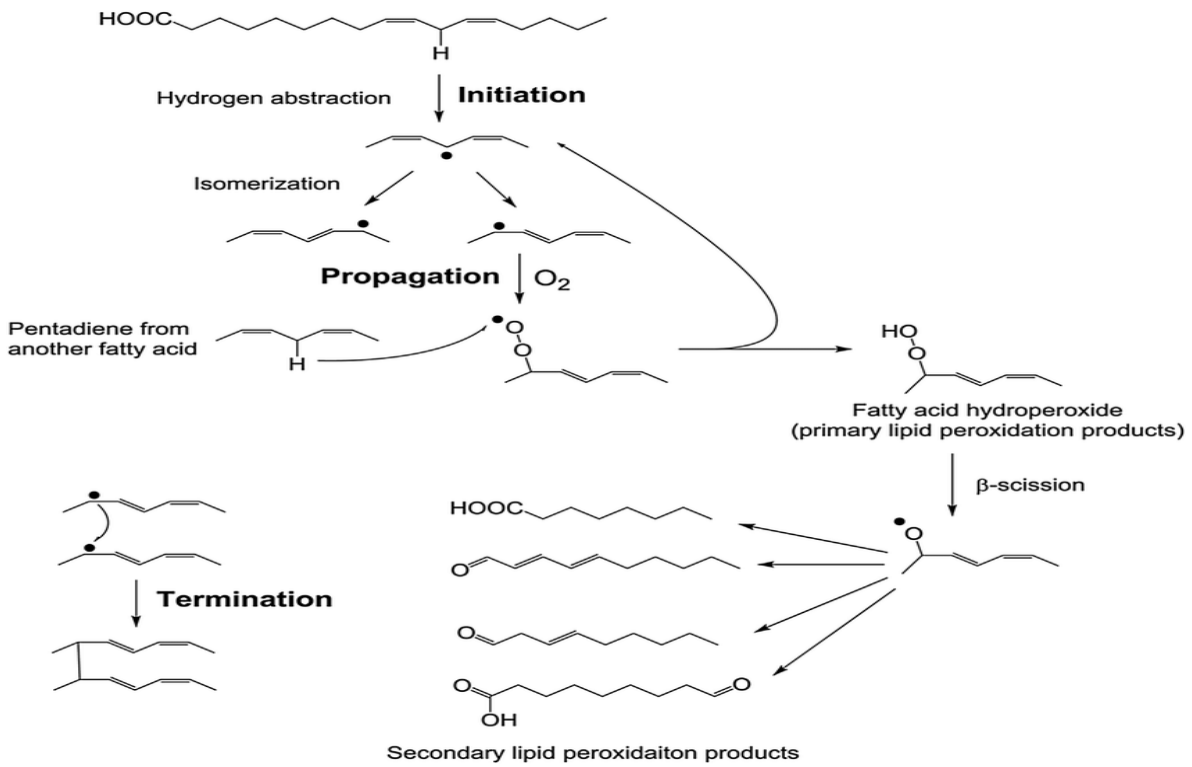


Figure 2.5. Initiation and propagation of linoleic acid. The figure was adopted from Wang et al. (2017).

Photooxidation is another non-enzymatic lipid oxidation pathway that occurs by several reaction between singlet oxygen (electrons in both orbitals) and unsaturated fatty acids. Singlet

oxygen is highly reactive and can attack unsaturated fatty acid directly to form lipid hydroperoxy radicals and, almost instantaneously, hydroperoxides. The key aspect of this mechanism is that singlet oxygen is generated by photoexcitation (e.g., ultraviolet light or photosensitizers-chlorophyll, riboflavin) (Shahidi & Abad, 2019). Thus, photooxidation can be restricted through better storage conditions, such as preventing light from contacting food (Kallenbech, 2016).

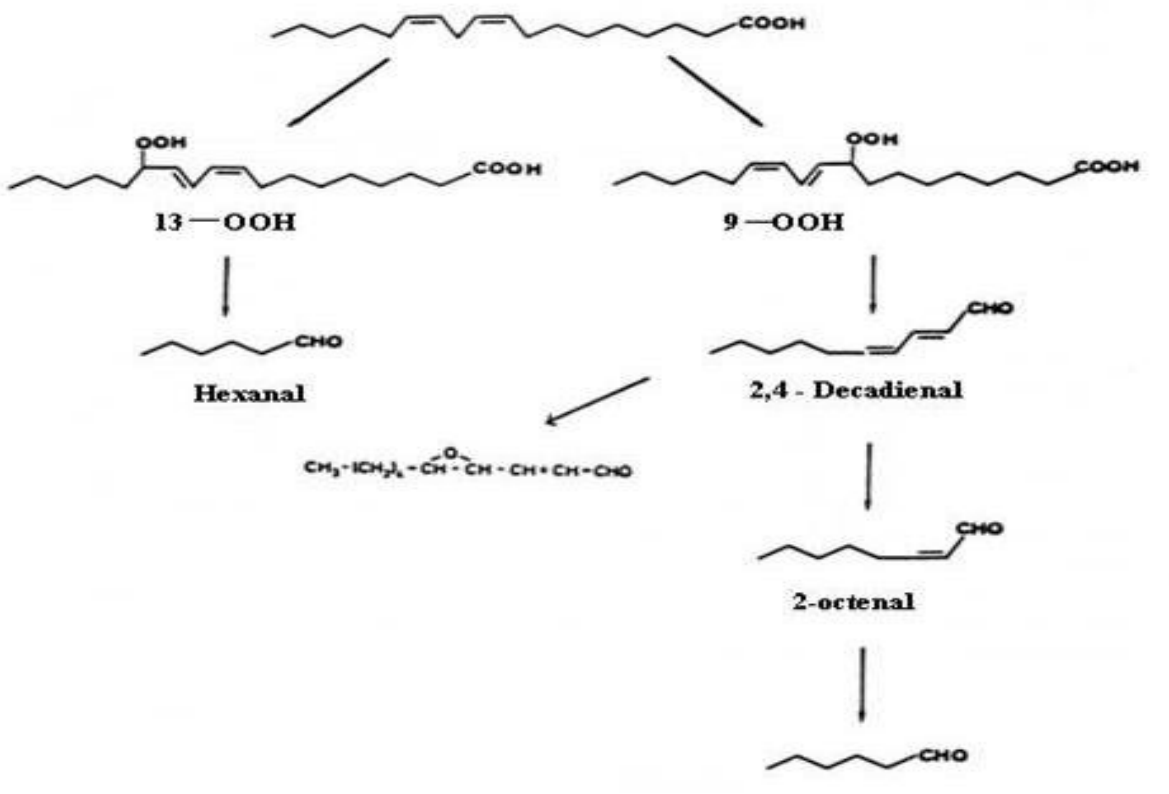


Figure 2.6. Formation of primary and secondary lipid oxidation products. The figure was adopted from Shi & Ho (1994).

The decomposition of lipid hydroperoxides into alkoxyl radicals (Figure 2.6) can be promoted by a variety of prooxidants (e.g., singlet oxygen, ascorbic acid), transition metals (e.g., $Cu^{1+}$, $Fe^{2+}$), light and high temperatures (e.g., thermal processing). When alkoxyl radicals are generated from lipid hydroperoxides, various reaction schemes (e.g., ß-scission reaction, alcohol formation reactions) occur and result in formation of various lower molecular weight volatiles depending on the fatty acid type and the location of the hydroperoxide (e.g., 9-OOH, 13-OOH).

Alkoxyl radical, an intermediate product of hydroperoxide oxidation, is more energetic than alkyl and peroxyl radicals. Thereby, it attacks its adjacent carbon-carbon bonds and cleaves the fatty acid chain to form smaller compounds. This reaction is referred to as "ß-scission reaction" (McClements & Decker, 2018).

Furthermore, highly energetic alkoxyl radical can attack other unsaturated fatty acids and pentadiene system within the same fatty acid and subsequently produces free fatty acids radicals. Thus, additional reactions take place in the presence of fatty acids radicals resulting in alcohols, carboxylic acids, ketones, and cyclic products. Overall, ß-scission reaction and additional reactions lead to the formation of many secondary decomposition products, including alcohols, aldehydes, ketones, that produce off-flavor compounds (McClements & Decker, 2018). For example, 1-hexanol is the product of subsequent reduction of hexanal by alcohol dehydrogenase (Maarse, 1991; Matoba et al., 1989).

Overall, predominantly secondary decomposition products of LOX-promoted unsaturated fatty acid oxidation and also autoxidation are present in the matrix of protein and carbohydrate (Heng, 2005). These secondary products, mostly developed during storage or processing (Azarnia et al., 2011a, Roland et al., 2017), are associated with the olfactory terms, "green", "grass", "hay-like", "earthy", and "mushroom"; thus, the combination of these generate significant unpleasant pea aroma (Vara-Ubol, Chambers, & Chambers, 2004; Azarnia et al., 2011a; Murat et al., 2013; Schindler, Zelena, Krings, Bez, Eisner, & Berger, 2012). In the literature, more than 130 VOCs have been reported in dry pea and its ingredients (Azarnia et al. 2011a; Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Murat et al., 2013; Wang, Guldiken, Tulbek, House, & Nickerson, 2020).

Among these VOCs, alcohols generated by fatty acid breakdown have been detected as the dominant VOCs in dry pea and its ingredients (Azarnia et al., 2011a; Jakobsen et al., 1998; Heng, 2005; Maarse, 1991; Murat et al., 2013; Vatansever & Hall, 2020; Wang et al., 2020). Specifically, 1-pentenol (pungent aroma), 1-hexanol (green and hay-like aroma), 1-octen-3-ol (mushroom aroma), 1-octanol (green and mushroom aroma), and 1-nonanol (green and citrus aroma) were reported as significant aroma contributors in pea flour (Heng, 2005; Murat et al., 2013; Vatansever & Hall, 2020). Similar pattern also was found in pea protein, containing mostly alcoholic aroma compounds, namely 1-pentanol, 1-penten-3-ol, 1-hexanol, 1-octen-3-ol, 1-octanol, and 1-nonanol (Murat et al., 2013; Wang et al., 2020).

Aldehydes formed by LOX-catalyzed oxidation of pea lipids also contributed significantly to off-aroma compounds in dry pea ingredients after the alcoholic aroma compounds. Predominantly, hexanal and nonanal have been detected and contribute to grassy and green, and citrus and solvent-like smells of dry peas, respectively. Hexanal was in the highest quantity in pea protein compared to other VOCs (Heng, 2005; Murat et al., 2013; Wang et al., 2020). The influence of cultivar, growing season and processing on changes in pea volatiles has been documented (Azarnia et al., 2011b).

#### *2.6.1.2. Alkyl methoxypyrazines from amino acids*

Pea flavor is attributed to a family of compounds called alkyl methoxypyrazines, which are inherently found and/or believed to be generated from amino acids in pea seed (Murray, Shipton, & Whitfield, 1970). Alkyl methoxypyrazines attribute an intensive unpleasant green pea perception at very low odor threshold in dry pea (Jakobsen et al., 1998; Heng 2005; Murray et al. 1970; 1976; Vatansever & Hall, 2020). Jakobsen et al. (1998) identified three predominant alkyl methoxypyrazines, which were 2-isopropyl-3-methoxypyrazine (pea aroma), 2-sec-butyl-3-

methoxypyranize (bell pepper aroma), and 2-isobutyl-3-methoxypyrazine (green, peapod aroma) in blanched pea.

Formation of alkyl methoxypyrazines from protein degradation is not completely known. The formation of pyrazines is likely from amino acid degradation, including Strecker degradation, in pea seeds requires a high temperature (at least 70 $^{o}$ C). Therefore, without heat treatment, pyrazines cannot be produced through protein degradation in pea seeds. However, several bacteria (e.g., *Bacillus natto*, *Lactococcus lactis*) are responsible for the pyrazine formation from the reaction between α-amino acids, which can be formed through protein degradation, and reducing sugar (Muller & Rappert, 2010). During biological formation, these bacteria may cause the occurrence of alkyl methoxypyrazines in pea pod and tissue. Maarse (1991) stated that 2-isopropyl-3-methoxypyrazine and 2-alkyl-3-methoxypyrazine in peas might be produced in plant roots and plant tissue by microorganisms.

### 2.6.2. Non-volatile organic compounds in dry peas

Non-volatile organic compounds (non-VOCs), attributed with bitterness taste in peas, are saponins and inherently present in the seeds (Heng et al., 2006; Roland et al., 2017). Heng et al. (2006) reported pea saponins as two groups, saponin B and saponin ßg, also known as DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) saponin, in 16 pea cultivars. Among these two saponins, DDMP saponin had higher bitterness intensity than saponin B. Furthermore, at the presence of ethanol, DDMP saponin can be converted into saponin B; thus, the sensory quality of dry pea can be improved (Heng et al., 2006). Besides bitterness perception of pea saponins, they may also be perceived as astringent and metallic flavor (Roland et al., 2017).

## 2.7. Improvement in the Flavor Profile of Pulse Ingredients

Better understanding in the development of off-flavor compounds as a limiting factor of the use of pulse ingredients has illustrated the necessity of flavor improvement through flavor modification. LOX might be controlled through plant breeding to prevent unsaturated fatty acid breakdown and improve sensory properties of pulse crops and their ingredients. However, plant breeding programs are long-term, around 5-10 years, and thus to generate a commercial pea cultivar that is free of the enzyme LOX will require a significant period of time. Therefore, deflavoring food processing technologies to improve pulse flavor could be a potential short-term solution. Up to now, various protocols have been applied to remove unpleasant off-flavors from pulse crops and their ingredients by many researchers. These protocols are cultivar selection, bioprocessing (e.g., fermentation, germination), heat treatment, water treatment, and solvent extraction (Chang, Stone, Green, & Nickerson, 2019; Roland et al., 2017).

Cultivar selection of pea with minimal off-flavor profile could be a potential method to select cultivars for production (Roland et al., 2017). The off-flavor profile of different pea cultivars and also cooked pea cultivar have been reported by Azarnia et al. (2011b) and Malcolmson et al. (2014). Through these findings, selected cultivars can be used for breeding programs. However, cultivar selection might also bring other issues, such as nutrition quality (high vs. low protein), limitation of pea varieties for producers and to utilize in foods. Therefore, an optimal emerging food processing technology might be more promising in the near-term.

Fermentation is a traditional bio-processing method, which has been used to produce healthier, more nutritious, and a unique and flavor rich foods (Simsek, Ozel, & Con, 2017). Fermentation have been applied naturally and through culture inoculation for production of various cereal and legume-based foods (e.g., tarhana, soy-sauce, sourdough bread) (Kaczmarska, Chandra-

Hioe, Frank, & Arcot, 2018; Ozel, Sabanoglu, Con, & Simsek, 2015; Schindler et al., 2012). Schindler et al. (2012) fermented pea protein extracts through lactic acid fermentation using *Lactobacillus plantarum* and *Pediococcus pentosaceus.* From this study, fermentation improved the overall sensory quality of pea protein through masking off-flavor compounds of the extract with production of pleasant volatiles. In comparison, fermented lupin flour through lactic acid fermentation using yoghurt culture exhibited an increased off-flavor compared to non-fermented lupin flour (Kaczmarska et al., 2018).

Similar to fermentation, germination has been traditionally used as an effective and non-expensive flavor and nutrient enrichment method (Kaczmarska et al., 2018; Xu, Jin, Jan, Rao, & Chen, 2019b). Kaczmarska et al. (2018) found that germinated lupin and soybean had relatively higher concentration of total volatile compared to their non-germinated counterparts. Particularly, germination increased characteristic off-flavor compounds (e.g., hexanal, 1-hexanol, alkyl methoxypyrazines) of soybean and lupin seeds along with production of new volatiles (e.g., dimethyl sulfide, 2-methylbutanal), that caused meaty and sulfur aromas. Likewise, Xu et al. (2019b) showed that different flavor profile was developed during germination of chickpea, lentil, and yellow pea seeds. Particularly, germinated lentil and yellow pea had higher off-flavor compounds, including increased hexanal, 1-hexanol, 2-pentylfuran, 2-methoxy-3-isopropyl pyrazine and others compounds. However, germination reduced the off-flavor intensity for chickpea seeds. Based on the findings of fermentation and germination, there is an inconsistency in the flavor profile of legumes. This might result from the type of legume seeds (pea vs. chickpea) and randomization of analytical methods, which might create more uncertainity, and requiring method optimization, such as optimum time period for fermentation and germination.

Water treatment, such as soaking and blanching, has been used to leach undesirable compounds of pulse crops as a low-cost method. Through soaking, water-soluble off-aroma compounds, such as alcohols and non-VOCs, can be reduced (Roland et al., 2017). Blanching, which is the combination of heat and water treatment can be used to inactivate LOX of pulse seeds (Jakobsen et al., 1998). Also, blanching can be used to decrease bitterness compounds in pulse crops (Roland et al., 2017). However, this method requires high water use and energy consumption. Also, blanching might cause irreversible changes in starch and protein structure and the resulting functionality.

Heat-steam treatment is a recent approach to reduce off-flavor compounds of pulse ingredients. Bourre et al. (2009) concluded that split yellow pea flour (SYPL) and whole navy bean flour (WNBF) treated at 120 and 140 ˚C with addition of steam (10%) had significant structural changes but had improved sensory quality compared to untreated pulse flours. Wheat breads fortified with heat-steam treated SPYL and WNBF had better sensory quality with less pea and beany aroma as well as less bitterness intensity, respectively (Bourre et al., 2019). Researchers showed that heat treatment without steam was less efficient for flavor improvement of pulse flours. In addition to these, greater temperatures, 160 ˚C for SYPL, and 160 and 180 ˚C for WNBF did not decrease pea and beany aroma for SYPL and WNBF, respectively. However, these authors did not reveal the changes in flavor profile after heat-steam treatment.

Solvent extraction using ethanol, isopropanol, and acetone have shown some promising findings for flavor improvement of pulse ingredients due to solubility of off-flavor compounds in organic solvents (Chang et al., 2019; Hillen, 2016; Wang, Guldiken, Tulbek, House, & Nickerson, 2020). Hillen (2016) evaluated the use of ethanol (95%) under high pressure extraction for the reduction of off-flavor compounds from yellow pea flour. The sensory acceptance of pea cake and

cookie produced with deflavored pea flour improved compared to non-deflavored pea flour. However, deflavored pea flours had higher hexanal content and non-significant changes for other standard off-aroma compounds (e.g., 1-hexanol, alkyl methoxy pyrazines). Therefore, sensory and chromatographic results in this study are not consistent.

Chang et al. (2019) investigated efficiency of diluted organic solvents (acetone, ethanol, and isopropanol at 35%, 55%, 75%, and 95%, v/v) for deflavoring lentil pea isolate (LPI). In this study, the researcher found that ethanol and isopropanol, except for at 95% (v/v), were efficient to reduce undesirable flavor compounds from LPI, but acetone increased off-aroma compounds in LPI. Overall, ethanol and isopropanol at 75% (v/v) were the most promising solvent for flavor improvement of LPI. Likewise, Wang et al. (2020) used ethanol and isopropanol at 20%, 50%, and 80% (v/v) for alcohol washing of pea protein enriched flour (PPEF) to decrease unpleasent pea flavor. From this study, both alcohol washing at 50 % and 80% were found effective for removal of pea flavor in PPEF, including changes in physicochemical and nutritional quality.

Among these deflavoring methods, solvent extraction and heat-steam treatment are promising for flavor modification of pea ingredients. But solvent extraction requires the use of more solvent and longer extraction time compare to other methods. Heat-steam treatment has not been evaluated extensively and physicochemical, morphological, and moisture properties of pulse flours from this method have not been fully presented. Furthermore, flavor profiles have not been characterized in relation to operation conditions. Therefore, using an emerging green technology to address flavor problem of pulse flour can be a promising alternative.

### 2.7.1. Supercritical carbon dioxide + ethanol extraction

A supercritical fluid is a fluid which has a state that exhibits properties of both liquid and gas when a certain pressure and temperature combination are reached. This state is considered as

a supercritical state (Figure 2.7). In this supercritical state, the density and diffusivity of supercritical fluid increases while its viscosity decreases (Mukhopadhyay, 2000).

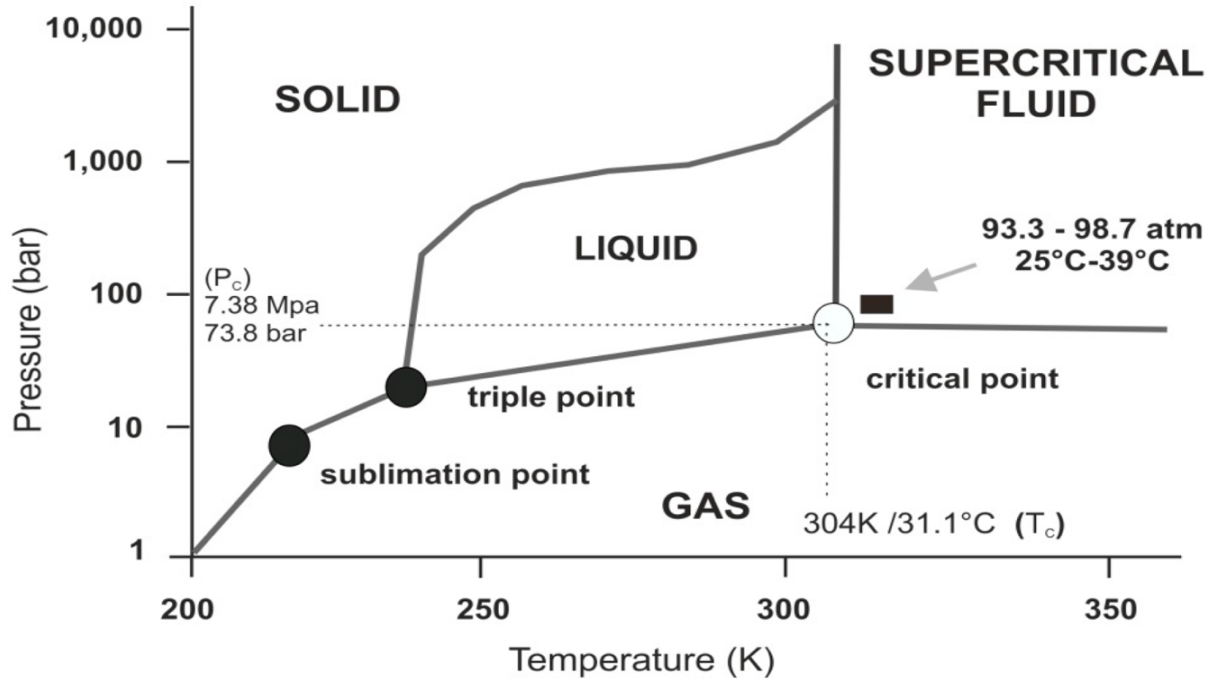


Figure 2.7. A phase diagram of different stages of matter at given pressure and temperature. The figure was adopted from Budisa & Schulze-Makuch (2014).

Supercritical extraction (SFE) is a green emerging technology. This extraction system can be manipulated easily by simply modifying temperature and pressure during extraction. As a result, natural substances extracted through SFE are diverse and include flavor and fragrance (e.g., essential oil), aroma extracts from fruit, spices, and herbs, natural antioxidants and food colors (e.g., carotenoids), plant and animal lipids and volatile compounds from plant materials (Gracia, Rodríguez, Garcia, Alvarez, & Garcia, 2007; Mukhopadhyay, 2000; Xu, Xu, Tao, Yuan, & Gao, 2015).

This technology gained a reputation in the last decade of 20$^{th}$ century as a green food processing method. However, industrial scale systems did not become a reality until the 21$^{st}$ century and more recently the advent of cannabis oil extraction by SFE have made intermediate scale system more affordable. Supercritical carbon dioxide is also considered non-explosive and

non-toxic, and also eco-friendly. SFE has several relative advantages compared to conventional solvent extraction methods, such as its high extraction rate and less extraction time requirement (Shao et al., 2014; Xu et al. 2015). In addition, uniqueness of this extraction system is that separation of specific compounds through easy manipulation of physical parameters, such as pressure, temperature, and co-solvent (Ciftci, Cahyadi, Guigard, & Saldaña, 2018) can be accomplished easily unlike conventional processing.

Carbon dioxide ($CO_2$) is the most popular supercritical fluid because of its low cost, availability, non-flammable and non-toxic nature (food grade), and moderate critical temperature (31.1 ˚C) and pressure (7.4 MPa) (Özkal, Yener, Salgın, & Mehmetoğlu, 2005; Ciftci et al., 2018; Vatansever & Hall, 2020). In addition, supercritical $CO_2$ exhibits a high selectivity and low viscosity, which allows for a high diffusivity into the plant matrix. It is feasible for extraction of non-polar volatile compounds, including alkenes and terpenes and for moderately polar volatile compounds, including aldehydes, ketones, and esters (Pourmortazavi, Sefidkon, & Hosseini, 2003). However, $CO_2$ is not a good solvent to extract polar compounds (e.g., alcohols) and/or high molecular components (e.g., saponins) from plant materials (Pourmortazavi et al., 2003). Therefore, addition of a co-solvent or modifier, (e.g., methanol, ethanol) into supercritical carbon dioxide (SC-$CO_2$) extraction is necessary to improve the extraction efficiency of polar compounds through modifying the solubility properties of the SC-$CO_2$ (Dobbs, 1986; Şanal, Bayraktar, Mehmetoğlu, & Çalımlı, 2005).

Ethanol has "Generally Recognized as Safe" (GRAS) status and is a favorable co-solvent for SC-$CO_2$ extraction for separation of polar compounds, such as carotenoids from corn meal (Cobb, Kallenbach, Hall, & Pryor, 2018), alcohols, phenolic compounds, and terpenoids from plant sources (Ciftci et al., 2018). Thereby, SC-$CO_2$ + ethanol (SC-$CO_2$+EtOH) can be an effective

method for fractionation of both non-polar and polar compounds from plant materials. Cobb et al. (2018) successfully used SC-$CO_2$+EtOH extraction for separation of luteins from corn gluten meal at the optimum conditions; which are temperature of 40 ˚C, pressure of 6820 psi, and ethanol of 15 % (by volume). Furthermore, Shao et al. (2014) showed that SC-$CO_2$ extraction can be effectively employed to extract VOCs from saffron (*Crocus sativus*) at optimum conditions (i.e., temperature at 44.9 ˚C, pressure at 34.9 MPa, total extraction for 150.2 min with $CO_2$ flow rate at 10.1 L/h). Also, maximum extraction of aroma compounds (e.g., esters, acids) from liquor vinasse was performed via SC-$CO_2$+EtOH extraction at a temperature of 51.28 ˚C, pressure of 24.98 MPa, and $CO_2$ flow rate of 13.38 L/h (Xu et al., 2015).

Overall, the SC-$CO_2$ extraction system with addition of ethanol can be an effective extraction method for removal of undesirable pea flavor compounds from pulse ingredients. Particularly, moderate critical conditions of SC-$CO_2$ might provide less damage during extraction, minimizing the effect on physicochemical properties of pulse ingredients. Also, its lipid extraction property might promote shelf-life stability of deflavored pulse ingredients. Cocero & Calvo (1996) stated that SC-$CO_2$ extraction using ethanol as a cosolvent was employed for sunflower oil extraction from the seeds. Researchers found that increasing ethanol concentration improved solubility during extraction and resulted in increased extraction of phospholipids from the seeds.

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# CHAPTER 3. FLAVOR MODIFICATION OF WHOLE YELLOW PEA FLOUR USING SUPERCRITICAL CARBON DIOXIDE + ETHANOL EXTRACTION AND RESPONSE SURFACE METHODOLOGY[1]

## 3.1. Abstract

Reduction of volatile aroma compounds that are the source of undesirable flavor is crucial for quality, acceptability, and marketability of products made with yellow pea (*Pisum sativum* L.) flour. Supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction was applicable for flavor modification of pea flour. Percentage ethanol, temperature, and pressure were optimized using a central composite rotatable design (CCRD) under response surface methodology (RSM). The minimum total volatile content (0.55 μg/g) was obtained significantly ($p < 0.05$) under optimum conditions, which were ethanol (22%), temperature (86 °C), and pressure (42.71 MPa). Furthermore, flour color was lighter when processed at optimum conditions. Through the response surface model, only ethanol, temperature, and quadratic term of ethanol were significant ($p < 0.05$). Principal component analysis (PCA) revealed total volatile (TV) content, sensory attributes, and color values were highly interrelated. The data support SC-$CO_2$+EtOH extraction as a viable method for removal of undesirable off-flavor from pulse flour.

## 3.2. Introduction

Pulses, the edible seeds of the legume family, have been noteworthy human food resources in the world. The year 2016 was declared as the International Year of Pulses by the United Nation to indicate the importance of these food crops in terms of their nutritional and health promoting

[1] Based on the article of Serap Vatansever & Clifford Hall published in *Journal of Supercritical Fluids* online Oct. 2019 (DOI:10.1016/j.supflu.2019.104659). Serap Vatansever was responsible for conceptualization, methodology, data collection and analysis, investigation, writing (original draft, reviewing and editing), and visualization of this chapter. Clifford Hall was responsible for resources, reviewing and editing, supervising.

benefits. Dry peas (*Pisum sativum* L.) are one of the most commonly produced high value pulse crops in the world (Hall, Hillen, & Garden-Robinson, 2017). The value of pulse crops is due to the high protein content (14-31%), which is a rich source of lysine (i.e., an essential amino acid), and are good sources of carbohydrate (e.g., fiber), folate, and minerals (Day, 2013; Hall et al., 2017). Recently, increasing consumer demands for nutritious foods have led to the development of foods with blended ingredients. However, creating new products with pulses generates concerns that pulse will impart off-flavors and affect texture. Dry peas, which is a highly nutritious food, are not extensively utilized due to the undesirable flavor. Enhancing sensory attributes of pea flour by reducing pea intensity (PI) will facilitate the wider use of nutrient dense pulses as ingredients in the food industry.

Volatile organic compounds (VOCs) and non-VOCs are responsible for the off-aroma, which cause strong pea aroma, and off-taste of pea flour due to bitterness compounds, respectively. The VOCs consist of alcohols, aldehydes, ketones, furans, and alkyl methoxypyrazines (Heng, 2005; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017) and are present in the matrix of protein and carbohydrate. Oxidation of unsaturated fatty acids, such as linoleic acid by lipoxygenase (LOX) causes pea off-flavor (Azarnia, Boye, Warkentin, & Malcolmson, 2011; Schindler, Zelena, Krings, Bez, Eisner, & Berger, 2012; Vara-Ubol, Chambers, & Chambers, 2004) and also secondary oxidation products increase during storage (Azarnia et al., 2011). The alcohols, 1-hexanol, 1-octanol, and 1-nonanol contribute to hay-like, mushroom, and earthy aromas of peas, respectively. Hexanal and nonanal, are aldehydes responsible for grassy and citrus odor, respectively (Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Murat, Bard, Dhalleine, & Cayot, 2013). Alkyl methoxypyrazines, 3-isopropyl-2-methoxypyrazine, 3-sec-butyl-2-methoxypyranize, and 3-isobutyl-2-methoxypyrazine are believed to be produced from

amino acids in the plant and are attributed to pea flavor even though they are found in extremely low concentrations (Heng, 2005; Jakobsen et al., 1998). Saponins in contrast are non-VOCs and are associated with bitterness of peas (Heng, 2005; Roland et al., 2017).

As with other pulses, a high concentration of linoleic acid makes pea susceptible to LOX that reduces the shelf-life of dry peas by facilitating development of pea off-flavor (Heng, 2005). This enzyme might be controlled using plant breeding. However, the problem is the time (e.g., 5-10 years) to produce commercial pea that is free of the enzyme LOX using plant breeding. Therefore, processing for deflavoring could be a more effective near-term solution. Besides VOCs, non-VOCs (i.e., saponins), are associated with bitterness of peas, which are responsible for bitter taste so significant contributor of pea off-flavor formation (Heng, 2005; Roland et al., 2017).

Supercritical carbon dioxide extraction (SC-$CO_2$), a green technology, has become a popular method compared to conventional extraction for producing a variety of natural product extracts (Bejarano, & del Valle, 2017; Ghasemi, Raofie, & Najafi, 2011; Gracia, Rodríguez, Garcia, Alvarez, & Garcia, 2007; Kazazi, Rezaei, Ghotb-Sharif, Emam-Djomeh, & Yamini, 2007; Mukhopadhyay, 2000; Saffarionpour & Ottens, 2018; Shao et al., 2014; Xu, Xu, Tao, Yuan, & Gao, 2015). The ease of manipulating physical parameters (e.g., pressure, temperature, co-solvent) allows for extraction of both moderately polar and non-polar compounds combined with short extraction times are reasons why this method is ideal for production of extracts (Kazazi et al., 2007; Özkal, Yener, Salgın, & Mehmetoğlu, 2005). Furthermore, carbon dioxide ($CO_2$) becomes a supercritical fluid above its critical points (temperature, 31.1 °C and pressure, 7.4 MPa), which provide less thermal damage to the product (Ciftci, Cahyadi, Guigard, & Saldaña, 2018; Ghasemi et al., 2011; Pourmortazavi, Sefidkon, & Hosseini, 2003; Ozkal et al., 2005; Trabelsi et al., 2016). However, $CO_2$ is not perfectly suitable for the extraction of polar organic compounds, such as

alcohols and/or high molecular components, such as saponins (Heng, 2005) and xanthophylls (Araus, Casado, del Valle, Robert, & Juan, 2019; Dobbs, 1986; Şanal, Bayraktar, Mehmetoğlu, & Çalımlı, 2005). Ethanol has been used as a co-solvent for the SC-$CO_2$ extraction due to improved solubility of polar compounds, such as polar-carotenoids (Araus et al., 2019; Şanal et al., 2005), alcohols, phenolic compounds, and terpenoids from plant materials (Ciftci et al., 2018).

The use of SC-$CO_2$ system has been applied to plant materials for separation of aroma compounds (Gracia et al., 2007; Shao et al., 2014; Xu et al., 2015), extraction of VOCs from pulse crops using SC-$CO_2$ system has not been reported. Therefore, the effects of experimental parameters (i.e., ethanol, temperature, and pressure) on the removal of VOCs (yield) from pulses must first be determined to provide the optimum conditions for this extraction. Response surface methodology (RSM) using a central composite rotatable design (CCRD) (Wang, Liu, Wei, & Yan, 2012) is an efficient and common statistical tool to optimize the experimental factors for the SC-$CO_2$ extraction (Bilgiç-Keleş, Şahin-Yeşilçubuk, Barla-Demirkoz, & Karakaş, 2019; Campone et al., 2018; Sharif et al., 2014; Trabelsi et al., 2016). This statistical procedure is superior to the classical methods because it requires less labor and time while allowing to investigate the impacts and interactions of the independent variables by fitting a second order polynomial model (Özkal, 2009; Sharif et al., 2014).

In this study, whole yellow pea flour was subjected to SC-$CO_2$+EtOH extraction for removal of VOCs to mitigate the intense pea flavor through deflavoring. The objectives of this study were (1) to determine the degree of volatile reduction in whole yellow pea flour using a headspace-solid-phase microextraction-gas chromatographic (HS-SPME-GC) system and sensory analysis, and (2) to optimize the experimental factors by the application of CCRD under the RSM.

## 3.3. Materials and Methods

### 3.3.1. Materials

Whole yellow dry peas were obtained from three different suppliers: Viterra (Minot, ND, USA), Specialty Commodities (Fargo, ND, USA), and SK Foods (Moorhead, MN, USA) and then manually blended. Blended yellow peas were hammer milled (Fitzpatrick, Elmhurst, IL, USA) using a 1.270 mm screen and hammer rotation of 102 m/s (7200 rpm). Milled pea flour was stored in sealed polyethylene bags at -20 ºC. Chromatographic grade $CO_2$ (99.99% purity) and ethanol (200 proof, undenatured) were used for SC-$CO_2$+EtOH extraction. The gases, nitrogen and helium, for the GC system were obtained from Praxair (Fargo, ND). The selected VOCs, which were used to prepare standard curve, included hexanal, nonanal, 1-pentanol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-heptanol, 1-octanol, 1-octen-3-ol, 1-nonanol, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine, 2-pentylfuran, γ-valerolactone (5-methyldihydro-2(3H)-furanone), γ-caprolactone (5-ethyldihydro-2(3H)-furanone), (Sigma-Aldrich, St. Louis, MO, USA) and kept at -20 ˚C until use. Cracker and corn starch, sensory training supplies, were purchased from local food distribution centers and caffeine powder was ordered from Sigma-Aldrich, St. Louis, MO, USA.

### 3.3.2. SC-$CO_2$+EtOH extraction

The SC-$CO_2$+EtOH extraction protocol was performed according to Cobb, Kallenbach, Hall, & Pryor (2018) with the following modifications. Briefly, an ISCO supercritical fluid extractor (Model SFX 2-10; Isco, Inc.) was used with the $CO_2$ as a main solvent and ethanol was a co-solvent. In the system, desired level of ethanol (Table 3.1) was pumped continuously into $CO_2$. Batches of 6 g of raw pea flour were placed in stainless steel vials with frits. The extraction ethanol, temperature, and pressure were performed at five levels (Table 3.1) to obtain maximum

removal of VOCs based on previous studies (Cobb et al., 2018; Shao et al., 2014; Xu et al., 2015) and a preliminary experiment. Ethanol, temperature, and pressure were set at 0, 10, 25, 40, and 50.2 %; 33.2, 40, 50, 60, and 66.8 °C; and 28.68, 31.03, 34.47, and 37.92 MPa respectively (Table 3.1). Each extraction was performed based on a run created by the CCRD (Table 3.2). The raw pea flour was subjected to a 40-min total extraction that included a 10-min static and a 30-min dynamic extraction at a flow rate of 2 mL/min. Afterward, the extracted sample was dried at 70 °C in a convection oven for 1 h to remove ethanol. The dried flour was stored in 2.5 mil Mylar bags (Uline; Pleasant Prairie, WI, USA) at -20 °C until analysis. The extraction efficiency was determined as the reduction of selected total volatiles (TV) and degree of pea intensity (PI) after processing.

### 3.3.3. HS-SPME-GC analysis of volatile compounds

Volatile detection of deflavored pea flours was measured using a gas chromatographer (Agilent 7820A) GC. A Zebron capillary GC column (60m x 0.25mm x 0.25µm) from Phenomenex (Torrance, CA, U.S.A) was used to separate the volatiles that were then detected by a flame ionization detector (FID) following the protocol described by Hall, Manthey, Lee, & Niehaus (2005) with modifications. In short, pea flour (1g) was added to 4-mL vials and sealed with a PTFE silicone Septa (Supelco, Bellefonte, PA, U.S.A.), which was heated at 150°C for 4 h before use. The vials were then heated in a 95 °C water bath for 10 min. The SPME filament (DVB/CAR/PDMS, 50/30 µm; Supelco, 57328-U, Bellefonte, PA, U.S.A), was placed in the headspace of the vial for 15 min to adsorb volatiles from the headspace of the vial, which was heated in a 90 °C water bath. Then, the SPME filament was manually inserted into the injection port of the GC and remained there for 7 min to desorb the volatiles from the fiber on to the GC column.

The volatile analysis was performed according to the following conditions: helium flow rate of 33.7 mL/min, initial oven temperature of 35 °C and ramped to 180 °C at 10 °C/min and maintained for 12 min at 180 °C. Each volatile compound was identified according to the retention time of chosen standards. Each volatile compound was identified according to the retention time of chosen standards and quantified (µg/g) using the standard curve. Then, the TV concentration in pea flour was obtained from the sum of selected VOCs (µg/g).

### 3.3.4. Standard curve preparation

The VOCs assessed were hexanal, nonanal, 1-pentanol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-heptanol, 1-octanol, 1-octen-3-ol, 1-nonanol, 2-pentylfuran, γ-valerolactone, γ-caprolactone, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine based on previous studies (Jakobsen et al., 1998; Murat et al., 2013; Hillen, 2016).

A standard curve was developed in a solid matrix (i.e., finely ground fresh saltine cracker) through dilution of the standards (Hillen, 2016) with modifications. The sample matrix for this study was a flour sample; therefore, the standard curve was prepared using the solid matrix. First, a concentrated standard was created with the following steps. The empty test tubes with screw caps were weighted and the mass was recorded. Fresh saltine cracker flour that was kept at -20 °C for few hours was then added to the test tubes (6 g of the cracker flour for each standard separately). Test tubes were weighed again, the mass was recorded, and then, tubes were returned to the freezer for 20 min. 10 µl of each VOC cold standard was pipetted into each test tube, labeled, and screw caps were immediately tightened to the test tubes. The test tubes were inverted for 10 min to minimize volatilization of standards into the headspace and then sonicated at 60 °C in an ultrasound bath for 60 min. The test tubes were dried and allowed to stabilize to room temperature for 60 min. After stabilization, the test tubes were weighted again, and the mass of each test tube

was recorded to calculate each standard added by subtraction from the test tube, including cracker. The test tubes were placed again in the freezer for 120 min, and then, another 6 g of cold cracker flour was added to each test tube, mixed, and sonicated at 60 °C in the ultrasound bath for 30 min. Test tubes were dried and allowed to equilibrate for three days. After equilibration, 1g of equilibrated standard was used to run the HS-SPME-GC analysis for three times to obtain the area corresponding to the determined concentration of each standard.

Standard concentrations included 0, 1, 5, 10, and 20 μg/g. The amount of each composite standard to dilute with the cold cracker at specific concentration was determined based on the average area obtained from the GC and the concentration of the composite standard. After mixing the composite standards with the cold cracker according to the desired dilution, the test tubes were sonicated at room temperature for 30 min then stabilized for three days in closed vials at room temperature. Thereafter 1 g of equilibrated standard was used to conduct the HS-SPME-GC analysis. The standard curve was developed by running each standard at each concentration three times. The mean value of the three runs was used to calculate concentration of the volatiles in the samples. The standard curve was set between area (y-axis) and concentration (x-axis). The $R^2$ values for chosen standards were ranged from 0.974 to 0.999.

**3.3.5. Sensory assessment**

Sensory analysis was performed according to Hillen (2016). The PI and bitterness of pea flour was evaluated using quantitative descriptive analysis (QDA) with eight trained healthy, and nonsmoking panelists (5 women and 3 men). Briefly, the panelists were first trained with the pea flour (bitterness-free) to detect pea intensity and with caffeine powder to detect bitterness along with corn starch (control) over a 5-day period for 60 min each day.

Attribute training was performed through specific control samples prepared at five levels using an unstructred line scale (147 mm), where the rating on the scale: lowest (0 mm), low (36.75 mm), medium (73.5 mm), high (110.25 mm), and highest (147 mm) for each attribute. Samples were diluted with corn starch to achieve desired attribute level. After the 3-day of attribute training, the panelists were trained with combinations of attributes with corresponding sensory supplement to reach the composition of pea flavor on the fourth day. The last day of training, proccessed pea flours prepared food grade were randolmly provided to panelists to evaluate the samples in terms of PI and bitterness using the scale. After training, processed flours (4 samples per day) with a control from training were given to the panelists to evaluate degree of PI and bitterness using the unstructred line scale (147 mm). The flour samples were a composite of three replicates of each processed flour. Standard and real samples were given in plastic cups, which were labeled with a random three-digit number. During each testing, unsalted crackers and purified water between samples were provided for panelists to prevent the crossover of flavors between samples.

### 3.3.6. Color analysis

The color analysis was applied to deflavored pea flours along with raw pea flour to measure the change in color values (L, a, and b values) using a MiniScan EZ Hunter Lab colorimeter (Reston, Virginia) according to Hall (2018). This analysis was carried out on each of the three replicates of processed flours.

### 3.3.7. Design of experiment for RSM

A $2^3$ full-fraction CCRD was applied for response surface fitting. This experimental design was set with three independent variables at five levels, including three replicates at the center point using JMP software (JMP 14.0.0, SAS Institute Inc., Cary, NC) (Table 3.1). The total experimental runs generated by the CCRD was 17 ($2^k$ + 2k +3, where k corresponds to the number of

independent variables (k =3) and 3 is the number of replicates at the center point) (Ciftci et al., 2018). The reason for using three replicates at the center was to measure the pure error (Ciftci et al., 2018; Wang et al., 2012). These 17 experimental runs were replicated three times both to measure the repeatability of the extractor and to provide reliable data for the GC and sensory analysis. Later, the mean data was used for response surface application. RSM was employed to optimize the SC-$CO_2$+EtOH extraction conditions for the maximum removal of undesirable flavor components from pea flour using JMP software. Ethanol addition level, temperature, and pressure were coded as $x_1$, $x_2$, and $x_3$, respectively (Table 3.1).

Table 3.1. Five level (coded and actual values) CCRD for three independent variables of the SC-$CO_2$+EtOH extraction of pea flour.

| Independent Variable | Symbol | Level | | | | |
|---|---|---|---|---|---|---|
| | | -1.68 | -1 | 0 | 1 | 1.68 |
| Co-solvent (ethanol) (%) | $x_1$ | 0 | 10 | 25 | 40 | 50.2 |
| Temperature (°C) | $x_2$ | 33.2 | 40 | 50 | 60 | 66.8 |
| Pressure (MPa) | $x_3$ | 28.68 | 31.03 | 34.47 | 37.92 | 40.27 |

The total volatile (TV) content ($y_1$) was the sum of the selected volatile compounds identified from processed flour via the GC system based on the standard curve. The second respond factor ($y_2$) was PI obtained from the quantitative descriptive analysis. The TV data was transformed by Box-Cox transformation at $\lambda$ = -1.199, which is exponent power, using JMP software to improve the normality of the TV data before application of the RSM (Osborne, 2010; Razavi et al., 2009). The transformed TV data was used to obtain optimum conditions. The second order polynomial model in three factor CCRD was explained by following equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

where $y$ is a response variable (yield); $x_1$, $x_2$, and $x_3$ are the independent variables; $\beta_0$ is an intercept coefficient; $\beta_1$, $\beta_2$, and $\beta_3$ are linear effect coefficients; $\beta_{11}$, $\beta_{22}$, and $\beta_{33}$ are quadratic effect coefficients; and $\beta_{12}$, $\beta_{13}$, and $\beta_{23}$ are interaction effect coefficients.

### 3.3.8. Statistical analysis

Univariate analysis of TV, PI, bitterness, and color values was evaluated by one-way analysis of variance (ANOVA). Means were separated using the least significance difference (LSD) and significance was accepted at $p \leq 0.05$. The principal component analysis (PCA) was performed using the mean data of TV, PI, bitterness, and color values of deflavored flours to interpret the relationship of these variables using an alpha of 0.05. The JMP Software was used to analyze the data.

## 3.4. Results and Discussion

### 3.4.1. Volatile compounds identified in pea flour

The amount of the selected TV in raw pea flour was 19.7 µg/g based on the standard curve. The TV consisted of 16.5 µg/g of alcohols, 1.2 µg/g of aldehydes, 1.7 µg/g of alkyl pyrazines, and 0.2 µg/g of furans. The three volatiles, (*Z*)-3-hexen-1-ol, 2-pentylfuran, and γ-valerolactone, were identified in raw pea flour but were not quantified due to low concentration, which was lower than the limit of quantification. Among the VOCs quantified in raw pea flour, alcohols were the dominant volatile compounds. Alcohols quantified were 1-pentanol (7.1 µg/g), 1-hexanol (1.5 µg/g), 1-heptanol (0.3 µg/g), 1-octanol (0.7 µg/g), 1-octen-3-ol (0.4 µg/g), and 1-nonanol (6.5 µg/g). Other VOCs quantified in raw pea flour were the aldehyde, nonanal (1.2 µg/g); alkyl pyrazines, 2-*sec*-butyl-3-methoxypyrazine (1.2 µg/g) and 2-isobutyl-3-methoxypyrazine (0.5 µg/g); and a furan, γ-caprolactone (0.2 µg/g). Similarly, alcohols were the most common in pea flours compared to other VOCs (Heng, 2005; Jakobsen, 1998; Murat et al., 2013). Furthermore, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-octanol, and 1-nonanol have been reported in high concentration (Heng, 2005; Murat et al., 2013).

The amount of TV for the 17 deflavored pea flours ranged between 1.4 and 7.1 µg/g (Table 3.2). The flour obtained from run 13 had the highest TV including all alcohols (except for 1-octen-3-ol), nonanal, and, 2-*sec*-butyl-3-methoxypyrazine. This extraction was performed without ethanol (%) and thus supports the use of ethanol during extraction. However, removal of volatiles from pea flour was the greatest for run 14. Addition of ethanol promoted the removal of polar volatile compounds (e.g., alcohols) (Campone et al., 2018), which may account for lower TV in run 14. In addition to ethanol, an increase of temperature was useful in reducing volatiles (Shao et al., 2014; Xu et al., 2015), likely due to enhanced VOC solubility caused by an increase in the solute vapor pressure (Ozkal et al., 2005; Shao et al., 2014).

Table 3.2. The total volatile (TV) concentrations and sensory attributes of deflavored pea flours via SC-$CO_2$+EtOH extraction.

| **Run** | $x_1$ | $x_2$ | $x_3$ | **A-TV**[A] | **T-TV**[B] | **PI** | **Bitterness** |
|---|---|---|---|---|---|---|---|
| | (%) | (°C) | (MPa) | (µg/g) | (µg/g) | (mm) | (mm) |
| 1 | 10 | 40 | 31.03 | $3.3 \pm 0.31^{bc}$ | 4.0 | $104.2 \pm 6.61^{b}$ | $39.4 \pm 36.84^{ab}$ |
| 2 | 10 | 40 | 37.92 | $3.5 \pm 0.79^{b}$ | 4.0 | $105.8 \pm 9.60^{ab}$ | $49.6 \pm 43.15^{ab}$ |
| 3 | 10 | 60 | 31.03 | $2.2 \pm 0.36^{d-h}$ | 3.2 | $54.8 \pm 12.75^{fgh}$ | $7.8 \pm 9.15^{de}$ |
| 4 | 10 | 60 | 37.92 | $1.7 \pm 0.70^{fgh}$ | 2.4 | $67.4 \pm 6.54^{d-g}$ | $16.8 \pm 14.96^{cde}$ |
| 5 | 40 | 40 | 31.03 | $2.5 \pm 0.38^{b-f}$ | 3.5 | $62.8 \pm 14.89^{e-h}$ | $9.6 \pm 7.22^{de}$ |
| 6 | 40 | 40 | 37.92 | $2.6 \pm 0.19^{b-f}$ | 3.5 | $61.2 \pm 19.95^{e-h}$ | $22.7 \pm 28.54^{b-e}$ |
| 7 | 40 | 60 | 31.03 | $2.6 \pm 0.47^{c-h}$ | 3.4 | $55.0 \pm 10.34^{fgh}$ | $3.3 \pm 5.19^{f}$ |
| 8 | 40 | 60 | 37.92 | $1.7 \pm 0.51^{fgh}$ | 2.4 | $51.6 \pm 12.07^{gh}$ | $16.7 \pm 19.36^{cde}$ |
| 9 | 25 | 50 | 34.47 | $1.9 \pm 0.46^{efh}$ | 2.9 | $81.4 \pm 10.45^{cd}$ | $16.6 \pm 6.50^{cde}$ |
| 10 | 25 | 50 | 34.47 | $1.5 \pm 0.63^{ghi}$ | 2.1 | $69.4 \pm 13.41^{def}$ | $13.6 \pm 5.59^{cde}$ |
| 11 | 25 | 50 | 34.47 | $1.6 \pm 0.36^{ghi}$ | 2.1 | $73.2 \pm 12.25^{de}$ | $25.6 \pm 16.00^{b-e}$ |
| 12 | 50.2 | 50 | 34.47 | $2.3 \pm 0.61^{efh}$ | 3.3 | $55.6 \pm 10.83^{fgh}$ | $8.1 \pm 11.09^{de}$ |
| 13 | 0 | 50 | 34.47 | $7.1 \pm 1.71^{a}$ | 4.7 | $122.0 \pm 14.53^{a}$ | $58.8 \pm 37.40^{a}$ |
| 14 | 25 | 66.8 | 34.47 | $1.4 \pm 0.19^{hi}$ | 1.8 | $30.2 \pm 15.17^{i}$ | $6.8 \pm 10.66^{e}$ |
| 15 | 25 | 33.2 | 34.47 | $3.0 \pm 0.08^{bcd}$ | 3.8 | $90.4 \pm 12.52^{bc}$ | $34.7 \pm 41.03^{ab}$ |
| 16 | 25 | 50 | 40.27 | $1.9 \pm 0.55^{fgh}$ | 2.7 | $67.2 \pm 19.71^{d-g}$ | $13.3 \pm 11.50^{cde}$ |
| 17 | 25 | 50 | 28.68 | $2.2 \pm 0.11^{d-h}$ | 3.2 | $70.0 \pm 12.31^{def}$ | $25.4 \pm 29.37^{b-e}$ |
| Optimum | 22 | 86 | 42.71 | $0.5 \pm 0.31^{i}$ | --- | $22.2 \pm 3.35^{i}$ | $0.6 \pm 0.93^{f}$ |

[A] Results of actual-TV (A-TV) (three replicates) and sensory attributes (eight replicates) were expressed as mean ± standard deviation and the samples with the same letter are not significantly different at $p > 0.05$.

[B] The transformed-TV (T-TV) data was transformed total volatile using the Box-Cox transformation at $\lambda = -1.199$.

Furthermore, opening the cell matrix might enhance the solute-solvent interaction causing increased VOC extraction (Cobb et al., 2018). Overall, pressure between 31.03 and 37.92 MPa did not significantly influence ($p < 0.05$) VOC removal (Table 3.2). However, increased pressure tends to increase fluid density, which increases the solubility of VOCs (Ghasemi et al., 2011). The deflavored pea flour obtained at optimum conditions as discussed later of the RSM had the lowest TV concentration (0.55 µg/g), which was composed of nonanal (0.5 µg/g) and 2-*sec*-butyl-3-methoxypyrazine (0.05 µg/g).

### 3.4.2. Sensory assessment

The PI was the main sensory attribute evaluated in the QDA and was used as a second response variable for model fitting. The degree of PI fell between 30.2 and 122 mm (Table 3.2), corresponding to the flours obtained from run 14 and 13, respectively. Sensory results followed the similar trend as the VOC data. Likewise, bitterness of the flour obtained from run 13 was the highest, but the least bitter flour was obtained from run 7. Bitterness is associated with saponins, which include saponin B (Heng, 2005) and 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one saponin (DDMP saponin) (Heng et al., 2006) in pea flour. The extraction conditions might decrease the stability of DDMP saponin. Heng et al. (2006) found that under high temperature (>65 °C) and the presence of ethanol, DDMP saponin was converted into saponin B and released maltol. Additionally, these saponins contain a sugar moiety, which has a polar nature, thus ethanol might promote their extraction along with the VOCs (Heng, 2005). The deflavored pea flour under the optimum conditions was chosen as bitterness-free flour by the trained panelists.

### 3.4.3. Color analysis

The deflavored pea flour under the optimum conditions resulted in a lighter product than the raw pea flour, where L*, a*, and b* values were 87.0 ± 0.06, 2.8 ± 0.02 and 21.2 ± 0.47,

respectively. The color pigments, which are mostly lutein and ß-carotene, might be removed due to ethanol, which might remove polar compounds and cause changes for the protein structure to release more carotenoids (Cobb et al., 2018). Cobb et al. (2018) stated similar results for carotenoid (e.g., lutein, ß-carotene) extraction from corn gluten meal. Therefore, the use of this co-solvent might promote the solubility of carotenoids and improve the extraction of these pigments from plant materials (Araus et al., 2019; Cobb et al., 2018). In the current study, the apparent difference in flour color values appeared between the runs with and without ethanol. Run 11 (i.e., included ethanol) and run 13 (i.e., ethanol-free) had L*, a*, and b* values of 89.2 ± 0.78 and 87.3 ± 1.04, 0.6 ± 0.09 and 2.0 ± 0.37, and 12.2 ± 0.84 and 19.8 ± 0.84, respectively. The co-solvent might promote a decrease of a- and b-values and an increase in L-value for flour from run 11 through removal of carotenoids. The reduction of the b-value resulted in a less yellow product. Across all experiments, the pea flour extracted at optimum conditions was the lightest, i.e. the highest L-value (90.64 ± 0.46), the lowest a-value (0.30 ± 0.15), and a moderately low b-value (12.85 ± 0.67). Araus et al. (2019) reported that ethanol addition to SC-$CO_2$ increased the solubility of the carotenoids, which are both non-polar and polar compounds, and contributed to the extraction of more carotenoids from petals of marigold flowers.

### 3.4.4. Optimization of SC-$CO_2$+EtOH extraction of pea flour

The CCRD was applied to optimize three independent variables at five levels. The second order polynomial model was fitted using the response variables, TV and PI. The response variables were determined based on the maximum removal of VOCs via supercritical fluid extraction and PI ratings. Experimentally obtained TV and PI ranged between 1.4 and 7.1 µg/g and 30.2 and 122 mm, respectively. When the TV concentration and degree of PI were subjected to response surface analysis, both response variables had a saddle point as the solution for the response surface. Thus,

the TV data was transformed using the Box-Cox transformation to improve data normality (Razavi et al., 2009); then the transformed data was used for model fitting. A significant model ($p < 0.05$) of the transformed TV had a minimum solution in the response surface application. The optimum conditions from the response surface for the transformed TV were obtained at $x_1$ (ethanol%) = 22%; $x_2$ (temperature) = 86 °C; and $x_3$ (pressure) = 42.71 MPa, which resulted in a predicted value of 1.2 µg/g total VOCs. The temperature and pressure obtained were outside of the range of values evaluated due to data transformation. Furthermore, the limitation of using only TV instead of the individual volatiles may also account for the optimal conditions falling outside the actual conditions tested.

The run at the optimum conditions was conducted to validate the predicted value with three replicates, and 0.55 ± 0.31 µg/g of total VOCs was obtained. Although this value was less than the predicted value, it was not significantly ($p > 0.05$) different than the predicted value. The value obtained from optimum conditions was lowest among all processing conditions created by the CCRD and was significantly ($p < 0.05$) lower than raw pea flour, which had 19.7 µg/g total VOCs. Furthermore, the GC results were supported by the sensory results, which indicated the lowest PI (22.2 mm) was in flours processed at optimum conditions. Furthermore, deflavored pea flour obtained under the optimum conditions had the lowest significant ($p < 0.05$) bitterness value (Table 3.2).

The response surfaces illustrate the effects of temperatures and ethanol on the TV at a fixed pressure of 34.47 MPa (Figure 3.1a). From this plot, an increase in temperature results in less TV. Similarly, significant ($p < 0.05$) the reduction in the TV resulted with increasing ethanol concentrations during the extraction, particularly between 20% and 30% ethanol. The effects of pressure and ethanol at a fixed temperature of 50 °C on the TV (Figure 3.1b) indicated that pressure

did not significantly impact ($p < 0.05$) the volatile loss. The effects of pressure and temperature at a fixed ethanol level of 25% on the TV (Figure 3.1c) illustrated that pressure did not influence the TV while the increase in temperature decreased significantly ($p < 0.05$) the TV from pea flour.

Ethanol co-solvent and temperature were important experimental factors that promote the extraction of VOCs from plant materials. Ethanol facilitates the extraction of alcohols and high molecular components via SC-$CO_2$ extraction. Campone et al. (2018) reported that ethanol as a co-solvent in SC-$CO_2$ extraction significantly increased the recovery of phenolic compounds. Xu et al. (2015) found SC-$CO_2$ extraction for recovering liquor aroma compounds was more efficient compared with other conventional extraction methods. SC-$CO_2$ extraction was more effective in extracting carbonyl compounds than alcoholic compounds because of non-polarity of $CO_2$ (Shao et al., 2014). An increase in temperature was important for removing aromatic compounds from a biological substance via SC-$CO_2$ extraction (Shao et al., 2014; Xu et al., 2015) by enhancing solubility of solutes based on increasing solute vapor pressure (Ciftci et al., 2018; Wang et al., 2012) and changes in the sample matrix, i.e., protein structure (Cobb et al., 2018).

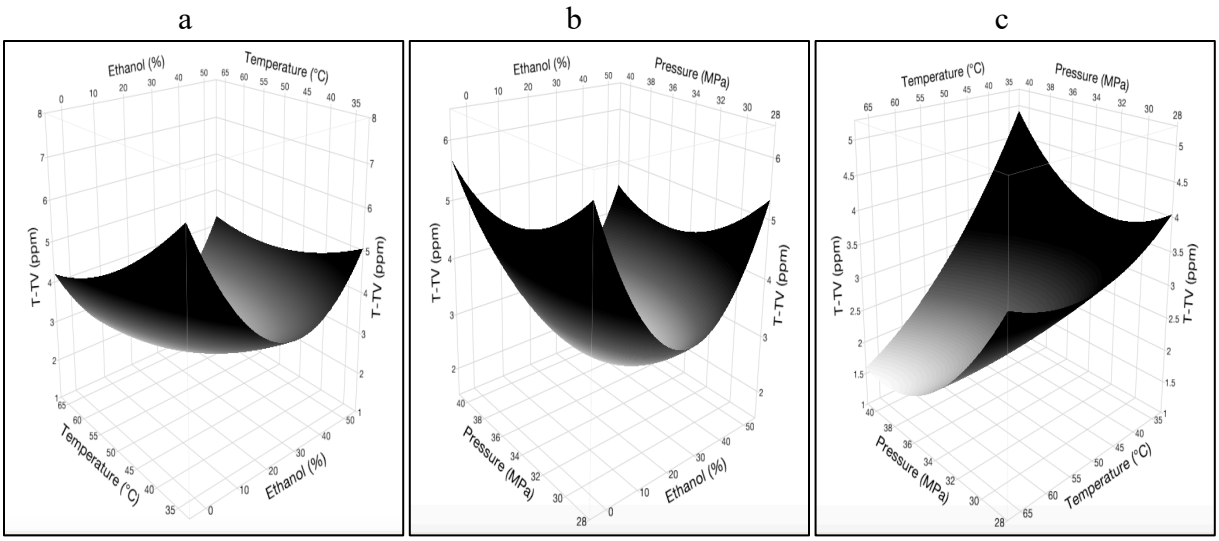


Figure 3.1. The response surfaces for the total volatile (TV) content of pea flour using SC-$CO_2$+EtOH extraction. Letters represent the following: a= effects of temperatures and ethanol on the TV at constant pressure; b = effects of pressure and ethanol on the TV at a fixed temperature of 50 °C; c= effects of pressure and temperature on the TV at a fixed ethanol level of 25%.

### 3.4.5. Model fitting

The TV ($y_1$) and PI ($y_2$) were used to calculate the second polynomial model of response surface (Table 3.3). The actual TV concentration had a low non-significant lack of fit value ($p$ = 0.0506). These results indicated that the model did not fit the experimental points. The solution for the response surface was obtained at the saddle point. When PI data for the 17 runs was subjected to the RSM, the model was significant ($p < 0.05$) with a non-significant lack of fit value ($p > 0.05$); however, the solution for the response surface was at the saddle point. Therefore, the transformed TV data for the 17 runs was employed in the RSM and resulted in a significant model fitting ($p <$ 0.05) with a non-significant lack of fit value ($p > 0.05$). Similarly, data normalization using the Box-Cox transformation was successfully applied by Razavi et al. (2009). This transformation helped improve both $R^2$ (0.90) and adjusted $R^2$ (0.79) (Table 3.3) of the TV since this fitted model explained 90% of the variability and 79% of the standard deviation (Campone et al., 2018).

The effects of independent variables on the TV were evaluated using the regression coefficients of the response surface model in the ANOVA (Table 3.4). Highly significant second order regression model of T-TV ($p$ = 0.007) sufficiently fixed the data points for independent variables (Xu et al., 2015). From this model, the linear term of temperature had the most significant influence on the response variable along with a greater regression coefficient (absolute value) supported by a smallest $p$-value (0.001) (Table 3.4) (Wang et al., 2012). The increase in temperature resulted in a decrease in the VOCs. Similar effect of temperature on the VOCs extraction was reported (Shao et al., 2014; Xu et al., 2015).

Furthermore, the optimum level of ethanol was about 22 % based on RSM. Addition of excessive ethanol might increase the polarity of the SC-$CO_2$, resulting in solubility and diffusivity that are less favorable for removal of VOCs (Wang et al., 2012). The interaction between temperature and ethanol was not significant though both parameters were important. Since the $p$-value of ethanol was close to the significance level, this parameter likely caused the interaction between two parameters not to be significant.

In addition to the effect of temperature, linear and quadratic term of ethanol had significant positive impacts on the TV. The proper amount of ethanol was essential for this extraction system; thus, over or under addition of ethanol was insufficient for VOCs extraction. Pressure did not significantly impact the TV from pea flour through this extraction (Table 3.4).

The second order polynomial equation of TV, which was created by re-running the analysis after removal of non-significant terms with lower $p$-values (<.0001, 0.0006, 0.0007, 0.0012), using significant independent variables (% ethanol ($x_1$), temperature ($x_2$)) is as follows:

$$y = 7.1193 - 0.1281x_1 - 0.0520x_2 + 0.0022x_1^2$$

Table 3.3. ANOVA of multiple regression model for response variables.

| Source | DF | SS | MS | F-value | *P*-value | $R^2$ | Adj. $R^2$ |
|---|---|---|---|---|---|---|---|
| **A-TV[a]** | | | | | | | |
| Model | 9 | 22.013 | 2.445 | 2.582 | 0.112 [ns] | 0.768 | 0.470 |
| Error | 7 | 6.632 | 0.948 | | | | |
| Lack of fit[b] | 5 | 6.496 | 1.299 | 19.076 | 0.051 [ns] | 0.995 | |
| Pure error[c] | 2 | 0.136 | 0.068 | | | | |
| **T-TV[d]** | | | | | | | |
| Model | 9 | 9.158 | 1.018 | 7.737 | 0.007** | 0.909 | 0.791 |
| Error | 7 | 0.921 | 0.132 | | | | |
| Lack of fit | 5 | 0.459 | 0.092 | 0.398 | 0.824 [ns] | 0.954 | |
| Pure error | 2 | 0.462 | 0.231 | | | | |
| **PI[e]** | | | | | | | |
| Model | 9 | 8011.318 | 890.146 | 16.860 | 0.001** | 0.956 | 0.899 |
| Error | 7 | 369.572 | 52.796 | | | | |
| Lack of fit | 5 | 294.345 | 58.869 | 1.565 | 0.434 [ns] | 0.991 | |
| Pure error | 2 | 75.2267 | 37.613 | | | | |

** $p < 0.01$ highly significant; *$0.01 < p < 0.05$ significant; ns: $0.05 < p$ not significant.
[a] A-TV = Actual-Total Volatile.
[b] Lack of fit is model error.
[c] Pure error is replicate error.
[d] T-TV=Transformed-Total Volatiles.
[e] PI =Pea Intensity.

Table 3.4. The regression coefficients of transformed total volatiles (TV) model.

| Variables | Coefficient (ß) | Standard Error | *t*-value | *P*-value |
|---|---|---|---|---|
| Constant | 7.0584 | 1.1292 | 6.25 | 0.0004** |
| $x_1$[a] | -0.0162 | 0.0065 | -2.47 | 0.0427* |
| $x_2$[b] | -0.0520 | 0.0098 | -5.29 | 0.0011** |
| $x_3$[c] | -0.0488 | 0.0285 | -1.71 | 0.1308[ns] |
| $x_1^2$ | 0.0026 | 0.0005 | 5.29 | 0.0011** |
| $x_2^2$ | 0.0015 | 0.0011 | 1.39 | 0.2062[ns] |
| $x_3^2$ | 0.0173 | 0.0091 | 1.90 | 0.0987[ns] |
| $x_1x_2$[d] | 0.0010 | 0.0009 | 1.15 | 0.2886[ns] |
| $x_1x_3$[e] | -0.0005 | 0.0025 | -0.20 | 0.8506[ns] |
| $x_2x_3$[f] | -0.0066 | 0.0037 | -1.77 | 0.1205[ns] |

** $p < 0.01$ highly significant; *$0.01 < p < 0.05$ significant; ns: $0.05 < p$ not significant.
[a] $x_1$ = Ethanol (%).
[b] $x_2$ = Temperature (°C).
[c] $x_3$ = Pressure (MPa).
[d] $x_1x_2$ = Interaction term of ethanol and temperature.
[e] $x_1x_3$= Interaction term of ethanol and pressure.
[f] $x_2x_3$ = Interaction term of temperature and pressure.

### 3.4.6. Principal component analysis of total volatile, pea attributes, and color values

The correlation matrix (Table 3.5) among the variables supports that PI and bitterness were highly correlated. As a result, the degree of PI explained by VOCs and bitterness expressed by non-VOCs were strongly associated. Also, these two sensory attributes were highly correlated with the TV. This result revealed the sensation of flavor was expressed as the combination of olfactory and taste (Noble, 1996).

The TV and sensory attributes were correlated with a-value and b-value positively and negatively with L-value. As the TV and sensory attributes decreased, a- and b-values decreased, and L-value increased. At the optimum conditions, a lighter pea flour resulted due to the impact of ethanol on the extraction of carotenoids present in pea flour. Cobb et al. (2018) stated that using the co-solvent increased the extraction of carotenoids. The apparent difference in color values of the runs 11 and 13 was stated in color analysis (3.4.3). The main difference between the two runs was that the run13 was carried out without ethanol. So, ethanol promoted certainly the reduction in a- and b-value. The reduction of b-value resulted in less yellow product.

Table 3.5. The sample correlation matrix of the response variables.

| Variable | TV[a] | PI[b] | Bitterness | L | a | b |
|---|---|---|---|---|---|---|
| **TV** | 1.0000 | 0.8056 | 0.8055 | -0.5938 | 0.8727 | 0.7843 |
| **PI** | 0.8056 | 1.0000 | 0.9116 | -0.4761 | 0.7907 | 0.6496 |
| **Bitterness** | 0.8055 | 0.9116 | 1.0000 | -0.4595 | 0.7915 | 0.6205 |
| **L** | -0.5938 | -0.4761 | -0.4595 | 1.0000 | -0.6586 | -0.5228 |
| **a** | 0.8727 | 0.7907 | 0.7915 | -0.6586 | 1.0000 | 0.7535 |
| **b** | 0.7843 | 0.6496 | 0.6205 | -0.5228 | 0.7535 | 1.0000 |

[a]TV = Total Volatile
[b]PI = Pea Intensity

For this PCA analysis, 80% of variation was considered; therefore, only one principal component significantly explained the variables. Collectively, 75.63% of the total variation was

explained by the first principal component. The PCA revealed TV content, sensory attributes, and color values were highly interrelated (Figure 3.2).

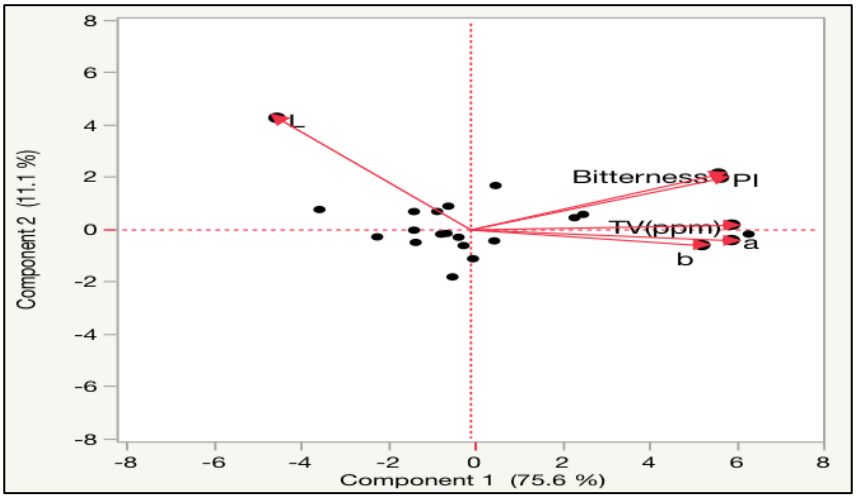


Figure 3.2. The biplot of two principal components.

### 3.5. Conclusions

Application of the CCRD under the RSM optimized the independent variables for the SC-$CO_2$+EtOH extraction. The RSM predicted the optimum extraction conditions as ethanol (22%), temperature (86 ˚C), and pressure (42.71 MPa). The response surface equation demonstrated that the linear term of temperature (negatively) and the linear and the quadratic terms of ethanol (positively) significantly influenced the TV content while the pressure had limited impact. The PCA illustrated that TV, sensory attributes, and color values were highly correlated. The data support the use of SC-$CO_2$+EtOH as a valuable method for removal of undesirable aroma and flavors from pea flour.

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# CHAPTER 4. CHEMICAL, FUNCTIONAL, AND MORPHOLOGICAL PROPERTIES, AND WATER SORPTION ISOTHERMS OF DEFLAVORED DIFFERENT PARTICLE SIZE PEA FLOURS USING SUPER CRITICAL FLUID EXTRACTION

## 4.1. Abstract

Pea (*Pisum sativum* L.) flour is ideal to fortify with cereals for nutritional enhancement and for producing gluten-free foods. However, unacceptable pea flavor restricts its utilization in food market. Supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction along with different particle size was employed to improve organoleptic attributes of pea flour. The extraction reduced moisture, resistant starch, damage starch, and lipid content of pea flours. Flour with coarse particles had lower protein, total starch, and starch damage than other flours. Flours with medium and fine particles had the highest protein and total starch, respectively. Pea flours became lighter after the extraction. Most viscosity parameters and water solubility index of flours decreased after deflavoring and varied based on particle size. Particle size distribution after the extraction did not change. Water sorption capacity of deflavored pea flours decreased with increased water activity. SC-$CO_2$+EtOH extraction and particle size had profound impacts on physicochemical, functional, pasting, and water sorption properties of pea flour. Effects of SC-$CO_2$+EtOH extraction with particle size on flour quality have not reported in the literature. Findings are crucial for application of this deflavoring technology for pulse ingredients and design of food processes.

## 4.2. Introduction

In recent years, the popularity of pulse crop ingredients has resulted in steady growth in the number of food product entering the marketplace. Their outstanding nutritional profile, along with significant contributions to food sustainability (e.g., providing a reliable and economical source of protein) and environmental aspects (e.g., decreasing pressure on natural sources through

nitrogen fixation capability) of food production are significant drivers. Furthermore, pulse crops adequately address the increasing global high-value food demands at the nexus of sustainable foods, environment concerns, and energy sources (Hall, Hillen, & Garden-Robinson, 2017; Tulbek, Lam, Wang, Asavajaru, & Lam, 2017).

These proteins are gluten-free, have low allergenicity, and notably rich in lysine (an essential amino acid) but low in sulfur-containing amino acids (cysteine and methionine) (Hall et al., 2017). Therefore, incorporation of pulse ingredients (e.g., pea flours, pea proteins) into cereal-based products is a promising option to obtain a nutritionally adequate complete protein profile (Kaiser et al., 2019; Xu, Jin, Simsek, Hall, Rao, & Chen, 2019). These flours are abundant in complex carbohydrates (e.g., dietary fibers, starch), vitamins such as folate, minerals, and antioxidants (Hall et al., 2017; Kaiser et al., 2019). Furthermore, pulses contain health-promoting components, such as dietary fibers, including high resistant starch (e.g., lowering glycemic index, boosting gut microbiome, and reducing risk of diabetes and colon cancer) along with the presence of bioactive compounds (e.g., carotenoids, phenolics, polyphenols) (Hall et al., 2017; Simons, Hall, & Vatansever, 2018). Currently, nutrient-dense pulse ingredients (e.g., pea flour, pea proteins) have gained more consumer interest in the current food market. Recently, pulse ingredients are being utilize for gluten-free, which is estimated to reach 7.6 billion U.S. dollars by 2020 (Li & Ganjyal, 2017; Xu et al., 2019), non-GMO, and protein-enriched functional foods. Furthermore, plant-based milk, meat, and seafood alternatives and addressing of different diet styles, such as vegan, vegetarian, and flexitarians have been targeted by the food industry (Kristiawan et al., 2018).

Dry pea (*Pisum sativum* L.), was one of the most produced pulse crops in the US in 2018 (Hall 2018). Peas are an economical, sustainable, and outstanding source of protein (14-31%),

starch (30-50%), and dietary fiber (3-27%), vitamins, minerals, and bioactive compounds (Hall et al., 2017; Tulbek et al., 2017; Zhou, Ma, Yin, Hu, & Boye, 2019). Pea protein is high in lysine but low in sulfur-containing amino acids (cysteine and methionine). In contrast, the low lysine but high sulfur amino acids in cereal flours is an ideal complimentary protein that results in a complete protein when the two protein sources are mixed (Hall et al., 2017; Kaiser, Barber, Manthey, & Hall, 2019). However, pea flour has not been extensively utilized in the current food system due to its unacceptable flavor (Azarnia, Boye, Warkentin, & Malcolmson, 2011; Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Murat, Bard, Dhalleine, & Cayot, 2013; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). Therefore, the deflavoring of pea flour is an ideal scenario to boost its utilization in the global food system. For this purpose, supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH), a green emerging technology, was used as a deflavoring tool for whole yellow pea flour in our previous study. SC-$CO_2$+EtOH extraction successfully improved sensory attributes of pea flour through the removal of pea aroma and taste compounds (Vatansever & Hall, 2020).

SC-$CO_2$+EtOH extraction has been employed to separate essential oil, lipid, aroma compounds, carotenoids, and other organic compounds from plant materials. Furthermore, SC-$CO_2$ extraction has been shown to modify starch and starch gelatinization properties (Braga, Moreschi, & Meireles, 2006; Ivanovic, Milovanovic, & Zizovic, 2016; Muljana, Picchioni, Heeres, & Janssen, 2009). Therefore, a greater understanding of SC-$CO_2$+EtOH extraction, employed as a deflavoring tool, on the physicochemical, pasting, and functional characteristics of treated flour is imperative for deflavored pea flour use in future food applications. Recently, significant impacts of particle size on the physicochemical and functional properties of pea flour have been previously reported (Kaiser et al., 2019; Maskus, Bourre, Fraser, Sarkar, & Malcolmson, 2016). Therefore,

determining the interaction effect between two factors and their impacts on the physiochemical, pasting, functional, moisture sorption isotherm, and morphological properties of yellow pea flour are useful for predicting food formulation, energy requirements for food processing, and shelf-life stability.

In this study, whole yellow pea flour (unsieved) and its fractions (coarse/large, medium, and fine/small) obtained by sieving and their deflavored counterparts treated by SC-$CO_2$+EtOH extraction were used. The objective of this study was to investigate the changes in composition, functional properties, and moisture sorption isotherms of pea flour samples after being treated with SC-$CO_2$+EtOH extraction and to determine the interaction effect between the extraction and particle size. The findings of this study will be useful to enhance the utilization of pea flour in the global food market and establish a new approach for deflavoring pea flour using a green processing technology.

## 4.3. Materials and Methods

### 4.3.1. Pea flour milling and particle size determination

Viterra (Minot, ND, USA), Specialty Commodities (Fargo, ND, USA), and SK Foods (Moorhead, MN, USA) were the source of whole yellow dry peas. The samples were of mixed cultivars and were manually blended to create a homogenous sample. The blended peas were hammer milled (Fitzpatrick, Elmhurst, IL) using a 1.270 mm screen and hammer rotation of 102 m/s to produce whole yellow pea flour (Kaiser et al., 2019). All samples were stored in sealed polyethylene bags at -20 ºC until analysis.

Sieving of whole yellow pea flour was performed using Rotap (W.S. Tyler, Mentor, OH, USA) sieve shaker with the following sieves: openings of 425 (40-mesh), 250 (60-mesh), 150 (100-mesh), 106 (140-mesh), and 53 (270-mesh) μm based on the approved method 55-60.01

(AACC International 2010). The fractions retained on the sieve were chosen as: coarse/large, >250µm (425 ≥ flour >250); medium, >150µm (250 ≥ flour >150); and fine/small, >106µm (150 ≥ flour >106). In addition to these three fractions, whole yellow pea flour (unsieved) was used in the study.

### 4.3.2. Supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction

The SC-$CO_2$+EtOH extraction technology was conducted to enhance sensory attributes of yellow pea flour (Vatansever & Hall, 2020). Briefly, 6 g of pea flour was deflavored using an ISCO supercritical fluid extractor (model SFX 2-10; Isco, Inc., Lincoln NE, USA) with two solvents, $CO_2$ as a main solvent and ethanol as a co-solvent. The extraction of pea flour samples was carried out at optimum conditions (22% ethanol, 86 $^{o}$C, and 42.70 MPa) for a total of 40-minute extraction (i.e., a 10-minute static and a 30-minute dynamic extraction) with the total solvent flow rate of ~ 2 mL/min. After the extraction process, the wet sample was dried at 70 $^{o}$C in a convection oven for 1 hour to remove residual ethanol and then stored in 2.5 mil Mylar bags (Uline; Pleasant Prairie, WI, USA) at -20 $^{o}$C until analysis could be completed.

### 4.3.3. Chemical composition

Moisture, protein, fat, and ash were determined based on approved methods 44-15.02, 46-30.01, 30-10.01, and 08-01.01 (AACC International 2010), respectively. Total starch, starch damage, and resistant starch analyses were performed using assay kits (Megazyme International Ltd., Wicklow, Ireland) following AACC International approved methods 76-13.01, 76-31.01, and 32-41.01, respectively (AACC International 2010).

### 4.3.4. Color determination

Color values of pea flour samples was conducted using a chroma meter CR-410 (Minolta, Tokyo, Japan) and results were recorded as L-, a-, and b-values (Vatansever & Hall, 2020).

### 4.3.5. Particle size determination

Particle size distribution curves and the d-values at d(0.1), the 10$^{th}$ percentile, at d(0.5), the 50$^{th}$ percentile, and at d(0.9), the 90$^{th}$ percentile of pea flour samples were determined using a Mastersizer 3000 Laser particle size analyzer (Malvern, Worcestershire, UK) including a solid powder dispersion unit. Mean particle size (μm) at the 10$^{th}$, 50$^{th}$, and 90$^{th}$ percentiles and volume weighted mean (μm) of the distribution curve were recorded.

### 4.3.6. Pasting properties

Pasting profiles of non-deflavored and deflavored pea flour samples were determined using a Rapid Visco Analyzer (RVA) (RVA 4500, Perten Instruments, Springfield, IL) based on the modified AACC International method 61-02.01. Briefly, the modifications included the weight for flour (3.5 g) and water (25 g) were adjusted for flour moisture content. Further, the temperature during a run started at 50 $^{o}$C and was raised to 95 $^{o}$C over 4 minutes and 42 seconds followed by a holding period until 7 minutes and 12 seconds into the run. Then, at 11 minutes the temperature was dropped to 50 $^{o}$C and remained at 50 $^{o}$C until the end of the 23-minute run. Peak time, hot and cold paste viscosities, and break down information were collected from the instrument.

### 4.3.7. Functional properties

Water absorption index (WAI) and water-soluble index (WSI) of pea flours were determined using the protocol described by Simons, Hall, & Tulbek (2012). Briefly, pea flour (2.5 g) was transferred to preweighed 50 mL centrifuge tubes and the mass was record. Then, 30 mL of distilled water was added and shaken vigorously to break lumps. Centrifuge tubes were stirred with stir bars for 30 min, then centrifuged at 3,000 rpm for 10 min. The supernatant was decanted into preweighed beakers. The tubes, including wet sediment, were weighted and recorded. Beakers

were placed in the oven at 110 °C for overnight before weighing the solids in the supernatant. The WAI (g/g) and WSI (%) were calculated using the following equations:

$$WAI = \frac{weight\ of\ the\ wet\ sediment\ (g)}{initial\ weight\ of\ the\ dry\ flour\ (g)}$$

$$WSI\ (\%) = \frac{weight\ of\ the\ solids\ in\ the\ superatant\ (g)}{initial\ weight\ of\ the\ dry\ flour\ (g)}\ x\ 100$$

Oil absorption capacity (OAC) of flour samples was determined using the protocol described by Maskus et al. (2016). Flour suspension was prepared with the combination of 2 g of pea flour and 20 g of canola oil, which were placed in preweighed 50 mL centrifuge tubes. Then, the suspension was mixed in a vortex mixer on high for 30 sec and then incubated at room temperature for 30 min. The samples were centrifuged at 3,000 rpm for 30 min. After centrifuging, the supernatant was discarded, and the tubes were kept inversely to drain for 10 min at room temperature. The final weight of the tubes, including gel, was recorded and OAC was determined as grams of oil bound/ gram of flour (dry weight basis) using following formula:

$$OAC\ (g/g) = \frac{final\ weight - tube\ weight - flour\ weight}{flour\ weight}\ x\ \frac{100}{100 - flour\ moisture\ content}$$

**4.3.8. Moisture sorption isotherm analysis**

Moisture sorption isotherms of pea flour samples were determined using a fully equipped vapor sorption analyzer (VSA, Decagon Devices, Inc., Pullman, WA) based on the dynamic vapor sorption protocol described by Syamaladevi et al. (2016) with some modifications. Briefly, 2000 mg of flour sample was placed into the instrument along with 20% of relative humidity until reaching a constant sample mass. The instrument was set up as followed: range of water activity ($a_w$) was 0.20-0.85 and total sorption stage was two (adsorption and desorption) with a resolution of 0.1 to generate water sorption curve at 25 $^{o}$ C. After reaching the sample equilibrium, water activity and moisture content were recorded by the instrument based on weight change data. Two

replications were done for each sample. The adsorption and desorption data were collected from the instrument to create the isotherms. The Double Log Polynomial (DLP) and Guggenheim Anderson-de Boer (GAB) models were chosen as sorption isotherm models to determine predictive moisture content (*m*, dry basis) using the SorpTrac$^{TM}$ Version 1.14 for AquaSorp Isotherm Generator. The equations of the two models are:

DLP equation:

$$m = b_3X^3 + b_2\mathrm{X}^2 + b_1\mathrm{X} + b_0$$

where *m* is the moisture in g/100 solids or g/g solids, X = *In [-In($a_w$)]* and $b_0$-$b_3$ are constants.

GAB equation:

$$m = \frac{ckm_0a_w}{(1-ka_w)(1-ka_w+cka_w)}$$

where *m* is the moisture in g/100 solids or g/g solids, *c* and *k* are constants in the range of 1 to 2000 and in the range of 0.70 to 1, respectively. Also, $m_o$ is the monolayer moisture content in the on the dry basis and $a_w$ is the water activity at moisture (*m*) (Nurtama & Lin, 2010).

**4.3.9. Scanning electron microscopy (SEM)**

The morphologic structure of pea flour samples was obtained using a scanning electron microscope (SEM) (JEOL Model JSM-6490LV, Peabody, MA, USA). Starch sample was placed to on an adhesive carbon tab on a cylindrical aluminum mount. A stream of nitrogen gas was employed to remove the excess sample. Later, the starch sample was coated with gold (Cressington 108 auto, Ted Pella, Redding, CA, USA) by sputtering. The micrographs were obtained at 500x, 1000x, and 2500x magnifications with an accelerating voltage of 15kV. The 1000x micrographs were used to exhibit each flour samples for further analysis.

### 4.3.10. Statistical analysis

For this study, a full factorial design including two factors, extraction (two levels) and particle size (four levels) with three replicates (n=3, N=24) for all analyses was used. The two main factors were considered as fixed effects. The analysis of variance was determined using JMP Software (JMP 14.0.0 Version 2018 SAS Institute Inc.). The mean separation of the eight-treatment means was conducted using a Tukey's test at 5% significance level. The correlations among the response variables and predictors were explained by principal component analysis using JMP Software (JMP 14.0.0 Version 2018 SAS Institute Inc.).

## 4.4. Results and Discussion

### 4.4.1. Proximate composition

The proximate composition of whole yellow pea flour (Table 4.1) was consistent with previous studies (Kaiser et al., 2019; Li & Ganjyal, 2017; Maskus et al., 2016; Rempel, Geng, & Zhang, 2019; Xu, Jin, Simsek, Hall, Rao, & Chen, 2019). The interaction effect between SC-$CO_2$+EtOH extraction and particle size was significant ($p < 0.05$) for all proximate compositions except total starch (Table 4.2). SC-$CO_2$+EtOH extraction did not influence ash and total starch contents but caused a significant reduction in the remaining proximate composition parameters except for protein in the coarse fraction. In contrast, particle size significantly impacted all proximate compositions (Tables 4.1 and 4.2).

The extraction significantly removed fat from pea flour (Table 4.1). Similarly, Kang et al. (2017) and Garcia Solaesa, Villanueva, Beltran, & Ronda (2019) used SC-$CO_2$ extraction as a successful defatting method for soy and quinoa flours, respectively. Furthermore, the reduction in the fat content of pea flour through this extraction might support shelf-life stability of pea flour by decreasing the substrate (i.e., fat) for lipoxygenase activity during the storage (Xu et al., 2019).

The significant reduction in the moisture content of pea flours might be associated with the drying effect of SC-$CO_2$ extraction. Likewise, this extraction has been employed as a faster drying method for foods (Brown, Fryer, Norton, Bakalis, & Bridson, 2008). Brown et al. (2008) found SC-$CO_2$ extraction removed moisture significantly ($p < 0.05$) from carrots. Also, these researchers stated that the addition of ethanol as a co-solvent improved the drying efficiency of the carrot samples due to increasing solubility of polar substances in SC-$CO_2$ via chemical interactions such as hydrogen and dipole-dipole bonding. Garcia Solaesa et al. (2019) reported the drying effect of SC-$CO_2$ extraction for defatted whole quinoa flour. Furthermore, a 1-h oven-drying period (at 70 $^{o}$ C) after each extraction of pea flour, which was applied to remove residual ethanol in the treated sample, might lead additional moisture removal.

Protein content did not change for whole pea flour but increased significantly ($p$ <0.05) in coarse fraction after the extraction. Similarly, a slight increase in protein content of corn gluten meal treated by SC-$CO_2$+EtOH extraction was previously observed (Cobb, Kallenbach, Hall, & Pryor, 2018). However, a significant reduction in protein contents of pea flours with medium and fine fractions was found after the extraction. The increased surface area of the flours with smaller particles might lead to the increased interaction with ethanol causing removal of some small molecular weight proteins, specifically ethanol soluble proteins. Also, these protein particles may dissolve in ethanol, resulting in slight protein reduction.

Extraction did not have a significant effect ($p > 0.05$) on the total starch content of all pea flour samples. A slight increase in total starch for flours with coarse and medium fractions was observed, but it was not significant. Similarly, Garcia Solaesa et al. (2019) reported a slight significant increase in the total starch content of whole quinoa flour treated with SC-$CO_2$ extraction, owing to the removal of lipids, which is relatively high in quinoa.

Unlike total starch, resistant starch and starch damage decreased significantly ($p < 0.05$) for all pea flour samples after the extraction. SC-$CO_2$+EtOH extraction might cause structural changes in starch granules. This extraction may induce the rearrangement of the pairs of double helices and thereby transform B-type polymorph (more resistant to enzyme hydrolysis) to an A-type polymorph (more accessible to enzymatic hydrolysis) (Jane et al., 2003). This transformation may have been the basis for the lower RS values in the deflavored pea flour samples. Significant reduction in starch damage for all pea flour samples might be associated with the loss of broken starch granules through ethanol solubilization. This result supports that the extraction did not cause an increase in starch damage.

Particle size had a significant impact on all proximate composition. Significant differences ($p < 0.05$) existed for protein, lipid, total starch, and starch damage among coarse (>250μm), medium (>150μm), and fine (>106μm) fractions (Tables 4.1 and 4.2). The highest protein and lipid contents were observed in the pea flours made up of the medium fraction. In contrast, pea flours with coarse fraction had the lowest percentage of protein, lipid and starch, which is likely due to the coarse bran particles from the hull. The higher total starch and starch damage in the pea flours with fine fraction likely resulted from the samples being derived from the cotyledon, which is mostly void of hull particles. The negative correlation between starch damage and particle size was reported previously (Kaiser et al., 2019; Maskus et al., 2016). Also, the increase in surface area with reducing particle size may lead to greater exposure of starch granules to starch degrading enzymes (Kaiser et al., 2019). Pea flours made up of the medium fraction had higher protein and lower starch concentration than flours with the fine fraction. Similarly, Maskus et al. (2016) indicated this reverse correlation between protein and starch concentration for yellow pea flour. Ash content was slightly higher in flours from medium and fine fractions. Although ash is typically

associated with hull and bran fractions of seeds, the ash in pea is embedded in the protein matrix and once this matrix was ground into small particles, the ash associated with the protein-rich fraction (i.e. medium fraction). This was supported by Rempel et al. (2019) whom indicated that protein content was higher in pea flours from medium and fine fraction compared to coarse fraction and that ash content was higher in finer fraction than coarse fraction of pea flours.

### 4.4.2. Color analysis

Color results (Table 4.1) of pea flour samples followed a similar trend with literature data (Kaiser et al., 2019; Vatansever & Hall, 2020). Significant main and interaction effects ($p < 0.05$) among the flours were determined for color values (Table 4.2). SC-$CO_2$+EtOH extraction significantly increased L* (lightness) and decreased a* (redness) and b* (yellowness) for all pea flour samples (Tables 4.1 and 4.2). Therefore, deflavored pea flour samples became lighter owing to the removal of carotenoids (e.g., mostly lutein and some ß-carotene) that are soluble in ethanol (Vatansever & Hall, 2020). Similarly, Cobb et al. (2018) reported the removal of carotenoids from corn gluten meal through SC-$CO_2$+EtOH extraction. Particle size had a significant impact for all proximate composition (Tables 4.1 and 4.2).

Significant differences for color values were observed for flours with different fractions and whole pea flour. Lightest pea flour contained fine fraction, whereas coarser fraction was darker with lower L* and higher a* and b* values, likely due to higher hull (seed coat) particles in the flour, which are rich in carotenoids (Marles, Warkentin, & Bett, 2013).

Table 4.1. Proximate composition, particle size distribution, and color values of pea flour samples [A]

| Flour Property | Non-deflavored | | | | Deflavored | | | |
|---|---|---|---|---|---|---|---|---|
| | Whole | >250[B] | >150 | >106 | Whole | >250 | >150 | >106 |
| Composition (%, db) | | | | | | | | |
| Moisture | 10.11 ± 0.22[a] | 9.94 ± 0.04[a] | 10.03 ± 0.05[a] | 10.03 ± 0.19[a] | 3.92 ± 0.33[b] | 2.61 ± 0.13[c] | 2.58 ± 0.43[c] | 2.81 ± 0.23[c] |
| Ash | 2.75 ± 0.03[ab] | 2.61 ± 0.10[ab] | 2.84 ± 0.11[a] | 2.82 ± 0.23[a] | 2.69 ± 0.08[ab] | 2.69 ± 0.04[ab] | 2.86 ± 0.05[a] | 2.49 ± 0.02[b] |
| Protein | 23.73 ± 0.16[c] | 20.84 ± 0.14[e] | 26.51 ± 0.31[a] | 23.73 ± 0.17[c] | 23.45 ± 0.41[c] | 22.17 ± 0.44[d] | 25.08 ± 0.28[b] | 21.83 ± 0.38[d] |
| Lipid | 2.01 ± 0.04[a] | 1.54 ± 0.05[b] | 2.01 ± 0.07[a] | 1.69 ±0.03[ab] | 0.84 ± 0.21[c] | 0.53 ± 0.10[c] | 0.69 ± 0.18[c] | 1.35 ± 0.32[b] |
| Total starch | 47.11 ± 2.09[b] | 34.97 ± 0.47[c] | 46.19 ± 1.05[b] | 54.77 ± 0.30[a] | 46.99 ± 0.99[b] | 36.89 ± 1.09[c] | 47.31 ± 0.64[b] | 54.44 ± 1.48[a] |
| Resistant starch | 2.03 ± 0.07[b] | 2.64 ± 0.34[a] | 2.72 ± 0.01[a] | 2.99 ± 0.20[a] | 1.65 ± 0.21[bc] | 1.10 ± 0.09[d] | 1.02 ± 0.03[d] | 1.40 ± 0.26[cd] |
| Starch damage | 1.37 ± 0.06[b] | 0.51 ± 0.03[d] | 0.87 ± 0.01[c] | 1.99 ± 0.05[a] | 0.72 ± 0.07[c] | 0.25 ±0.02[e] | 0.41 ± 0.03[d] | 1.37 ± 0.10[b] |
| Particle size distribution (μm) [C] | | | | | | | | |
| d (0.1) | 13.93 ± 0.15[d] | 229.33 ± 1.15[a] | 28.73 ± 1.84[c] | 9.92 ± 0.08[d] | 19.63±0.15[cd] | 227.67 ± 0.57[a] | 68.50 ± 5.46[b] | 13.17 ± 0.06[d] |
| d (0.5) | 169.00 ± 3.60[d] | 356.33 ± 0.57[a] | 166.67 ± 2.08[d] | 32.40 ± 0.17[e] | 180.67 ± 4.16[c] | 346.67 ± 1.52[b] | 163.33 ± 3.21[d] | 37.43 ± 0.38[e] |
| d (0.9) | 514.33 ± 4.04[b] | 539.00 ± 1.00[a] | 275.67 ± 2.31[c] | 113.67 ± 1.15[e] | 516.67 ± 6.80[b] | 519.00 ± 3.00[b] | 275.00 ± 4.16[c] | 137.33 ± 3.78[d] |
| Color | | | | | | | | |
| L* (lightness) | 89.97 ± 0.05[d] | 78.94 ± 0.11[g] | 84.61 ± 0.07[e] | 89.05 ± 0.04[b] | 90.64 ± 0.46[a] | 83.25 ± 0.03[f] | 87.5 ±0.05[c] | 91.02 ± 0.02[a] |
| a* (redness) | 2.76 ± 0.02[c] | 4.99 ± 0.04[a] | 4.01 ± 0.01[b] | 1.70 ± 0.01[d] | 0.30 ± 0.15[g] | 1.46 ± 0.01[e] | 1.01 ± 0.02[f] | 0.32 ± 0.01[g] |
| b* (yellowness) | 21.16 ± 0.52[c] | 29.73 ± 0.10[a] | 27.88 ± 0.12[b] | 19.01 ± 0.01[d] | 12.85 ± 0.67[f] | 18.18 ± 0.06[de] | 17.44 ± 0.02[e] | 12.51 ± 0.02[f] |

[A] Values of mean ± standard deviations (n=3) with same letter are not significantly different at $\alpha = 0.05$.
[B] Coarse/large, >250μm (425 ≥ flour >250); medium, >150μm (250 ≥ flour >150); and fine/small, >106μm (150 ≥ flour >106) and whole is unsieved pea flour.
[C] At d (0.1), the 10th percentile, 10% of the volume of pea flour particles are the indicated size (μm) or smaller; at d (0.5), the 50th percentile, 50% of the volume of pea flour particles are the indicated size (μm) or smaller; and at d (0.9), the 90th percentile, 90% of the volume of pea flour particles are the indicated size (μm) or small.

Table 4.2. F- and p-values for proximate composition, particle size distribution, and color values of pea flours using a fixed full factorial model [A]

| Flour Property | Extraction | | Particle Size | | Extraction*Particle Size | |
|---|---|---|---|---|---|---|
| | F-value | *p*-value | F-value | *p*-value | F-value | *p*-value |
| Composition (%, db) | | | | | | |
| Moisture | 5206.98 | <.0001 | 12.53 | 0.0002 | 8.85 | 0.0011 |
| Ash | 2.88 | 0.1091[ns] | 4.86 | 0.0137 | 4.45 | 0.0186 |
| Protein | 20.33 | 0.0004 | 203.08 | <.0001 | 32.38 | <.0001 |
| Lipid | 214.95 | <.0001 | 10.28 | 0.0005 | 10.82 | 0.0004 |
| Total starch | 1.94 | 0.1826[ns] | 269.09 | <.0001 | 1.29 | 0.3131[ns] |
| Resistant starch | 282.32 | <.0001 | 4.61 | 0.0166 | 16.13 | <.0001 |
| Starch damage | 499.17 | <.0001 | 647.42 | <.0001 | 16.55 | <.0001 |
| Particle size distribution (μm) [B] | | | | | | |
| d (0.1) | 58.44 | <.0001 | 4458.33 | <.0001 | 37.79 | <.0001 |
| d (0.5) | 0.60 | 0.4479[ns] | 11958.34 | <.0001 | 15.48 | <.0001 |
| d (0.9) | 0.4672 | 0.5041[ns] | 10063.61 | <.0001 | 20.99 | <.0001 |
| Color | | | | | | |
| L* (lightness) | 2202.94 | <.0001 | 3366.98 | <.0001 | 54.98 | <.0001 |
| a* (redness) | 12743.52 | <.0001 | 1868.66 | <.0001 | 403.16 | <.0001 |
| b* (yellowness) | 5380.03 | <.0001 | 1050.4 | <.0001 | 80.65 | <.0001 |

[A] ns: non-significant at $\alpha = 0.05$ and df =1, 3, 3 for extraction, particle size, and interaction, respectively with N=24.

[B] At d (0.1), the 10$^{th}$ percentile, 10% of the volume of pea flour particles are the indicated size (μm) or smaller; at d (0.5), the 50$^{th}$ percentile, 50% of the volume of pea flour particles are the indicated size (μm) or smaller; and at d (0.9), the 90$^{th}$ percentile, 90% of the volume of pea flour particles are the indicated size (μm) or smaller.

### 4.4.3. Particle size distribution

The mean particle size at d (0.1), d (0.5), and d (0.9) representing the $10^{th}$, $50^{th}$, and $90^{th}$ percentiles of the particle size distribution, respectively, are presented in Table 4.1. The d (0.1), d (0.5), and d (0.9) were significantly associated with the interaction between SC-$CO_2$+EtOH extraction and particle size fractions ($p < 0.05$; Table 4.2). The particle size distribution of hammer milled whole yellow pea flour was in agreement with previous reports (Kaiser et al., 2019; Maskus et al., 2016).

Particle size distribution curves of non-deflavored (N) and deflavored (D) pea flour samples (Fig. 4.1) showed that whole (unsieved), and the fine fraction of pea flours had a distinct bimodal distribution. In contrast, coarse and medium fractions of pea flours exhibited a more unimodal distribution. Likewise, Maskus et al. (2016) determined a bimodal distribution for hammer milled whole yellow pea flour. Furthermore, Rempel et al. (2019) reported that rotor milled yellow split pea flour (parent flour) had a bimodal distribution, but its coarse and fine fractions obtained through re-milling and air classification showed a unimodal distribution. After SC-$CO_2$+EtOH extraction, significant particle size reduction occurred in coarse fraction, but overall the changes in the particle size distribution of pea flour samples were relatively negligible (Fig. 4.1).

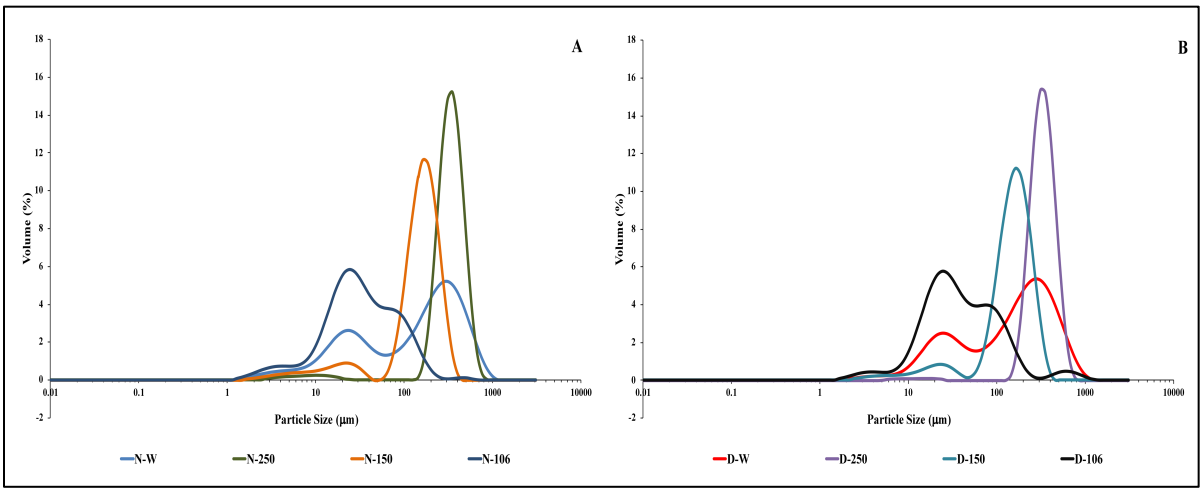


Figure 4.1. Particle size distribution curves for pea flour samples. Letters represent the following: N-W: Non-deflavored whole (unsieved); D-W: deflavored whole (unsieved); N-250: Non-deflavored coarse; D-250: deflavored coarse; N-150: Non-deflavored medium; D-150: Deflavored medium; N-106: Non-deflavored fine; D-106: deflavored fine pea flour.

### 4.4.4. Pasting properties

Pasting properties of pea flour are crucial factors to uniquely utilize in cereal, pulse, meat, and gluten-free formulations applications in the food industry (Tulbek et al., 2017). Therefore, pasting properties of pea flours were determined (Table 4.3). The main effects and interaction effect between SC-$CO_2$+EtOH extraction and particle size were found significant for pasting properties of flour samples (Table 4.4).

Pasting properties of whole yellow pea flour exhibited similar behavior with previous reports (Abdel-Aal, Ragaee, Rabalski, Warkentin, & Vandenberg, 2019; Maskus et al., 2016). Based on the viscosity characteristics of pea flours, a significant decrease ($p < 0.05$) of peak and hot paste (i.e., trough viscosity) viscosities were observed for deflavored pea flours. These parameters are closely associated with the conversion of pea starch granules into a hot paste through hydration and swelling in the presence of excessive water at increasing temperature. Lower peak viscosity of deflavored pea flours compared to native pea flours indicated a reduction in water absorption capacity by the starch granules, thereby resulting in a slow hydration and less

swelling along with the separation of the polymer (e.g., amylose leaching) during heating (Marta & Tensiska, 2017; Simons, Hall, & Vatansever, 2018). The conditions of SC-$CO_2$+EtOH extraction, particularly high temperature (86 $^{o}$ C), might cause an increase in molecular mobility and attribute structural changes in pea starch causing less swelling and a reduction in pasting parameters (Garcia Solaesa et al., 2019). A similar pattern was determined by Marta & Tensiska (2017) and Kim, Oh, & Chung (2017) for heat-moisture treated sweet potato starch and brown rice flour, respectively. In contrast, Garcia Solaesa et al. (2019) reported a similar pasting profile for defatted quinoa grits treated by SC-$CO_2$ extraction and native quinoa grits. The extraction of quinoa at a relatively lower temperature (40 $^{o}$ C) without the addition of a co-solvent may account for differences we observed in our study.

An increase in the breakdown was determined for deflavored pea flours compared to native flours. In general, pea starch exhibits a much lower breakdown tendency due to its high amylose content, providing higher resistant starch and also its high resistance to collapse owing to less shear-thinning behavior (Kaiser et al., 2019; Simsek, Tulbek, Yao, & Schatz, 2009; Tulbek et al., 2017). SC-$CO_2$+EtOH extraction may cause the structural changes in pea starch, such as a reduction in amylose content and resistant starch, which are less soluble and resist shear thinning (Delcour & Hoseney, 2010). The reduction in resistant starch by SC-$CO_2$+EtOH extraction (Table 4.1) lends partial support to the observed viscosity breakdown. Furthermore, deflavored pea flours (except flours with fine fraction) exhibited significantly higher pasting temperatures, which indicate higher energy input required for cooking and improved stability against heat. Increased stability of starch granules, in the SC-$CO_2$+EtOH treated samples, against heat-induced changes might be associated with the transformation of B-type polymorph to A-type polymorph that is more resistant to heat due to tighter double helix arrangement (Marta & Tensiska, 2017).

Retrogradation tendency is associated with the final (cold paste) and setback viscosity. When hot paste is exposed to cooling, reassociation between starch chains (mainly amylose chains) through hydrogen bonding occurs, and thereby, a viscosity of the paste begins to increase corresponding to the formation of a gel (Marta & Tensiska, 2017). This process is referred to as setback, and increased viscosity during cooling is final viscosity. Deflavored pea flours had significantly lower ($p < 0.05$) setback (except for the coarse fraction) and final viscosity than native pea flour (Table 4.3). Reduction in these parameters indicates the tendency for reassociation between starch chains is weak, owing to slower hydrogen bonding due to possible starch modification induced by SC-$CO_2$+EtOH extraction. Also, decreased retrogradation tendency of deflavored pea flours might result from tightly adhered protein remains on the surface of starch granules created by the extraction (Fig. 4.3). The protein remains on the starch granule might inhibit reassociation of amylose and amylopectin chains during cooling and eventually lead to the formation of a weaker gel (Wang, Li, Copeland, Niu, & Wang, 2015).

Besides the effects of SC-$CO_2$+EtOH extraction on the pasting properties of pea flours, particle size significantly ($p < 0.05$) affected pasting properties among pea flours (Table 4.3). Notably, flours with fine fraction exhibited a high pasting curve compared to other pea flour samples owing to its higher total starch content compared to others (Table 4.1). Additionally, smaller particles have a greater surface area to which starch can be exposed to water during pasting, resulting in faster water absorption and swelling, subsequently greater amylose leaching and higher peak and final viscosity. Also, a negative correlation between pasting properties (e.g., peak, breakdown, and final viscosity) and particle size was reported for yellow pea flour by Kaiser et al. (2019) and Indian lentil flour by Ahmed, Taher, Mulla, Al-Hazza, & Luciano (2016).

### 4.4.5. Functional properties

Information about the changes in functional properties of pea flour, along with its pasting properties, is essential for a better understanding of its applications in food production. Therefore, WAI, WSI, and OAC of pea flours were determined (Table 4.3). The main effects and interaction effect between SC-$CO_2$+EtOH extraction and particle size were significant for the functional properties of pea flours (Table 4.4).

The WAI indirectly measures the volume occupied by flour components (e.g., starch, protein) after swelling in excess water (Kaur, Sandhu, & Singh, 2007; Sharma, Singh, Hussain, & Sharma, 2017). WAI for native and deflavored pea flours ranged between 2.26-3.04 g/g and 2.53-3.46 g/g, respectively. The WSI, associating with the presence of soluble biomolecules (e.g., starch, fibers, sugar, proteins) in excess water (Sharma et al., 2017), for native and deflavored pea flours ranged between 15.35-22.56% and 10.67-14.73%, respectively. Kaur et al. (2007) reported relatively higher WAI (4.84-5.01 g/g) than this study. Nevertheless, their findings for WSI (19.8-20.06%) were similar to this study. The inconsistency for WAI with the literature data might be related to the procedure and also other factors (e.g., milling type, particle size, defatting process, and pea variety). Furthermore, Kaur & Sing (2005) and Simons (2013) reported similar trends for different chickpea flours (WAI: 2.39-2.66 g/g; WSI: 20.42-22.89%) and whole pinto bean flour and its high starch fraction (WAI: 2.92 and 2.69 g/g; WSI: 24.66 and 14.10%), respectively.

SC-$CO_2$+EtOH extraction significantly increased WAI and notably decreased WSI in pea flours ($p < 0.05$; Table 4.3). Possible modification of starch, protein, and fibers (e.g., increasing the availability of hydrophilic groups to bind more water) and also removal of lipids during the extraction may promote water absorption of biomolecules and thereby result in higher WAI. Similarly, Simons (2013) showed increased WAI after extrusion cooking owing to structural

changes of starch and other components. The decrease in WSI might result from the conversion of B-type to A-type crystallinity, causing lower water solubility with increased thermal stability (Crochet, Beauxis-Lagrave, Noel, Parker, & Ring, 2005) and further support the higher gelatinization temperature of processed flours (Table 4.3). Additionally, possible structural alterations of pea proteins during the extraction might be another reason for reducing WSI due to aggregation of unfolded proteins, which reduces protein solubility (Sashikala, Sreerama, Pratape, & Narasimha, 2015).

Particle size had a significant impact on WAI and WSI. Among flours, coarse fraction had the lowest WAI, whereas medium fraction exhibited the highest WAI owing to its lower starch damage content. Sharma et al. (2017) reported that less damaged polymer chains might provide greater availability of hydrophilic groups, which might bind more water molecules, subsequently resulted in a higher value of the WAI. Flours with coarse fraction had significantly lower starch damage than flours with medium fraction. However, it's starch, and protein contents were also lower than others, and thereby, it might exhibit the lowest WAI. Thus, other components of flour, particularly protein, might have an impact on WAI since medium fraction had the highest level of protein. WSI was significantly lower in flours with coarse fraction than other flours. Its lower starch and protein content might be reasons for less soluble components. Also, decreased surface area with larger particles may provide less exposure of components to water, thus decreasing its solubility due to reducing the site accessibility for chemical reactions (Angelidis, Protonotariou, Mandala, & Rosell, 2016). Overall increased WAI and decreased WSI for deflavored pea flours indicate that these flours might be suitable in food applications requiring high viscosity (Bryant, Kadan, Champagne, Vinyard, & Boykin, 2001). Additionally, particle size had a profound impact on WAI and WSI.

Table 4.3. Pasting and functional properties of pea flour samples [A]

| Flour Property | Non- deflavored | | | | Deflavored | | | |
|---|---|---|---|---|---|---|---|---|
| | Whole | >250 [B] | >150 | >106 | Whole | >250 | >150 | >106 |
| Pasting properties | | | | | | | | |
| Peak Viscosity (cP) | 1544 ± 26.52$^{c}$ | 689 ± 17.01$^{e}$ | 1745 ± 12.72$^{b}$ | 2120 ± 55.62$^{a}$ | 582 ± 40.50$^{f}$ | 253 ± 30.41$^{g}$ | 929 ± 32.61$^{d}$ | 1602 ± 35.81$^{c}$ |
| Through (cP) | 1458 ± 18.51$^{c}$ | 664 ± 20.60$^{e}$ | 1686 ± 15.23$^{b}$ | 1854 ± 6.62$^{a}$ | 449 ± 47.70$^{f}$ | 199 ± 31.22$^{g}$ | 460 ± 36.50$^{f}$ | 1295 ± 5.6$^{d}$ |
| Breakdown (cP) | 87 ± 8.12$^{cd}$ | 25 ± 4.40$^{d}$ | 59 ± 2.54$^{cd}$ | 266 ± 49.72$^{b}$ | 133 ± 23.17$^{c}$ | 54 ± 27.82$^{cd}$ | 469 ± 68.69$^{a}$ | 307 ± 30.91$^{b}$ |
| Final Viscosity (cP) | 2561 ± 32.81$^{c}$ | 1020 ± 16.31$^{e}$ | 3178 ± 36.92$^{b}$ | 4383 ± 34.71$^{a}$ | 728 ± 44.93$^{f}$ | 501 ± 34.16$^{g}$ | 573 ± 35.50$^{g}$ | 1981 ± 12.50$^{d}$ |
| Setback (cP) | 1103 ± 17.42$^{c}$ | 356 ± 16.62$^{e}$ | 1476 ± 33.44$^{b}$ | 2529 ± 43.51$^{a}$ | 286 ± 33.52$^{e}$ | 303 ± 35.67$^{e}$ | 109 ± 12.18$^{f}$ | 686 ± 11.80$^{d}$ |
| Peak Time (min) | 5.2 ± 0.15$^{b}$ | 7.0 ± 0.12$^{a}$ | 5.2 ± 0.10$^{b}$ | 5.2 ± 0.10$^{b}$ | 5.6 ± 0.31$^{b}$ | 6.5 ± 0.40$^{a}$ | 6.5 ± 0.20$^{a}$ | 4.6 ± 0.10$^{c}$ |
| Pasting Temp. ($^{o}$C) | 75.5 ± 0.54$^{b}$ | 75.3 ± 0.63$^{b}$ | 75.1 ± 0.10$^{b}$ | 75.0 ± 0.10$^{b}$ | 79.5 ± 0.60$^{a}$ | 79.5 ± 0.40$^{a}$ | 79.0 ± 0.10$^{a}$ | 73.8 ± 0.55$^{c}$ |
| Functional properties | | | | | | | | |
| WAI (g/g) [C] | 2.26 ± 0.01$^{e}$ | 2.73 ± 0.17$^{cd}$ | 3.04 ± 0.14b$^{c}$ | 2.24 ± 0.09$^{e}$ | 2.87 ± 0.09$^{cd}$ | 3.46 ± 0.08$^{a}$ | 3.25 ± 0.05$^{ab}$ | 2.53 ± 0.25$^{de}$ |
| WSI (%) [D] | 21.10 ± 0.06$^{a}$ | 15.35 ± 0.22$^{b}$ | 22.56 ± 1.27$^{a}$ | 22.44 ± 1.29$^{a}$ | 14.02 ± 0.99$^{b}$ | 10.67 ± 0.70$^{c}$ | 14.73 ± 0.58$^{b}$ | 13.97 ± 0.66$^{b}$ |
| OAC (g/g) [E] | 0.83 ± 0.04$^{b}$ | 1.10 ± 0.03$^{a}$ | 0.89 ± 0.02$^{b}$ | 1.08 ± 0.03$^{a}$ | 0.89 ± 0.02$^{b}$ | 1.06 ± 0.02$^{a}$ | 0.79 ± 0.01$^{bc}$ | 1.06 ± 0.02$^{a}$ |

[A] Values of mean ± standard deviations (n=3) with same letter are not significantly different at $\alpha = 0.05$.
[B] Coarse/large, >250µm (425 ≥ flour >250); medium, >150µm (250 ≥ flour >150); and fine/small, >106µm (150 ≥ flour >106) and whole is unsieved pea flour.
[C]WAI: Water Absorption Index, [D]WSI: Water Solubility Index, [E]OAC: Oil Absorption Capacity

Table 4.4. F- and p-values of main (extraction and particle size) and interaction effects for pasting and functional properties of pea flours [A]

| Property | Extraction | | Particle Size | | Extraction*Particle Size | |
|---|---|---|---|---|---|---|
| | F-value | *p*-value | F-value | *p*-value | F-value | *p*-value |
| Pasting properties | | | | | | |
| Peak Viscosity (cP) | 2445.41 | <.0001 | 1754.36 | <.0001 | 80.51 | <.0001 |
| Through (cP) | 5636.7 | <.0001 | 1870.94 | <.0001 | 279.08 | <.0001 |
| Breakdown (cP) | 87.15 | <.0001 | 71.97 | <.0001 | 43.29 | <.0001 |
| Final Viscosity (cP) | 8463.04 | <.0001 | 2503.74 | <.0001 | 551.49 | <.0001 |
| Setback (cP) | 3110.06 | <.0001 | 869.05 | <.0001 | 441.44 | <.0001 |
| Peak Time (min) | 2.83 | 0.1117[ns] | 85.58 | <.0001 | 27.81 | <.0001 |
| Pasting Temp. ($^{o}$C) | 273.17 | <.0001 | 78.18 | <.0001 | 64.18 | <.0001 |
| Functional properties | | | | | | |
| WAI (g/g) [B] | 72.32 | <.0001 | 49.47 | <.0001 | 5.47 | 0.0088 |
| WSI (%) [C] | 421.81 | <.0001 | 57.96 | <.0001 | 5.88 | 0.0066 |
| OAC (g/g) [D] | 17.14 | 0.0008 | 189.9 | <.0001 | 9.47 | 0.0008 |

[A] ns: non-significant at $\alpha = 0.05$ and df =1, 3, 3 for extraction, particle size, and interaction, respectively with N=24.
[B] WAI: Water Absorption Index, [C] WSI: Water Solubility Index, [D] OAC: Oil Absorption Capacity.

The OAC is associated with the surface availability of hydrophobic amino acids and non-polar chains of carbohydrates (e.g., dietary fibers) found in flour. OAC of native and deflavored pea flours was in the range of 0.83-1.10 g/g and 0.89-1.06 g/g and was agreement with previous reports (Ettoumi & Chibane, 2015; Maskus et al., 2016). SC-$CO_2$+EtOH extraction did not change OAC for pea flours, but flours with fine and coarse fractions exhibited significantly higher OAC ($p < 0.05$; Table 4.3). Higher OAC of flours with fine fraction might be related to its increased surface area and accessibility of the oil to hydrophobic sites in the flour. In contrast, higher OAC in the flours with coarse fraction might be associated with its higher hull content, which can increase fiber content and improve oil binding capacity (Martens, Nilsen, & Provan, 2017).

### 4.4.6. Moisture sorption properties

Moisture adsorption of pea flours exhibited a sigmoidal shaped isotherm, while their moisture desorption isotherms displayed a more straight-line relationship (Fig. 4.2). Moisture sorption isotherms of pea flours followed a typical type II behavior, which is widely seen for cereal-based foods. This behavior is associated with the occurrence of sorption multilayers, where small pores are saturated at lower water activities; conversely, large pores are saturated at higher water activities (Syamaladevi et al., 2016). Similar findings for moisture sorption isotherms of wheat, pulse, and yam flours have been previously reported (Nurtama & Jin, 2010; Syamaladevi et al., 2016; Xu et al., 2019). SC-$CO_2$+EtOH extraction reduced water adsorption and desorption capacity of all pea flours (Fig. 4.2), whereas particle size did not change sorption isotherms among pea flours. Particularly, flours with medium and fine fractions displayed almost the same sorption curves (Fig. 4.2).

The reduction in sorption isotherms of deflavored pea flours caused an increase in water activity owing to weaker bindings between water and hydrophilic sites of protein, starch, and other components of pea flour via hydrogen bonding. Relatively high operating temperature (86 $^{o}$C) of SC-$CO_2$+EtOH extraction might induce the structural changes in the biomolecules of flour, which may decrease water binding sites of biomolecules. Thus, the bound water level in the flour may reduce and may cause more available water in the flour. Syamaladevi et al. (2016) stated that wheat flour treated at 80 $^{o}$C exhibited lower sorption isotherms along with increased water activity at the same water content compared to wheat flour treated at lower temperatures due to weaker interaction with water. Furthermore, a similar finding was reported for yam flour (Nurtama & Jin, 2010).

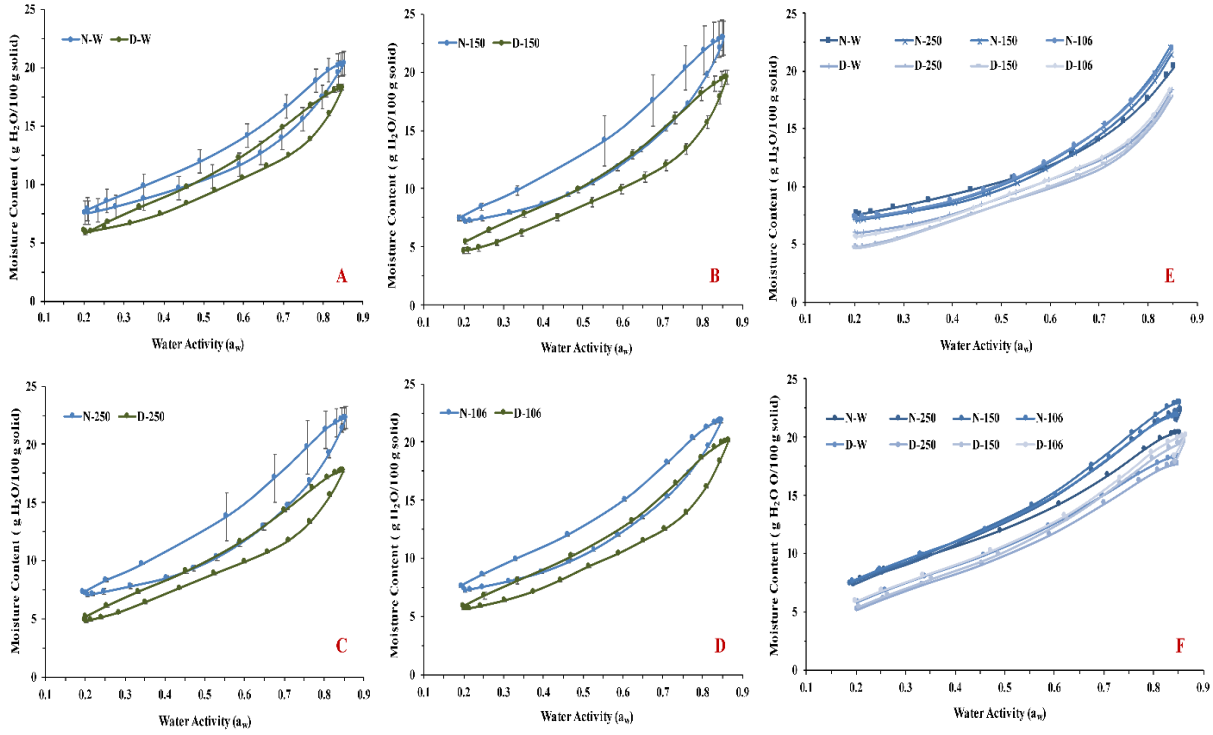


Figure 4.2. The effect of SC-$CO_2$+EtOH extraction and particle size on moisture sorption isotherms of pea flours. Letters represent the following: W: Whole (unsieved), N: Non-deflavored, and D: Deflavored flour, graphs of A-D: full sorption isotherms, E: Adsorption, and F: Desorption isotherm for all samples. Curves were plotted using the mean value of two replications (for all graphs) with standard deviation (for A-D).

The experimental full sorption data of pea flour samples determined was fitted using means of DLP and GAP models. GAP model displayed a higher error of prediction (EP) and a lower coefficient of determination ($R^2$) values than DLP (Table 4.5). Thus, the DLP model was found more appropriate for the moisture sorption isotherms of pea flour samples. Predicted moisture contents of deflavored pea flour were relatively lower than non-deflavored pea flours at four values of water activities (Table 4.5). The basis for relates to SC-$CO_2$+EtOH extraction causing a reduction in water binding sites of flour components to water molecules by reflecting physical and chemical modifications of biomolecules during extraction. Nurtama and Jin (2010) showed a decrease in the predictive moisture content of yam flour at a given water activity as the increasing temperature in adsorption and desorption isotherms of yam flour.

Table 4.5. Full sorption isotherm model parameters and predicted moisture content values of pea flour samples at 25 °C.

| Pea flour* | Double Log Polynomial (DLP) Model | | | | | | Guggenheim Anderson-de Boer (GAB) Model | | | | | $m$ predicted # | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $b_0$ | $b_1$ | $b_2$ | $b_3$ | $R^2$ | EP& | $c$ | $k$ | $m_o$ | $R^2$ | EP | $a_w$=0.2 | $a_w$=0.6 | $a_w$=0.8 | $a_w$=0.85 |
| **Adsorption** | | | | | | | | | | | | $m$ adsorption (db%) | | | |
| N-W | 8.762 | -3.454 | 1.346 | -0.076 | 1.000 | 0.050 | 11073.560 | 0.809 | 6.142 | 0.998 | 0.195 | 7.41 | 11.71 | 17.23 | 19.94 |
| D-W | 7.467 | -3.740 | 0.515 | -0.379 | 0.998 | 0.189 | 111.040 | 0.841 | 5.156 | 0.996 | 0.256 | 5.76 | 10.33 | 15.51 | 18.23 |
| N-250 | 8.654 | -3.405 | 1.927 | 0.152 | 1.000 | 0.057 | 12925.733 | 0.818 | 6.051 | 0.998 | 0.175 | 7.49 | 11.76 | 17.58 | 20.29 |
| D-250 | 6.791 | -4.285 | -0.404 | -0.758 | 0.999 | 0.146 | 19.677 | 0.846 | 4.994 | 0.996 | 0.276 | 4.58 | 9.72 | 14.86 | 17.79 |
| N-150 | 8.374 | -3.852 | 2.655 | 0.282 | 0.999 | 0.054 | 13034.538 | 0.876 | 5.744 | 1.000 | 0.113 | 7.17 | 12.07 | 19.17 | 22.45 |
| D-150 | 6.719 | -4.538 | -0.376 | -0.758 | 0.999 | 0.163 | 17.225 | 0.866 | 4.918 | 0.996 | 0.313 | 4.39 | 9.83 | 15.24 | 18.27 |
| N-106 | 8.517 | -3.979 | 2.556 | 0.372 | 0.999 | 0.055 | 13354.389 | 0.861 | 5.898 | 0.999 | 0.097 | 7.24 | 12.23 | 18.98 | 21.95 |
| D-106 | 7.343 | -4.050 | 0.359 | -0.488 | 0.999 | 0.186 | 57.411 | 0.862 | 5.056 | 0.997 | 0.286 | 5.44 | 10.37 | 15.87 | 18.81 |
| **Desorption** | | | | | | | | | | | | $m$ desorption (db%) | | | |
| N-W | 9.281 | -5.502 | 2.314 | 0.891 | 0.999 | 0.183 | 12.923 | 0.746 | 8.319 | 0.999 | 0.232 | 7.28 | 13.75 | 19.73 | 21.57 |
| D-W | 8.334 | -5.701 | 1.850 | 1.059 | 1.000 | 0.096 | 6.827 | 0.630 | 9.806 | 0.998 | 0.228 | 6.15 | 12.68 | 17.47 | 18.45 |
| N-250 | 9.890 | -5.721 | 3.525 | 1.614 | 1.000 | 0.129 | 8.624 | 0.672 | 10.477 | 0.996 | 0.346 | 8.14 | 14.83 | 20.95 | 22.24 |
| D-250 | 7.650 | -5.430 | 2.230 | 1.170 | 1.000 | 0.095 | 5.502 | 0.637 | 9.618 | 0.998 | 0.225 | 5.70 | 11.95 | 16.86 | 17.86 |
| N-150 | 10.065 | -5.784 | 3.828 | 1.712 | 0.999 | 0.152 | 9.385 | 0.691 | 10.307 | 0.996 | 0.379 | 8.36 | 15.16 | 21.58 | 22.95 |
| D-150 | 7.832 | -5.749 | 2.490 | 1.183 | 1.000 | 0.115 | 5.296 | 0.661 | 9.780 | 0.998 | 0.277 | 5.79 | 12.46 | 18.07 | 19.40 |
| N-106 | 10.293 | -5.843 | 3.113 | 1.528 | 0.999 | 0.131 | 10.033 | 0.662 | 10.588 | 0.997 | 0.314 | 8.38 | 15.16 | 20.91 | 22.02 |
| D-106 | 8.360 | -5.455 | 2.741 | 1.229 | 1.000 | 0.132 | 6.679 | 0.676 | 9.446 | 0.997 | 0.287 | 6.52 | 12.89 | 18.56 | 19.95 |
| **Hysteresis (Adsorption + Desorption)** | | | | | | | | | | | | $m$ (adsorption + desorption) (db%) | | | |
| N-W | 9.391 | -4.358 | 1.557 | 0.321 | 0.972 | 0.795 | 90.112 | 0.776 | 7.005 | 0.973 | 0.791 | 7.70 | 12.92 | 18.35 | 20.52 |
| D-W | 7.749 | -4.515 | 1.102 | 0.157 | 0.972 | 0.794 | 20.981 | 0.797 | 6.143 | 0.972 | 0.793 | 5.87 | 11.23 | 16.47 | 18.65 |
| N-250 | 8.504 | -4.387 | 3.177 | 0.785 | 0.969 | 1.077 | 50.966 | 0.843 | 6.365 | 0.969 | 1.089 | 7.22 | 12.65 | 19.58 | 22.25 |
| D-250 | 7.072 | -4.837 | 0.545 | -0.084 | 0.974 | 0.792 | 11.560 | 0.802 | 5.988 | 0.974 | 0.791 | 4.88 | 10.59 | 15.84 | 18.16 |
| N-150 | 8.834 | -4.654 | 3.358 | 0.906 | 0.972 | 1.022 | 46.939 | 0.841 | 6.662 | 0.971 | 1.041 | 7.48 | 13.20 | 20.31 | 22.94 |
| D-150 | 7.063 | -5.059 | 0.753 | -0.039 | 0.971 | 0.965 | 10.581 | 0.819 | 6.002 | 0.971 | 0.965 | 4.82 | 10.81 | 16.47 | 18.97 |
| N-106 | 9.062 | -4.767 | 2.922 | 0.846 | 0.971 | 0.958 | 40.400 | 0.819 | 6.922 | 0.971 | 0.971 | 7.55 | 13.33 | 19.93 | 22.30 |
| D-106 | 7.615 | -4.608 | 1.400 | 0.182 | 0.973 | 0.914 | 20.006 | 0.825 | 5.968 | 0.973 | 0.913 | 5.76 | 11.29 | 17.06 | 19.52 |

# $m$ predicted: Predicted moisture content using DLP model, & EP: Error of Prediction; * N (Non-deflavored); D (Deflavored); W (whole); 106, 150, 250 (Fractions associated particles)

### 4.4.7. Scanning electron microscopy (SEM)

A mixture of pea starch and protein-rich particles were observed in all micrographs of pea flours (Fig. 4.3). Protein-rich particles adhered to the surface of starch granules. Starch granules were spherical to oval in shape with different sizes (e.g., 10 to 20 μm), as previously reported (Rempel et al., 2019). However, more tightly adhered protein remains on the surface of starch granules were observed in deflavored pea flour exhibiting a tighter protein matrix, where starch granules are embedded (Fig. 4.3). This might be due to operating conditions (e.g., pressure, temperature) of the SC-CO2+EtOH extraction through disruption of the starch-protein matrix and creating an adhesion (e.g., via hydrogen bonding) characteristic of protein, which resulted in more protein adhering to the starch granules in deflavored pea flours (Fig. 4.3). Flours with fine fractions, before and after extraction, had damaged starch granules, but extraction decreased the level of starch damage and did not disrupt the integrity of pea starch granules (Fig. 4.3).

Particle size showed certain differences among micrographs of pea flours. Flours with coarse fractions had relatively less starch granules along with lower protein-rich fractions compared to flours with medium and fine fractions (Fig. 4.3). Coarse fraction had more non-starch carbohydrate structures such as hull. Fine fraction indicated more starch granules, containing small protein particles on the surface. Whereas starch granules were mostly embedded in the protein matrix in the medium fraction and caused a more difficult separation of starch granules from the protein matrix.

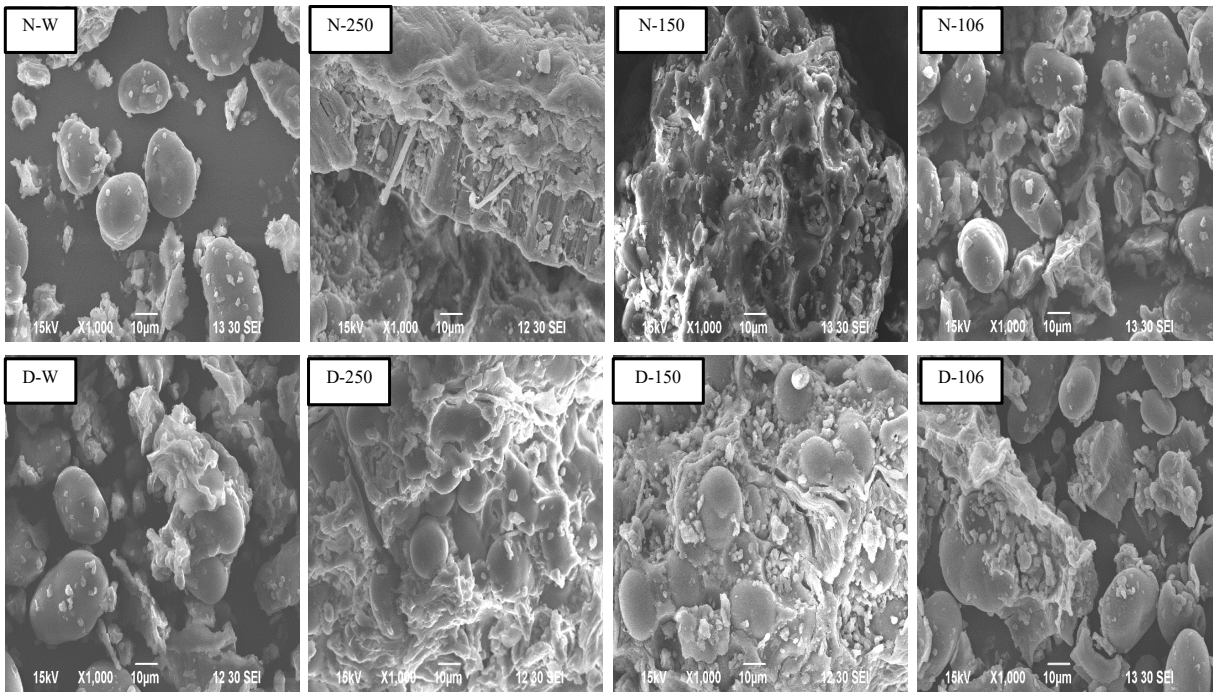


Figure 4.3. SEM micrographs of pea flour samples. Letters represent the following: N-W: Non-deflavored whole (unsieved); D-W: deflavored whole (unsieved); N-250: Non-deflavored coarse; D-250: deflavored coarse; N-150: Non-deflavored medium; D-150: Deflavored medium; N-106: Non-deflavored fine; D-106: deflavored fine pea flour.

## 4.5. Conclusion

Findings of the present study indicate that the SC-$CO_2$+ethanol extraction technology influenced whole pea flour quality. This technology was a significant method for releasing fats and carotenoids of pea flour in addition to improving its flavor. This technology might cause starch and protein modification and eventually, changed functional, pasting, and moisture sorption properties of pea flour. Particle size had a significant effect on physicochemical, functional, and pasting properties, and particle size distribution of pea flour. Pasting and functional properties were affected most by both SC-$CO_2$+ethanol extraction and particle size and overall, interaction effects existed in both factors for most of response variables.

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# CHAPTER 5. IMPROVEMENT OF ORGANOLEPTIC PROPERTIES OF PEA FLOUR THROUGH FLAVOR MODIFICATION

## 5.1. Abstract

A green emerging technology, supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction, was employed as a deflavoring method to improve sensory properties of pea flours. Furthermore, the impacts of particle size along with extraction on volatile profile and sensory attributes of pea flours were investigated using multiple approaches. These included, headspace solid-phase microextraction-gas chromatography (HS-SPME-GC), GC-olfactometry (GC-O), and quantitative descriptive analysis (QDA) using a trained sensory panel. Total volatile contents of non-deflavored and deflavored whole pea flour and its fractions were in the range of 7.1 to 18.1 μg/g and 0.4 to 2.7 μg/g, respectively. The GC-O system showed the total volatile intensity was in the range of 14.5 to 22.0 and 0 to 3.5, for non-deflavored and deflavored pea flours, respectively. Analytical methods indicated that 1-hexanol, 1-octanol, 1-nonanol, nonanal, and 2-alkyl methoxypyrazines were major off-aroma compounds associated with green, mushroom, earthy, and pea aroma in non-deflavored pea flours. After SC-$CO_2$ extraction, most off-aroma compounds were not detected in treated pea flours. Also, the sensory evaluation revealed less pea intensity and bitterness and higher acceptability of deflavored pea flours. Particle size had a profound impact on sensory attributes of pea flours. Increasing particle size of non-deflavored and deflavored pea flours resulted in less off-aroma compounds based on the GC data. However, these findings did not coincide with the sensory results. SC-$CO_2$+EtOH extraction at optimum conditions can be a potential technology to improve organoleptic properties of pulse ingredients. Additionally, particle size can be an approach to improving the sensory quality of pulse flour.

## 5.2. Introduction

As global awareness for healthy lifestyles increases, there has been an increasing demand for healthier (e.g., high fiber), nutritious plant-based (e.g., high protein and fiber), and gluten-free foods. The rapid growth in human population, expecting to reach 9.5 billion by 2050, combined with increased disposable income are drivers for growing consumer demands for nutrient-dense foods (Alves & Tavares, 2019; Nadathur, Wanasundara, & Scanlin, 2017; Pojic, Misan, & Tiwari, 2018). Addressing these demands have created challenges for food scientists to reformulate food products with appropriate sustainable food ingredients, have an outstanding nutritional profile and have acceptable sensory quality (Malcolmson, Frohlich, Boux, Bellido, Boye, & Warkentin, 2014).

Flour prepared from pea (*Pisum sativum* L.) is an attractive gluten-free and non-GMO food ingredient that has an outstanding nutritional profile (e.g., high protein, good complex carbohydrates, high folate and micronutrient contents) and potential health benefits (Hall, Hillen, & Garden-Robinson, 2017; Maskus, Bourre, Fraser, Sarkar, & Malcolmson, 2016). However, pea flour, like other pulse flours, has been underutilized due to its unacceptable flavor or off-flavor usually described as “beany,” “pea,” “earthy,” “green,” and “bitter” (Murat, Bard, Dhalleine, & Cayot, 2013; Nosworthy, Tulbek, & House, 2017; Vatansever & Hall, 2020). Thereby, this off-flavor limits the potential utilization of pea ingredients in the food system and mitigates their market value (Malcolmson et al., 2014; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017).

Off-flavor of dry peas can be either present naturally or developed during harvesting, processing, and storage (Azarnia, Boye, Warkentin, & Malcolmson, 2011; Murray, Shipton, Whitfield, & Last, 1976; Sessa & Rackis, 1977). Pea off-flavor is the combination of off-aroma compounds, i.e., volatile organic compounds (VOCs), imparting strong pea aroma, and off-taste

compounds, and non-VOCs that cause, for example, bitterness. Different VOCs identified in dry peas can develop through lipid oxidation and amino acid degradation pathways (Roland et al., 2017; Xu, Jin, Lan, Rao, & Chen, 2019). The degradation of pea lipids and unsaturated fatty acids (e.g., linoleic acid), through enzymatic (i.e., hydrolytic and oxidative processes) and non-enzymatic (i.e., autoxidation) reactions generates significant amount of alcohols, aldehydes, ketones, and furans, causing off-flavor in field peas (Azarnia et al., 2011; Murray et al. 1976; Sessa et al., 1977). Lipase hydrolyzes lipids to free fatty acids, which are then oxidized by lipoxygenase (LOX) and autoxidation pathways (Azarnia et al., 2011). The secondary products of lipid oxidation possess distinct undesirable aroma. Alcohols, such as 1-hexanol, contribute a green aroma while grassy and citrus odor are caused by the aldehydes hexanal and nonanal, respectively (Murat et al., 2013, Vatansever & Hall, 2020). Alkyl methoxypyrazines, 3-*sec*-butyl-2-methoxypyrazine and 3-isobuthyl-2-methoxypyrazine, are produced from amino acids in the seed (Murray, Shipton, & Whitfield, 1970). These methoxypyrazines are important contributors of "perceived green pea aroma" with extremely low olfactory thresholds (Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Heng 2005; Murray et al. 1970; 1976). The bitterness of peas is associated with non-VOCs, saponins, which contribute to off-taste development (Heng et al., 2006).

The renewed interest in supercritical carbon dioxide (SC-$CO_2$) extraction is based on desire by the food industry to use sustainable or green technology for extraction of natural substances (e.g., flavor and fragrance extracts, natural antioxidants, lipids, and VOCs) from plant materials (Shao, Huang, Zhou, Guo, Zhang, & Wang, 2014; Vatansever & Hall, 2020). The uniqueness of SC-$CO_2$ extraction is the speed at which physical parameters (e.g., pressure, temperature, co-solvent) and polarity of the extractant can be adjusted. This allows for separation of moderately polar (e.g., aldehydes, ketones, and esters) and non-polar compounds (e.g., alkenes and terpenes)

in short time with less energy requirement. The temperature of critical stage of $CO_2$ is relatively low, which results in minimal thermal damage of plant materials. Furthermore, this extraction system can be assisted with a polar co-solvent (e.g., ethanol, methanol) to enhance the solubility of SC-$CO_2$ for extraction of polar organic compounds (e.g., alcohols, saponins, xanthophylls, phenolics). Ethanol (EtOH) has been favored as a co-solvent to separate polar compounds (Cobb, Kallenbach, Hall, & Pryor, 2018; Vatansever & Hall, 2020).

Optimized SC-$CO_2$+EtOH extraction was successfully employed to improve organoleptic attributes of yellow pea flour through the removal of off-flavor compounds (Vatansever & Hall, 2020). Flavor modification of pea flour using SC-$CO_2$+EtOH extraction at optimum conditions was conducted in less time and using less ethanol (Vatansever & Hall, 2020) compared to other conventional solvent-based deflavoring methods for pea ingredients (Chang, Stone, Green, & Nickerson, 2019; Wang, Guldiken, Tulbek, House, & Nickerson, 2020). Furthermore, the efficiency of this extraction for flavor modification might be more promising than bio-processing approaches, such as fermentation (Schindler, Zelena, Krings, Bez, Eisner, & Berger, 2012) and germination (Xu, Jin, La, Rao, & Chen, 2019).

Significant effects of particle size on the physicochemical and functional properties of pea flour have been previously reported (Kaiser, Barber, Manthey, & Hall, 2019). However, impacts of particle size on the volatile profile and sensory properties of pea flours have not been examined. Therefore, the objective of the present study was to assess the applicability of SC-$CO_2$+EtOH extraction for deflavoring pea flour with different particle sizes and to determine the interaction effect between the two factors (i.e., SC-$CO_2$+EtOH and particles size) on the volatile profile and sensory quality of yellow pea flours. For this purpose, instrumental analyses, headspace-solid phase microextraction-gas chromatography (HS-SPME-GC), and GC-Olfactory (GC-O) detection

along with the quantitative descriptive analysis (QDA) sensory evaluation technique were applied to determine changes in selected off-aroma compounds and sensory attributes in pea flours.

## 5.3. Materials and Methods

### 5.3.1. Materials

Viterra (Minot, ND), Specialty Commodities (Fargo, ND), and SK Foods (Moorhead, MN) were-the source of whole yellow pea used in this study. The samples were manually blended prior to being hammer milled (Fitzpatrick, Elmhurst, IL) using a 1.270 mm screen and hammer rotation of 102 m/s (7200 rpm). Milled whole pea flour was stored in sealed polyethylene bags at -20 ºC until required for deflavoring. Information on the carbon dioxide used for extraction, VOCs used to make standard curves and sensory supplies can be found in a previous publication (Vatansever & Hall, 2020).

Standard curve was prepared using selected VOCs, including hexanal, nonanal, 1-pentanol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-heptanol, 1-octanol, 1-octen-3-ol, 1-nonanol, 2-pentylfuran, γ-valerolactone, γ-caprolactone, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine (Sigma-Aldrich, St. Louis, MO, USA) and kept at -20 ºC until use. Cracker and corn starch, sensory training supplies, were purchased from local food distribution centers, and caffeine powder was ordered from Sigma-Aldrich, St. Louis, MO, USA.

### 5.3.2. Particle size determination and SC-$CO_2$+EtOH extraction

Particle size separation of yellow pea flour obtained from hammer milling was performed using a Rotap (W.S. Tyler, Mentor, OH, USA) with a series of sieves having openings of 425 (40-mesh), 250 (60-mesh), 150 (100-mesh), 106 (140-mesh), and 53 (270-mesh) μm based on the approved method 55-60.01 (AACC International, 2010). The particles size obtained were classified as fractions consisting of several particles: 425 μm ≥ flour >250 μm (coarse/large), 250

μm ≥ flour >150 μm (medium), and 150 μm ≥ flour >106 μm (fine/small). Unsieved yellow pea flour (hereafter referred to as whole flour since all particle were present in this flour) was used for further analyses along with coarse, medium, and fine fractions.

The three fractions and whole yellow pea flour were subjected to SC-$CO_2$+EtOH extraction, separately, using the optimum deflavoring conditions (22% ethanol, 86 °C, and 42.70 MPa) described by Vatansever & Hall (2020) without modification. The deflavored pea flours were stored in 2.5 mil Mylar bags (Uline; Pleasant Prairie, WI, USA) at -20 ° C until needed.

**5.3.3. HS-SPME-GC analysis of selected volatile compounds**

Volatile detection of non-deflavored and deflavored pea flours (whole, coarse, medium, and fine) was measured using HS-SPME-GC (Agilent 7820A) with FID following the protocol described by Hall, Manthey, Lee, & Niehaus (2005) with some modification. Briefly, 1 g of pea flour was added to a 4-mL vial and sealed using PTFE silicone Septa (Supelco, Bellefonte, PA, U.S.A.). The sample was heated in a 95 °C water bath for 10 minutes. The SPME filament (DVB/CAR/PDMS, 50/30 μm; Supelco, 57328-U, Bellefonte, PA, U.S.A) was inserted for 15 minutes while the sample was heated at 90 °C. Then, the filament was transferred to the injection port of the GC and remained to desorb for 7 min. The volatile analysis was performed under the following conditions: helium flow rate of 33.7 mL/min, initial oven temperature of 35 °C, and ramped to 180 °C at 10 °C/min, then, maintained for 12 minutes at 180 °C.

Each VOC was identified by comparing the retention time of chosen standards and quantified (μg/g) using the standard curve. Then, the total volatile (TV) concentration in pea flour was obtained from the sum of VOCs (μg/g), which were selected based on previous studies (Azarnia et al., 2011; Heng, 2005; Hillen, 2016; Jakobsen et al., 1998; Murat et al., 2013). A standard curve was constructed in a solid matrix (i.e., finely ground fresh saltine cracker) through

dilution of the standards based on the protocol described by Vatansever & Hall (2020) without any modification. The $R^2$ values of chosen standards had a range of 0.974 to 0.999.

### 5.3.4. GC-O training

A specific GC-O training composed of vocabulary, reference mixture, and real sample training with five healthy, nonsmoking trainees (1 male and 4 females) were completed based on the protocols of Vene, Seisonen, Koppel, Leitner, & Paalme (2013) with some modifications. These trainees were informed before analysis to abstain from alcoholic drinks, spicy meals, and other strong flavorful foods. Additionally, the trainees did not have access to chromatogram results and did not communicate with one another during testing to produce reliable results (Xu et al., 2019).

The vocabulary training reported by Vene et al. (2013) took place using fourteen standard aroma compounds, which were used for the standard curve preparation (Vatansever & Hall, 2020). The stock solutions, which were 2.8 mg/ml for each standard except for γ-valerolactone, which was 3.5 mg/ml, were diluted with methanol to prepare 1 mg/ml and 0.5 mg/ml for each standard. Then, the samples were prepared using sniffing strips (1 cm) dipped into the solutions (i.e., 0.5 mg/ml, 1.0 mg/ml, and 2.8 mg/ml or 3.5 mg/ml for the fourteen compounds). After the removal of methanol residue, strips were placed into screw-cap tubes (20 ml). The training section was arranged as a group discussion. Trainees smelled the solution in the vials, including sniffing strips, to determine the experimental descriptor and also to assess the degree of intensity of each standard for three concentrations using a five-point scale, where 1: very weak, not identifiable; 2: weak, but identifiable; 3: moderate; 4: strong; and 5: highly strong. After vocabulary training, trainees continued sniffing the compounds for two weeks to memorize each VOC.

Seven randomly selected standards were used to make a reference mixture for training base on methods of Vene et al. (2013) and Xu et al. (2019) with some modifications. The reference mixture consisted of 0.1 ml of each of the seven randomly selected standards; which resulted in 0.7 mL total volume. Considering that the volatiles selected have different detection threshold, normalization of the reference mixture was done by selecting standard concentrations (0.14 to 0.5 mg/ml) based on the intensity rating by the trainees during vocabulary development. All standards used in the reference mixture were rated as moderate intensity (~3.0 to 3.5) by trainees. Overall, two reference mixtures were prepared using fourteen selected standards and used to train panelists on the GC-O protocol. The data produced by each trainee was evaluated based on detected peaks.

In the last training session, pea flour composed of the target and other compounds were used to train the trainees. The odor intensity of a VOC was determined using a posterior intensity method including a 4-point scale, where 1: weak, but detectable; 2: moderate; 3: strong; and 4: highly strong. Through this method, the intensity of the recognized compound was identified and compared with mass spectrum obtained from GC/MS to evaluate the results of each trainee. After conducting each training session, trainees were informed about their results and comments were provided (Xu et al., 2019).

**5.3.5. HS-SPME-GC/MS-Olfactory analysis**

Non-deflavored and deflavored pea flours were analyzed by GC-O. Three batches of each flour treatment (i.e., after SC-$CO_2$+EtOH extraction) were blended to create a homogeneous sample for this analysis (Murat et al., 2013). Briefly, blended pea flour (2 g) was placed in 20-mL vials and sealed with a screw cap with PTFE silicone Septa (Supelco, Bellefonte, PA, U.S.A.) and transferred to the Agilent Technologies 7890B GC system with a ZB-Wax column (60 m x 0.25 mm and 0.25 µm thickness) using the injection port in splitless mode (Xu et al., 2019). The analysis

was performed according to Hall et al. (2005) with some modifications. The sample was heated for 10 min at 93 °C. The SPME fiber (DVB/CAR/PDMS, 50/30 µm; Supelco, 57328-U, Bellefonte, PA, U.S.A) was placed in the vial for 15 minutes at 93 °C and then, inserted into the GC and remained for 5 minutes to desorb the volatiles. The HS-SPME-GC/MS-O analysis followed these conditions: helium flow rate of 2 mL/min, initial oven temperature of 35 °C ramped to 180 °C at 10 °C/min then, maintained for 12 minutes at 180 °C and increased to 200 °C at 9 °C/min and to 250 °C at 45 °C/ml then held for 3 min.

The MS-olfactory analysis was completed based on the protocol described by Xu et al. (2019) without modification. Briefly, the column effluent (1/3) was split to the 5977A mass detector and analyzed using the following conditions: electron impact (EI) ionization port at 70eV, ion source temperature at 230 °C, scan time segments from 4.00 to 17.89 min, and scanning from m/z 40 to 350.

The remaining column effluent (2/3) was split to the olfactory detection port (ODP3; Gerstel, Mulheim an der Ruhr, Germany). The conditions used for the olfactory analysis are as follows: heating transfer line to the ODP3 at 200 °C, humidifying air of the sniffing port at 30 ml/min, measuring the intensity using a specific remote-control button for quantifying intensity with the 4-point scale, and recording the experimental descriptor corresponding to each odor using the Gerstel ODP recorder program including an active microphone to record the data from each panelist when the odor was detected. At the same time, the corresponding peak area to odors perceived was obtained through the mass spectrum and experimental descriptors of each compound were recorded by each panelist. The peak intensity was measured as the mean of two repetitions for each panelist. Then, all mean intensity scores for each treatment were summed to obtain the total intensity.

### 5.3.6. Sensory assessment

Sensory evaluation of non-deflavored and deflavored pea flours was completed using QDA technique described by Vatansever & Hall (2020) without modification. The degree of pea intensity (PI), bitterness, and acceptability of flour samples was measured by eight trained panelists. Each flour sample was replicated three times.

### 5.3.7. Statistical analysis

A full factorial design including two factors, extraction (two levels) and particle size (four levels) for all analyses was used. The HS-SPME-GC and sensory analyses were performed with three replicates including five and eight observations within each replicate, while GC-O was completed with two replicates with five panelists. The two main factors were considered as fixed effects. The mean separation of the eight-treatment means was conducted using a Tukey's test at 5% significance level. A Tukey's test at 5% significance level was applied for mean separation. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed on the mean values VOCs determined by HS-SPME-GC and GC-O analyses and on the mean values of sensory attributes obtained by QDA. All statistical analyses were completed using the JMP software (JMP 14.0.0 Version 2018 SAS Institute Inc.).

## 5.4. Results and Discussion

### 5.4.1. Volatile compounds identified in pea flour using HS-SPME-GC analysis

SC-$CO_2$+EtOH extraction resulted in a significant reduction in total off-aroma compounds determined in pea flours. The TV concentrations (Table 5.1) of non-deflavored and deflavored pea flours composed of 6 alcohols, 1 aldehyde, 2 alkyl methoxypyrazines, and 1 furan (Fig. 5.1). The range of TV was from 7.1 to 18.1 µg/g and 0.4 to 2.7 µg/g for non-deflavored and deflavored pea flours, respectively (Table 5.1). Significant main and interaction effects ($p$ <0.05) among pea flours

were found for the total amount of volatile compounds (Table 5.2). The TV concentration in deflavored whole yellow pea flour was in agreement in the previous report (Vatansever & Hall, 2020). In addition to these VOCs, (Z)-3-hexen-1-ol, 2-pentylfuran, and γ-valerolactone were determined in non-deflavored pea flours but were not quantified due to their concentrations falling below the lower limit of quantification. Hexanal, a primary lipid oxidation product of linoleic acid catalyzed by LOX (Murat et al., 2013), was not detected in pea flours through the HS-SPME-GC system. Similarly, Murray et al. (1976) reported a low concentration of hexanal in peas where the hexanal: hexanol ratio was 1:200. The low hexanal concentration might be related to the strong aldehyde binding ability of proteins (Heng, 2005). High concentrations of hexanal has been reported in pea protein products (Heng, 2005; Murat et al., 2013; Wang et al., 2020) and lentil protein isolate (LPI) (Chang et al., 2019). In contrast, hexanal concentration was relatively low in pea flour (Murat et al., 2013). The high hexanal concentrations in protein concentrates and isolates likely relate to the aldehyde bind potential of protein and the inability of the pretreatment (e.g., heating) step of the analytical method to facilitate the release of hexanal. Additionally, hexanal can be reduced to 1-hexanol (e.g., green, hay-like aroma) in the presence of alcohol oxidoreductase (Jakobsen et al., 1998; Murray et al., 1976). In whole pea flour, 1-hexanol was quantified as one of the most abundant alcohols after 1-pentanol and 1-nonanol. Likewise, Murat et al. (2013) and Wang et al. (2020) obtained 1-hexanol as a major alcohol in pea flour and protein-enriched pea flour (PPEF), respectively.

Table 5.1. Sensory parameters, total volatile, and total volatile intensity of pea flours.

| Treatment | | Acceptability | Pea intensity | Bitterness | HS-SPME-GC (TV)[A] | GC-O (TVI)[B] |
|---|---|---|---|---|---|---|
| | | | mm | | μg/g | degree of intensity |
| Non-deflavored | Whole [C] | 32.8±5.77$^{b}$ | 112.3±4.69$^{a}$ | 53.4±4.25$^{ab}$ | 18.1±0.95$^{a}$ | 19.0±1.47$^{a}$ |
| | ≥250 | 24.9±6.93$^{b}$ | 106.1±6.38$^{ab}$ | 65.5±4.88$^{a}$ | 7.7±0.18$^{c}$ | 14.5±1.82$^{b}$ |
| | ≥150 | 48.3±8.04$^{b}$ | 87.9±3.64$^{b}$ | 38.5±4.75$^{bc}$ | 7.1±0.28$^{c}$ | 18.0±1.71$^{ab}$ |
| | ≥106 | 54.3±6.00$^{b}$ | 63.0±5.44$^{c}$ | 26.7±2.94$^{c}$ | 10.3±0.25$^{b}$ | 22.0±1.19$^{a}$ |
| Deflavored | Whole | 119.7±5.51$^{a}$ | 18.3±3.54$^{d}$ | 4.5±1.73$^{d}$ | 1.4±0.20$^{de}$ | 2.0±0.10$^{c}$ |
| | ≥250 | 114.5±8.66$^{a}$ | 13.0±3.70$^{d}$ | 9.7±2.39$^{d}$ | 0.4±0.11$^{e}$ | 0.0±0.00$^{a}$ |
| | ≥150 | 109.7±8.69$^{a}$ | 12.0±3.54$^{d}$ | 8.1±2.81$^{d}$ | 0.8±0.10$^{e}$ | 2.0±0.90$^{c}$ |
| | ≥106 | 101.2±11.12$^{a}$ | 29.5±5.86$^{d}$ | 6.1±2.17$^{d}$ | 2.7±0.38$^{d}$ | 3.5±1.01$^{c}$ |

[A] TV: Total volatile in pea flour detected by HS-SPME-GC. [B] TVI: Total volatile intensity (degree of intensity) in pea flour detected by GC-O. [C] Whole is unsieved pea flour. Data points were given as mean ± standard deviation and different letters indicate significantly differences ($p < 0.05$).

Table 5.2. F- and p-values of main (extraction and particle size) and interaction effects for sensory parameters, total volatile, and total volatile intensity (olfactory) of pea flours [A]

| Response Variable | Extraction | | Particle Size | | Extraction*Particle Size | |
|---|---|---|---|---|---|---|
| | F-value | *p*-value | F-value | *p*-value | F-value | *p*-value |
| Acceptability | 166.65 | <.0001 | 0.57 | 0.6367$^{ns}$ | 3.5 | 0.018 |
| Pea Intensity | 491.94 | <.0001 | 6.84 | 0.0003 | 17.94 | <.0001 |
| Bitterness | 257.24 | <.0001 | 13.59 | <.0001 | 11.17 | <.0001 |
| HS-SPME-GC (TV)[B] | 1109.05 | <.0001 | 91.45 | <.0001 | 72.19 | <.0001 |
| GC-O (TVI)[C] | 622.28 | <.0001 | 11.67 | <.0001 | 1.62 | 0.2112$^{ns}$ |

[A] ns: non-significant at $\alpha = 0.05$ and df =1, 3, 3 for extraction, particle size, and interaction, respectively.
[B] HS-SPME-GC (TV): Total Volatile by HS-SPME-GC [C] GC-O (TVI): Total Volatile Intensity by GC-O

Alcohols (e.g., 1-pentanol, 1-hexanol, 1-octanol, and 1-nonanol) were in the highest concentrations in non-deflavored pea flours, ranging from 4.9 to 13.9 μg/g. In contrast, after employing SC-$CO_2$+EtOH extraction, the aldehyde nonanal was the most predominant VOC in deflavored pea flours, ranging from 0.3 to 1.1 μg/g, compared to other VOCs (Fig. 5.1). Likewise, ethanol washing of PPEF (Wang et al., 2020) and LPI (Chang et al., 2019) showed removal of alcohols were greater than aldehydes (e.g., hexanal and nonanal) through ethanol-washing that employed 50% and greater ethanol concentrations. In SC-$CO_2$+EtOH extraction, the addition of

ethanol was sufficient to increase the polarity of the system, leading to the removal of alcohols through disruption of hydrogen bonds (Vatansever & Hall, 2020). Significant removal of 2-*sec*-butyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and γ-caprolactone of pea flours were obtained during SC-$CO_2$+EtOH extraction ($p < 0.05$, Fig. 5.1).

Particle size had a significant impact ($p < 0.05$) for the volatile profile of pea flours and also for the efficiency of SC-$CO_2$+EtOH extraction. Selected VOCs, namely 1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol, 1-nonanol, nonanal, 2-*sec*-butyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and γ-caprolactone, except for 1-pentanol were identified in all non-deflavored pea flours (Fig. 5.1). Previous reports showed similar trends with relatively higher alcohol concentrations compared to other VOCs (Heng, 2005; Murat et al., 2013). Among non-deflavored pea flours, whole pea flour had a significantly greater TV concentration than did the flours from the various fractions (Table 5.1). Particularly, 1-pentanol, 1-hexanol, 1-nonanol, and alkyl methoxypyrazines were relatively higher in whole pea flour. For non-deflavored flour fractions, the fine fraction had greater volatile concentrations than medium and coarse fractions, which may be due to the enhanced removal of VOCs from flour samples with high surface area, such as in the fine particles, during the analytical procedure.

Similarly, enhanced extraction of VOCs from finely ground coffee samples compared to coarse counterparts, having reduced contact area, was reported (Cordoba, Pataquiva, Osorio, Moreno Moreno, & Yolanda Ruiz, 2019). Several VOCs (e.g., 1-hexanol, 1-octanol, 1-nonanol, and 2-*sec*-butyl-3-methoxypyrazine) were predominant in pea flour of the fine fraction (Fig. 5.1). These compounds might be embedded in the protein-starch matrix of pea flour and by disruption of this matrix leads to an increased accessibility of these VOCs to extraction. The finer the particles, the greater disruption of the matrix would be anticipated.

Significant differences ($p$ <0.05) were obtained for the TV concentration in deflavored pea flours based on varying of sieve fractions and whole pea flour. Within these samples, TV was significantly higher in the fine fraction; though the highest TV concentration was found in whole pea flour of non-deflavored samples. This finding indicated that the reduction in particle size decreased the effectiveness of SC-$CO_2$+EtOH extraction. This was in contrast to what was expected. In theory, finer particles provide a larger surface area that reduces the diffusion path, thus resulting in diminished intra-particle diffusion resistance and subsequently facilitates the extraction process and efficiency (Khaw, Parat, Shaw, & Falconer, 2017). Ozkal & Yener (2016) reported that reducing the particle size of flaxseed resulted in higher oil yield by SC- $CO_2$ extraction. However, Khaw et al. (2017) reported that excessive particle size reduction caused a decrease in extraction yield due to likely agglomeration, which might lead to $CO_2$ flows only through micro-channels with diminished surface area (Khaw et al., 2017) and subsequently results in lower extraction of VOCs. SC-$CO_2$+EtOH extraction removed most selected VOCs from all pea flours except for the fine fraction owing to possible agglomeration that caused a reduction in extraction efficiency. Nonanal was the only VOC detected in all deflavored pea flours, which is supported by other researchers. Nonanal might be tightly bound in the starch-protein complex via hydrogen bonding or dipole-dipole interactions; therefore, making it difficult to remove from the starch-protein matrix.

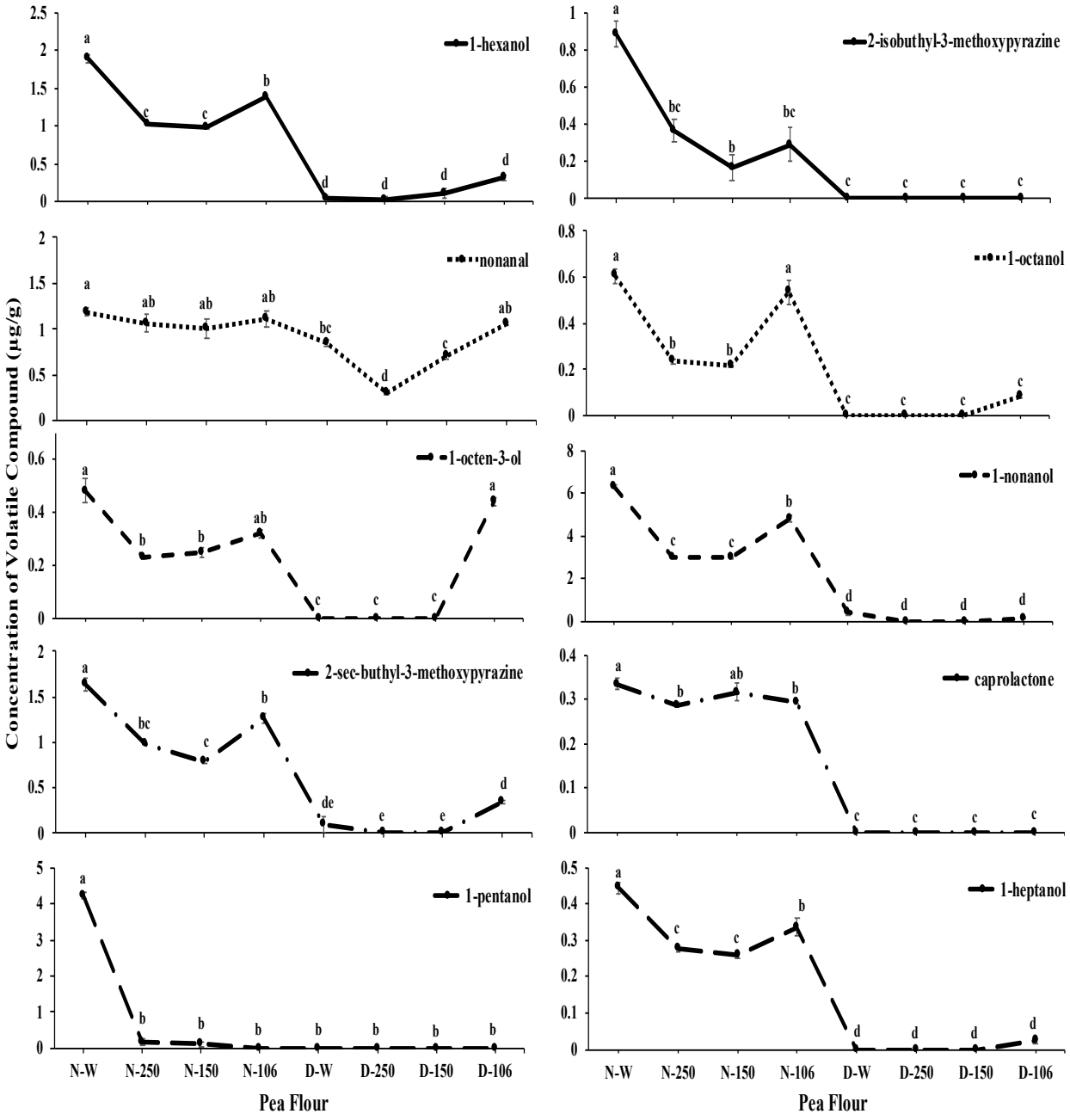


Figure 5.1. Selected volatile compounds of pea flour detected by HS-SPME-GC. Letters represent the following: N-W, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively while D-counterparts are deflavored pea flours. Data points are means ± standard error. Different letters indicate significantly differences ($p < 0.05$).

### 5.4.2. HS-SPME-GC/MS-O analysis of volatile compounds

The TVI values of pea flours based on GC-O fell between 0 and 19 (Table 5.1) and supported by the changes in selected standard compounds based on processing (Fig. 5.2). Significant main effects ($p < 0.05$) among pea flours were obtained, but the interaction effect between the two factors was non-significant ($p > 0.05$) for TVI of selected VOCs (Table 5.2). Additionally, experimental odor descriptors (e.g., green, lemon, bell pepper, pea, mushroom, sweet) recorded through GC-O analysis for each VOC (Table 5.3) complied with previous reports (Murat et al., 2013; Xu et al., 2019).

GC-O results (Table 5.1 and Fig. 5.2) illustrated that SC-$CO_2$+EtOH extraction significantly decreased selected off-aroma compounds in pea flours. The TVI of non-deflavored and deflavored pea flours, contained 4 alcohols, 1 aldehyde, 2 alkyl methoxypyrazines, and 1 furan (Fig. 5.2), were in the range of 14.5 and 22, and 0 and 3.5, respectively (Table 5.1, Fig. 5.2). These selected VOCs were previously detected through GC-O in pea flours (Murat et al., 2013, Xu et al., 2019). Deflavored pea flours had relatively low TVI, which supported TV results. In comparison to HS-SPME-GC, 1-pentanol and 1-heptanol were not detected by GC-O panelists in non-deflavored pea flours; likely due to their quantity and threshold level. Previously, 1-pentanol was recorded in pea flour through GC-O analysis, but in the same study, 1-heptanol was not detected (Murat et al., 2013). However, Xu et al. (2019) reported the detection of 1-heptanol in germinated pulse flours by GC-O analysis, unlike non-reporting 1-pentanol in the same samples through GC-O analysis.

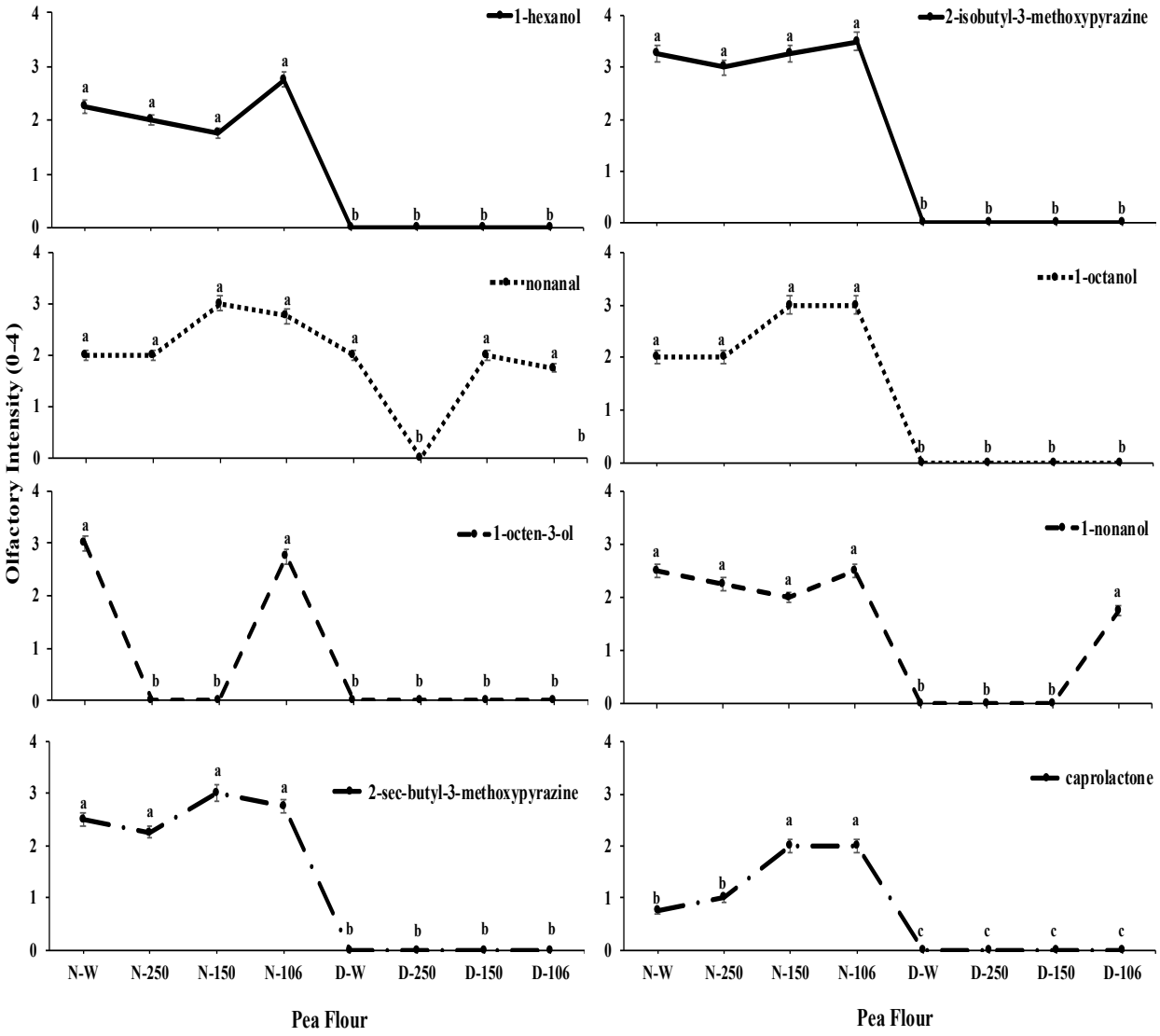


Figure 5.2. Selected volatile compounds of pea flour detected by GC-O. Letters represent the following: N-W, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively while D-counterparts are deflavored pea flours. Data points are means ± standard error. Different letters indicate significantly differences ($p < 0.05$).

The intensity of selected VOCs varied based on particle size, contributing a significant impact on the TVI value ($p < 0.05$, Table 5.1). The results of GC-O were highly consistent with the HS-SPME-GC results, but differed from sensory results based on varying particle size. Non-deflavored and deflavored coarse fraction exhibited significantly lower TVI than other samples. In contrast, fine fraction had higher TVI value, which was not significantly different from whole

flour and the medium fraction (Table 5.1). However, non-significant difference for TV values between coarse and medium fractions was observed in the HS-SPME-GC data.

For all non-deflavored pea flours, both alkyl methoxypyrazines (Fig. 5.2) were the most recognized compounds by the GC-O panelists; owing to low sensory threshold values, such as 3 ppt in air for 2-*sec*-butyl-3-methoxypyrazine (Neta, Miracle, Sanders, & Drake, 2008). Methoxypyrazines have been reported as musty, green, and earthy off-aroma contributor of various plants (e.g., peas, asparagus, potatoes) with many descriptions (e.g., bell pepper, peapod, earthy, green, vegetable, nutty smell) (Murat et al., 2013; Neta et al., 2008). Nonanal was the only compound detected by GC-O for most deflavored flours, confirming the HS-SPME-GC analysis. Murat et al. (2013) also reported this VOC in pea flour.

Table 5.3. Aroma compounds identified in non-deflavored pea flours by GC-O panelists [A].

| Compound | CAS | Theoretical Descriptors | Experimental Descriptors | Origin [B] |
|---|---|---|---|---|
| 1-Hexanol | 928-96-1 | Green, hay-like odor | Floral, green, grain, hay-like | Lipid oxidation |
| Nonanal | 124-19-6 | Waxy, citrus | Lemon, citrus, green | Lipid oxidation |
| 1-Octen-3-ol | 3391-86-4 | Mushroom, earthy, broccoli | Broccoli, mushroom, earthy | Lipid oxidation |
| Alkyl Pyrazine 1[D] | 24168-70-5 | Green, bell pepper, peapod | Green, vegetable, bell pepper, cilantro | Natural/Protein[H] |
| Alkyl Pyrazine 2[E] | 24683-00-9 | Green, peas, bell pepper | Bell pepper, broccoli, pea | Natural/Protein |
| 1-Octanol | 111-87-5 | Mushroom, green, vegetable | Grainy, vegetable, mushroom, musty | Lipid oxidation |
| 1-Nonanol | 143-08-8 | Peas, vegetable, green, | Green, bell pepper | Lipid oxidation |
| γ-Caprolactone [F] | 695-06-7 | Candy, coconut, sweet | Sweet, coconut | Natural |

[A] Percentage level based on the detection level identified by olfactory panelists.
[B] Origin of VOCs based on following literature: Azarnia et al. (2011), Jakobsen et al. (1998), Murat et al. (2013), Roland et al. 2017, and Vatansever & Hall (2020).[C] N-W: Non-deflavored whole (unsieved). [D]Alkyl Pyrazine 1: 2-*sec*-butyl-3-methoxypyrazine [E]Alkyl Pyrazine 2: 2-isobutyl-3-methoxypyrazine. [F] γ-Caprolactone: 5-ethyldihydro-2(3H)-furanone. [H] Natural/Protein: The origin of this compound is either natural or from protein degradation.

The degree of intensity of other selected VOCs depended on the particle size. Particularly, coarse fraction was relatively low in the degree of intensity for all VOCs owing to potentially its higher bran content and less starch and protein components (Vatansever & Hall, 2019), the latter

cause more volatile binding and result in higher off-flavors (Heng, 2005; Murat et al., 2013; Wang et al., 2020). Among deflavored pea flours, GC-O analysis showed that coarse fraction had zero degrees of intensity; likewise, this fraction had the lowest TV based on the HS-SPME-GC analysis.

### 5.4.3. Quantitative descriptive analysis for sensory evaluation

Sensory evaluation of pea flours using QDA was conducted to confirm the efficiency of SC-$CO_2$ extraction with different particle size pea flours on the removal of off-flavors (Table 5.1). Both factors and their interaction showed significant effects ($p < 0.05$) on sensory attributes except acceptability for particle size (Table 5.2).

The significant reduction ($p < 0.05$) in pea intensity and bitterness of deflavored pea flours demonstrated that SC-$CO_2$+EtOH extraction effectively removed selected VOCs and agreed with HS-SPME-GC and GC-O findings (Table 5.1). Furthermore, there is the possibility that saponins were removed. As a result, increased acceptability of pea flours was observed. The range of pea intensity and bitterness in non-deflavored pea flours were between 63 to 112.3 mm and 26.7 to 65.5 mm, whereas those in deflavored pea flours were between 12 to 29.5 mm and 4.5 to 9.7 mm, respectively, based on a 147 mm line scale. These findings supported previous sensory data (Vatansever & Hall, 2020). Furthermore, Malcolmson et al. (2014) showed that moderate pea aroma and slightly bitter intensity in cooked yellow peas through QDA. Researchers also determined various aroma descriptions (e.g., pea, metallic, grainy, earthy, vegetable, hay-like) for cooked peas.

Undesirable bitter taste in dry peas is mostly associated with saponins, including saponin B and saponin ßg (or called as DDMP saponin) (Heng et al., 2006; Roland et al., 2017). SC-CO2+EtOH extraction might promote the extraction of these saponins at the presence of high temperature and ethanol, thereby resulted in decreased bitterness intensity for deflavored flours.

In addition, likely conversion of DDMP saponin into less bitter saponin B might reduce bitterness intensity (4.5 to 9.7 mm) in deflavored pea flours in the presence of ethanol and high temperature. Similar pattern was observed by Heng et al. (2006). Heng et al. (2006) showed conversion of DDMP saponin, exhibiting higher bitterness intensity, into saponin B and maltol at >65 $^{o}$C with ethanol. Relatively low bitterness intensity (4.5 to 9.7 mm) of deflavored pea flours indicated conversion of DDMP saponins into less bitter saponin B and also their removal through this extraction, particularly with ethanol, owing to their polar nature (Heng 2005). Heng et al. (2005) estimated threshold levels of saponin B and saponin mixture (80% saponin DDMP and 20% saponin B) using QDA (on a 150 mm line scale) based in bitterness intensity. The bitterness intensity of saponin B and saponin mixture at 2-12 mg/L was ranged ~18-40 mm and ~45-105 mm, respectively. Therefore, deflavored pea flour might have a little saponin concentration (< 2 mg/L) with probably including mostly saponin B. Meantime, these saponins might be perceived as astringent and metallic aroma (Roland et al., 2017).

Particle size has significant impacts on pea and bitterness intensity. Relatively higher pea intensity and bitterness, thus lower score for acceptability, were recorded for non-deflavored coarse fraction compared to medium and fine fractions (Table 5.1). These findings were negatively correlated with instrumental analyses. Potentially, panelists might chew coarse fraction for a longer time to reduce particle size, resulting in increased chance that off-flavor compounds will be perceived compared to other fractions. Thus, it may influence the perception and result in higher intensity. The coarse fraction might contain more hulls than other samples owing to larger particle size since saponins in dry peas were reported at high concentrations in the hulls (Heng, 2005). The greater bitterness intensity in coarse fractions might be due to the higher hull content in this fraction. Furthermore, metallic and astringent perception of saponins (Roland et al., 2017) may

enhance pea intensity ratings for non-deflavored coarse fractions. Malcolmson et al. (2014) reported metallic aroma for cooked yellow peas.

Compared to other flour samples, non-deflavored fine fraction had the lowest pea intensity and bitterness with the greatest acceptability. However, instrumental analyses indicated relatively higher TV and TVI amounts in non-deflavored fine fraction (Table 5.1). This fraction is relatively higher in starch with a moderately high protein level (Vatansever & Hall, 2019). Possibly, flavor binding capacity of protein and starch might reduce the perception of off-aroma and might cause a longer time for perception of VOCs during oral processing. In this case, fine fraction had smaller particles; therefore, panelists may swallow this fraction relatively faster than coarse fraction with less chewing and comminution. Subsequently, bolus formation, involving jaw movement and saliva secretion, might be deficient and causes minimal interaction of particle with saliva, and the oral cavity (Liu, Deng, Sha, Hashem, & Gai, 2017); resulting in shorter retention time of fine fraction in the mouth. In addition, fine and medium fractions were relatively lower in hull particles due to their higher starch and protein contents, thereby lower bitterness intensity for these fractions were expected compared to coarse and whole pea flours.

### 5.4.4. PCA and HCA of response variables from instrumental and sensory analyses

Changes in selected VOCs identified by HS-SPME-GC and GC-O, and in sensory attributes determined by QDA was subjected to PCA and HCA. PCA was employed on the correlation matrix to determine complex interrelationships among response variables via principal components (PCs) produced by reducing the dimensions of data with maximizing the variance. HCA was applied to identify the specific response variable (i.e., flavor compounds and sensory attributes), accounting for division of eight groups of pea flours in detail. The results of PCA and HCA are presented in Figures 5.3-5.5.

The PCA of VOCs from HS-SPME-GC (Fig. 5.3) illustrated that 82.7% of the total variance was explained by the first PC, while PC1 and PC2 explained 90.5% of the total variance. Based on this finding, PC1 explained all VOCs in one dimension, indicating a high correlation among VOCs. Similarly, the VOCs determined by GC-O (Fig. 5.4) exhibited that PC1 was responsible for most variation (80.1%) across the samples, while PC2 explained only 10%.

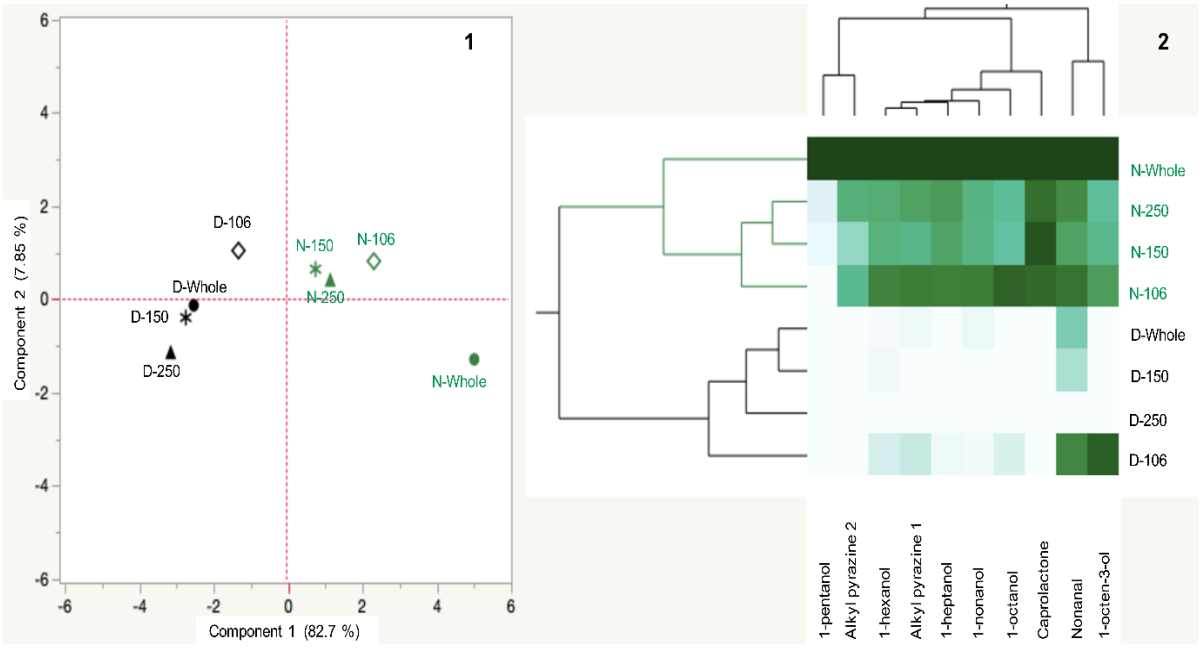


Figure 5.3. Principal component analysis (PCA; 1) and hierarchical cluster analysis (HCA; 2) of volatile compounds detected by HS-SPME-GC analysis. Letters and numbers represent the following: 1: score plots of principal component 1 and 2, and 2: Hierarchical cluster dendrograms, respectively. The color box presents the mean value of each response variable given on the x-axis. White to dark green color represents low to high level of response. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively while D-counterparts are deflavored pea flours.

Overall, VOCs also illustrated a high correlation between volatiles. VOCs of non-deflavored pea flours were positively correlated with the first PC in contrary to those of deflavored pea flours. Likewise, Chang et al. (2019) reported that the volatile profiles of ethanol (95%)-washed LPI had a negative correlation with PC1, whereas VOCs of ethanol (35-75%)-washed LPIs was positively correlated with PC1 due to requiring higher alcohol concentration for effective removal of volatiles. Furthermore, Wang et al. (2020) presented that VOCs of deflavored PPEF

samples via ethanol (50 and 80%) washing were negatively correlated with extracted PCs in contrary to the volatile profile of control, and 20% of ethanol washed the PPEF sample. Compared to instrumental analyses, sensory attributes had a relatively higher correlation, and its PC1 (Fig. 5.5) accounted for almost all total variance (97.7%) across the samples. Score plots of PCA showed that there is an absolute separation between non-deflavored and deflavored pea flours.

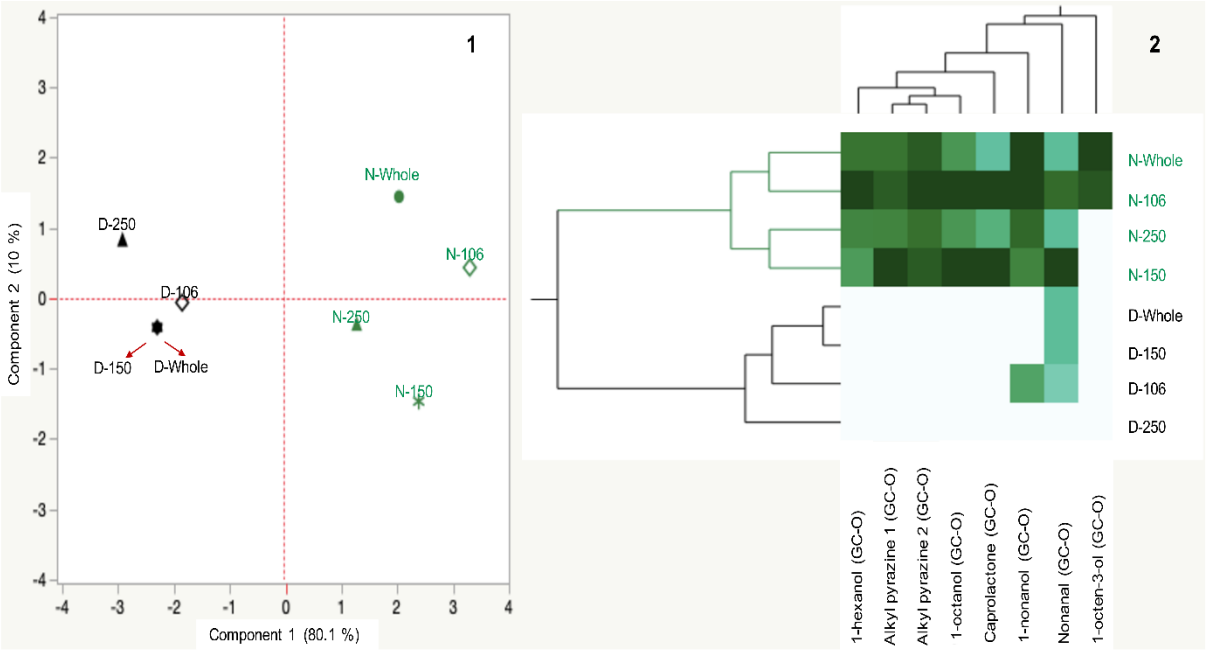


Figure 5.4. Principal component analysis (PCA; 1) and hierarchical cluster analysis (HCA; 2) of volatile compounds detected by GC-O analysis. Letters and numbers represent the following: 1: score plots of principal component 1 and 2, and 2: Hierarchical cluster dendrograms, respectively. The color box presents the mean value of each response variable given on the x-axis. White to dark green color represents low to high level of response. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively while D-counterparts are deflavored pea flours.

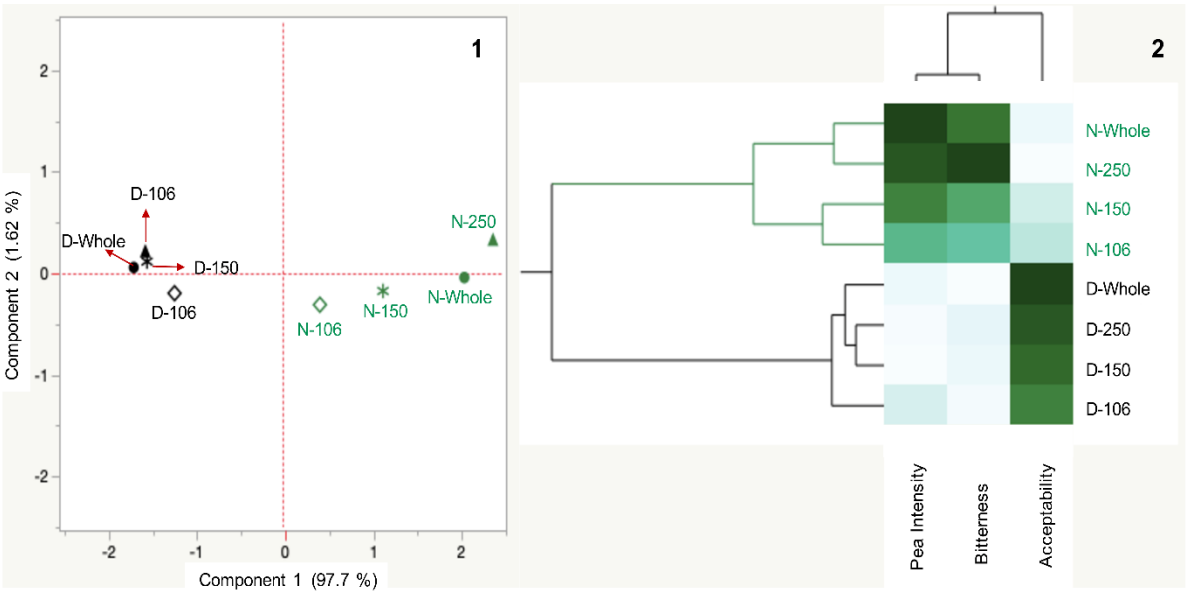


Figure 5.5. Principal component analysis (PCA; 1) and hierarchical cluster analysis (HCA; 2) of sensory evaluation using QDA. Letters and numbers represent the following: 1: score plots of principal component 1 and 2, and 2: Hierarchical cluster dendrograms, respectively. The color box presents the mean value of each response variable given on the x-axis. White to dark green color represents low to high level of response. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively while D-counterparts are deflavored pea flours.

The cluster analyses of VOCs detected by HS-SPME-GC and GC-O, and of sensory attributes obtained by QDA showed that the non-deflavored and deflavored pea flours were broadly characterized into two groups based on the dendrograms 2 of Figures 5.3-5.5, respectively. Darkest green block represents the highest values of response variables, while the light (white-like) block is non-value detected by the analysis. SC-$CO_2$+EtOH extraction completely removed selected aroma compounds from the coarse fraction and also diminished the most VOCs for other pea flours (Figure 5.3). Chang et al. (2019) and Wang et al. (2020) demonstrated similar findings for cluster analyses of LPI and PPEF after ethanol washing treatment. Nonanal was the only VOC that did not entirely get remove through extraction (except coarse fraction) and is represented in lighter green columns in deflavored flours, but it had relatively dark green columns in non-

deflavored pea flours (Fig. 5.3 & 5.4). Similarly, nonanal was recorded at high amounts after ethanol washing treatment of PPEF based on the cluster analyses (Wang et al., 2020).

The HCA of sensory attributes exhibited that pea and bitterness intensity were demonstrated with relatively darker green blocks for non-flavored whole flour and coarse fraction, while lighter green blocks were observed for medium and fine fractions. For deflavored pea flour, pea and bitterness intensity were almost white-like blocks for all samples. However, acceptability was oppositely positioned with other attributes for pea flour samples. Since after extraction, pea flours became more acceptable and resulted in dark green blocks in the dendrogram (Fig. 5.5).

**5.5. Conclusion**

This study showed the impacts of SC-$CO_2$-EtOH extraction and particle size on the flavor profiles of pea flours. Both factors had significant interaction effects for sensory attributes and instrumental outputs. SC-$CO_2$-EtOH extraction significantly decreased off-aroma and off-taste compounds of all pea flour samples. Different particle size had significant importance on aroma profile. Smaller particle size had higher off-aroma compounds, but larger particle size had higher bitterness intensity. HS-SPME-GC and GC-O findings agreed with each other for non-deflavored and deflavored pea flours. However, the findings of instrumental analyses for non-deflavored pea flour were opposite of data from the sensory analysis. PCA revealed that volatiles were highly correlated to each other. Also, pea and bitterness intensity had a high positive correlation. Cluster analysis revealed that non-deflavored and deflavored flours were separated based on the dendrograms. Findings of the presented study showed that flavor studies require multiple approaches to provide reliable results due to differences of human flavor perception. SC-$CO_2$+ETOH extraction could be used an efficient green technology to enhance organoleptic properties of pulse ingredients.

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# CHAPTER 6. OVERALL SUMMARY AND CONCLUSION

## 6.1. Conclusion

Supercritical carbon dioxide + ethanol (SC-$CO_2$+EtOH) extraction was an effective eco-friendly deflavoring method to improve organoleptic properties of yellow pea flours. Response surface methodology with central composite rotatable design significantly ($p$<0.05) fit the model to optimize operating conditions of SC-$CO_2$+EtOH extraction at 22% ethanol, 86 °C of temperature and 42.71 MPa of pressure to reduce off-flavor compounds. Deflavored pea flour extracted at optimum conditions had relatively lower off-aroma compounds along with improved sensory attributes with lower pea intensity and bitterness. Particularly, major off-aroma compounds, 1-hexanol, 1-octanol, 1-nonanol, nonanal, and 2-alkyl methoxypyrazines, which cause green, mushroom, pea, and earthy aroma (Heng, 2005; Murat, Bard, Dhalleine, & Cayot, 2013), were either not identified or found at very low concentration in deflavored pea flours based on analytical methods. Particle size showed a significant interaction effect with the extraction on flavor profile. Fine fraction had higher volatile intensity based on instrumental analyses than other fractions. Similarly, deflavored fine fraction had a greater off-flavor concentration than deflavored flours. Coarse fraction was higher in bitterness than other flours, but extraction significantly reduced bitterness intensity for all deflavored flours. Heng et al. (2006) previously reported that bitterness intensity of pea ingredients was reduced in the presence of ethanol at high temperature (over > 65 °C). Particularly, highly bitter DDMP saponin might be converted into less bitter saponin thus bitterness intensity of pea ingredients can be decreased. Among off-aroma compounds, nonanal was only detected in deflavored pea flours except for coarse fraction. Likewise, Wang, Guldiken, Tulbek, House, & Nickerson (2020) stated a higher nonanal quantity in alcohol-washed protein enriched pea flour. Significant interaction effect between SC-

$CO_2$+EtOH extraction and particle size found on the chemical composition (except for total starch), pasting and functional properties, and particle size distribution. Deflavored pea flours had lower moisture, resistant starch, damage starch, and lipid content compared to non-deflavored counterparts. Flours with coarse particles had lower protein, total starch, and starch damage than other flours. Medium and fine fractions had greater protein and total starch, respectively. SC-$CO_2$+EtOH extraction caused a reduction in viscosity parameters and water solubility index. Furthermore, those variables were significantly different based on particle size. SC-$CO_2$+EtOH extraction had certain impact on predicted moisture contents of pea flours. Water sorption capacity of deflavored pea flours decreased with increased water activity.

Overall, findings of multi-approached flavor research and physicochemical study confirmed that the SC-$CO_2$ + EtOH extraction technology is a reliable deflavoring technology for removal of unpleasant flavors from pulse flour. This extraction system might be a potential sustainable deflavoring system for flavor improvement of dry plant materials in food technology as follows: (1) contribution to environment by using SC-$CO_2$ as a main solvent and requiring relatively less amount of co-solvent compared to conventional solvent extractions; (2) reduction of processing cost through recycling option of used solvents and through shorter extraction time compared to other deflavoring methodologies; (3) improvement of shelf-life stability of pulse ingredients by removal of fats besides off-flavor compounds; (4) possible modification of starch and protein components of pulse flour thereby better applicability of these ingredients in further food developments (e.g., pasta, bread, meat alternatives, dairy alternatives); (5) likely preservation of pulse ingredients through decreasing microorganisms; (6) applicability of this emerging green technology for the utilization of dry food waste in foods through flavor and structure improvements.

## 6.2. Future Direction

In this study, SC-$CO_2$+EtOH extraction was effectively used as a deflavoring technology at optimum conditions to improve sensory quality of pea flour. Additionally, effects of particle size on flavor profile, extraction efficiency, physicochemical properties, and moisture sorption isotherms of pea flours were studied. Important findings were obtained through study for the targeted variables.

In the future, SC-$CO_2$+EtOH extraction at optimum conditions can be used as a potential technology to improve organoleptic properties of other pulse ingredients. Additionally, particle size can be an approach to improving the sensory quality of pulse flour. For the future direction, deflavored pulse flours treated by SC-$CO_2$+EtOH extraction can be utilized for food applications, particularly for breads, cookies, and crackers to reveal the effect of this extraction on the end product-quality. Furthermore, better understanding of changes in starch and protein structure due to SC-$CO_2$+EtOH extraction are necessary to provide knowledge with regards to food applications. Also, deflavored starch and protein from pulses need to be tested for techno-functionality analyses and applications, such as pasta, bakery, alternative-meat and other foods.

In this study, carotenoid and saponin analyses using HPLC system were not conducted, but lighter flour with lower bitterness intensity indicated a reduction in both carotenoids and saponins. Therefore, analysis of carotenoids and saponins can be added for the future testing to determine the actual amounts before and after extraction. In the meantime, the recovery of saponins and carotenoids during the extraction could provide processors with another ingredient as these components have been found to have health benefits such as cholesterol lowering and antioxidant activity. Mineral and vitamins analyses might be useful to confirm the effect of this extraction

system on micronutrient profile of deflavored pulse ingredients using this extraction system. Additionally, this study was performed using pea flour from whole pea seeds.

For the future study, dehulled pulse flours, including different particle size, might be deflavored using this extraction system and could be compared with deflavored whole pulse flour samples to determine the efficiency of this system in creating different ingredients. In addition to pulse flours, protein enriched pulse and legume flours (e.g., soy flour), starch enriched pulse and legume flours (e.g., soy flour), and other dry ingredients from different crops, which have off-flavor issues, might be treated with SC-$CO_2$+EtOH extraction to determine the efficiency of this extraction system as a deflavoring method.

SC-$CO_2$ extraction has been reported for reducing microbes in foods. Also, ethanol has a lethal effect on the microorganisms. Thus, microbiological analyses might be added to highlight the effect of this processing system on the food safety concerns of pea flour.

**6.3. References**

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## APPENDIX A. SCALING SCORE SHEET FOR QUANTITATIVE DESCRIPTIVE ANALYSIS OF PEA FLOUR

**Pea Flour Evaluation**

Code _____ Date _____

You will be given 4 flour samples, one sample at a time. Please taste each sample then rate it using the following scales. Each sample will be evaluated on a separate sheet. Comments are desired.

**Sample Code** ABC

**Flavor**

Unacceptable Acceptability

Weak Pea Strong Pea

Weak bitterness Strong bitterness

Comments:

# APPENDIX B. IRB CONSENT FORM

NDSU NORTH DAKOTA STATE UNIVERSITY

September 11, 2017

Dr. Clifford Hall
Plant Sciences

Re: IRB Determination of Exempt Human Subjects Research:
Protocol #AG18027, "Enhancing Pulse Utilization Through Flavor Modification"

Co-investigator(s) and research team: Serap Vatansever, Madison Gohl, Mary Niehaus, Katelyn Schmoll
Certification Date: 9/11/2017 Expiration Date: 9/10/2020
Study site(s): various
Sponsor: n/a

The above referenced human subjects research project has been certified as exempt (category #6) in accordance with federal regulations (Code of Federal Regulations, Title 45, Part 46, Protection of Human Subjects). This determination is based on the original protocol submission (received 8/14/2017) with revised cover letter (received 8/21/2017).

Please also note the following:
• If you wish to continue the research after the expiration, submit a request for recertification several weeks prior to the expiration.
• The study must be conducted as described in the approved protocol. Changes to this protocol must be approved prior to initiating, unless the changes are necessary to eliminate an immediate hazard to subjects.
• Notify the IRB promptly of any adverse events, complaints, or unanticipated problems involving risks to subjects or others related to this project.
• Report any significant new findings that may affect the risks and benefits to the participants and the IRB.

Research records may be subject to a random or directed audit at any time to verify compliance with IRB standard operating procedures.

Thank you for your cooperation with NDSU IRB procedures. Best wishes for a successful study.
Sincerely,

Kristy Shirley

Digitally signed by Kristy Shirley
DN: cn=Kristy Shirley, o=NDSU, ou=Institutional Review Board, email=kristy.shirley@ndsu.edu, c=US
Date: 2017.09.11 14:18:14 -05'00'

Kristy Shirley, CIP, Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult http://www.ndsu.edu/research/integrity_compliance/irb/. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

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