FLAVOR MODIFICATION OF PEA FLOUR USING ETHANOL-BASED DEODORIZATION

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Title

ENHANCING PULSE UTILIZATION THROUGH FLAVOR MODIFICATION

By

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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

MASTER OF SCIENCE

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ABSTRACT

Peas are rich in protein and dietary fiber and can be used to create specialty products; however, flavor issues are one of the primary concerns regarding utilization. Sensory evaluations indicated the optimal treatment utilized aqueous ethanol at a concentration of 47.5%, extraction time of 63 min, and no pressure. Decreased ($P<0.05$) moisture and ash content, with no loss of protein or starch, were observed after treatment. Foaming properties were poor, indicating protein modification. Increased water absorption impacted WAI, WSI, setback, and peak time observations. Remaining pasting profile values were unchanged ($P<0.05$). While some volatiles were released via changes in protein and starch structure, total ppm decreased. Treated pea flour products had significantly ($P<0.05$) higher flavor acceptance scores. Texture results suggested treated flour imparted softness of baked items. Shelf-life measurements were improved for both cookies and crackers using treated pea flour.

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LIST OF ABBREVIATIONS

AACCI.....American Association of Cereal Chemists International

AAFCO.....Association of American Feed Control Officials

ANOVA.....Analysis of Variance

C.....Celsius

cm.....Centimeter

FAO.....Food and Agriculture Organization

FDA.....Food and Drug Administration

g.....Gram

GC.....Gas Chromatography

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

GI.....Glycemic Index

kcal.....Kilocalorie

kg.....Kilogram

MIFA.....Methylene Interrupted Fatty Acids

mg.....Milligram

min.....Minute

mL.....Milliliter

mm.....Millimeter

NASS.....National Agricultural Statistics Service

NCI.....Northern Crops Institute

NDSU.....North Dakota State University

ppm.....Parts per Million

RCBD.....Randomized Complete Block Design

rpm.....Revolutions per Minute

RVA .....Rapid Visco Analyzer

SPME .....Solid Phase Microextraction

QDA .....Qualitative Descriptive Analysis

µg .....Microgram

µl .....Microliter

v/v .....Volume to Volume

VSA.....Vapor Sorption Analyzer

USDA.....United State Department of Agriculture

WAI.....Water Absorption Index

WSI .....Water Solubility Index

LIST OF SYMBOLS

° .....Degrees

> .....Greater than

< .....Less than

% .....Percentage

α .....Alpha

β .....Beta

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1. INTRODUCTION

1.1. General Introduction

Pulses, such as field pea (*Pisum Sativum L.*) produce a potential to create novel, nutrition-dense snacks and baked goods when milled into a flour. Common in eastern Asian countries such as India, China, and Japan, as well as part of Africa, pulses are a historical staple food in many parts of the world (Derbyshire, 2011). Specifically, field pea is high in protein (21 to 33%) and fiber (14 to 26%) (Dahl, Foster, & Tyler, 2012). Dry peas are an excellent source of iron, zinc, magnesium, and selenium (Hall, 2017). Folate, a vitamin critical in preventing neural tube defects in infants, is rich in peas (Wald, 2004). Furthermore, dry peas have a low glycemic-index, meaning they are digested over a longer period of time, maintaining blood glucose levels.

Despite the health benefits of pulses, such as field pea, the poor flavor acceptance is a major limiting factor in its utilization as an ingredient within the food industry. In a study conducted to evaluate the public's perception of peas, 60% of consumers rejected the flavor, with perceptions including "green" and "pea" constituting the majority of the disapproval (Saint-Eve, Granda, Legay, Cuvelier, & Delarue, 2019). A combination of lipid oxidation and protein degradation is suggested to produce the off-flavor compounds, referred to as volatiles, present in peas (Azarnia, Boye, Warkentin, & Malcolmson, 2011; Maarse, 1991; Schindler et al., 2012; Vara-Ubol, Chambers E., & Chambers D., 2004). A total of 66 volatiles have been found in pea flour (Murat, Bard, Dhalleine, & Cayot, 2013). In response to the general disapproval of pea flavor, researchers have begun to investigate methods to deodorize flavors using methods that target the causes of flavor production.

Cultivar selection has been suggested as a pre-harvest technique to reduce unwanted flavor. However, this method limits producers to use only select varieties. Flavor modification

post-harvest provides producers more freedom in the purchasing of raw ingredients. Some of these techniques include germination (Shanmugasundaram, 2003; Troszyńska et al., 2011), fermentation (Roland, Pouvreau, Curran, Velde, & Kok, 2017; Schindler et al., 2012), distillation (Berk, 2013), water treatment (Lv, Song, Li, Wu, & Guo, 2011; Roland et al., 2017), and solvent extraction (Chang, Stone, Green, & Nickerson, 2018; Xu & Chang, 2007). Hillen (2016) and Roland et al. (2017) determined ethanol solvent extraction was one of the most viable options to flavor reduction. Combinations of ethanol and water allow for the extraction of both water and ethanol soluble compounds (Do et al., 2014). Therefore, a reduction in pea flavor may be attributed to the removal of water and ethanol soluble volatiles. Oftentimes, the use of high-pressure extraction is combined with aqueous ethanol (Hillen, 2016). However, there is limited research on the optimization of these techniques, as well as its impact on the functionality of the pulse flour. Therefore, objectives of this study were to select an optimal ethanol extraction treatment; determine chemical composition, physiochemical properties, and volatile quantification of treated pea flour; and evaluate cookie and cracker acceptance.

1.2. Overall Methodology

Whole, yellow peas from three various sources were combined to create a composite sample. The composite sample was hammer milled into flour for evaluation. Ethanol extraction was conducted to treat the pea flours, with the objective of reducing the pea flavor. Preliminary sensory evaluations were utilized to determine the optimal treatment that would be evaluated further. Chemical composition, physiochemical properties, and volatile content were evaluated on raw and treated pea flours. Furthermore, cookies and crackers produced with raw and treated pea flours were evaluated using physical and sensory methods.

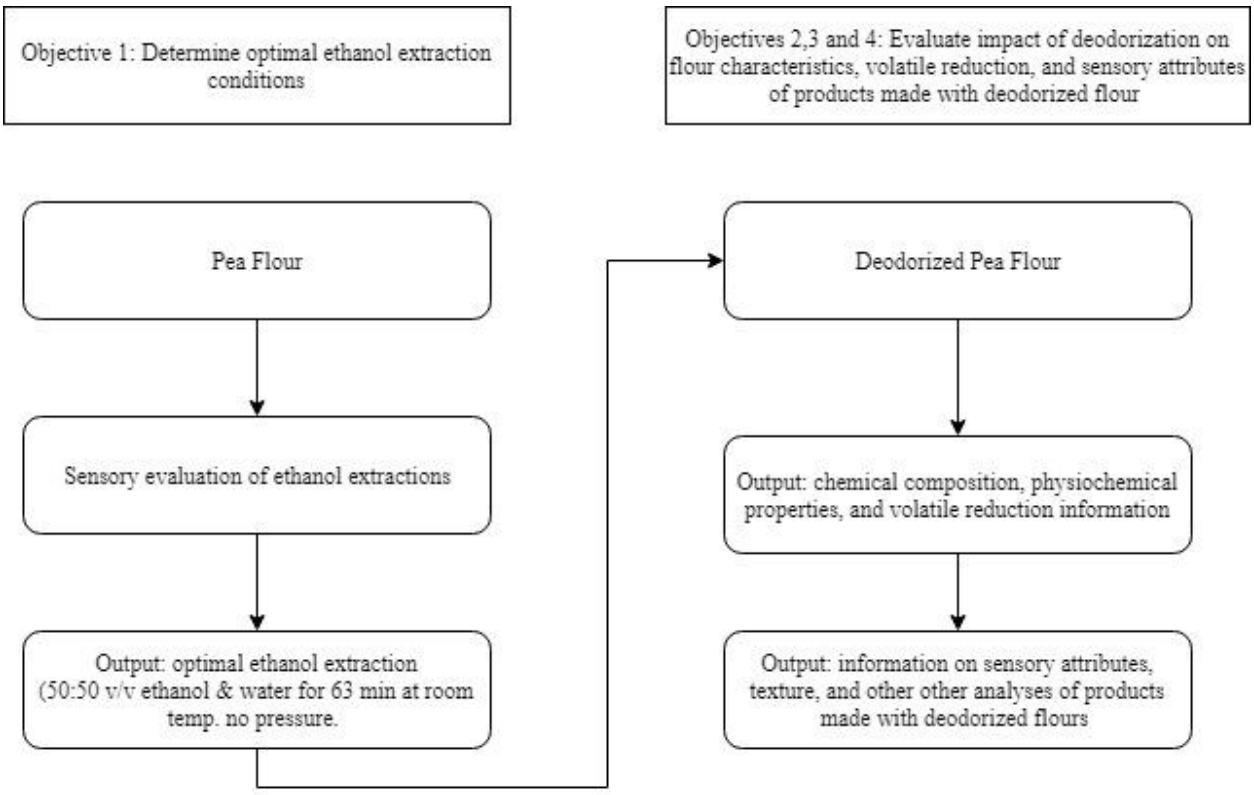

Figure 1.1. Overall scheme for the selection and evaluation of the optimally treated pea flour

2. LITERATURE REVIEW

2.1. Classification of Field Peas and Pulses

Pulses are defined as the seeds of legumes used for human consumption (FAO, 1994). The Food and Agriculture Organization (FAO) further specifies that the term "pulse" refers to crops harvested solely for dry grain. Based on this classification, legumes used for oil extraction, such as soybeans or peanuts are excluded as pulses (Mudryj, Yu, & Aukema, 2014). There are 11 pulses defined by the FOA including bean, broad bean, pea, chickpea, cowpea, pigeon pea, lentil, bambara bean, vetche, lupin, and pulses nes. Dry pea (*Pisum Sativum L.*), also known as the field pea or smooth pea accounts for 8 to 14.6% of the total world production of pulses (Joshi & Rao, 2017). Field pea differs from a fresh pea, which is typically marketed as a fresh vegetable for human consumption (Sell, 1993).

Field pea is typically well adapted to cool, semi-arid climates with optimal growing temperatures between 13 °C (55°F) and 18 °C (65°F) (Sell, 1993). Native to Southwest Asia, the field pea was first commercially grown in the United States in the Palouse region of Northern Idaho (Muehlbauer & Rhoades, 1993; Sell, 1993). In recent years, North Dakota and Montana have surpassed the Palouse region as the major field pea growing regions (NASS, 2018a,b). Planting is traditionally completed in the spring with the field pea reaching maturity 95 to 100 days after emergence (Sell, 1993). Harvest occurs when the seeds are hard and fully mature, usually at 16 to 18% moisture. Approximately 2.8 million acres of pulses were harvested across 40 states in 2017, with peas accounting for an estimated 1.3 million acres (Hall, 2017).

2.2. Composition of Field Peas

As a pulse, field pea is known to provide a composition beneficial to human health. The composition of the dry pea is high in protein (21 to 33%), starch (36 to 49%), and dietary fiber

(14 to 26%) (Dahl et al., 2012). Pea flour contains over twice as much protein and dietary fiber as unenriched wheat flour, with minimal lipids (USDA 2018a,b). A comparison of pea flour to wheat flours indicates that pea flour has approximately 2x and 8x more protein and fiber, respectively (Table 2.1).

Table 2.1. Nutritional information for ¼ c. serving of pea flour and whole wheat flour, unenriched (USDA 2018a,b)

Nutrient	Unit	Pea Flour	Wheat Flour, unenriched
Energy	Kcal	104.0	155.0
Protein	G	10.42	5.13
Total lipid (fat)	G	0.0	0.71
Carbohydrate, by difference	G	18.75	31.05
Fiber, total dietary	G	8.3	1.0
Sugars, total	G	2.08	0.13
Sodium, Na	Mg	4.0	1.0

2.2.1. Starch

The primary constituents of field pea are starch, dietary fiber, protein, lipids, vitamins, and minerals. The most abundant constituent of field pea is starch, making up 36.9-49.0% of the total composition (Dahl et al., 2012). Starch is the primary polysaccharide used by plants to store glucose (Carpi, 2003). Traditionally, this polysaccharide is stored in the plastids of most plants in the form of granular storage bodies (Donald & Richmond, 1997). Readily digested, starch is a major energy source for most diets (Donald & Richmond, 1997). All naturally occurring starches are made from a mixture of amylose and amylopectin. The total amylose content of field pea starch is 48.8 to 49.6%, with the remaining content comprised of amylopectin. Included in the total starch content is resistant starch. Resistant starch is non-digestible by mammalian enzymes

and can be used as a functional fiber (Sajilata, Singhal, & Kulkarni, 2006). Approximately 2.45% of the total starch is resistant starch (Almeida Costa, Silva Queiroz-Monici, Pissini Machado Reis, & Oliveira, 2006). As an ingredient, starch contains soluble macromolecules that provide functionalities such as high viscosity, adhesion, and surface coating (Donald & Richmond, 1997).

2.2.2. Dietary Fiber

The composition of field pea is 14 to 26% (on a dry weight basis) dietary fiber, a portion of which is resistant starch (Almeida Costa et al., 2006; Dahl et al., 2012). The Food and Drug Administration (FDA) defines dietary fiber as the "non-digestible soluble and insoluble carbohydrates and lignin that are intrinsic and intact in plants" (FDA, 2018). Components that make up dietary fiber include cellulose, hemicellulose, pectins, hydrocolloids, and lignin (McKee & Latner, 2000). The consumption of dietary fiber has been linked to the protection against heart disease and cancer (McKee & Latner, 2000).

2.2.3. Protein

The second most abundant component of field pea is protein, accounting for 21.2 to 32.9% of the total composition on a dry weight basis (Dahl et al., 2012). Protein is a macronutrient made up of amino acids linked together into long chains. The majority of the protein in legumes is made up of salt-soluble globulins, which are storage proteins synthesized during seed development (Wang & Arntfield, 2016). The remainder of the existing proteins are albumins. Albumins include proteins that serve functions inside the seed, including lectins and lipoxygenases (Wang & Arntfield, 2016). Pulses do not contain prolamin or gliadin protein fractions associated with the allergenic response of gluten-containing crops such as wheat, rye, or barley (Casper & Atwell, 2014). Furthermore, proteins function to build and repair cells and

body tissue and provide energy for the human body (Rennie, 2005). There are 9 essential amino acids required for adequate health that are not synthesized by the human body and therefore must be consumed in the diet (Havel, Calloway, Gussow, Walter, & Nesheim, 1989). Pulses are incomplete proteins, which means they do not contain all 9 essential amino acids in sufficient quantities. (Mai, Owl, & Kersting, 2005). While low in sulfur-containing amino acids, pulses are high in the essential amino acid lysine (Hall, 2017). When combined, cereal crops and pulses can create a complementary amino acid profile or complete protein (Awika, Rose, & Simsek, 2018). As an ingredient, proteins provide functional properties relating to gelation, emulsifying and foaming behavior (Wang and Arntfield, 2016).

2.2.4. Lipids

The lipid content of field pea ranges from 1 to 4% (Hall, 2017). Moreover, the lipid content can be divided into saturated fatty acids (15 to 20%), monounsaturated fatty acids (27 to 37%), and polyunsaturated fatty acids (42 to 57%) (Villalobos, Patel, Orstat, Singh, & Lefsrud, 2013). Villalobos et al. (2013) found palmitic and steric were the most common of the saturated lipids, with oleic and linoleic being the primary monounsaturated and polyunsaturated lipids, respectively. Oleic acid is the most common unsaturated fatty acid in plants and a precursor to many other polyunsaturated fatty acids (Akoh & Min, 2008). The degradation of polyunsaturated lipids is thought to produce the off-flavors present in pulses (Roland et al., 2017). Nevertheless, these polyunsaturated lipids are favorable for human health. Both linoleic and oleic lipids promote good cardiovascular health by increasing high-density lipoprotein (HDL) levels and decreasing low-density lipoprotein (LDL) levels (Akoh & Min, 2008).

2.2.5. Minerals & Vitamins

Apart from their main constituents, dry peas are rich in a variety of minerals and vitamins (Hall et al., 2017). Accounting for approximately 1.04% of the total weight, potassium is the most abundant mineral of field peas (Dahl et al., 2012). Phosphorus is the second most abundant mineral (Dahl et al., 2012; Hall, 2017). Field pea is an excellent source of iron (Fe), zinc (Zn), magnesium (Mg), and selenium (Se); which were in excess of 10% of the RDA (Hall, 2017). While not as predominant as the previously mentioned minerals, Han and Tyler (2003) found the concentration of the vitamin folate range from 23.7 to 55.6 ug/100g DM in the yellow pea. The consumption of folate is very important for women who are pregnant or may become pregnant. As little as 0.4 mg per day can reduce the chance of neural tube defects during pregnancy by 35% (Wald, 2004). A semi-unfavorable compound to consider when evaluating the mineral content of pulses are phytates. Phytates can bind with Fe, Zn, and Mg to reduce their bioavailability (Dahl et al., 2012). However, phytate at high concentrations has been reported to prevent hydroxide radical formation, acting as an antioxidant (Graf, Empson, & Eaton, 1987).

2.3. Nutritional Benefits

2.3.1. General Health Benefits

The protein, dietary fiber, and mineral contents of pulses are favorable for improving human health. The USDA recommends a 1 ½ c. serving of beans and peas per week for a person consuming a 2,000 calorie diet (USDA, 2015). To ensure adequate protein consumption, this recommendation is higher for vegetarians and vegans. Legumes are an incomplete protein, lacking in the essential amino acid methionine (Galili & Amir, 2013). This can be overcome by combining with a secondary incomplete protein such as a cereal grain, which contains methionine but is lacking in legume having abundant lysine. Combinations such as rice and

beans or wheat crackers with peanut butter are examples of combinations that create a complete protein.

Legumes are high in dietary fiber with low lipid content, providing cardiovascular benefits when properly consumed (Dahl et al., 2012). Moreover, dietary fiber alone can positively improve digestive health. Constipation affects many children and elderly creating mild to severe discomfort. Dahl, Whiting, Healey, Zello, & Hildebrandt (2003) served 3 to 4 foods with a 1-3 g serving of pea hull fiber to patients each day in an elderly home, which produced significant improvements in bowel movements, with a decrease in the amount of prune based laxatives required for each patient. In a similar study, Flogan and Dahl (2010) found that snacks foods fortified with pea hull fiber in combination with inulin fiber supplements increased bowel movement frequency for young children affected by constipation.

In addition to the macronutrients, valuable micronutrients and minerals are present in pulses. For instance, field pea is an excellent source of Fe, Zn, Mg, and Se (Hall, 2017). Dueñas, Estrella, & Hernández (2004) found phenolic compounds in the seed coat and cotyledon of the field pea. Phenolic compounds are bioactive compounds found in plants that act as natural antioxidants (Ho, 1992). Antioxidants have been proposed as a preventative measure for diseases associated with free radicals (Thompson, 1994). The composition of field pea from macromolecules to micronutrients promote the advantages of its incorporation into new food products.

2.3.2. Glycemic Index

A major benefit of pulses is their low-glycemic index. Glycemic index (GI) is an indicator of the impact a carbohydrate has on blood sugar, or glucose levels after eating (Brand-Miller, 2017). The index follows a scale of 0 to 100. Foods with a high GI are rapidly digested

and raise glucose levels quickly. In contrast, low GI foods are digested over a longer period of time, generating smaller fluctuations in blood glucose and insulin levels. The starch and fiber profile of the pea is thought to contribute to the low-glycemic index of field pea (Trinidad, Mallilin, Loyola, Sagum, & Encabo, 2009). Marinangeli, Kassis, & Jones (2009) confirmed the benefits of utilizing pea in low glycemic products by comparing whole yellow pea flour (WYPF) banana bread, biscotti, and pasta with whole wheat flour (WWF). Banana bread and biscotti made from the WYPF reduced glycemic responses compared with the WWF products. Similarly, Fujiwara, Hall, & Jenkins (2017) evaluated the in vivo glucose response of panelists consuming pulse fortified products such as crackers, snacks, cookies, and muffins. Results indicated that all pulse-fortified products fell into the low GI category, with a GI variant of 4.8 ± 26 between the control and pulse variants. Furthermore, yellow pea flour and pea starch have been reported to have better glucose responses than maize starch (Seewi, Gnauck, Stute, & Chantelau, 1999). Evaluations on the strength of low GI foods to promote weight loss have provided mixed results. Nevertheless, a large scale study facilitated by Larsen et al. (2010) found a modest reduction in the GI of diets led to better maintenance of weight loss compared with other dieting methods, suggesting the potential for the use of pulses for weight management. While weight management continues to play an important role in many lives, the rise of preventable health conditions such as type 2 diabetes has begun to gain considerable attention recently.

In 2015, an estimated that 9.4% of the U.S. population was living with diabetes (CDC, 2016). Type 2 Diabetes is caused by an insulin resistance where the body is unable to produce enough insulin to keep blood glucose levels controlled (American Diabetes Association, 2015). Unlike Type 1 diabetes, which is developed at a young age by an immune system defect that destroys the cells that release insulin, Type 2 diabetes is preventable (Anonymous, 2018).

Oftentimes, Type 2 diabetes is treated with lifestyle changes, oral medications, insulin, or a combination of the three. Low-glycemic foods are often suggested as a preventative measure or treatment to Type 2 diabetes (CDC, 2016). A survey found that 68% of dietetics recommend legumes for patients with diabetes (Desrochers & Brauer, 2001). In brief, pulses are rich in protein, dietary fiber, vitamins, and minerals providing the potential to meet the needs of our growing population.

2.4. Agricultural Importance

Pulses prefer cool, dry environments making production well suited for western and northern North Dakota and eastern Montana (Coon et al., 2015). The production of pulses has the potential to provide great monetary and environmental benefits to these areas. Declining prices of North Dakota's primary crops such as soybeans, corn, and wheat, suggest that pulses may provide greater profits than traditional crops. Consequently, an increase in pulse production affects the economy in ND and eastern MT from farm-level production to final product processing (Coon et al., 2015). Coon found these economic impacts to be quite significant. In 2015, North Dakota brought in $115.7 million in pulse related expenditures including sales and personal incomes, with 67.8% of these expenditures derived from field pea. Included in this value are the profits made from the transportation and processing of pulses. Furthermore, an increase in the use of pulse flour as an ingredient may create opportunities for the expansion or creation of new milling and processing facilities.

In addition to the monetary benefits, pulses have the potential to improve the soil health of a field. Pulses, such as field pea and dry bean are often used as break crops in North Dakota (Kirkegaard, Christen, Krupinsky, & Layzell, 2008). Break crops provide environmental benefits such as improving disease control, increasing soil nitrogen levels, and lowering water utilization.

The increase in nitrogen input from legumes removes the need for fertilizers produced from non-renewable energy sources (Evans, McNeill, Unkovich, Fettell, & Heenan, 2001). Furthermore, Kirkegaard et al. (2008) reported that adding break crops such as pulses to a field produced a mean yield increase of 20% or more for wheat planted the next season. An increase in the incorporation of pulses into food products creates a demand for acreage, providing benefits to the economy and environment of pulse growing regions, such as North Dakota.

2.5. Development of Off-Flavors

Off-flavors of a pulse can be found naturally or are developed during harvesting, processing, and storage (Sessa & Rackis, 1977). The most predominant mechanisms of off-flavor development are lipid oxidation and protein degradation. The breakdown of the field pea lipids and proteins produces compounds called volatiles that are responsible for the off-flavor.

2.5.1. Lipid Oxidation

Field pea is comprised of 1 to 4% oil (Hall 2017). The oxidation of these lipids is believed to produce the dominant off-flavors found in pulse flour (Azarnia, Boye, Warkentin, Malcolmson, 2011; Schindler et al., 2012; Vara-Ubol, Chambers, E., & Chambers, D., 2004). Lipid oxidation begins either with enzymatic degradation, which then follows the autoxidation pathway, or begins directly with autoxidation. The main fatty acid in pea flour is linoleic acid, which can be oxidized by enzymes leading to the formation of hydroperoxides (Figure 2.1) (Murat, Bard, Dhalleine, & Cayot, 2013). Lipoxygenase and lipase are the two primary enzymes associated with lipid oxidation (Maarse, 1991). Lipoxygenase is directly responsible for plant oxidation, while lipase produces fatty acids that can readily undergo oxidation. In addition to their role in beany flavor intensification, lipoxygenase and lipase have been found to reduce the shelf life of peas (Wilson, 1996). Lipoxygenase initiates lipid oxidation by abstraction of

hydrogen from methylene interrupted fatty acids (MIFA) (Maarse, 1991). Linoleic and linolenic acid are examples of MIFAs and are the most abundant lipids found in field pea (Villalobos et al., 2013). The abstraction of hydrogen and subsequent oxygen addition results in hydroperoxides during lipoxygenase-promoted oxidation. Autoxidation is another pathway common in plant tissues that produce hydroperoxides (Maarse, 1991; Murat et al., 2013).

Autoxidation is initiated by lipid peroxide radicals that are formed during enzymatic lipid oxidation (Maarse, 1991). This reaction is self-propagating and is terminated when two radicals react with each other. Unstable lipid hydroperoxides participate in secondary reactions that form off-flavor compounds (Maarse, 1991; Murat et al., 2013). The type of compounds formed depends on the type of hydroperoxide formed, temperature, and amount of available oxygen (Maarse, 1991).

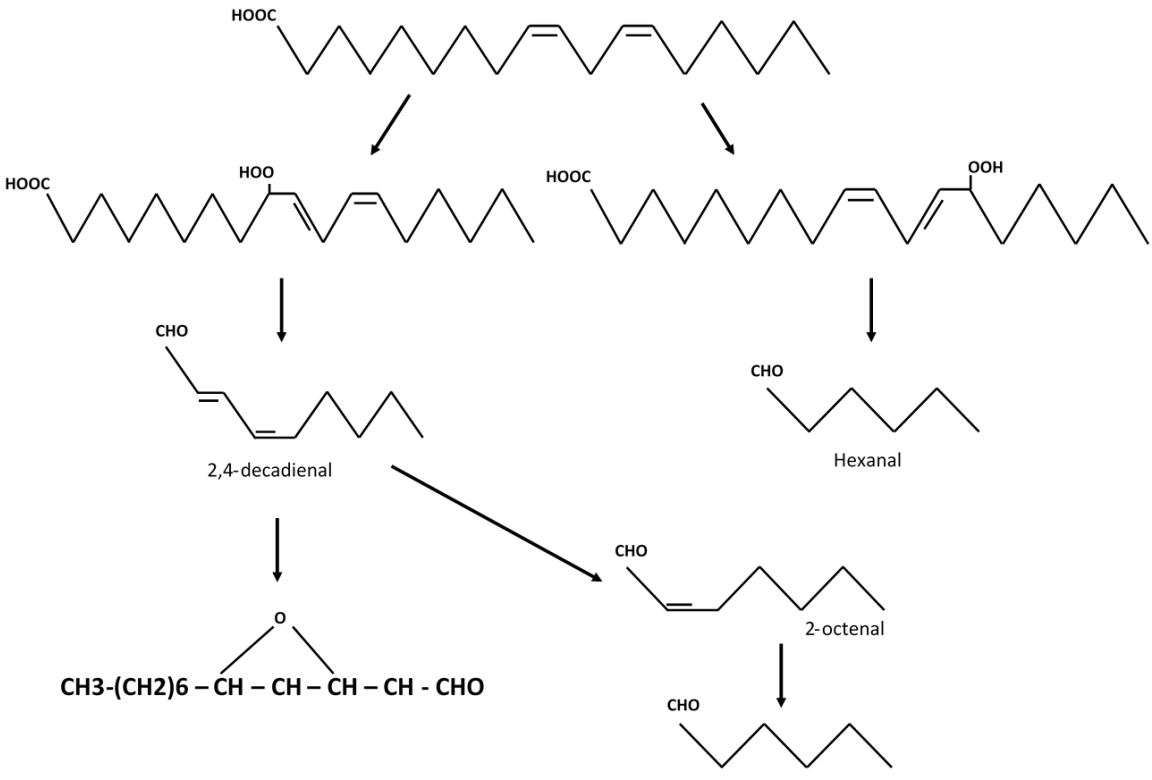

Figure 2.1. Oxidation and degradation products of linoleic acid

The degradation products of enzyme lipid oxidation and autoxidation are volatiles. Volatiles are aromatic compounds of low molecular weight with low boiling points (Fleming-Jones & Smith, 2003). When evaporated, these compounds provide distinct odors, many of which have been classified using gas chromatography-olfactometry (GC-O). Collectively, over 130 volatiles have been found in peas (Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Azarnia et al., 2011b; Murat, Gourrat, Jerosch, & Cayot, 2012; Murat et al., 2013). When evaluating blanched peas, researchers found that the majority of these compounds were degradation products of free fatty acids (Jakobson et al., 1998). Until recently, the majority of volatile research was conducted on fresh or blanched peas rather than dry peas (Azarnia et al., 2011a; Murat et al., 2012, 2013). Sixty-six volatiles have been found specifically in pea flour, with the majority being alcohols, ketones, and carboxylic acids (Murat et al., 2013). Additional responsible compounds include aldehydes, pyrazines, and sulfur compounds (Roland et al., 2017). It is rare for only one compound to be responsible for an odor or flavor (Maarse, 1991). Therefore, it is confidently assumed that a combination of volatiles, rather than a single volatile, are responsible for the beany, earthy flavor of peas (Malcolmson et al., 2014).

The predominant off-flavors of peas are associated with the terms green, beany, pea, earthy, and hay-like (Roland et al., 2017). Hexanol was previously found to be the most abundant compound in pea flour headspace (Jakobsen et al., 1998), which is a grassy, floral scent (Murat et al., 2013). Hexanal produces a similar green odor (Jakobsen et al., 1998). 1-Nonanol is known to provide an odor described as pea, vegetable, and earthy (Murat et al., 2013). Others, such as 1-octen-3-one and 1-octen-3-ol produce a mushroom, vegetable odor (Jakobsen et al., 1998; Murat et al., 2013). Not all volatile odors coincide with the common off-flavors associated with peas. In some cases, when isolated, the volatile may contribute a pleasant or unrelated smell

that is thought to contribute to the overall pea flavor when combined with other volatiles. Examples include octanal, 5-pentyldihydro-2(3H)-furanone, and 4-hydroxy-3-methoxy-benzaldehyde, which provide sweet orange, coconut, and vanilla odors, respectively (Jakobsen et al., 1998; Murat et al., 2013). Nonanal has a solvent-like odor that may additionally influence the pea flavor (Murat et al., 2013).

2.5.2. Protein Degradation

Protein degradation can produce alkyl-methoxypyrazines, which are additionally attributed to pea flavor. 3-Isopropyl-2-methoxypyrazine, 3-sec-butyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine are considered the three most important pyrazines associated with pea flavor (Jakobsen et al., 1998). Murray, Shipton, & Whitefield (1970) found that these methoxypyrazines create significant aroma even at low concentrations. The three volatiles are similar in odor with descriptors such as pea, bell pepper, and green (Jakobsen et al., 1998). While not as well understood as the mechanisms of lipid oxidation, the amidation of amino acids is thought to produce the alkoxypyrazines (Maarse, 1991). Shu (1998) proposes that this mechanism occurs when α-amino acids and reducing sugars react to generate compounds that after rearrangement, undergo Strecker degradation where the final products are condensed into pyrazines. The thermal degradation of phenolic acids and thiamine are hypothesized to further promote the off-flavors of pulses (MacLeod & Ames, 1988). Furthermore, proteins can affect pea flavor by binding volatiles (Wang and Arntfield, 2016), making them difficult to remove during processing.

2.6. Odor Removal Methods

A deeper understanding of the mechanisms and compounds that contribute to the off-flavors found in pulses has created the groundwork needed to develop flavor modification

methods. While some methods occur pre-harvest, such as cultivar selection, the majority occur as a processing step during production. Biological methods, such as germination and fermentation alter the environment of the pulse to remove flavor and/or create a masking effect. Additional treatments include distillation, water treatment, and solvent extraction.

2.6.1. Cultivar Selection

Selecting cultivars with a low pea flavor profile can be used as a pre-harvest odor reduction technique. Selected cultivars have a lower presence of precursors or enzymes that support off-flavor development (Roland et al., 2017). Researchers in Canada, which used gas chromatography to evaluate the volatile flavor compounds of five different pea cultivars grown in the same location, found significant differences between cultivars indicating the potential for flavor reduction through breeding (Azarnia et al., 2011b). While cultivar selection and plant breeding may help in reducing the pea flavor, these methods limit the varieties of pea a production facility can purchase and utilize. Ultimately, finding an optimal processing method provides producers more freedom in the purchasing of raw ingredients.

2.6.2. Germination

As a post-harvest odor removal method, many Asian producers store legumes at ambient temperatures after soaking in water to promote germination, thereby activating enzymes that partially hydrolyze proteins, starch, and oligosaccharides (Shanmugasundaram, 2003). Bitterness is reduced during this process by degrading tannins. Furthermore, germination has been found to decrease antinutritional factors, including the undesirable flavors caused by lipid oxidation (Simons, 2011). As an added benefit, germination has the potential to increase the levels of phytonutrients such as vitamins, phytosterols, saponins, and phenolics (Simons, 2011). A drawback of germination is that as inherent off-flavors are reduced, new undesirable flavors may

arise. Troszyńska et al. (2011) examined the impact of germination on the flavor profile of lentils. After 7 days of germination, the beany and green flavor intensity decreased while an increase in intensity was observed for off-odor, bitterness, and astringency. Overall, the sensory profile was not improved after treatment (Troszyńska et al., 2011). The increase of bitterness and astringency was attributed to the production of tannins and catechin during germination (Vidal-Valverde et al., 1994). While the practice of germination provides benefits to some Asian producers, this process appears limited as a deodorization method for pea flour.

2.6.3. Fermentation

Fermentation is an anaerobic process where microorganisms are used to convert the carbohydrates in food into alcohol or organic acids. Fermentation is most commonly used to reduce bitterness by modifying the structure of saponins (Roland et al., 2017). Lactic acid fermentation of legume protein extracts has shown the potential to improve the aroma of pea protein extracts by reducing the n-hexanal content and masking off-flavors (Schindler et al., 2012). Nevertheless, the impact of the alcohols and organic acids produced during fermentation on the final sensory acceptance must be considered.

2.6.4. Distillation

Distillation can be used to separate the volatiles that produce undesirable odor and flavor (Berk, 2013). The technique separates a solution via boiling. Volatile compounds have lower boiling temperatures than other compounds, therefore, when the substance is boiled, volatile compounds will separate from the liquid as vapor. This can be a continuous or batch process. Continuous flash distillation is the most common method for deodorization in the food industry. The feed mixture is preheated then introduced into the vaporization chamber. Feed immediately comes to a boil and a portion evaporates as the distillate, while the remaining leaves the chamber

as a liquid. Hillen (2016) found that the distillation of pea flour mixed with water at 50, 60, 70, 80, and 85 °C gelatinized the starch, resulting in a pasta-like dough that was unable to be milled back into flour, consequently dismissing distillation as a potential flavor removal method.

2.6.5. Soaking and Blanching

The use of water is a simple, low-cost method of attempting to decrease undesired flavor. Soaking can be used to leach unwanted compounds into the water (Roland et al., 2017). The addition of heat into the soaking system may further improve the flavor. Macleod et. al (1988) suggested blanching as a technique to inactive lipoxygenase, the enzyme found responsible for off soy flavors. Blanching is a process where a food ingredient or finished product is placed in boiling water for a short period of time, then cooled in ice water to discontinue the cooking process. Lv et al. (2011) found that hot water blanching for up 10 min to was able to successfully reduce the lipoxygenase activity of soymilk. During the treatment, non-beany flavors were reduced, which may be undesirable for soymilk, but beneficial for producers that desire a neutral pulse ingredient. The use of water treatments in processing requires simple equipment and the use of resources most likely already available to the processor; however, the high energy costs of heating water is something a processor must consider when evaluating the sustainability of their facility. Furthermore, research is limited regarding the sensory impact soaking and blanching have on the acceptability of the final pulse product.

2.6.6. Solvent Extraction

The final method of consideration is solvent extraction. Organic solvents have been found to be effective in removing phenolic compounds from legumes (Roland et al., 2017). Combinations of ethanol and water allow for the extraction of both water and ethanol soluble compounds (Do et al., 2014). Therefore, a reduction of pea flavor may be attributed to the

removal of water and ethanol soluble volatiles. Chang et al. (2018) evaluated the use of acetone, ethanol, and isopropanol at 35 to 95% (v/v) to treat lentil protein isolate. Aqueous ethanol and isopropanol treatments at 75% (v/v) removed significant amounts of volatile compounds with the lowest overall impact on the physiochemical and functional properties of the isolate. Previous research on the sensory acceptance of pea flour treated using solvent extraction has been completed. Hillen (2016) found that 1:1 and 3:1 v/v high-pressure ethanol extraction significantly ($P<0.05$) improved cake and cookie acceptability scores. Yet, there is a need for further analysis of the impacts the treatment has on the pea flour composition and functionality. The objective of the current study is to fill these gaps and confirm the results of Hillen.

2.7. Pulse Acceptability in Food Products

2.7.1. Fortification with Pulse Flour

Fortification can be used to increase the nutritive benefits of products without the complete replacement of traditional flour. The opportunities for fortification include wheat-based products such as baked goods, soups, and extruded products. Current literature on pulse-containing products has indicated that the fortification of snacks and desserts may be more accepted than bread or pasta. Fujiwara et al. (2017) found sensory scores of pulse fortified crackers, cookies, and granola bars were not significantly ($P>0.05$) different from the control at levels of 9-11%. Furthermore, Qayyum et al. (2017) found biscuits could be acceptably fortified with pea flour up to 20%. The results of Fujiwara et al. (2017) and Qayyum et al. (2017) suggest, that in low amounts, the fortification of products with raw pulse flour is generally accepted. When fortification levels increase, issues in acceptability begin to rise. For instance, Jeyanthi (2016) found that cupcakes fortified with green gram (mung bean) were similarly accepted to the control at 25%, but significantly disliked at levels above 50%.

A staple food across many cultures, bread has many opportunities for fortification; however, it is a rather simple product with neutral palate ingredients, making it difficult to mask the pulse flavor. Additionally, gluten is integral to proper dough development, so fortification with non-gluten ingredients can quickly become detrimental to the final product. For these reasons, the impact of fortification may differ slightly from other food types. For instance, Kamaljit, Baljeet, & Amarjeet (2010) found that bread fortified with pea flour at 5% did not differ from the control, yet when increased to 10%, a significant decrease in acceptability occurred. Furthermore, when evaluating wholemeal bread enriched with pea flour, Mastromatteo et al. (2015) found a significant decrease in overall quality at 5% enrichment compared with the control. Secondary formulations mitigated quality issues by incorporating guar gum at 2%, suggesting that additives play an important role in the formulation. The type of bread can further impact acceptance. For instance, Fujiwara et al. (2017) produced focaccia bread fortified with 16% pulse that did not differ ($P>0.05$) from the control, indicating that breads with a more complex flavor profile are able to mask some of the pulse flavors. A gap found within the literature is a lack of large scale acceptance testing. The majority of the current literature results provided were from panel sizes of under 15, which may not be enough to fully understand the scope of the general public's acceptance of pulse fortified products.

2.7.2. Complete Pulse Flour Replacement

A complete replacement can be made where 100% of the flour used is pulse. Oftentimes, this is to create a gluten-free product. Marinangeli et al. (2009) evaluated banana bread, biscotti, and pasta made from 100% whole yellow pea flour (WYPF) and 100% whole wheat flour (WWF) based on appearance, taste, texture, smell, and overall acceptance of the product. The replacement of WWF with WYPF in banana bread and biscotti produced no significant ($P>0.05$)

differences in all five attributes. In contrast, the smell of the WYPF pasta was ranked as poor, leading to a significantly lower overall acceptance score. Marinangeli et al. (2009) suggested that the neutral flavor of pasta was not sufficient enough to cover the pea odor and flavor compared with the banana bread and biscotti, which contained more sugar and have a stronger flavor. Furthermore, Jeradechachai (2012) found that, while the optimized gluten-free bread produced with yellow pea flour had a longer shelf life, the acceptability was significantly lower ($P<0.05$) compared with the commercial premix bread product. The results of Marinangeli et al. (2009) and Jeradechachai (2012) present issues regarding complete pulse flour replacement in neutrally flavored products such as bread and pasta.

From an industry standpoint, pea flavor issues are considered one of the top concerns regarding increased pulse utilization. Modification of pulse flour has been shown to improve the acceptability of pulse flour, therefore allowing the complete fortification of products. Hillen (2016) extracted pea flour using high-pressure solvent extraction (HPSE) to reduce pea flavor. Products were produced using raw and treated pea flour and evaluated on a 9-point hedonic scale. Results indicated that cake produced from 100% pea flour was improved from an average of 3.8 for the raw pea flour to a value of 6.5 and 6.4 for the 1:1 and 3:1 HPSE treated flours, respectively. The reduction of pea flavor improved the acceptance of sugar cookies in a similar manner (Hillen 2016). Likewise, pre-cooked flours have been found to have lower pulse flavor than the raw counterpart, although complete elimination of off-flavors may not occur (Roland et al., 2017). The treatment of pulse flours may facilitate the improvement in acceptance of 100% fortified pulse products.

2.8. Gluten-Free Products

2.8.1. Gluten-Free Market

Gluten is a protein complex made when the protein fractions glutenin and gliadin are mixed together in the presence of water (Gallagher, Gormley, & Arendt, 2004). Sources of gluten include wheat, kamut, spelt, rye, and barley (Hüttner & Arendt, 2010). The gluten-free sector is rapidly growing, increasing from 2.8% to 6.5% of the food market share from 2013 to 2015 (Statistica, 2018). A typical gluten-free consumer is someone with celiac disease, gluten intolerance, or a belief that gluten is harmful to their health. Celiac disease is an autoimmune disorder where the consumption of gluten damages the small intestine (Celiac Disease Foundation, 2018). As medical technologies advance, the number of patients being diagnosed with celiac disease has increased (Casper & Atwell, 2014). Consumers with celiac disease and gluten intolerances remain consistent members of the gluten-free market. Consumers who live a gluten-free lifestyle by choice that are the most unpredictable segment of the market (Casper and Atwell, 2014). From 2009 to 2010, this segment of consumers increased from 0.52 to 1.69% (Kim et al., 2016). By 2020, the gluten-free market is expected to be worth $7.59 billion (Statistica, 2018). Primary products included in the gluten-free market are those traditionally made with wheat flour, such as bread, pasta, or baked goods. Other "hidden" sources of gluten include thickened sauces, soups, pudding, and sausages (Hüttner & Arendt, 2010). An important note is that the gluten-free market does not always coincide directly with the wheat-based product market. For example, while bread has remained the largest product segment for years within the wheat-based market, it was replaced in volume by pasta, crackers, cookies, pizza, and pancake mix in the gluten-free market (Casper & Atwell, 2014). The goals of the gluten-free

market are to replace diet staples made from conventional wheat while keeping in mind the evolving needs and desires of the consumer.

2.8.2. Gluten-Free Baking Challenges

Products made from gluten-free ingredients produce a variety of challenges for producers and consumers. Gluten is the required structural protein for breadmaking and is responsible for the elastic properties of dough, bread appearance, and crumb structure (Gallagher, Gormley, & Arendt, 2004). Rice, corn, potato, and tapioca are common gluten-free replacements to wheat flour (Casper & Atwell, 2014). Oftentimes, the replacement of wheat with gluten-free ingredients produces low quality, poor mouthfeel product (Gallagher, Gormley, & Arendt, 2004). The market has pushed for innovation from researchers to combat these issues. Gluten plays an important role in gas retention which develops loaf volume. A lack of gas retention leads to improper rising of the dough. Gallagher, Gormley, & Arendt (2004) has proposed the use of gels to mitigate this issue. Available gels include rice starch, gums, and hydrocolloids, such as xanthan, guar gum, and cellulose (Casper & Atwell, 2014; Gallagher et al., 2004). Hydrocolloids work to increase the viscosity of the dough (Casper & Atwell, 2014). Furthermore, Selinheimo, Autio, Kruus, & Buchert (2007) found that enzymes laccase and tyrosinase improve gas retention while softening the bread crumb texture. Results were further improved when used in combination with xylanase.

Another issue with gluten-free products is a reduction in water retention, which leads to a dry product with poor crumb quality. In addition to their gas retention abilities, hydrocolloids can additionally improve water retention (Casper & Atwell, 2014). Oftentimes, the synergetic relationship of hydrocolloids shows the improvements made from a combination is far greater than a sum of two hydrocolloids used separately (Saha & Bhattacharya, 2010). Dairy products

have the potential to improve water retention but are limited as an ingredient due to the lactose intolerances experienced by celiac patients (Gallagher, Gormley, & Arendt, 2004).

Much of the grains, pseudocereals, oilseeds, and tubers used as gluten-free ingredients are first ground and isolated into a starch (Casper & Atwell, 2014). The use of refined starches creates a product lacking fiber and nutrients, therefore nutrition is an important aspect to consider when formulating gluten-free products (Casper & Atwell, 2014; Gallagher et al., 2004). Furthermore, (Mariani et al., 1998) found that adolescent children with celiac disease consumed more protein and lipids than others in their age group. The use of inulin as an ingredient to improve baking quality has an added benefit of functioning as soluble prebiotic fiber (Juszczak et al., 2012). Finding ingredients that maintain baking quality while improving nutrition is important to the health of those following a strict gluten-free diet.

3. PROBLEM STATEMENT

3.1. Objectives

Objective 1: To determine the optimal conditions of ethanol extraction (ethanol concentration, pressure cycles, extraction time) that removes the most pea flavor.

Objective 2: To characterize the chemical composition and physiochemical properties of the deodorized pea flour.

Objective 3: To examine and compare the changes in the volatile composition of the treated and untreated (raw) pea flour using gas chromatography (GC).

Objective 4: To evaluate the sensory attributes and shelf-life of cookies and crackers produced from raw and ethanol extracted pea flours.

3.2. Hypotheses

Objective 1: Given the parameters tested, an optimal treatment that reduces the pea flavor most effectively will be found.

Objective 2: Ethanol extracted pea flour will have a similar chemical composition, physiochemical properties and shelf life stability of raw pea flour.

Objective 3: Ethanol extracted pea flour will have reduced volatiles.

Objective 4: The sensory acceptance of cookies and crackers produced from ethanol extracted pea flour will be greater than that of the raw pea counterparts.

4. OPTIMAL SOLVENT EXTRACTION DETERMINATION

4.1. Abstract

A central issue delaying an increase in pea utilization is the low acceptability rating of pea flavor. The objective of this research was to determine the optimal solvent extraction parameters that would result in flour with the lowest pea flavor profile. Extraction solvent was non-denatured 95% ethanol and distilled water, which produced aqueous ethanol at concentrations of 9.5, 47.5, and 90.3%. Pressure cycles were either 3 or 6 min, resulting in extraction times of 27 to 93 min. A combination of qualitative and quantitative sensory evaluation was used to evaluate the pea flavor intensity of the extracted pea flours. Optimal conditions were based on sensory results that supported minimal pea flavor. Results suggested that ethanol at 9.5% concentration was not sufficient enough to remove pea flavor; while the 90.3% concentration produced adverse flavors, which panelists perceived as adding to pea flavor. Ranking results indicated that a treatment utilizing 47.5% aqueous ethanol, 3 min compression time, and 63 min extraction time was perceived to have a significantly ($P<0.05$) lower pea flavor intensity compared with other treatments. Moreover, an additional treatment completed without the aid of pressure was found to further decrease pea flavor. Therefore, optimal treatment parameters included aqueous ethanol at a concentration of 47.5%, an extraction time of 63 min, and no pressure.

4.2. Introduction

Pulses, such as peas can be used to create specialty products that differ from traditional wheat-based goods. Pea flour is considered a gluten-free, low-glycemic ingredient. Dry peas are rich in protein (21.2 to 32.9 %) and dietary fiber (14 to 26 %) (Dahl, Foster, & Tyler, 2012). Apart from their main constituents, dry peas are rich in minerals, folate, and amino acids,

including lysine, which is limiting in cereal crops (Hall, Hillen, & Garden-Robinson, 2017). Despite the benefits of field peas, flavor issues are one of the primary concerns regarding pea utilization. Volatiles produced during lipid degradation are believed to be the cause of pea flavors (Vara-Ubol, Chambers, & Chambers, 2004; Azarnia et al., 2011b; Schindler et al., 2012). Cultivar selection has been suggested as a pre-harvest technique to reduce unwanted flavor. However, this method limits producers to use only select varieties. Flavor modification post-harvest provides producers more freedom in the purchasing of raw ingredients. Some of these techniques include germination (Shanmugasundaram, 2003; Troszyńska et al., 2011), fermentation (Roland, Pouvreau, Curran, Velde, & Kok, 2017; Schindler et al. 2012), distillation (Berk, 2013), water treatment (Lv, Song, Li, Wu, & Guo, 2011; Roland et al., 2017), and solvent extraction (Chang, Stone, Green, & Nickerson, 2018; Xu & Chang, 2007). Hillen (2016) and Roland et al. (2017) determined ethanol solvent extraction was one of the most viable options to flavor reduction. Combinations of ethanol and water allow for the extraction of both water and ethanol soluble compounds (Do et al., 2014). Therefore, a reduction in pea flavor may be attributed to the removal of water and ethanol soluble volatiles. Oftentimes, the use of high-pressure extraction is combined with aqueous ethanol (Hillen, 2016). However, there is limited research on the optimization of the combination of these techniques. Thus, the objective of this research was to determine the best treatment parameters for high-pressure ethanol solvent extraction (HPSE). Parameters included % solvent concentration, pressure cycle time, and extraction time. A combination of qualitative and quantitative sensory panels was used to establish the treatment that produced the lowest pea flavor profile.

4.3. Materials

Dry yellow peas (Pisum Sativum L.) were obtained from Viterra and Special Commodities located in North Dakota and SK Foods (Moorhead, MN). Materials utilized for creating quantitative descriptive analysis standards included rice flour and corn starch, obtained from Food Service of America (Fargo, ND), and quinine (Sigma-Aldrich).

4.4. Experimental Design and Statistical Analysis

Raw pea flour was treated in triplicate using a randomized complete block design (RCBD). Triplicate samples were blended at equal ratios prior to sensory evaluation. An RCBD model was used for all sensory evaluations. Qualitative descriptive analysis results were evaluated by analysis of variance (ANOVA) and least significant differences (LSD) methods using the JMP program (Genomics 14). A confidence level of 95% ($P<0.05$) was used to establish significant differences among the data. Ranking results were evaluated using a Freidman analysis (Traynham, Myers, Carriquiry, & Johnson, 2007) at the confidence level of 95% ($P<0.05$).

4.5. Methods

4.5.1. Milling

Peas (50 kg) from the three sources were combined using a paddle mixer located at the Northern Crops Institute (Fargo, ND) to create a composite sample (150 kg). The composite sample was stored at 15ºC until milling. A pilot-scale hammer mill (Fitzpatrick, Elmhurst, IL) was used to mill the composite pea sample into flour using a 1.270 mm screen with a hammer rotation of 102 m/s. Pea flour was stored in a 32-gallon Brute utility container lined with a sealed polyethylene bag at -20ºC until needed for extraction and sensory analysis.

4.5.2. High-Pressure Solvent Extraction

Methodology was based on previous research that used ethanol extraction as a method to reduce pea flavor (Hillen, 2016). A Timatic Micro Series Extractor (Supercritical Fluid Technologies Inc., Newark, DE) was used for the extraction (Figure 4.1). The extraction solvent was a combination of non-denatured 95% ethanol and distilled water in ratios of 10:90, 50:50, and 95:5 v/v, ethanol: distilled water. This produced aqueous solvent with ethanol concentrations of 9.5, 47.5, and 90.3%. Pressure cycles included 3 or 6 min of compression followed by a 6 min decompression. Extraction times were set using the number of cycles during an extraction. Due to the limitation of not being able to directly set the extraction time, cycles were set to achieve extraction times as close to 30, 60, and 90 min as possible (Figure 4.1). The pressure was set from 0.41 to 0.62 Bar (6 to 9 psi) to allow the solvent to flow through the flour.

Table 4.1. Total treatments and extraction parameters

Treatment	Ethanol Concentration %	Compression Time (min)	Decompression Time (min)	# Cycles	Total Time (min)
Raw	0	0	0	0	0
1	9.5	3	6	3	27
2	9.5	6	6	3	36
3	9.5	3	6	7	63
4	9.5	6	6	5	60
5	9.5	3	6	10	90
6	9.5	6	6	8	96
7	47.5	3	6	3	27
8	47.5	6	6	3	36
9	47.5	3	6	7	63
10	47.5	6	6	5	60
11	47.5	3	6	10	90
12	47.5	6	6	8	96
13	90.3	3	6	3	27
14	90.3	6	6	3	36
15	90.3	3	6	7	63
16	90.3	6	6	5	60
17	90.3	3	6	10	90
18	90.3	6	6	8	96

A mesh bag held 150 g of raw pea flour in the extraction chamber. Aqueous ethanol was added to the mesh bag until almost full and stirred to ensure a complete mixture. The mesh bag was sealed, and the remainder of the chamber was filled with the aqueous ethanol. In total, approximately 1.5 L of aqueous ethanol was utilized. After extraction, the chamber was drained under pressure. Additional solvent was removed by compressing the mesh bag prior to spreading the extracted mixture thinly in 22.9 x 33.0 cm foil pans.

Figure 4.1. HPSE Timatic Micro Series extractor (Supercritical Fluid Technologies Inc.)

4.5.3. Room Temperature Solvent Extraction

Following the HPSE method development, room temperature solvent extraction processing occurred. All steps, equipment and time parameters remained the same, apart from pressure, which was removed as a treatment condition.

4.5.4. Vacuum Oven Drying

After the extraction, foil pans containing the treated samples were placed in a vacuum oven (Buflovak, Buffalo, NY, USA) to remove any remaining solvent. A total of 8 foil pans, or treatments, could be dried at one time. Samples were dried at 65 ± 2 °C and 1.24 ±0.14 Bar (18 ± 2 psi) for 16 h. Prior to milling, the dried flour samples were transferred to 16.5 x 39 cm

metallic, zipper sealed bags (Pacific Bag, Des Moines, IA) and placed in the freezer (-10 to -15°C) until needed for subsequent testing.

Figure 4.2. Vacuum oven (Buflovak, Buffalo, NY, USA) used for drying of samples

4.5.5. Milling Treated Samples

After drying, samples were re-milled using a Retsch Z-Mill (Ultra Centrifugal Mill ZM 100, Haan, Germany) at 14,000 rpm. The treated flour samples (150 g) were first milled using a 3.0 mm screen, then divided into two subsamples (≈75 g) that were milled with a 0.5 mm screen. The temperature of the lid was monitored to ensure the mill was not overheating. As soon as the lid became warm to the touch (55 ± 5 °C), milling was paused until the lid reached 21 ± 1 °C. Additionally, a 20 min break was taken after 3 samples were milled. These precautions were set to ensure the heat of the mill did not significantly affect the functionality of the pea flour or alter the volatiles. Milled samples were transferred to 16.5 x 39 cm metallic, zipper sealed bags (Pacific Bag, Des Moines, IA) and stored in the freezer at (-10 to -15 °C) until needed.

Figure 4.3. Retsch Z-Mill (Ultra Centrifugal Mill ZM 100, Haan, Germany)

4.5.6. Sensory Evaluation Methodology

A combination of qualitative and quantitative methods was used to determine the optimal extraction treatment. The alternating use of these methods is illustrated below (Figure 4.4).

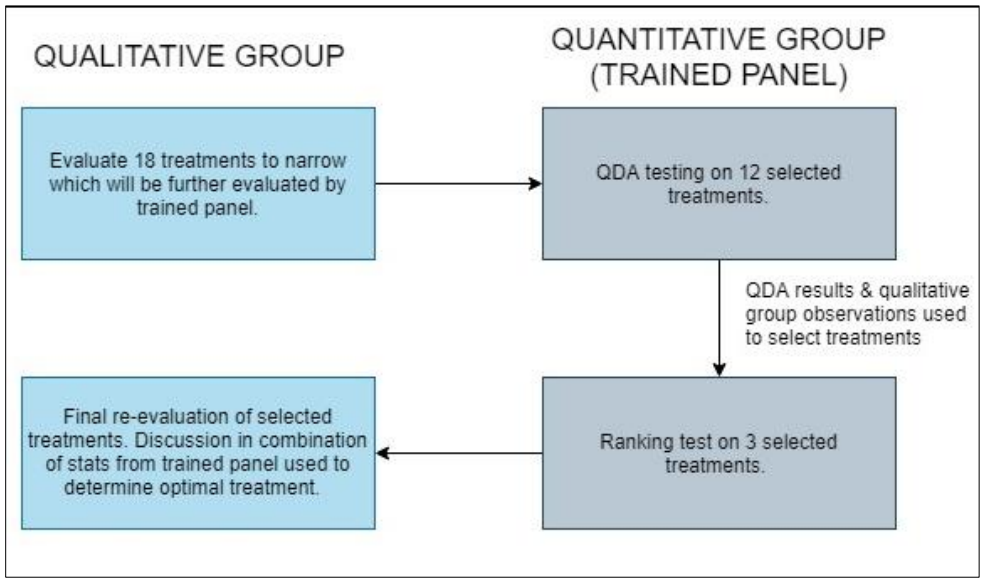

Figure 4.4. Overall sensory evaluation scheme for optimized treatment determination

4.5.6.1. Quantitative (trained panel) group

Trained panelists were used to determine the quantitative results of the pea flour treatments (Figure 4.5). Quantitative descriptive analysis (QDA) and ranking tests were completed to provide a numeric analysis to compare with the evaluations of the qualitative group.

Panelists were selected from the cereal science research group at North Dakota State University. Panelists were initially trained in QDA over five, one hour sessions. Samples containing combinations of pea flour, rice flour, and quinine were used to train panelists on pea flavor, cardboard, and bitterness, respectively. Corn starch was used as a neutral ingredient to transfer diluted amounts of the mentioned flavors. If more than two months had passed since the initial training then panelists were re-trained with five, half-hour sessions.

Upon training, five trained panelists participated in a four-day QDA panel. Three treated samples with a standard were evaluated during each session. The standard consisted of a mixture of either corn starch, raw pea, rice flour or quinine that was previously seen by panelists during training. Standards were used to assess the accuracy and consistency of the panelists. Samples were presented in plastic cups labeled with a random three-digit number. Pea flavor, bitterness and cardboard flavor intensities were quantified using a 156 mm scale (Figure 4.5). Panelists were provided unsalted oyster crackers and purified water between samples to prevent the crossover of flavors between samples. The QDA test served the purpose of determining the highest and lowest rated treatments that would advance to further testing.

Sample Code XXX

Flavor

Undesirable — Desirable

Weak Pea — Strong Pea

Weak bitterness — Strong bitterness

Cardboard not detected — Strong Cardboard

Comment

Figure 4.5. Qualitative descriptive analysis pea flour scaling score sheet

Subsequently, panelists participated in a pea flavor intensity ranking test. Three selected treatments and a raw sample were evaluated during a one day panel. Again, samples were presented in plastic cups labeled with a random three-digit number. Sensory sheets (Figure 4.6) instructed panelists to rank samples from the highest to the lowest pea flavor intensity. Again, panelists received oyster crackers and water to clean the palate between samples.

Pea Intensity Ranking

Directions:

1. Please rank the samples for pea flavor intensity. Rank the **highest pea flavor intensity as first** and the sample with the **lowest pea flavor intensity as last**.

2. Eat a cracker and have a sip of water between samples to prevent carry-over between samples.

3. Be sure to disperse the sample throughout the mouth when tasting to get the full flavor.

Taste the samples in the following order: 817 425 512

#1: _____

#2: _____

#3: _____

Comments:

Figure 4.6. Pea intensity ranking score sheet used by trained panelists

4.5.6.2. Qualitative group

A subgroup of four trained panelists, led by a senior panelist, were used as the qualitative group that discussed the off-flavors and intensities of the treated pea flour. Prior to QDA analysis, the original 18 treatments were evaluated by the qualitative group to narrow the number of treatments to be evaluated. Three sessions were held to test the flours from the six treatments within the 9.5, 47.5, and 90.3% ethanol concentrations. Sensory sheets (Figure 4.7) were provided to guide the panel discussion. The impact of pressure cycles and time were used to determine the treatments for QDA analysis. Following the QDA analysis, the treatments that were the lowest and highest rated treatments during the QDA analysis, in addition to the treatment most approved by the qualitative group, were subjected to a ranking evaluation.

Extracted Pea Flour Sensory

Group 1: 9.5% ethanol used for extraction

Treatment	Time (minutes)	Comments? Difference b/w other similar times?
13	27	
15	63	
16	60	
17	90	

Figure 4.7. Sensory sheets used for the qualitative panel to narrow down the original 18 treatments. The sheet was modified to also be used for the 9.5, 47.5, and 90.3 % ethanol concentrations evaluated

Upon completion of the ranking test by the trained panelists, the three selected treatments were re-evaluated by the qualitative group. Observations from the qualitative group were compared with the statistical differences recognized by the trained panel during the ranking test to ensure the treatment with the lowest pea flavor was being advanced. Furthermore, a final evaluation was completed by the qualitative group to determine if the absence of pressure had an impact on the perceived pea flavor intensity. Results from this final session determined the finalized treatment parameters that would be used for further evaluation as presented in Chapters 5 and 6.

4.6. Results and Discussion

4.6.1. Treatment Selection

As previously stated, the preliminary sessions of the qualitative group determined which of the original 18 treatments would advance to the QDA analysis. A focus was placed on whether or not there were significant differences between samples extracted for similar times. A consensus from the qualitative group found the 27 and 36 min treatments, as well as the 90 and 96 min treatments, were indistinguishable. This suggested that compression time did not have a significant impact on the effectiveness of pea flavor removal. In order to decrease processing times, treatments with the lowest extraction time (27 and 90 min) were selected to advance. Slight differences were detected between the 60 and 63 min extraction times, advancing both to the QDA analysis. These similarities and differences between time points remained consistent at all ethanol to water ratios. Based on these results, a total of 12 treatments were selected for QDA testing. It was important that all ethanol concentrations be evaluated. Therefore, the parameters tested included ethanol concentrations of 9.5, 47.5, and 90.3%, as well as 27, 60, 63, and 90 min extraction times.

4.6.2. Qualitative Descriptive Analysis with Qualitative Input

The objective of the QDA test was to determine the treatment that reduced the off-flavors to the greatest extent. Bitterness and cardboard intensities were found to fluctuate significantly ($P<0.05$) between panelists. As a result, these attributes were removed from the analysis in order to focus on the removal of pea flavor, which was the primary objective of the extraction. A general trend indicated that pea intensity decreased with decreasing percentage of ethanol, however, a few treatment outliers made this observation non-significant ($P >0.05$). The impact of extraction time on pea flavor intensity was not significantly different ($P>0.05$).

Treatment 4 (9.5% ethanol concentration, 6 min compression, 60 min extraction time) and 17 (90.3% ethanol concentration, 3 min compression, 90 min extraction time) were scored as having the lowest and highest pea flavor intensities, respectively. The purpose of re-evaluating treatments with the lowest and highest pea intensity scores via ranking test was to differentiate an effective process from one that was ineffective. The addition of a treatment ranked as having a high pea intensity was used to compare two samples that underwent the same processing, placing a focus on potential off-flavors that may develop during processing.

In agreement with Treatment 17, the remainder of the flours extracted at 90.3% ethanol concentration were scored as having notably higher pea intensities compared with the 9.5 and 47.5% ethanol concentrations. The lack of pea flavor reduction observed in treatments extracted at 90.3% ethanol suggests that in excess, ethanol may produce adverse flavors that panelists perceived as contributing to overall pea flavor intensity.

Treatment 9 (47.5% ethanol concentration, 3 min compression, 63 min extraction time), which was described as having a “neutral flavor, no bitterness” by the qualitative group, contrasted with the QDA results when it was not ranked as the highest rated sample. Therefore,

both Treatment 9 and Treatment 4 were included in the ranking test as a means of confirming the pea flour with the lowest pea flavor intensity through a secondary testing method. Consequently, the addition of Treatment 9 ensured that treatments from all ethanol and distilled water ratios were being evaluated.

4.6.3. Ranking Analysis

Ranking analysis was used to finalize the treatment results. All three solvent extracted samples, Treatment 4, Treatment 9, and Treatment 17, had significantly lower ($P<0.05$) pea flavor than the raw pea flour. Results from the ranking test confirmed the assessments of the qualitative group. Treatment 9 was ranked as having the lowest pea intensity, which was significantly lower ($P<0.05$) than Treatment 4, 17, and the raw pea flour sample (Table 4.2). Ranking tests are often easier for panelists to complete because ranking comes more naturally than scaling (Valentin, Chollet, Lelivevre, & Abdi, 2012). Therefore, the results of the ranking test were accepted as providing the best estimate of pea flavor intensity. Furthermore, upon completion of the ranking test, the qualitative panel blindly re-evaluated flours subjected to Treatment 4, 9, and 17 using three-digit codes, confirming the original assessments. Literature suggests that solvent ratios ranging from 50 to 75% were optimal at removing volatiles from pea flour (Chang et al., 2018; Hillen, 2016), which again confirms the determination of the qualitative group as well as the ranking results.

Table 4.2. Pea intensity ranking results of raw and treated pea flours

Treatment	Rank Sum**
47.5% aqueous ethanol, 3 min compression, 63 min extraction (Trt 9)	54a*
9.5% aqueous ethanol, 6 min compression, 60 min extraction (Trt 4)	40b
90.3% aqueous ethanol, 6 min compression, 90 min extraction (Trt 17)	32b
Raw (untreated)	14c

*Sums not connected by the same letter are significantly different ($P<0.05$) from each other.
**Number of panelists = 14. Ranking value of 1 = highest pea flavor intensity, ranking value of 4 = lowest pea flavor intensity. Therefore, the sample with the highest rank sum was considered the sample with the least pea flavor.

4.6.4. Finalized Optimal Treatment

The ranking test in combination with the qualitative group evaluation confirmed that Treatment 9 (47.5% ethanol concentration, 3 min compression, 63 min extraction time) produced the lowest pea flavor intensity of the evaluated treatments. A final evaluation was conducted to determine if the removal of pressure from the treatment parameters would impact the overall flavor. An overwhelming agreement from the qualitative group indicated that a lack of pressure further decreased the pea flavor intensity of the sample. Removal of pressure from the treatment generates savings for processors through a reduction in energy and equipment costs. Therefore, the final optimized treatment utilized aqueous ethanol at a concentration of 47.5% for 63 min at room temperature (21 ± 1 °C).

4.7. Conclusion

The use of solvent extraction for the treatment of pea flour indicated overall positive results regarding the reduction of pea flavor. Pressure, solvent ratio and extraction times were set with the intention of testing a variety of parameters. A series of qualitative and quantitative sensory sessions were used to evaluate the effectiveness of the treatment parameters in producing

the lowest pea flavor profile. While high-pressure solvent extracted samples were significantly ($P<0.05$) lower in pea flavor than the raw flour, removing pressure further improved the flavor of the flour. As a result, pressure was determined not to be critical for pea flavor removal. A final review determined the best treatment utilized aqueous ethanol at an ethanol concentration of 47.5%, extraction time of 63 min, and no pressure. Primary research indicated the treatment successfully removed pea flavor, but further evaluation is needed to determine the practicality of its utilization within the food industry.

5. IMPACT OF SOLVENT EXTRACTION ON THE COMPOSITION AND PHYSIOCHEMICAL PROPERTIES OF PEA FLOUR

5.1. Abstract

While previous research has confirmed the ability of solvent extraction to reduce off-flavors in pea flour, there is lacking knowledge of its impacts. Therefore, the objective of this study was to compare the chemical composition and physiochemical properties of raw pea flour with treated pea flour. Treated samples were subject to 1.5 L of 47.5% aqueous ethanol (diluted with distilled water) for 63 min. Results indicated a significant decrease ($P<0.05$) in moisture and ash content after treatment, with no loss of protein, total starch, or resistant starch. An increase in water absorption impacted WAI, WSI, setback, and peak time observations. Remaining pasting profile values were unchanged ($P<0.05$). Foaming capacity (56% to 27%) and foaming stability were reduced compared to raw pea flour, indicating that proteins were potentially altered during treatment.

5.2. Introduction

Field pea is a protein and dietary fiber-rich crop with a low-glycemic index (Dahl, Foster, & Tyler, 2012; Trinidad, Mallilin, Loyola, Sagum, & Encabo, 2009). Unfortunately, the health benefits of utilizing field pea as flour are hindered by the low acceptance of pea flavor (Saint-Eve, Granda, Legay, Cuvelier, & Delarue, 2019). Previous research has presented solvent extraction as a successful option in removing the off-flavors found in pulse flour (Hillen, 2016; Roland, Pouvreau, Curran, Velde, & Kok, 2017; Chang, Stone, Green, & Nickerson, 2018). While the combination of ethanol and water has been found to reduce pulse flavor, the effects of this treatment on composition and physiochemical properties have not been studied extensively. Furthermore, there is a gap of knowledge regarding the impacts of ethanol extraction on the

functionality of the pea flour. In an attempt to fill in these gaps, the composition and physiochemical properties will be evaluated for both raw pea flour and the treated flour counterpart. An objective of this study was to determine the moisture, protein, ash, lipid, total starch, and resistant starch contents. To evaluate the physiochemical properties of the flour, analyses including water absorption index, water solubility index, moisture isotherms, foaming properties, and pasting profiles were assessed. In summary, the objective of this research was to determine the impact of ethanol solvent extraction on the final functionality of the pea flour based on a collection of testing parameters.

5.3. Materials

Three replicates of the raw pea flour were extracted with solvent consisting of 47.5% of ethanol (i.e. aqueous ethanol) for 63 min at room temperature. With each extracted treatment, a corresponding untreated replicate was created by transferring 150 g of raw sample into 16.5 x 39 cm metallic, zipper sealed bags (Pacific Bag, Des Moines, IA). Raw and treated pea flour was stored in the freezer (-10 to -15 °C) until utilized.

5.4. Experimental Design and Statistical Analysis

A randomized complete block design (RCBD) was utilized as the experimental design. Raw pea flour was extracted in triplicate, over three separate days. Quality tests were additionally run in triplicate, with three replications per raw or extracted sample. Data was evaluated using two-way analysis of variance (ANOVA) and least significant differences (LSD) was used to determine differences between treatments using the JMP program (Genomics 14). A confidence level of 95% ($P<0.05$) was used to establish significant differences among the data.

5.5. Methods

5.5.1. Pea Flour Treatment

Raw pea flour was extracted using ethanol at 47.5% concentration for 63 min, the best treatment as determined in Chapter 4. In brief, 150 g of pea flour was soaked with approximately 1.5 L of aqueous ethanol, vacuum oven dried overnight for 16 h at 65 ± 2 °C, then re-milled using a 0.5 mm screen in a retsch z 200 mill.

5.5.2. Chemical Composition

5.5.2.1. Moisture content

Moisture content was obtained using the official AACCI method 44-15.02 (AACCI, 2016a). This method is based on the moisture content as a loss in weight of a sample when heated under specified conditions. Samples (3 g) were transferred to metal tins, weighed, and placed in the oven at 130 °C for one hour before removing. Samples were covered and left to cool to room temperature in the desiccator before weighing. The desiccator ensured that no moisture was re-absorbed into the flour. Moisture content was determined with the following formula:

$$Moisture\ Content\ (\%) = \left(\frac{original\ sample\ wt. - dry\ sample\ wt.}{original\ sample\ wt.}\right) * 100 \quad (1)$$

5.5.2.2. Protein content

Protein content was obtained using the official AACCI method 46-30.01 (AACCI, 2016b). A LECO FP628 (LECO, St. Joseph, MI) nitrogen analyzer located at the Northern Crops Institute (Fargo, ND) was utilized. Pea flour (0.25 g) was initially transferred to a small piece of foil. The foil was sealed so that the sample was concentrated at the bottom, with the top twisted tight. Samples were placed in the LECO where they underwent combustion to determine total nitrogen. Nitrogen was converted to protein using a conversion factor of 6.25.

5.5.2.3. Lipid content

Lipid content was obtained using the official AACCI method 30-10.01 (AACCI, 2016c). The formula used for calculations was as follows:

$$\text{As is } Oil\ \% = \left(\frac{oil\ wt.(g)}{flour\ sample\ wt.(g)}\right) * 100 \quad (2)$$

Prior to oil extraction, the samples were dried in an oven at 130 °C for 4 h. Subsequently, the filter paper was folded and approximately 2 g of the sample was added into the envelope. The exact mass of the filter paper and sample were recorded. The top of the envelope was folded three times to ensure the flour was secured in the envelope. Four envelopes were inserted into each thimble of the Soxhlet apparatus (Figure 5.1), which contained a total of 6 thimbles. Hexane acted as the solvent, which removed the oil from the flour. Flasks containing the hexane were placed on a heater while cold water ran through the condensers. The entire apparatus ran overnight, approximately 15 h. Samples were removed from the Soxhlet and placed in a 40 °C vacuum oven for one hour to remove any residual hexane. Upon cooling, samples were weighed.

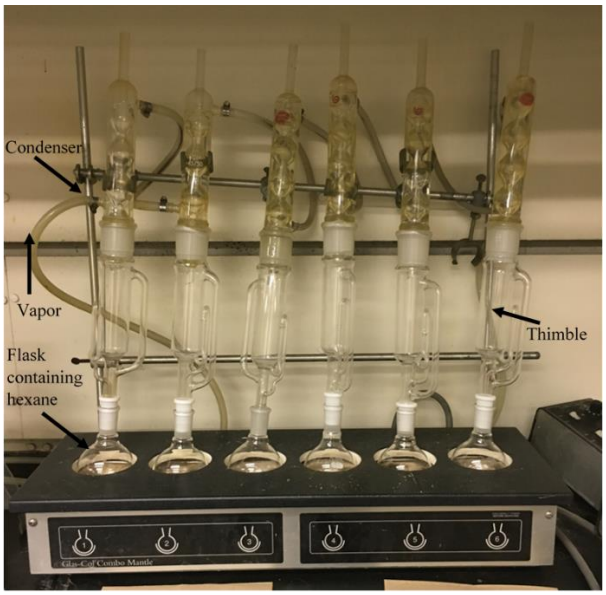

Figure 5.1. Soxhlet apparatus set-up (Pilot Plant, NDSU)

5.5.2.4. Ash content

Ash content was obtained using the official AACCI method of 08-01.01 (AACCI, 2016d). Ash is made up of inorganic matter, mostly minerals present in the flour sample. The principle of the test is to heat samples at high temperatures until only ash residue remains. To prevent burning of the sample, the oven was first brought to 350 °C for 1h, then 450 °C for 1h, before being left at 590 °C overnight. The ash content was determined using the following formula:

$$Ash\ Content\ (\%) = \left(\frac{ash\ wt.}{original\ sample\ wt.}\right) * 100 \quad (3)$$

5.5.2.5. Total and resistant starch contents

Total starch and resistant starch were obtained using the official methods 76-13.01 and 32-40.01, respectively (AACCI, 2016e,f). K-TSTA and K-RSTCL kits from Megazyme International (Bray International) were used for the analyses. The total starch procedure was specific for α-glucans, which included starch and non-resistant maltodextrins (Megazyme 2019). The K-RSTCL resistant starch kit contained additional steps that further allowed for determining the amount of the total starch was resistant to mammalian enzymes.

5.5.3. Physiochemical Properties

5.5.3.1. Particle size distribution

Flour sample (100 g) was sieved into 600, 500, 425, 250, 150, 100, 50, and <50 µm using a Retsch AS 200 basic vibratory sieve shaker (Newton, PA). The sieve shaker was set for 3 min, with 15-sec intervals. The amount of flour (g) on each sieve tray was weighed.

5.5.3.2. Damaged starch

Starch damage was evaluated using official methods 76-31.01 (AACC, 2016g). A K-SDAM kit from Megazyme International (Bray International) was used for analysis. Damaged

starch was quantified via hydrating and hydrolyzing granules into maltosaccharides and α-limit dextrins via fungal α-amylase. Obtained values are presented as a percentage of flour weight on an "as is" basis, which was converted to dry basis for analysis.

5.5.3.3. Water absorption index & water solubility index

Water absorption index (WAI) and water solubility index (WSI) were obtained using a modified method of Simons, Hall, & Tulbek (2012). Flour (2.5 g) was added to centrifuge tubes with 30 mL of distilled water and shaken vigorously to break lumps. Centrifuge tubes were placed on a magnetic stirrer with stir bars for 30 min, then centrifuged at 3,000 rpm for 10 min. The supernatant was decanted into beakers and the container was weighed. Beakers were placed in the oven at 110 °C overnight prior to weighing the solids in the supernatant. WAI (g/g) and WSI (%) were calculated from the following equations:

$$WAI = \frac{weight\ of\ the\ wet\ sediment\ (g)}{initial\ weight\ of\ the\ dry\ flour\ (g)} \quad (4)$$

$$WSI\ (\%) = \frac{weight\ of\ the\ solids\ in\ the\ superatant\ (g)}{initial\ weight\ of\ the\ dry\ flour\ (g)}\ x\ 100 \quad (5)$$

5.5.3.4. Pasting profiles

Pasting profiles were measured using an RVA 4500 (Perten Instruments). The peak, breakdown, hot paste viscosity, setback, final viscosity, pasting temperature, and peak time information was collected. The pasting profile parameters followed that of Hillen (2016). The temperature profile started at 50 °C and was raised to 90 °C after 4 min and 42 sec. The temperature was held until 7 min and 12 sec, where it was gradually dropped back to 50 °C by 11 min. The temperature remained at 50 °C for the remainder of the 23 min run. The speed of the paddle rotation started at 960 rpm for 10 sec, then was lowered to 160 rpm for the remainder of the run.

5.5.3.5. Foaming capacity and stability

Foaming capacity and stability were evaluated using a modified method of Periago et al. (1998). Flour (6 g) and 200 mL of distilled water were homogenized in a commercial laboratory blender (Torrington, Connecticut Model HGB7WTG4) for 1 min. Blended samples were poured into graduated cylinders and the volume (mL) was measured at 0, 5, 10, 30, and 60 min. The foaming capacity was measured as the percent increase in volume with the following formula:

$$Foaming\ Capacity\ (\%) = \frac{volume\ immediatly\ after\ blending\ (mL) - initial\ volume\ (mL)}{initial\ volume\ (mL)} x\ 100 \quad (6)$$

5.6. Results and Discussion

5.6.1. Chemical Composition

5.6.1.1. Moisture

The moisture content of the raw pea flour (10.6%) was consistent with current literature (Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015; Wani & Kumar, 2014). Treatment of the pea flour significantly ($P<0.05$) reduced the amount of moisture within the flour (Table A.1). This reduction was quite substantial, with the moisture content dropping about 4% on average from 10.6 to 6.6%. Hillen (2016) reported similar results after treating raw pea flour with 3:1 and 1:1 v/v ratios of ethanol and water using HPSE. The vacuum oven removes some of the original moisture through evaporation during drying. Furthermore, the use of ethanol as a solvent is thought to provide additional drying effects.

5.6.1.2. Protein

Protein content remained unchanged during processing (Table 5.1). The protein content of the raw and treated pea flours (23.8% on a dry basis) was both within the reported average protein content of field pea (21.2 to 32.9%) (Dahl et al., 2012). These results are important since

the treatment did not negatively impact protein content. Further research is needed to evaluate the influence treatment has on the specific amino acid profile of the protein content.

Table 5.1. Proximate composition (dry weight basis, d.w.b) for raw and treated pea flours

	Protein	Lipid	Ash	Total Starch	Resistant Starch
	----------%----------				
Raw	23.8a	1.4a	2.7a	47.8a	2.4a
Treated	23.8a	1.3a	2.4b	51.8b	2.3a

*Composition that have different letters are significantly different (P<0.05) from each other.

5.6.1.3. Lipid

The lipid content results of the raw and treated pea flour (1.4 and 1.3%, respectively) were within the range (1.4 to 2.4%) reported by Dahl et al. (2012). The oil content did not differ (P>0.05) between the raw and treated pea flour (Table 5.1). Ethanol is amphipathic, meaning it contains both polar and non-polar functional groups (Bigge, 2018). While statistically non-significant, the slightly lower lipid content of the treated flour suggests that slight removal via aqueous ethanol may have occurred.

5.6.1.4. Ash

Ash content on a dry weight basis of the raw pea flour (2.7% d.w.b.) was within reason of results reported by Hall (2017) in the pulse survey (2.0 to 3.2%, on an “as is” basis). Treatment significantly (P<0.05) decreased ash content by 0.3% (Table 5.1). Ash content is defined as the amount of minerals in the flour after milling (King Arthur Flour, 2019). Results indicate that minerals were able to leach into the ethanol and water during treatment. Moreover, Kajihausa, Fasasi, & Atolagbe (2014) found that an increase in soaking time decreased the ash content of sesame flour, suggesting that water alone may impact the removal of minerals from the flour.

5.6.1.5. Total starch

The total starch contents of the treated flour samples were significantly ($P<0.05$) higher than the raw flour, with values of 51.8 and 47.8% d.w.b., respectively (Table 5.1). Total starch values of raw pea flour were within the upper range (36.9 to 49.0%) of those reported by Dahl et al. (2012). Total starch accounted for all plant starch, maltodextrins, maltose, and isomaltose present (Megazyme, 2019). There is no mechanism where starch could have been added to the flour, so it is assumed that this significant increase in total starch content is due to the loss of moisture and ash that occurred as a result of treatment.

5.6.1.6. Resistant starch

Resistant starch was relatively unchanged ($P>0.05$) between raw and treated pea flours, with values of 2.4 and 2.3% d.w.b., respectively (Table 5.1). Resistant starch values were on the lower end of what had been previously reported for field pea (2.1 to 6.3%) (Dahl et al., 2012). A functional fiber, resistant starch is not digestible by mammalian enzymes (Sajilata, Singhal, & Kulkarni, 2006), providing insight into the total dietary fiber content of the flour. Results support that fiber was not lost during treatment. The consumption of dietary fiber has been linked to the protection against heart disease and cancer (McKee & Latner, 2000).

5.6.2. Physiochemical Properties

5.6.2.1. Particle size distribution

Particle size distribution of the raw and treated pea flours indicated that treated pea flour had a smaller distribution, with 61.2% of flour collected via a single sieve size (Table 5.2). In contrast, raw pea flour was distributed over a wider range of sieve sizes. Average particle size of the raw and treated pea flours were approximately 174 and 77 µm, respectively. To observe the impact of re-grinding, the raw pea flour was additionally re-grinding in the same manner as the

treated pea flour. The average particle size was slightly higher than the treated pea flour, with a value of 86.8 µm, however, the overall distribution was similar.

Table 5.2. Particle size distribution of raw and treated pea flours

Sieve Size µm	Raw Pea Flour*	Treated Pea Flour	Raw Pea Flour**
	----------%----------		
600	1.1	0.0	0.0
500	1.7	0.0	0.0
425	3.1	0.0	0.0
250	22.2	4.7	7.6
150	43.4	12.8	16.2
100	23.2	11.9	12.1
50	4.2	61.2	41.6
<50	0.0	6.3	21.2

*Raw flour obtained from hammer mill screen size 1.27 mm.
**Raw flour obtained from re-mill using retsch mill 0.5 mm screen size.

5.6.2.2. Starch damage

Starch damage was significantly higher (P>0.05) in the treated pea flour compared with the raw pea flour, with values of 1.9 and 1.6% d.w.b., respectively. Starch damage of the raw pea flour was slightly higher than reported hammer milled flours (1.0%) (Maskus, Bourre, Fraser, Sarkar, & Malcolmson, 2016). When compared with the total starch content of the pea flours, the % starch damage in respect to total starch content was 3.2% and 3.6% for the raw and treated pea flours, respectively. While statistically significant, it is suggested that in practice, this difference is quite minimal. Damaged starch is a starch granule that has been physically broken or fragmented, which is characterized by increased water absorption (Arya, Sadawarte, & Waghmare, 2015). Starch damage often occurs during the milling process via a combination of heat generation and physical force. The development of slight starch damage within the pea is suggested to have occurred during the re-grinding process of the flour post-extraction. Likewise,

Okada, Negishi, & Nagao (1986) reported a direct relationship between the amount of starch damage and the number of re-grinding cycles.

5.6.2.3. Water absorption index & water solubility index

Treatment of the pea flour significantly ($P<0.05$) increased WAI from 2.2 to 2.7 g/g. Both the WAI of the raw and treated pea flours were lower than those reported in the literature, i.e. 4.9 to 5.0 g/g (Maninder, Sandhu, & Singh, 2007; Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015). However, with a P-value of <0.001 between reps, it was determined that the consistency of the results provided data that could be sufficiently used to analyze the differences between raw and treated pea flour despite the discrepancies with outside sources.

Table 5.3. Water absorption index and water solubility index of raw and treated pea flour samples

	WAI	WSI
	---------g/g---------	----------%----------
Raw	2.2a*	20.7a
Treated	2.7b	10.0b

*Values in columns not connected by the same letter are significantly different ($P<0.05$) from each other.

WAI is the volume starch occupies after swelling in excess water (Maskan & Altan, 2012), indicating water absorption. Oftentimes, a decrease in particle size is suggested to increase water absorption due to the increases in surface area. This was disputed as a primary impact by evaluating the WAI values of raw pea flour used for the current study with raw pea flour that was re-milled using a retsch mill on a screen size of 0.5 mm, which found no significant ($P>0.05$) differences between raw pea flours at different particle sizes (Table 5.2). Therefore, it is suggested that the increase in WAI may be attributed to structural changes in the starch and protein that occurred during treatment. Furthermore, the slight increase in starch

damage may impact the WAI of the flour. As a result of starch damage, particles are broken into smaller particles that can absorb water more easily. (Arya et al., 2015).

The average WSI for raw pea flour was 20.7%, which is consistent with current literature values of 19.8 to 20.6% (Maninder et al., 2007; Soria-Hernández et al., 2015). Treatment has a substantial impact on the WSI of the treated pea flour, dropping by over half to 10.0% (Table 5.3). The WSI can be used to indicate the solubility of biomolecules, such as starch, water-soluble fibers, protein, and sugars (Sharma, Singh, Hussain, & Sharma, 2017). Structural transformations of protein and starch are believed to be the primary sources behind the decrease. Based on the foaming characteristics of the treated pea flour, it was observed that the protein structure might have been altered during treatment. Unfolded proteins are known to aggregate, leading to a decrease in solubility can impact the WSI (Sashikala, Sreerama, Pratape, & Narasimha, 2015). Furthermore, the impact of starch is believed to be quite significant. Zhang, C., Zhang, H., Wang, & Qian (2014) have reported that WSI is oftentimes negatively correlated with starch content, therefore, suggesting that the increase in total starch of the treated pea flour impacted WSI. Furthermore, gelatinization may decrease WSI (Zhang et al. 2014). In the presence of heat and water, it is possible treated starch was pre-gelatinized during treatment. Furthermore, ethanol is occasionally used in pre-gelatinization processes (Jackowski & Czuchajowska, 2002).

In agreement with the results of the current study, Miladinov and Hanna (2001) found the use of ethanol during maize starch extraction significantly lowered the WSI from the control. Despite the significant decrease in WSI, Miladinov and Hanna (2001) found that WAI was not significantly affected during extraction. While our results indicated there was a significant

(P<0.05) increase in the WAI of the treated pea flour, it is clear that solvent extraction has a more notable impact on the WSI compared with the WAI of treated flours.

The WAI and WSI values are utilized as a tool to predict the behavior of binders, stabilizers, and protein sources for nutritional products (Oikonomou & Krokida, 2012). The higher WAI and lower WSI of the treated pea flour indicate it will swell easier, providing greater viscosity (Choi et al., 2012). Bryant, Kadan, Champagne, Vinyard, & Boykin (2001) suggest that flour with higher WAI and lower WSI values may be best utilized for products where the main priority is high viscosity.

5.6.2.4. Pasting profiles

The average raw pea pasting profile of this study was similar to that reported by Hillen (2016). Little difference (P>0.05) was detected between pasting temperatures of raw and treated pea flours, with values of 70.1 and 68.2 °C, respectively (Table 5.4). Pasting temperature indicates the minimum temperature required to cook the provided sample, most commonly used to estimate energy costs (Perten, 2019). There was no significant difference (P>0.05) observed in peak viscosity or breakdown values between pea flours. Peak viscosity, peak time, and breakdown values are all linked to the gelatinization process where, in the presence of excess water, starch granules hydrate and swell, causing them to burst and transform into a paste (hot-paste viscosity) (Wang, Li, Copeland, Niu, & Wang, 2015). Peak times were significantly (P<0.05) different between raw and treated pea flours, with values of 5.7 and 6.7 min, respectively. While non-significant, the peak viscosity was notably higher in the treated pea flour. A higher peak viscosity in combination with an increased peak time is often linked with increased water absorption (Fox, Visser, Skov, Meijering, & Manley, 2014). In agreement with

this, the water absorption values were increased from 2.21 to 2.68 g/g during treatment (Table 5.3).

Table 5.4. Pasting profiles of raw and treated pea flour samples

	Peak Visc. (cP)	Hot Paste Viscosity (cP)	Breakdown (cP)	Setback (cP)	Final Visc. (cP)	Pasting Temp (°C)	Peak Time (min)
Raw	1868a	1794a	74a	1073a*	2867a	70.1a	5.7a
Treated	2239a	2132a	107a	774b	2906a	68.2a	6.7b

*Pasting values in columns are not connected by the same letter are significantly different ($P<0.05$) from each other.

Setback was significantly ($P<0.05$) lower in treated pea flour compared to the raw, with values of 774 and 1073 cP, respectively. Setback refers to the difference between the hot-paste viscosity and final viscosity that occurs during the retrogradation process (Perten, 2019). Decreases in setback have been linked to starch damage and decreased particle size, both present within the treated pea flours (Elliot, Dang, & Bason, 2019; Song, 2007).

Final viscosity results were not significantly ($P>0.05$) different between treatments, contradicting Hillen (2016) who found 50:50 ethanol/water HPSE treatment to substantially decrease the final viscosity from 2821 to 1941 cP. Contrary to the treatment used for this study, Hillen (2016) utilized pressure, which in agreement with other reports, appeared to negatively impact the final viscosity of the treated pea flour (Ahmed, Zafar, & Arfat, 2016). Final viscosity is oftentimes used as the primary parameter to predict the quality of the final product, indicating the ability of the flour to form a paste or gel after cooking and cooling (Perten, 2019). In brief, the treatment did not significantly impact values such as hot paste viscosity, breakdown, pasting temperature, and final viscosity. The increase in water absorption of the ethanol extracted flours influenced the peak time, peak viscosity, and setback of the pea flour.

5.6.2.5. Foaming properties

The foaming capacity of the raw pea flour and treated pea flour were significantly ($P<0.05$) different with values of 56 and 27%, respectively. Related literature found the foaming capacity of raw pea flour to be 39% (Wani & Kumar, 2014). The discrepancy may be associated with differences in variety and/or protein content of field peas used for the analysis. Foaming is dependent on the interfacial film formed by proteins (Wani & Kumar, 2014). The film keeps the air bubbles suspended while slowing the rate of breakdown. The presence of lipids can prevent protein-protein interactions, reducing foaming properties (Shevkani, Singh, Kaur, & Rana, 2014; Wang et al., 2015). The treated pea flour had a lower lipid content (Table 5.1) compared with the raw pea flour, therefore, removing lipids as a possible factor in the poor foaming results. Protein content was not lost during processing (Table 5.1), therefore the disruption in foaming ability is most likely attributed to changes in protein structure that occurred during treatment, which inhibited the formation of protein-protein interactions, thus a proper film did not occur.

The treated pea flour had a much lower initial foam volume (Figure 5.2) The stability of the treated pea flour was poor, with a considerable volume dissipating within the first 5 min. Almost no foam was left after one hour. In the presence of ethanol, proteins have been found to unfold and re-fold into α-helices, losing their biological functions (Pace, Treviño, Prabhakaran, & Scholtz, 2004). In agreement with these results, Chang et al. (2018) observed a decrease in solubility and increase in surface hydrophobicity of lentil protein isolates treated with aqueous organic solvents, which was attributed to partial denaturation during treatment. It is unclear whether a single processing step or a combination of steps influenced the final protein structure of the treated pea flour. It is evident, however, that processing had a significant effect on the proteins of the treated pea flour, producing poor foaming capacity and foaming stability.

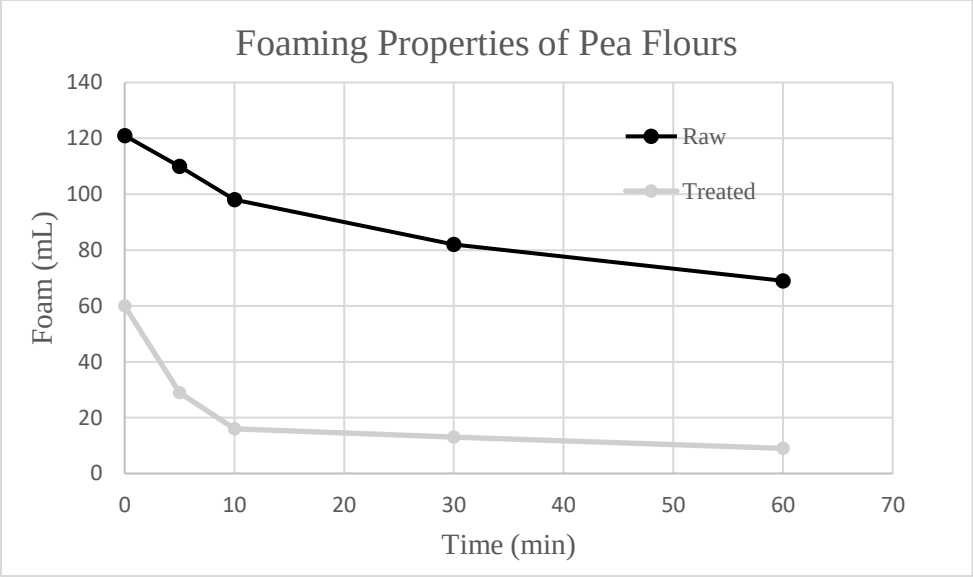

Figure 5.2. Foaming stability of raw and treated pea flour

5.7. Conclusion

The objective of the current study was to evaluate the impact of ethanol solvent extraction on the functionality of the treated pea flour. A significant reduction of moisture and ash content was observed. Treatment produced no loss of protein, total starch, or resistant starch. An increase in water absorption impacted WAI, WSI, setback, and peak time observations. Remaining pasting profile values were unchanged ($P<0.05$). Treated pea flour provided poor foaming capacity and stability, indicating proteins were altered during processing. This proves disadvantageous to protein functionality and may hinder the potential for the use of treated pea flour in some products. Results from this study may be used by processors to understand the composition and functionality of the treated pea flour; therefore, allowing them to determine which products are best suitable when using treated pea flour as an ingredient.

6. SENSORY EVALUATION OF COOKIES AND CRACKERS

6.1. Abstract

The volatile profile of deodorized pea flour treated via ethanol extraction was quantified using GC analysis. Furthermore, the application and shelf-life of deodorized pea flour in crackers and cookies was evaluated using sensory testing. GC results indicated ethanol extraction significantly ($P<0.05$) impacted the presence of volatiles within the pea flours. The concentration of 1-pentanol, 1-hexanol, 1-octen-3-ol, 2-sec-β-3-methoxypyrazine, and 1-nonanol were significantly decreased, while others such as hexanal, nonanal, 2-isobutyl-3-methoxypyrazine, and 1-octanol were released during processing. The data indicates that the type of volatile present may be more important to pea flavor than the total concentration of volatiles present. Cookie and cracker sensory results indicated that treated pea flour products had significantly ($P<0.05$) higher flavor acceptance scores compared with the raw. Texture results suggested treated flour is best used for softer products. Shelf-life results indicated treated pea flour maintained cookie softness, while preventing brittleness in crackers.

6.2. Introduction

Despite their health benefits, pulses are underutilized in commercial products due to low flavor acceptance. A combination of volatiles produced from lipid oxidation and protein degradation are believed to be the cause of these off-flavors (Azarnia, Boye, Warkentin, & Malcolmson, 2011a; Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Maarse, 1991; Vara-Ubol, Chambers E., & Chambers D.). The majority of volatiles associated with pea flavor are alcohols, ketones, and carboxylic acids (Murat, Bard, Dhalleine, & Cayot, 2013). Previous research has presented solvent extraction as a successful option in removing the off-flavors of pulse flours (Hillen, 2016; Roland, Pouvreau, Curran, Velde, & Kok, 2017; Chang, Stone, Green,

& Nickerson, 2018). It is hypothesized that a reduction of pea flavor will increase its acceptability as an ingredient in products. The first objective of this study was to investigate the impact of treatment on the volatile composition of the pea flour. Based on previous research, the selected volatiles included hexanal, 1-pentanol, 1-hexanol, nonanal, 1-octen-3-ol, 2-sec-β-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 1-octenol, 1-nonanol, and 2-Pentylfuran (Jakobsen et al., 1998; Murat et al., 2013; Roland et al., 2017). The second objective was to evaluate the impact of treatment on the overall acceptance of products produced with pea flour, such as cookies and crackers. Moreover, volatile profiles were compared with results of the sensory acceptance tests as a means to develop correlations between the two. In a final evaluation, shelf-life studies assessed the influence of treated pea flour on product hardness over time.

6.3. Materials

Three replicates of the raw pea flour were extracted using aqueous ethanol at an ethanol concentration of 47.5% for 63 min at room temperature. With each extraction, a corresponding untreated replicate was created by transferring 150 g of raw sample into 16.5 x 39 cm metallic, zipper sealed bags (Pacific Bag, Des Moines, IA). Raw and treated pea flour was stored in the freezer (-10 to -15°C) until utilized.

6.4. Experimental Design and Statistical Analysis

A randomized complete block design (RCBD) was utilized as the experimental design for extraction, acceptance testing, and product analysis. Raw pea flour was extracted on three replication days, then GC analysis was conducted in triplicate on each of the reps. Data was evaluated using two-way analysis of variance (ANOVA) and least significant differences (LSD)

was used to determine differences between treatments using the JMP program (Genomics 14). A confidence level of 95% ($P<0.05$) was used to establish significant differences among the data.

6.5. Methods

6.5.1. Volatile Reduction

6.5.1.1. Gas chromatography

Volatiles were evaluated using an Agilent gas chromatography (GC) system. During GC analysis, compounds were injected into the gas chromatograph and flash evaporated onto the column (Phenomenex ZB Wax column, Figure 6.1) (Osweiler & Imerman, 2012). Compounds are separated on the column depending on how they interact with the stationary and mobile phase.

Pea flour samples (1 g) were placed in 4 ml vials and sealed using a Teflon faced silicone septa. Septa were baked at 180°C for 4 h to ensure they were volatile free prior to their use. Sample vials were placed in a 95°C water bath for 10 min, then transferred to a 90°C water bath where the solid phase microextraction (SPME) filament that absorbs the volatiles, was placed in the vial for 15 min. The SPME filament was then transferred to the GC to desorb for 11 min. The total run time for the GC was set to 22.7 min. The pressure was set to 39.9 psi. The GC first began at 35°C and was held for 7 min. The temperature was raised at the rate of 20°C/min until it reached 250°C, where it was held for 5 min. Ultra-high purity hydrogen was used as the carrier gas.

Volatiles quantified included hexanal, 1-pentanol, 1-hexanol, nonanal, 1-octen-3-ol, 2-sec-β-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 1-octenol, 1-nonanol, and 2-Pentylfuran. The selection was determined based on previous research (Jakobsen et al., 1998; Murat et al., 2013; Roland et al., 2017).

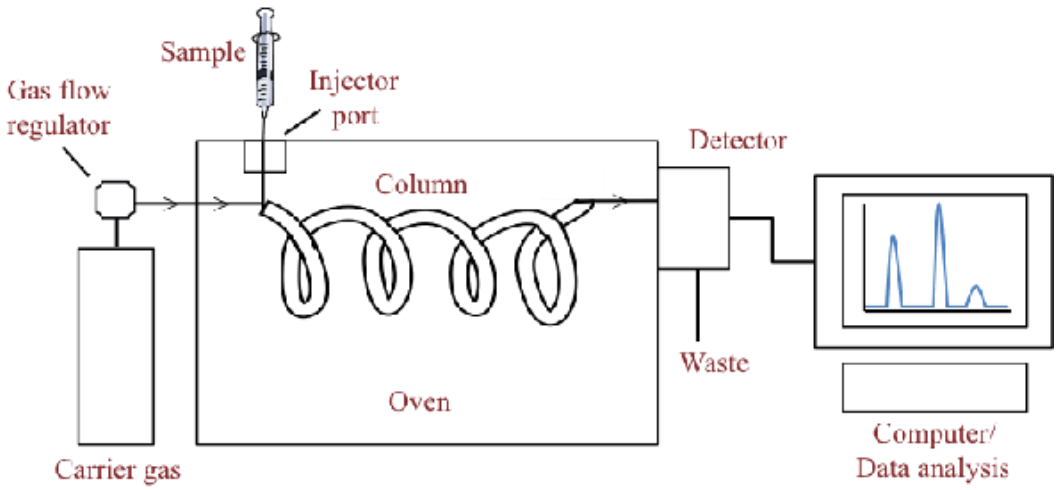

Figure 6.1. Gas chromatography diagram (Wang, Zhang, Liu, & Sun, 2014)

6.5.1.2. Preparation of standards

Separate standards were created for each volatile of interest. Test tubes were labeled, and the weight recorded. Ground saltine cracker (6 g) was added to each test tube and the exact weight was recorded. Test tubes were placed in the freezer until cold, about 20 min. Cold standard (10 µl) was pipetted into each test tube. Screw caps were tightened before inverting the tubes at room temperature to minimize vaporization into the headspace. Tubes were then sonicated in a water bath at 60 °C for 60 min. Upon sonication, test tubes were dried and allowed to stabilize at room temperature for one hour before the weight was again recorded. This final weight was used to calculate the amount of standard added by subtracting the difference. Test tubes were then placed in the freezer (-13 to -15 °C) for a minimum of 2 h. An additional 6 g of ground saltine cracker was added and sonicated at 60°C for 30 min. Test tubes were dried and allowed to cool to room temperature, approximately one hour. Samples were kept at room temperature for 3 days until equilibrium was reached. Equilibrated standards were kept in the freezer (-13 to -15 °C) until evaluated using the GC.

6.5.1.3. Creation of standard curves

The 10 µl standards were run on the GC, providing the average peak time and area for each of the 10 standards. Based on these results, the amount of pure volatile to pipet into cracker filled test tubes to create standards of 1, 5, 10, and 20 ppm was determined using the following formula:

$$amount\ of\ 10\ ul\ to\ add = \frac{(target\ concentration)(area\ of\ curve)(grams\ of\ clean\ cracker\ used)}{concentrated\ ppm\ at\ 10\ ul} \quad (7)$$

All 10 volatiles of interest were pipetted into a single cracker standard (Table 6.1). The 1, 5, 10, and 20 ppm standards were prepared. Once prepared, samples were sonicated at room temperature for 1 h. Samples were allowed to equilibrate at room temperature for 3 days. Standards were stored in the freezer (-13 to -15 °C) until evaluated using GC.

Table 6.1. Standard volatile preparation for standard curve development

	1 ppm	5 ppm	10 ppm	20 ppm
Volatile	----------Amount (g) ----------			
Hexanal	0.045	0.095	0.18	0.18
1-Pentanol	0.05	0.082	0.195	0.193
1-Hexanol	0.04	0.082	0.157	0.156
Nonanal	0.045	0.093	0.177	0.177
1-octen-3-ol	0.05	0.102	0.195	0.194
2-sec-β-3-methoxypyrazine	0.032	0.066	0.125	0.125
2-Isobutyl-3-methoxypyrazine	0.033	0.069	0.131	0.131
1-Octanol	0.009	0.018	0.034	0.034
1-Nonanol	0.025	0.053	0.1	0.1
2-Pentylfuran	0.021	0.042	0.079	0.078
Cracker	49.65	20.298	18.627	8.632
Grand Total	50	21	20	10

Standards of 1, 5, 10, and 20 ppm were run in quadruplicate on the GC. Standard curves were created for each volatile by graphing the ppm (x-axis) by the area (mm^2) (y-axis). Linear regression was added to the graph, with the equation used to determine the ppm of each volatile in the raw and treated pea flours.

6.5.2. Pea Flour Application

6.5.2.1. Cookie preparation

Gluten-free cookies were made following the procedure of Hillen (2016) with a slight reduction in vanilla content. This was adjusted to ensure the strong flavor of the vanilla was not obscuring the underlining pea flavors of the cookie. Room temperature butter and sugar were creamed together in a KitchenAid Commercial mixer (Benton Harbor, MI) at speed 4 for 1 min 30 sec. Eggs and butter were then added and mixed on speed 4 for 30 sec. Once the wet ingredients were mixed, the dry ingredients were added. To prevent the loss of dry ingredients, the mixer was started at speed 1 for 30 sec, then increased to speed 4 for 90 sec. Upon preliminary baking, it was observed that the treated raw cookies produced no spread (Figure B.2). Therefore, the treated cookies and raw cookies were rolled and cut using different methods to create cookies of similar width and height for comparative acceptance testing. It was important to ensure that panelists were not able to easily differentiate between the samples, visually. Raw pea flour cookies were rolled using a rolling pin that had 6.35 mm end rings to ensure an even thickness of the dough. The cookies were cut using a circular cookie cutter (24 mm). Treated cookies were rolled using a rolling pin to 4.0 mm then cut using a slightly larger circular cookie cutter (33 mm). The cookies were baked at 177 °C (350 °F) for 5 min. Cookies were transferred to cooling trays for 30 min, then placed in sealed plastic zip-lock bags. Bags were left at room temperature overnight and used for sensory and quality testing the following day.

Table 6.2. Cookie formulation

Ingredient	%
Granulated Sugar	25.1
Butter	26.2
Vanilla	0.6
Egg	5.7
Pea Flour	41.9
Baking Powder	0.2
Baking Soda	0.3
Total	100.0

6.5.2.2. Cracker preparation

Gluten-free crackers were made using a modified method of Kallenbach (2016). Ingredients (Table 6.3) were mixed in a KitchenAid Commercial mixer (Benton Harbor, MI) at speed 2 for 6 min. The dough was placed on a 2.0 mm raised platform and rolled using a rolling pin that had 3.175 mm end rings to ensure an even spread of the dough. The platform was used to produce a sheet thinner than the end rings available in the lab, therefore, producing a 1.175 mm thick sheet through subtraction. The sheet was rolled once, then folded over and rolled a second time to create a laminating effect. Laminating is used to create air within the middle of the cracker, allowing it to puff slightly. Crackers were cut using a circular cookie cutter (24 mm). The dough was baked at 232 °C (450 °F) for 3.5 min. Crackers were transferred to cooling trays for 30 min, then placed in sealed plastic zip-lock bags. Bags were left at room temperature overnight and used for sensory and quality testing the following day.

Table 6.3. Cracker formulation

	Raw	Treated
	----------%	----------
Pea Flour	65.2	50.0
Shortening	4.2	3.2
Salt	1.1	0.8
Baking Powder	1.1	0.8
Water	28.4	45.2

6.5.3. Evaluation of Pea Flour Products

6.5.3.1. Consumer acceptance sensory evaluation

Panelists (72 and 50) participated in the cookie and cracker sensory panels, respectively. Score sheets utilized a 9-point hedonic scale for testing that included appearance, texture, flavor and overall acceptance (Figure 6.2).

Panelists completed acceptance testing in Harris Hall room 11 (Fargo, ND). The panels were made up of students, staff, and faculty from NDSU. The IRB Protocol #AG18027, "Enriching Pulse Utilization Through Flavor Modification" was followed for sensory evaluations. IRB consent forms were provided for each individual panelist (Figure B.1). Panelists were separated using cardboard booth separators (Figure 6.3). Each testing location contained a cup of water, unsalted oyster crackers, napkins, and a pencil.

SAMPLE NUMBER: XXX

Please evaluate the cookie/cracker sample for the following qualities: Flavor, Texture, Appearance and Overall Acceptability (i.e. liking). Make an X on the appropriate line. After completing two samples, raise your hand for the additional samples (you will have six total)

APPEARANCE:

---------- like extremely
---------- like very much
---------- like moderately
---------- like slightly
---------- neither like nor dislike
---------- dislike slightly
---------- dislike moderately
---------- dislike very much
---------- dislike extremely

COMMENTS:

FLAVOR:

---------- like extremely
---------- like very much
---------- like moderately
---------- like slightly
---------- neither like nor dislike
---------- dislike slightly
---------- dislike moderately
---------- dislike very much
---------- dislike extremely

COMMENTS:

TEXTURE:

---------- like extremely
---------- like very much
---------- like moderately
---------- like slightly
---------- neither like nor dislike
---------- dislike slightly
---------- dislike moderately
---------- dislike very much
---------- dislike extremely

COMMENTS:

OVERALL ACCEPTABILITY:

---------- like extremely
---------- like very much
---------- like moderately
---------- like slightly
---------- neither like nor dislike
---------- dislike slightly
---------- dislike moderately
---------- dislike very much
---------- dislike extremely

COMMENTS:

Figure 6.2. Consumer acceptance score sheets used for cookie and cracker sensory

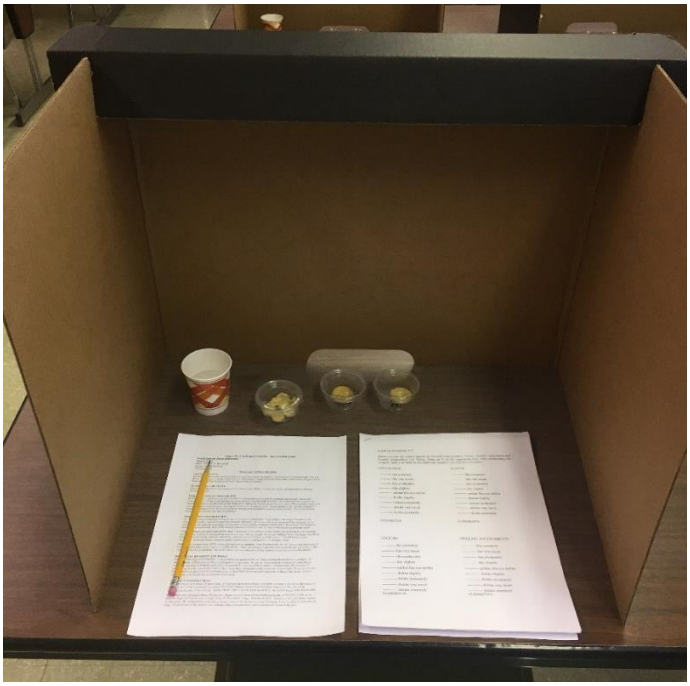

Figure 6.3. Panelist booth set-up for cookie acceptance testing

Cookies and crackers were evaluated on two separate days. During each session, six products were evaluated: three made with raw pea flour and three made from the three replications of treated flour. Panelists were provided two samples to taste at a time to avoid analysis fatigue. Upon completion, panelists were instructed to raise their hand to receive the next two samples. This was repeated until all six samples were evaluated. A randomized complete block design was utilized; therefore, all panelists acted as replicates that received all six samples in random order. Each sample was assigned a 3-digit code, provided on labeled cups (Figure 6.4, Table A.2).

Figure 6.4. Raw (left) and treated (right) pea flour crackers in cups labeled with three-digit numbers

6.5.3.2. Physical parameters

The physical parameters of the cookies and crackers were measured as a means to compare the products. Six samples from each product (3 raw pea flour, 3 treated pea flour) were used for physical evaluation. Weight was recorded and diameter and height were measured in mm using a clear ruler for cookie and cracker samples.

6.5.3.3. Cookie and cracker shelf-life

Cookie and cracker shelf life was determined using the official texture analysis method 10-54.01 (AACCI, 2016h). The hardness of the cookies was evaluated on days 1, 3, 6, and 14. Cookies and crackers were sealed in zip-lock bags stored in a dark, dry drawer at room temperature between analysis. The force needed to break a cookie in half was evaluated using a texture analyzer (TA.XT. Plus, Texture Technologies) with the TA-92N attachment at the 2" width. Settings included a pretest speed of 2.5 mm/s, test speed of 2.0 mm/s, post-test speed of 10 mm/s, a distance of 6 mm, trigger type of 20 g, tare rate automatic, and data acquisition rate was 200 pps. This method is a three-point break that measures the amount of force (N) needed to break the cookie in half. Six samples from each product (3 raw pea flour, 3 treated pea flour) were evaluated to account for the variability between and within batches. Texture analysis was run in triplicate on all batches.

6.5.3.4. Cookie Sensory Replication

As previously stated, the preparation differed slightly between cookies made from raw pea flour and treated pea flour to ensure that panelists were not able to easily differentiate between the samples, visually. A secondary cookie acceptance panel was conducted where both treated and raw cookies followed the original methods of the raw pea flour cookies. A total of 20 panelists were provided two cookie samples, made from raw and treated pea flours. Methods of the sensory panel followed those previously discussed in section 6.5.4. Results were compared with those of the larger sensory panel to determine if preparation differences contributed a significant impact on the perception of flavor.

6.6. Results

6.6.1. Volatile Reduction

A total of 10 major volatiles including hexanal, 1-pentanol, 1-hexanol, nonanal, 1-octen-3-ol, 2-sec-β-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 1-octenol, 1-nonanol, and 2-Pentylfuran were evaluated in raw and treated pea flours (Table 6.4). While the majority of these volatiles decreased significantly ($P<0.05$), four were released during processing, therefore, increasing in concentration. Consequently, the volatile composition was different between pea flours. In the raw pea flour, the most abundant volatiles, by concentration, were 1-pentanol, 2-sec-β-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and 1-nonanol. All but 2-isobutyl-3-methoxypyrazine were significantly ($P<0.05$) lower in treated samples. Post-treatment, the most abundant volatiles, by concentration, were hexanal, nonanal, 2-isobutyl-3-methoxypyrazine, and 1-octanol, all of which significantly increased ($P<0.05$) during treatment. 2-pentylfuran was not detected in either the raw or treated pea flour. Literature provides mixed results on the presence of 2-pentylfuran in pea flour. Results from the current study agree with Heng (2005) who did not

detect 2-pentylfuran in peas, contradicting others who perceived its presence (Jakobsen et al., 1998; Xu, Jin, Lan, Rao, & Chen, 2019). Consequently, this volatile may not be classified as a consistent contributor to pea flavor.

Table 6.4. Volatile concentrations of raw and treated pea flours

Volatile	Raw ppm	Treated ppm
Hexanal	Not detected	3.19
1-Pentanol	2.02a*	Not detected
1-Hexanol	0.38a	0.19b
Nonanal	0.67a	2.48b
1-octen-3-ol	0.47a	0.16b
2-sec-β-3-methoxypyrazine	1.40a	0.44b
2-isobutyl-3-methoxypyrazine	1.17a	2.45b
1-octanol	0.70a	0.87b
1-nonanol	3.92a	0.42b
2-pentylfuran	Not detected	Not detected

*Volatiles not connected by the same letter are significantly different ($P<0.05$) from each other.

Volatiles that were reduced were extracted from the flour through the ethanol and water or released during the vacuum oven drying process. The majority of the volatiles removed were alcohols, which are reported to be associated with food via hydrogen bonding to heteroatoms (McGorrin & Leland, 1996). The hydroxyl groups of the ethanol and water readily form hydrogen bonds, which may have interrupted the hydrogen bonding between volatiles characterized as alcohols and the pea flour matrix in the presence of aqueous ethanol.

Volatiles can interact with the food matrix through binding with proteins (Guichard, 2002; Wang et al., 2014) and/or amylose inclusion complexes that provide hydrophobic regions where lipophilic volatiles can be stored (Guichard, 2002; McGorrin & Leland, 1996). Volatiles that are firmly bound within a food matrix are “hidden” and do not appear during GC analysis.

Altered environments, such as a drastic change in pH or protein/starch structural changes can release bound volatiles (Heng, 2005). Previous foaming property results of the current study (Figure 5.2) indicated that proteins were altered during treatment that may have weakened the interactions of the volatiles, hexanal, nonanal, 2-isobutyl-3-methoxypyrazine, and 1-octanol with protein and starch, which resulted in higher concentrations of these volatiles in treated flour. Furthermore, slight starch damage physically broke the starch granule, creating the potential for associated volatiles to be released.

Heng (2005) found similar results when evaluating pea flours at pH levels of 4 and 8. At pH 4 and 8, both hexanal and nonanal were released in the greatest amounts. Likewise, both Heng (2005) and Jakobsen et al. (1998) found hexanal to account for the highest concentration of bound volatiles with values of 35% and 55%, respectively. In agreement with these previous studies, hexanal accounted for just under 50% of the total concentration of volatiles that increased during treatment. Furthermore, under similar processing conditions, Hillen (2016) saw a significant ($P<0.05$) increase in hexanal concentration after treatment. While not as predominant as the others, Heng (2005) found that 1-octanol was additionally released, similar to results found in the current study.

While the quantification of volatiles provides an insight into the flavor profile of pea flours, there are still many gaps in understanding the true scope of how all volatiles and pea flour constituents interact. Proteins, lipids, and starch play a role in how the volatile interacts with the food matrix (McGorrin and Leland, 1996; Guichard, 2002; Roland et al., 2017). Furthermore, the synergistic relationships between volatiles are thought to create the flavor, yet the exact combinations are unknown (Maarse, 1991). The treated pea flour of the current study was found to have a significantly ($P<0.05$) lower pea flavor intensity than the raw sample. Yet, the total

concentration of volatiles evaluated was decreased only 0.5 ppm, from 10.7 to 10.2 ppm. Therefore, it must be suggested that the type of volatile present contributes more to the overall flavor than the total concentration of volatiles present.

6.6.2. Cookie Quality

Physical cookie quality parameters evaluated included weight, diameter, and height. Visual observations were additionally discussed. The cookies made with raw and treated pea flour were slightly distinguishable (Figure 6.5). Treated cookies lacked the cracks often associated with sugar cookies, referred to as top grain. Top grain develops from the recrystallization of sucrose at the surface of the cookie (Barak, Mudgil, & Khatkar, 2014). Higher water absorption of the treated pea flour directed water away from the sugar, hindering recrystallization.

Figure 6.5. Sugar cookies made with raw (left) and treated (right) pea flour

Treated cookies were heavier ($P<0.05$) than that of the raw due to the fact that the cookies were made with a higher concentration of dough to account for the lack of spread. The spread of the raw cookies created a lighter cookie that contained more air pockets than the treated pea flour cookies. The diameter was slightly ($P<0.05$) wider for the treated cookies compared with the raw cookies. While statistically significant, this observation is minimal when visually comparing the cookies (Figure 6.5). Differences in height were non-significant ($P>0.05$). Methodology ensured that all cookies rose to a similar height. This attribute was maintained by

rolling the treated cookie dough into a thinner sheet and cutting with a larger diameter cutter to counterbalance the lack of spread compared with the raw cookies.

Table 6.5. Physical parameters of cookies made with raw and treated pea flour

	Weight (g)	Diameter (mm)	Height (mm)
Raw Cookie	2.6a*	31.7a	4.3a
Treated Cookie**	4.5b	33.5b	4.7a

*Physical parameters not connected by the same letter are significantly different ($P<0.05$) from each other.
**Treated cookie was obtained from the modified cookie method

6.6.3. Cracker Quality

Physical cracker quality parameters evaluated included weight, diameter, and height. Crackers produced from treated pea flour were slightly heavier ($P<0.05$) in weight compared with crackers made from raw pea flour (Table 6.6). The higher water absorption of the treated pea flour led to re-formulation, which increased the presence of moisture within the crackers, therefore, increasing total mass. Differences in diameter and height were non-significant ($P<0.05$) between crackers made from raw and treated pea flours.

Table 6.6. Physical parameters of crackers made with raw and treated pea flour

	Weight (g)	Diameter (mm)	Height (mm)
Raw Cracker	0.5a*	21.6a	1.3a
Treated Cracker	0.7b	21.1a	1.0a

*Physical parameters not connected by the same letter are significantly different ($P<0.05$) from each other.

6.6.4. Sensory Acceptance Results

6.6.4.1. Cookies

The application of treated pea flour in sugar cookies was evaluated using a 9-point hedonic scale acceptance test. Upon evaluation of acceptance scores that utilized both a modified and unmodified cookie preparation (Tables A.4 and A.5), it was determined that preparation had minimal impact on the acceptance scores of the treated pea flour cookies. Therefore, values from both replications were averaged to account for both methodologies. Flavor and overall acceptance scores were significantly ($P<0.05$) different among cookies. Appearance of the cookies was slightly distinguishable, with the raw pea flour cookie containing greater top grain (Figure 6.5). Despite the lack of top grain, this did not influence the acceptability scores of the treated sugar cookie appearance (Table 6.7).

Table 6.7. Acceptance results for cookies made with raw and treated pea flours

	Appearance**	Texture	Flavor	Overall
Raw Cookie	6.9a	6.3a	5.7a*	5.7a
Treated Cookie	7.0a	6.0a	6.6b	6.4b

*Attributes not connected by the same letter are significantly different ($P<0.05$) from each other.
**Results based on 71 panelist responses

Furthermore, the acceptability of cookie texture was not significantly ($P>0.05$) impacted by the incorporation of treated pea flour versus raw pea flour. When examining panelist comments, subtle differences in mouthfeel were observed. Panelists labeled cookies made from raw and treated pea flours as “crunchy” and “soft”, respectively. It was presumed that the panelist’s preference in texture characteristics determined which cookie was rated with the higher acceptance score.

A major objective of this study, flavor was one of the most important acceptance parameters evaluated. Sugar cookies made from treated pea flour had significantly higher flavor acceptance scores ($P<0.05$), suggesting that the flavor differences observed between pea flours when evaluated individually were maintained with the added ingredients and processing.

Finally, results indicated that the overall acceptance was significantly higher ($P<0.05$) for cookies made with treated pea flour compared to the raw pea flour counterpart. With minimal differences of appearance and texture scores between cookies, it can be assumed that flavor played a major factor in the overall acceptance score.

6.6.4.2. Crackers

Appearance scores of the crackers made from raw pea flour were higher ($P<0.05$) than the crackers made from treated pea flour, with values of 6.3 and 4.9, respectively (Table 6.8). Likely the cause of the higher appearance acceptance scores (Figure 6.6), raw pea flour produced crackers with a more uniform color. Treated pea flour produced crackers with fewer surface cracks, however, this did not appear to improve appearance scores.

Figure 6.6. Crackers made with raw (left) and treated (right) pea flour

Table 6.8. Acceptance results for cookies made with raw and treated pea flours

	Appearance	Texture	Flavor	Overall
Raw	6.3a*	5.3a	4.4a	4.8a
Treated	4.9b	2.9b	5.0b	3.9b

*Attributes not connected by the same letter are significantly different ($P<0.05$) from each other.

Texture acceptance scores were significantly ($P<0.05$) reduced by 2.4 points when using the treated pea flour. The physiochemical properties altered during treatment promoted the treated pea flour to absorb more water. Therefore, a higher amount of water was required during re-formulation to promote the formation of dough, rather than small hydrated dough balls, which were present during the original formulation. Despite efforts to remove moisture before severe browning occurred, texture was nonetheless much softer for the treated crackers. Desiring a crisp cracker, panelists rated the texture of the treated pea crackers as poor, which was further confirmed through panelist comments.

Panelists were able to differentiate ($P<0.05$) the perceived pea flavor of the raw pea crackers from the treated pea crackers, with flavor acceptance scores of 4.4 and 5.0, respectively. An important distinction in panelist acceptability that was not represented in the quantitative results, but rather the comments, was observed. When raw crackers were rated poorly, comments were associated with terms such as “pea, bitter, off-flavor”. On the other hand, when treated crackers were rated poorly, comments were associated with terms such as “bland, no flavor”. Therefore, we can conclude that while a lack of flavor was discovered by panelists, it may not match with what consumers desire in a cracker. Furthermore, the lower shortening content in the treated cracker formulation that occurred as a result of the increase in added water should be considered. Shortening has been shown to have flavor masking properties. Specifically, lipids in water can bind amphiphilic bitter compounds (Coupland & Hayes, 2014). The impact of shortening in combination with panelist’s desire for a non-neutral flavored product may have narrowed the gap between flavor scores from what was observed during cookie acceptance testing.

Despite a higher flavor acceptance of the treated crackers, it was clear from panelists comments that while flavor was noticeably improved, texture was a major deciding factor in the overall acceptance. Therefore, the overall acceptance was significantly ($P<0.05$) lower in crackers made from treated pea flour, from 4.8 to 3.9. While the reduction in pea flavor associated with the treated pea flour crackers was significant ($P<0.05$), texture results indicate that crispy products may not be an ideal candidate for treated pea flour products. This further confirms the suggestion made by Bryant, Kadan, Champagne, Vinyard, & Boykin (2001) indicating that flours with an increased WAI and decreased WSI may be better suited for products that require a higher final viscosity, such as pudding or soup.

6.6.5. Shelf-Life

6.6.5.1. Cookies

Texture is an important parameter associated with the shelf-life and consumer acceptance of cookies. The variance in hardness between raw and treated pea flour cookies was non-significant until day 14, however, by day 7 a steep increase in hardness was observed for the raw pea flour cookies (Table 6.9). At day 14, this difference was significant ($P<0.05$), with the raw pea flour, and treated pea flour cookies having hardness values of 28.17 and 22.66 N, respectively. Firmness measurements are oftentimes used as a method for evaluating the variation in cookie hardness associated with ingredients and storage conditions. A three-point break, or “snapping” method was used where a blade slowly deforms the product before snapping, providing hardness and brittleness results (Gaines, 1991). This method was preferred for this study because it most closely mimics the mechanism of biting.

Table 6.9. Pea flour cookie hardness (N) recorded over time

	Day 1	Day 3	Day 7	Day 14
Raw Cookie	21.62a	21.77a	28.48a	28.17a*
Treated Cookie	21.28a	21.62a	22.03a	22.66b

*Values within a day not connected by the same letter are significantly different ($P<0.05$) from each other.

It has been reported that the presence of pre-gelatinized starch may improve the shelf-life of baked products by slowing the rate of moisture loss (Seyhun, Sumnu, & Sahin, 2005). Pre-gelatinized starch is defined by the USP26/NF21 as "starch that has been chemically and/or mechanically processed to rupture all or part of the granules in the presence of water and subsequently dried" (USP, 2003). Treated pea flour is exposed to both water and drying during processing, suggesting the potential for pre-gelatinization. Similar to damaged starch, pre-gelatinized starch absorbs more water, as observed during WAI evaluation (Table 5.3). Furthermore, the lack of spread during baking can indicate the presence of pre-gelatinized starch (Donelson & Gaines, 1998).

In agreement with these results, Seyhun, Sumnu, & Sahin (2005) found the use of pre-gelatinized tapioca starch statistically reduced ($P<0.05$) the staling of microwave baked cakes compared with conventional microwaves cakes made from 100% wheat flour. The cookie shelf-life study indicates positive results regarding the use of treated pea flour for improving the shelf-life of baked products made with pea flour.

6.6.5.2. Crackers

In agreement with the texture observed by the panelists, the hardness of the crackers made from raw pea flour was higher than those made from treated pea flour, with values of 12.01 and 9.49 N, respectively (Table 6.10). Over time, the crackers equilibrated to similar hardness

values during the first week of the shelf-life study. Initial moisture of the treated pea flour cookies was higher, creating a faster rate of moisture loss observed between day 1 and 3.

Table 6.10. Pea flour cracker firmness (N) recorded over time

	Day 1	Day 3	Day 7	Day 14
Raw Cracker	12.01a	15.6a	16.9a	10.04a*
Treated Cracker	9.49a	16.6a	16.2a	18.19b

*Values within a day not connected by the same letter are significantly different ($P<0.05$) from each other.

Interestingly, the average hardness of the raw pea flour crackers decreased quite substantially after two weeks. The peak force (N) is used to evaluate the hardness of the sample, while the slope can estimate brittleness (Gaines, 1991). Comparing the 3-point break curves provides a better understanding of the decrease in hardness observed in the raw pea flour crackers. The treated pea flour crackers snap quickly in about two separate actions (Figure 6.7). On the other hand, crackers made from raw pea flour produced graphs such as the one on the right, where multiple curves were present. This suggests brittleness, which was confirmed simply by observing that many crackers would break apart during handling from storage to the texture analyzer. An explanation for this behavior is the lack of initial moisture of the raw pea flour crackers. By day 14, moisture became too low to sufficiently keep the matrix bound together.

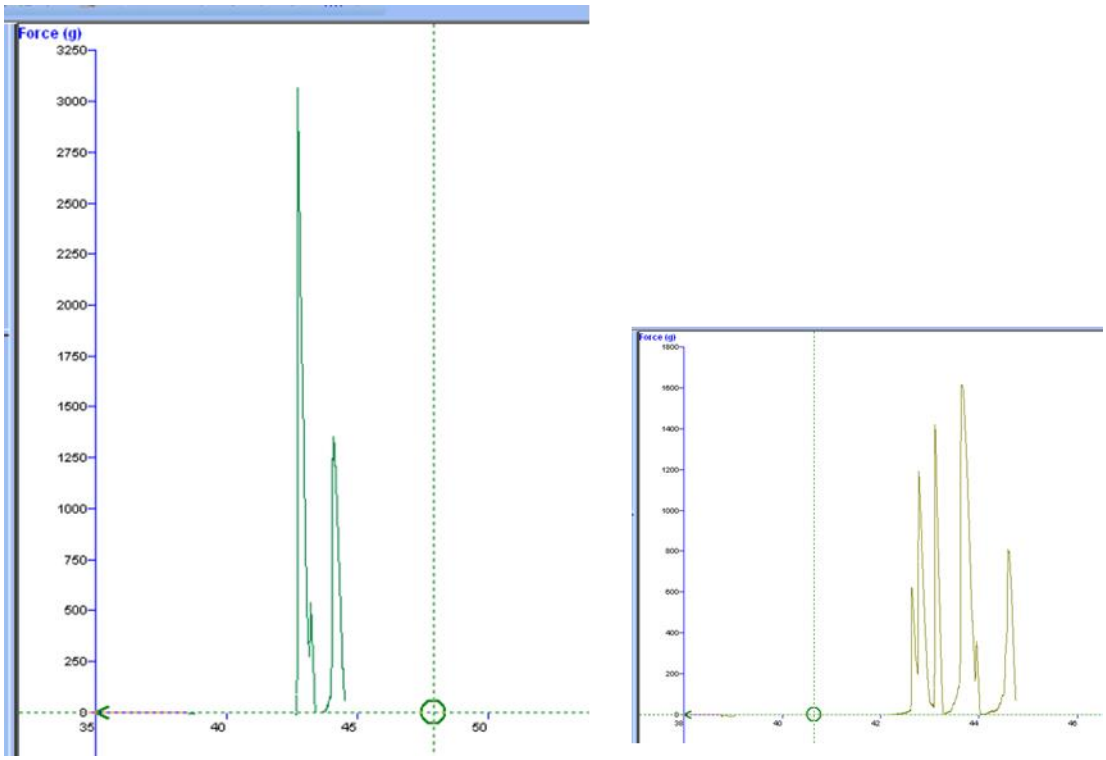

Figure 6.7. 3-Point break curves of crackers made from treated (left) and raw (right) pea flour evaluated on day 14

Results indicate that the shelf-life of the crackers made with raw pea flour may be poor if excessive brittleness is to occur during storage or handling. The presence of pre-gelatinized starch did not influence the reduction of staleness as observed with the cookies (Table 6.9), most likely due to the differences in initial moisture between crackers. The loss of moisture observed via an increase in hardness after day 3 suggests that a holding period may be a viable option in improving texture acceptance scores of crackers made with treated pea flour. With a controlled holding period at room temperature, moisture can be removed without the visual issues of browning.

6.7. Conclusion

The objectives of this study were to quantify volatiles present in raw and treated pea flours, while assessing the application of pea flour in products, such as cookies and crackers. GC

results indicated that while five volatiles, primarily alcohols, significantly ($P<0.05$) decreased in concentration, four others were released from the protein-starch matrix and therefore, increased in concentration. Results indicate the types of volatiles present may have a greater impact on pea flavor than the total volatile concentration. Acceptance testing of cookies and crackers made from raw and treated pea flours indicated that treatment produced products with significantly ($P<0.05$) higher flavor acceptance scores. Texture acceptance scores suggest that treated pea flour is best used for softer products, such as sugar cookies. Shelf-life measurements were improved for both cookies and crackers using treated pea flour. Furthermore, reformulations such as the use of seasonings in the crackers may further improve products made with treated pea flour.

7. OVERALL CONCLUSION

Objectives of this study were to select an optimal ethanol extraction treatment; determine chemical composition, physiochemical properties, and volatile quantification of the optimally treated pea flour; and evaluate cookie and cracker acceptance using raw and treated pea flours. A series of qualitative and quantitative sensory sessions investigated the effectiveness of the treatment parameters to produce the lowest pea flavor intensity. While high-pressure solvent extracted samples were significantly ($P<0.05$) lower in pea flavor than the raw flour, removing pressure further lowered the pea flavor intensity of the treated flour. A final review determined the best treatment was 47.5% ethanol concentration extraction at room temperature for 63 min.

Significant reductions of moisture and ash content were observed. Treatment produced no loss of protein, total starch, or resistant starch. An increase in water absorption impacted WAI, WSI, setback, and peak time was observed. Remaining pasting profile values were unchanged ($P<0.05$). Treated pea flour provided poor foaming capacity and stability indicating proteins were altered during processing.

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8. FUTURE WORK

Results indicated that solvent extraction may be a viable method for removing pea flavor from pea flours. Knowing that 47.5 % ethanol concentration was optimal based on the three parameters evaluated, evaluating additional concentrations within the 25 to 75% range may identify an improved treatment method. Further research needs to be conducted to evaluate the complexities of volatile quantification, as well as the full application of treated pea flour in products.

Volatile quantification is a complex study that requires much analysis. Continuing this research may help define the volatile combinations that impact pea flavor the most. Using gas chromatography-olfactometry, researchers can determine the intensity that each of the evaluated volatiles emits. Expanding the number of volatiles evaluated may further expand the knowledge of pea flavor origination. Furthermore, assessing the volatile profile of protein extracts could assist in determining which volatiles are most associated with the protein matrix, as well as providing insight into the impacts of protein extraction on bound volatiles. In regards to final product evaluation, quantifying the volatile concentrations in the cookies and crackers produces the opportunity to discuss how the baking process may impact the volatile profile of the raw and treated pea flours.

In regard to furthering product assessments, there are three primary recommendations. The first recommendation is to optimize formulations for the crackers and cookies made from treated pea flour. Results from the cracker sensory indicated that panelists reacted poorly to a neutral flavored, bland cracker. The addition of seasonings may mitigate this issue. Furthermore, because hardness values (N) increased during storage at room temperature for one week, a suggested “holding time” may further improve texture approval. By optimizing formulations, the

applications of treated pea flour may be expanded. A final recommendation is to assess the application of treated pea flour in a high-viscous product, such as pudding, may aid in recommendations as to the optimal products for its usage.

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APPENDIX A. TABLES

Table A.1. "As is" proximate composition of raw and treated pea flour

	Moisture	Protein	Lipid	Ash	Total Starch	Resistant Starch
	----------%----------					
Raw	10.6a*	21.3a	1.2a	2.5a	42.1a	2.2a
Treated	6.6b	22.2b	1.2a	2.2b	48.4b	2.2a

*Composition that have different letters are significantly different ($P<0.05$) from each other.

Table A.2. Three-digit codes used for cookie & cracker large scale sensory testing

Product	Code	Pea Flour
Cookie	231	Treated, Rep 1
	805	Treated, Rep 2
	361	Treated, Rep 3
	324	Raw, Rep 1
	598	Raw, Rep 2
	959	Raw, Rep 3
Cracker	957	Treated, Rep 1
	773	Treated, Rep 2
	141	Treated, Rep 3
	163	Raw, Rep 1
	768	Raw, Rep 2
	256	Raw, Rep 3

Table A.3. Three-digit codes used for cookie sensory replication

Product	Code	Pea Flour
Cookie	193	Treated
	834	Raw

Table A.4. Cookie acceptance results made from raw and treated pea flours using a modified method that ensured consistent diameter and thickness between cookies

	Appearance	Texture	Flavor	Overall
Raw Cookie	6.97a*	6.32a	5.80a	5.74a
Treated Cookie	7.08a	5.91a	6.58b	6.32b

*Values not connected by the same letter are significantly different (P<0.05) from each other.

**Results based on 51 panelist responses.

Table A.5. Acceptance results of sugar cookies made from raw and treated pea flours using and unmodified method

	Appearance	Texture	Flavor	Overall
Raw Cookie	6.6a*	6.1a	5.0a	5.4a
Treated Cookie	6.6a	6.6a	6.9b	7.0b

*Attributes not connected by the same letter are significantly different (P<0.05) from each other.

**Results based on 20 panelist responses.

Table A.6. Water absorption index and water solubility index of hammer milled (1.27 mm) and re-milled retsch (0.5 mm) pea flour samples

	WAI	WSI
	---------g/g---------	----------%----------
Hammer	2.19a	20.30a
Hammer & Retsch**	2.20a	20.41a

*Values in columns not connected by the same letter are significantly different (P<0.05) from each other.

**Flour milled on the retsch mill was originally milled on the hammer mill with a 1.27 mm screen

Table A.7. Pasting profiles of hammer milled (1.27 mm) and re-milled retsch** (0.5 mm) raw pea flour samples

	Peak Visc. (cP)	Hot Paste Viscosity (cP)	Breakdown (cP)	Setback (cP)	Final Visc. (cP)	Pasting Temp (°C)	Peak Time (min)
Hammer	1892a*	1683a	209a	1117a	2878a	67.4a	5.04a
Hammer & Retsch	1819a	1686a	133a	1403a	3005a	67.0a	5.18a

*Pasting values in columns are not connected by the same letter are significantly different
**Flour milled on the retsch mill was originally milled on the hammer mill with a 1.27 mm screen

APPENDIX B. FIGURES

North Dakota State University
Plant Sciences
Dept. 7670, P.O. Box 6050
Fargo, ND 58108-6050
701-231-6359

Pea Flour Fortified Products

Dear Sensory Panelist,
You are invited to participate in a research study to evaluate the sensory characteristics of products made with pea flours. This project is being conducted by Clifford Hall III from the in the Cereal and Food Sciences program in the Department of Plant Sciences at North Dakota State University.

PURPOSE OF THE STUDY:
The primary objective of the research is to study the feasibility of using pea flour as an ingredient in crackers, cookies and cake.

EXPLANATIONS OF PROCEDURES:
In this sensory evaluation, you will be asked to taste products with and without pulse ingredients. During the evaluation you will be given samples on plates marked with different numbers. The instructions to complete the sensory evaluation are provided on the form given during the sensory panel. In short, you will be asked to mark on a scale the degree of acceptance. The 9 point scale is in increments from "Like Extremely" to "Dislike extremely" based on acceptability or liking of the flavor, texture, and appearance sensory characteristics. The entire sensory evaluation should take less than 15 minutes.

PARTICIPANT INFORMATION:
All the information obtained during the test will remain confidential. Your identity will not be revealed in the experiment results. Instructor-supervisor/student influence will be avoided as no names will be collected on the scoresheets and thus the instructor will not know which student evaluated the product. In addition, the instructor will not be present at the time of the sensory panel and therefore will not know who participated in the panel.

You may choose not participate in this study. However, if you choose to participate your participation is voluntary and you may withdraw from participation at any time without penalty. If you do withdraw from this study, it will not affect your relationship with the instructor, unit, the services it may provide to you, or North Dakota State University. Your assistance would be greatly appreciated in making this a meaningful study.

Only group comparisons will be made and reported in summary form. Furthermore, we will not collect signatures on the score sheet thus preserving your confidentiality. Only the research team will have access to the score sheets after you complete the evaluations. The score sheets will be collated and thus further increasing your confidentiality.

POTENTIAL BENEFITS AND RISKS:
Results of this test will be helpful in determining the acceptability of using pea ingredients in food products. No direct benefit will be received from participation in the study. However, improvements in product formulations would be expected to help the population in general. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known risks. If you are known to be sensitive to any food or food ingredient, or have had violent allergic reactions to drugs, chemicals, or food ingredients, you should not participate in this study.

CONTACT INFORMATION:
You have rights as a research participant. If you have questions about your rights or complaints about this research, you may talk to the researcher or contact the NDSU Human Research Protection Program at 701.231.8908, ndsu.irb@ndsu.edu, or by mail at: NDSU HRPP Office, NDSU Dept 4000, P.O. Box 6050, Fargo, ND 58108-6050.

If you have any questions about this project, please contact me at clifford.hall@ndsu.edu or 701-231-6359, or by mail at: Dept of Plant Sciences, Dept 7670, PO Box 6050, Fargo, ND 58108-6050. Thank you for your participation in this study. We will provide you with a second copy of the informed consent to keep if you would like to receive a copy. Completion of the sensory test indicates that you consent to participate in this research project.

Figure B.1. IRB Consent Form

Figure B.2. Sugar cookies made from raw (left) and treated (right) pea flour using the same dough height and cutter diameter.