SENSORY AND QUALITY ATTRIBUTES OF DEODORIZED PEA FLOUR USED IN GLUTEN-FREE FOOD PRODUCTS

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MASTER OF SCIENCE

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ABSTRACT

Yellow pea (*Pisum sativum* L.) flour was deodorized using high-pressure solvent extraction (HPSE) with ethanol and water as solvent. These flours were evaluated in gluten-free (GF) baked goods. Sensory evaluation, volatile analysis, and laboratory quality testing were completed to determine the impact on flavor, aroma, and flour quality. The HPSE pea flours obtained from 1:1 and 3:1 ethanol and water were tested against a control pea flour in cake and cookie sensory tests. Both showed significantly higher ($P<0.05$) acceptance scores for flavor, texture, and overall acceptance, but acceptance was not significantly different. Texture of cookies did not change significantly over 5 days, but significant differences ($P<0.05$) between the three flour treatments in cookies and cakes were observed. The protein of the HPSE treated flours were significantly higher than the control. The headspace analysis showed some significant differences between control and treatments for the three standards of interest.

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LIST OF ABBREVIATIONS

ANOVA ..... Analysis of Variance

CD ..... Celiac Disease

GC ..... Gas Chromatography

GNAP ..... Great Northern Agriculture Plaza

HPSE ..... High-Pressure Solvent Extraction

RVA ..... Rapid Visco Analyzer

SPME ..... Solid Phase Microextraction

SC ..... Specialty Commodities

GF ..... Gluten-Free

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1. GENERAL INTRODUCTION

Growth in the number of sufferers of Celiac Disease (CD) has been recorded as knowledge about the disease is discovered. Approximately 1% of the world population suffers CD (Eisner and others 2014). Adults between 18-59 were surveyed, and approximately 26% said they were avoiding eating gluten. This percentage increased from 24% in 2010 (Glazer 2013). The industry is growing, yet the product quality is lacking and the sensory attributes of the products are undesirable. CD is an immune reaction that occurs after the consumption and digestion of gluten (Dessi and others 2013). This reaction causes many symptoms including nausea, diarrhea, abdominal pain, and many others.

Gluten is a protein matrix created from the gluten-forming protein glutenin and gliadin when mixed with water. Glutenins provide elasticity to the dough while gliadins provide extensibility to the dough. These unique characteristics of the proteins is why gluten is such a unique matrix in baked goods (Eisner and others 2014). Grains such as wheat, rye, barley, and contaminated oats contain gluten and individuals with CD must avoid these products (Dessi and others 2013). Products that can be safely eaten by sufferers of CD must be gluten-free, including pulses. Dry field peas (*Pisum sativum* L.) are a pulse that have many nutritional benefits and are gluten-free.

Declaration by the United Nations making 2016 the Year of Pulses demonstrates the importance of pulses as a food source (United Nations 2015). Pulses are unique as they are nutrient dense and healthy (Udahogora 2012). Interest in these commodities is growing because they have nutritional characteristics, are gluten-free, and can be used for soy replacers in food products. Gluten-free and high quality protein content are two reasons for the interest in pea flour, but the off-flavors and aromas detract from the appeal to bakers (Sudha and Leelavathi

2011). Ingredient replacements that minimally impacts the flavor profile and improves the nutrition and quality are desired in the industry today. However, pea flour is not a suitable substitute in food products due to the strong flavor and aroma it gives the product.

This research had three main objectives, to deodorize pea flour, evaluate the sensory and quality characteristics of the flours in baked goods, and finally to evaluate the volatile compounds present in the flours and baked products. The deodorization process used was high pressure solvent extraction (HPSE). Pea flour was tested in GF cookies and GF yellow cake. Sensory analysis was conducted with three panels per product and an average of 50 consumers attended. Headspace analysis was used to evaluate the flavor and aroma compounds of interest, and those that were determined to be present in majority of the samples were then quantified. Other quality tests were conducted on the flour and baked goods including protein, rapid-visco analyzer (RVA), texture, cookie and cake dimensions, and moisture content determination.

2. HYPOTHESES

Cake and sugar cookies made with extracted pea flour will have less intense bitter and grassy flavors along with less intense earthy and green aromas. The products prepared with extracted pea flour will be favored to those prepared with the raw pea flour in terms of flavor and acceptability. The product quality will be minimally impacted by using extracted pea flour versus untreated pea flour. The starch RVA profile of the extracted flour will be slightly altered due to the slight degree of gelatinization that can occur in the vacuum oven when the extracted flour is dried, but not to the extent that would make it incompatible for baking applications.

3. OBJECTIVES

1. Develop a deodorization technique and deodorized pea flour.
2. Evaluate acceptance of baked products made with deodorized pea flour.
3. Evaluate quality and flavor characteristics of deodorized pea flour.

4. LITERATURE REVIEW

4.1. Celiac Disease

4.1.1 Introduction

Celiac disease (CD) is an autoimmune disease that causes inflammation of the small intestine and damages the villi that line the walls of the small intestine when gluten is ingested (Eisner and others 2014). Villi absorb nutrients in the small intestine during digestion. When they are damaged, nutrients pass through the body and can cause malnutrition. Villi damage can be prevented by removing dietary gluten, which is found in wheat, barley, rye, contaminated oat, and several other grains (Eisner and others 2014). These grains possess prolamin protein fractions that can trigger an immune response in individuals with CD (Chirado and others 2002). The significance and prevalence of this disease is quite high, affecting up to 1% of the world population (Mustalahti and others 2002).

4.1.2. Mode of action and symptoms

Symptoms of CD include diarrhea, steatorrhea, weight loss, bloating, flatulence, constipation, intestinal discomfort, malnourishment, and other non-gastrointestinal abnormalities. Steatorrhea is when an excess amount of fat is released with feces due to the reduction of fat absorption in the intestines. Some celiac patients do not experience gastrointestinal problems, but may develop a rash or similar symptoms (Bizzaro and others 2012). These symptoms are caused by the inability to properly digest food due to the damage caused to the villi (Murray 1999). Although gluten has been associated with CD, the actual cause is prolamin proteins, which are rich in proline, and considered especially toxic to patients with CD (Weiser and Koehler 2008). The amino acid sequences vary and due to the variance, can trigger a variety of responses in the body. The amino acid sequences results from the digestion of

gluten. These responses cause apoptosis, lysis of the cell, of the intestinal cell wall and other issues (Weiser and Koehler 2008).

Other symptoms have been observed in people including neurological disorders that cause neuropathy (Zone 2005). Several of these disorders include dementia, myopathy, which causes muscle weakness, and multiple sclerosis (Volta and Giorgia 2010). Individuals suffering CD with prolonged exposure to gluten can suffer more serious complications such as osteoporosis (Rashtak and Murray 2012). People who suffer from CD should avoid eating gluten to prevent the occurrence of gastrointestinal and neurological disorders.

4.1.3. Gluten structure and function

Gluten is a structure that is formed when two gluten-forming proteins, gliadin and glutenin, are mixed in water. Gluten-forming proteins, gliadin and glutenin, consist of about 60-85% of the total protein content in wheat, and are very important to the baking structure of bread and other baked goods (Eisner and others 2014). The glutenin proteins provide the elasticity to dough, while the gliadin proteins provide the extensibility to dough. A good ratio of these proteins gives dough the viscoelastic properties that allow it to rise and entrap air in gas cells. This structure is most evident in products including bread and rolls. Without the presence of gluten in these food products, many challenges are faced due to the lack of the key structural components.

4.2. Pulses and the food industry

4.2.1. Industry interest in pulses

Yellow peas, which are dry seeds and are typically dicots, are a member of the pulse family. Growing interest in pulse crops is very evident in the food industry today, with emphasis due to the United Nations. United Nations, declaration of 2016 as the International Year of

Pulses. The declaration was intended to help increase awareness of pulses and the inclusion of them in the diet (United Nations International Years 2015). Growing interest in pulses is due to their high nutrient density (Table 1) and health-promoting compounds. Pulses have approximately double the protein content of cereal grains. Pulse protein is high in the amino acid, lysine, which is the limiting amino acid in cereals (Udahogora 2012). They also are low on the list of allergens and are seen as a potential protein and flour replacer for soy. Soy is used in a many food products, but also one of the eight primary food allergens. The occurrence of soy allergies is growing, and food manufacturers are looking to different ingredients to replace soy in their formulations (Eigenmann and others 2008). Protein purified from pulses, including peas, could present a solution to several problems including gluten-free food quality, soy substitution, and fortification to increase nutritional value.

Pulses promote food security because they are a cheap source of protein. On average the cost per cup serving is less than $0.50 (Stewart and others 2011). Pulses can be classified both as protein food sources and vegetables because of the nutritional composition. Protein, fiber, vitamins, and minerals allow them to be included in the bean and pea subgroup of vegetables. The recommended serving is one to two cups per week (U.S. Department of Health and Human Service 2015).

Pulses also have the potential to reduce the chance of developing chronic diseases such as heart disease, diabetes, and cancer (Mudryj and others 2012; Dahl and others 2012). Due to the high fiber content, which helps with the function of the immune and digestive systems, they aid in disease prevention (Anderson and others 2009). Pulses act against cancer specifically because of their antioxidant, fiber, micronutrient, and antinutrient content (Mudryj and others 2014).

Table 1. Nutritional information for dry split peas.

Nutrient	Unit	Amount (100g)
Water	g	8.62
Energy	kcal	352
Protein	g	23.82
Total Lipid	g	1.16
Ash	g	2.66
Carbohydrate (by difference)	g	63.74
Fiber	g	25.5
Sugar	g	8
Minerals		
Calcium	mg	37
Iron	mg	4.82
Magnesium	mg	49
Phosphorus	mg	321
Potassium	mg	823
Sodium	mg	15
Zinc	mg	3.55
Copper	mg	0.815
Magnesium	mg	1.22
Selenium	µg	4.1
Vitamins		
Ascorbic Acid	mg	1.8
Thaimin	mg	0.726
Riboflavin	mg	0.215
Niacin	mg	2.889
Pantothenic Acid	mg	1.758
Vitamin B-6	mg	0.174
Folate Total	µg	274
Beta Carotene	µg	89
Alpha-Tocopherol	mg	0.09
Gamma Tocopherol	µg	2.09

Vegetarians following strict plant-based diets can benefit from consuming pulses (Leterme 2002). When consumed with grains such as rice or wheat-based products, complete

proteins and complementing amino acids are consumed. Consumption of pulses can be beneficial for all populations, including vegetarians (Darmadi-Blackberry and others 2004).

4.2.2. General nutrient compounds of peas

Dry peas are of interest due to their nutrient density. Peas are primarily starch (46%) and have a wide range of protein (13.7-30.7). The fat content in peas is very low, with majority of the calories coming from starch and protein. They are good sources of protein, calories, fiber, vitamins, and minerals (Table 1). Specifically, the protein is high in lysine, which is a deficient amino acid in cereal grains. Though peas are a substantial source of lysine, they are low in methionine and cysteine (Bahnassey and others 1986).

Yellow peas contain a high level of minerals including calcium, manganese, magnesium, zinc, and phosphorus (Warkentin and others 1997). Some of the compounds that provide health benefits include phytosterols, which are thought to reduce the cancer risks as well as help to reduce LDL cholesterol (Rochfort and Panozzo 2007). Other compounds include resistant starch, which is considered part of dietary fiber as it does not break down as it passes through the gastrointestinal tract (Rochfort and Panozzo 2007). In addition, pulses have a low glycemic index. Glycemic index is a rating between 1 and 100 on how food items impact blood sugar. This is important to individuals who may suffer from diabetes and need to watch their blood sugar levels or individuals who are trying to lose weight.

Peas are very high in B-vitamins, including folate. Folate concentrations were found between 41-55μg /100g (Sen Gupta and others 2013). Peas are also good sources of Thiamin and Riboflavin, as well as Niacin (El-Adawy 2002). Vitamins are very susceptible to decomposition during boiling or pressure cooking (Dang and others 2000). This presents a significant challenge

to individuals who use them in cooking processes if they want to maintain their source of vitamins. Folate is an important nutrient and thus has been a focus in pulses.

Folate is essential to prevent birth defects and is found in high concentration in pulses. The most abundant forms of folate found in pulses include 5-methyltetrahydrofolate (5-MTHF) and 5-formyltetrahydrofolate (5-FTHF) (Rychlik and others 2007; Jha and others 2015). Folate content in yellow peas ranges between 41-55 μg/ 100 g. Field peas have an average range of 26-202 μg/ 100 g (Sen Gupta and others 2013; Jha and others 2015). 5-MTHF concentration in peas is the most abundant form in peas (Dang and others 2000). Thaimin and riboflavin also are found in high concentrations in peas. Niacin also is found in pulses (El Adawy 2002; Erbas and others 2005). These vitamins are all susceptible to decomposition under boiling or cooking conditions (Dang and others 2000). Therefore, proper cooking or preparation is important to maximize the vitamin retention.

Pulses are typically high in potassium, magnesium, iron, and manganese (Ray and others 2015). The average composition of potassium, magnesium, iron, zinc, and selenium are 10.4 mg/g, 1.17 mg/g, 54 μg/g, 31 μg/g, 13 μg/g, and 47 μg/g, respectively, with only trace amounts of copper and nickel (Ray and others 2015, Dahl and others 2012). Minerals, specifically potassium and magnesium, tend to leach from peas when cooked or soaked (Wang and others 2010). However, no changes in zinc, iron, and calcium were observed. Increases in phosphorus and manganese were observed after cooking (Wang and others 2009).

4.2.3. Protein content and functionality

Protein content of dry peas is approximately 24.6% (USDA 2016), with a window of variance for the influence of environmental factors. Albumins represent between 15-25%, and globulin represents 50-60% of the total protein (Gueguen and Barbot 1988, Rubio and others

2014, Crevieu and others 1994). The peptides that comprise the albumins range in molecular mass of ~6,000 Dalton to 25,000 Dalton (Rao and others 1989). The major globulin protein fractions are legumins and vicilin (Gueguen and Barbot 1988). There is a strong interest in high protein foods in the diet today, as well as eating more plant based foods. There are amino acids present in peas that make them a complement to cereal grains, specifically lysine (Table 2).

Increased protein in gluten-free foods could help to improve the firmness, viscosity, stability, and elasticity due to the disulfide cross-linking (Buchert and others 2010). These disulfide bonds assist in many different aspects of product quality by forming a protein network. Protein networks provide firmness to products, stability, and the ability to resist proteolysis (Buchert and others 2010). More cross-linking can improve the structure with gelation, as well as help with foam formation. This allows for more give without breaking of dough. They also can help to improve the quality by improving the browning and flavor, through Maillard browning. In baked goods such as bread, loaf volume is increased, the crumb has more consistent cell distribution, and sensory properties are improved compared to pulse-fortified products (Crockett and others 2011).

Table 2. Amino acid composition of yellow, dry, split peas.

Amino Acids	Unit	Amount (/100 g)
Tryptophan	g	0.275
Threonine	g	0.872
Isoleucine	g	1.014
Leucine	g	1.76
Lysine	g	1.772
Methionine	g	0.251
Cysteinie	g	0.373
Phenylalanine	g	1.132
Tyrosine	g	0.711
Valine	g	1.159
Arginine	g	2.188
Histidine	g	0.597
Alanine	g	1.08
Aspartic Acid	g	2.896
Glutamic Acid	g	4.196
Glycine	g	1.092
Proline	g	1.014
Serine	g	1.08

4.2.4. Carbohydrate content and functionality

Carbohydrate content of peas is approximately 20% of the seed composition (Cerning-Beroard and others 1976). The mono- and oligosaccharides account for less than 15% of the seed weight. Oligosaccharides contain β-glycosidic bonds, which link monosaccharides together (Chilomer and others 2010). Oligosaccharides are not digestible by humans due to these bonds. Approximately 2.6% of the seed composition is sucrose and stachyose, and approximately 1% of the seed weight is raffinose (Fan and others 2015). The insoluble and soluble fiber content of peas range between 8.7-12.9% and 0.6-3.7%, respectively (Stoughton-Ens and others 2010). A majority of pea fiber is insoluble, with the abundant compounds being cellulose (55%),

hemicellulose (23%), and pectin-type polysaccharides (8%) (Brummer and others 2015). Resistant starch in peas is approximately 4.7% (Brummer and others 2015). When peas are cooked, the amount of resistant starch dropped by 23% (Costa and others 2006).

Pea starch granules vary in size from 2 to 40 μm (Ratnayake and others 2002). Amylose comprises between 33.1-57.0% of the total starch in dry peas (Ratnayake and others 2001). Gelatinization occurs when starch is heated with water. The crystalline structure of starch is disturbed due to water bonding via hydrogen bonds with the polysaccharides of starch including amylose and amylopectin. When starch granules absorb water, they swell and lose their crystalline order. The ability of starch to absorb water is impacted by the ratio of amylose to amylopectin (Hoover and Hadziyev 1981; Ratnayake and others 2002). Gelatinization temperatures for pea flour include onset of 61°C, midpoint of 67°C, and end gelatinization temperature of 76°C (Ratnayake and others 2001).

Pasting properties (Figure 1) are determined by the Rapid Visco Analyzer. The RVA subjects the flour and water to temperature profiles and shear rates, and resistance is measured in the formation of a curve. Heating causes the crystalline structure to melt and break down, which forms a gel (Batey 2007). The peak viscosity relates to the maximum amount of water a granule can uptake. When the granules reach their largest size prior to breaking down, the maximum viscosity is reached. From there, the granules begin to break down and the viscosity decreases. The broken starch structures begin to re-associate in a process known as retrogradation, during the cooling phase, which is indicated by the setback value or total difference between the breakdown and final viscosity (Saunders and others 2011).

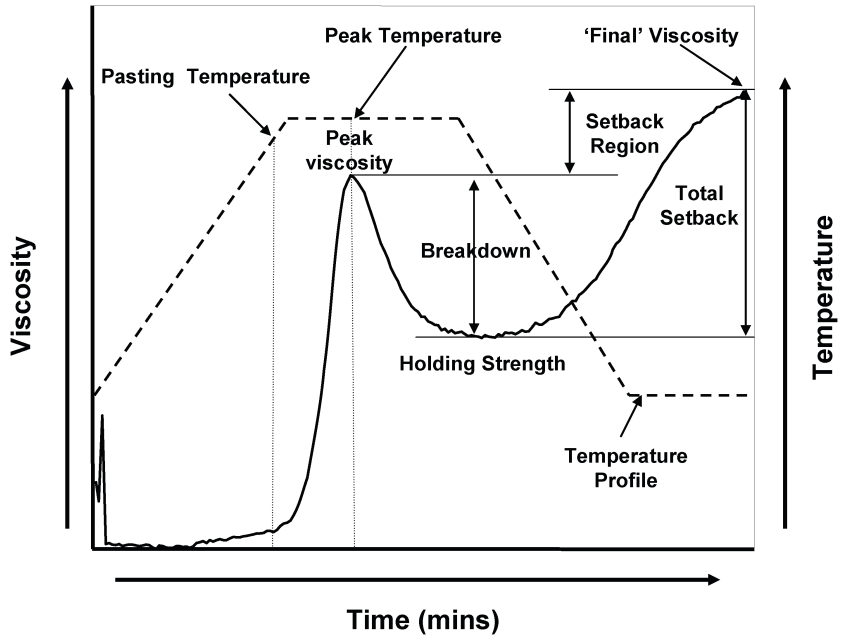

Figure 1. Typical pasting curve obtained from the RVA (Saunders and others 2011).

Figure 1 is a typical RVA curve of corn starch. RVA parameters of wheat and pea flours are very different (Table 3). These parameters show drastic differences between the starch pasting properties of pea and wheat flours. The pasting temperatures are very similar, but peak viscosities are very different (Hoover and Manuel 1996). The breakdown of the pea is minimal and the setback is also very low, whereas the wheat is high. This means that the pea flour viscosity changes less as the gelled flour sits opposed to wheat, where a drastic change in viscosity can be expected (Chung and others 2008, Chung and others 2012). The setback value indicates the stage during cooling where retrogradation occurs, which is the reassociation of glucan chains from starch to form gel. The breakdown value indicates the point of decreasing viscosity once the peak viscosity is reached.

Starch gelatinization presents challenges for baking because gelatinization can impact baking quality and digestibility of the starch. Starch granules break down in water forming a solution of polymers (Ratnayake and Jackson 2008). Starch gelatinization is the swelling of

starch crystals and leaching of soluble polysaccharides. Starch molecules, amylose and amylopectin, act with water causing the crystal to swell and break apart (Tester and Morrison 1984). This forms a gel in solution, which can be beneficial to baking quality characteristics such as cake viscosity.

Table 3. Comparison of pasting properties between pulses and wheat.

Sample	Pasting Temperature (°C)	Peak Viscosity (cP)	Breakdown (cP)	Setback (cP)	Final Viscosity (cP)
Pea[1]	69.6	1214.3	143.3	650.7	1772
Wheat[2]	68.5	5310	2212	2364	5458

[1]Chung and others 2008, [2]Chung and others 2012.

4.2.5. Fiber content and functionality

Total dietary fiber is composed of insoluble dietary fiber and soluble dietary fiber. Most of the fiber in whole peas is insoluble (between 63-92%). The breakdown of pea fiber includes cellulose (55%), hemicellulose (23%), and 8% polysaccharides similar to pectin (Vose and others 1976). Galacturonic acid in peas ranged from 15.6-18.4% (Brummer and others 2015). Dietary fiber can help reduce the risk of colon cancer, coronary heart disease, stroke, diabetes, hypertension, and other common diseases (Anderson and others 2009). Increased dietary fiber can reduce cholesterol and blood pressure. Consumers today do not consume enough fiber and increased consumption of peas can help increase daily fiber intake (Clemens and others 2012).

4.2.6. Phytochemical compounds

Pulses also contain antioxidants such as carotenoids, tannins, flavonoids, polyphenols, and phenolic acid, which are believed to help prevent cancer. These antioxidants are found in high quantities with phenolic acid being the most abundant, contributing up to 92% of the total phenolic content in field peas (Udahogora 2012). Antioxidants work by reducing the effects of

free radicals by donating an electron. This prevents the free radical from abstracting the electron from DNA or a functional protein, which is how cancer and other problems arise (Perron and others 2007).

While the total carotenoid content in peas ranges from 0.6 to 2.7 mg / 100 g, β-carotene content in peas was 0.16 mg/ 100 g and comprises of 1% of the carotenoid content in yellow peas (Augustin and Klein 1989; Holasova and others 2009; Ashokkumar and others 2014). Lutein accounted for 96% of the carotenoids in yellow peas (Ashokkumar and others 2015). Similar content of free phenolic acids (trans-ferulic, trans-ρ-coumaric, and syringic acids) in pulses was observed (1.8-16.3 mg/ 100 g), with the highest concentration in the hull fraction of the seed (Sosulski and Dabrowski 1984). The predominant phenolic acids in peas include protocatechuic acid, vanillic acid, and hydroxybenzoic acid (Lopez-Amoros and others 2006). Flavonoid content in cooked yellow peas ranges from 321 to 2404 μg/ 100 g, and phenolic acid content was approximately 230 μg/ 100 g (Duenas and others 2016). The total tocopherol content in peas ranges from 90.4 to 97.3 mg/ g depending on the cultivar (Yoshida and others 2007a). Tocopherol content in cooked pulses drop from that of native or non-cooked pulses (Kalogeropoulos and others 2010). α- (<6.7%) and δ-Tocopherols (<8.0%) were found in very small amounts and γ-Tocopherol (85.4%) was the most abundant.

4.2.7. Lipid content

The lipid content of pulses is lower than other grains, ranging from 1.0 to 2.8% for peas (Chung and others 2008). The distribution of lipid in peas includes phospholipids (52-61%), triacylglycerols (31-40%), and minor amounts of steryl esters (0.8-2.4%), free fatty acids (1-3%), and diacylglycerols (2-3%) (Yoshida and others 2007a,b). Protein content in peas is reported to be approximately 24%, but ranges between 20 and 25% (Northern Pulse Growers Association

(NPGA) 2016, Wang and Daun 2004). The common fatty acid in legumes is linoleic acid (18:2), at a concentration of about 31.2% (Grela and Gunter 1995). Due to the high concentration of unsaturated fatty acids, the lipid in peas is susceptible to oxidation (Domoney and others 1990). Lipoxygenase facilitates the oxidation of polyunsaturated fats into hydroperoxides (Casey and others 1996). These enzymes are present in peas (Domoney and others 1990). Therefore, pea flours may be susceptible to enzymatic oxidation and must be monitored for oxidation problems.

4.3. Pea flour food applications

Yellow pea flour has been evaluated as a replacement for wheat flour in gluten-free foods such as bread, crackers, and pasta (Sudha and Leelavathi 2011). Pea flour is ideal because it has a high protein content, which may help with the functionality or structure problems that have been observed in gluten-free alternatives. Since gluten containing ingredients cannot be used in these products, and alternatives to gluten are lacking, high quality protein content is important to the quality of these food products (Bahnassey and others 1986). The potential for pea flour use in the industry has been limited due to sensory characteristics related to the strong odor of peas (Sudha and Leelavathi 2011). Products that pea flour could be utilized in include bread, snack foods or extruded snacks, soups, pasta, tortillas, cookies, cake, crackers, and others (Asif and others 2013, Han and others 2010, Petitot and others 2010). Peas are a high quality and economical protein. Though they are low in the essential amino acid, methionine, they are high in lysine, which can balance the deficiencies that are seen in cereals (Bahnassey and others 1986). This demonstrates the importance of using pulses in fortification applications.

Fortification in baked goods is an application for pea flour due to the improved nutrient content of baked products. Blending pea flour with wheat flour is a great option for improving the protein quality and content in bread due to the abundance of lysine, which is the limiting

amino acid in cereal grains (Udahogora 2012). Bread is low in the amino acid lysine, due to it being the limiting amino acid in wheat. Lysine is abundant in peas. The addition of pea flour to bread allows for the limiting amino acid concentration to increase, therefore making a higher concentration of complete protein (Udahogora 2012).

Blending wheat and pea flour to make breads is of interest, but in order to make any sort of nutrition claims, an estimated 25% blend is needed. Issues arise when blending pea flour in wheat bread such as lack of gluten-forming proteins (glutenin and gliadins), the darkening that occurs in breads with blends of pea flour, and the presence of off-flavors (Raidl and Klein 1983). The significant color change is due to the high amount of starch present in the pea flour. The starch is hydrolyzed by the action of amylase during the fermentation step of baking bread, resulting in an increased abundance of glucose in the dough. Once the baking process starts and kills the yeast, the remaining glucose participates in Maillard browning reactions with proteins or in caramelization reactions (Raidl and Klein 1983). Lysine in the protein further enhances browning reaction rates, thus causing a darker color (Bertram 1953).

Pea proteins and concentrates can be used as egg replacers in food products. Pea protein is a possible egg replacer due to functionalities of the protein and the allergenicity and no cholesterol in peas (Hoang 2012). Cake and cookies made with pea proteins as an egg replacer were rated higher than those made with eggs, showing the potential significance pea protein could play in the food industry (Hoang 2012).

4.4. Flour treatments

Treating the flour with extraction methods is important to remove flavor compounds but minimizing the nutrient loss or leaving the flour as close to native state as possible is necessary.

Extraction methods include soaking whole peas and drying in vacuum oven, high pressure solvent extraction (HPSE), supercritical fluid carbon dioxide extraction (SCFE), and distillation.

4.4.1. Vacuum oven

Soaking in water is one approach to removing aroma-causing volatiles that bind to water (Lei and others 2013). Drying under vacuum reduces the boiling temperature for water and other solvents, which potentially reduces the nutrient degradation. Vacuum ovens provide a higher quality product in a shorter time at a lower temperature (Chen 2014). These ovens also decrease drying time by removing moisture in the oven. Apple slices dried in a reduced pressure oven had fewer quality issues (Lei and others 2013). Thus, vacuum ovens are ideal for drying without reducing the quality of a product.

4.4.2. Distillation

Distillation processes are methods of deodorization where a distillate can be collected (Berk 2009). A common method used for laboratory procedures is a batch (differential) distillation. This method is not continuous, and a liquid mixture is boiled in an enclosed vessel. As the water boils and vaporizes to steam, volatiles are carried with water, which condenses in another flask. Though this works best in a lab, in industry a continuous flash distillation has been most commonly used because it is a continuous process, making it highly efficient (Berk 2009).

Distillation technology is a relatively new procedure that is being used because of the energy efficiency, separation and purification abilities, and the elimination of non-volatile components (Ozel and others 2005). Steam distillation removes many compounds due to lack of selectivity in the process, but it is relatively cheap, making it appealing to industry (Ozel and others 2005). A major issue with distillation when working with powders is the potential for gelatinization of starch, which directly impacts the quality of the flour.

4.4.3. High pressure solvent extraction (HPSE)

High pressure solvent extraction (HPSE) is a method of accelerated solvent extraction. Solvent is subject to varying cycles of high and low pressure (500-3000 psi) at room temperature, allowing it to move through and interact with the sample (Richter and others 1996). The use of pressure allows solvent to travel into areas of the matrix that would not have been previously accessible. It also allows the solvent to contact the surface of the matrix more rapidly, allowing for more contact in the same period of time (Richter and others 1996). Utilizing different solvent combinations, non-polar, weak, and strong polarity compounds can be extracted (Shouqin and others 2004). Though it works well to leave the sample in good quality, there are some issues that occur, including protein denaturation. Pressure extraction methods are less invasive and destructive that most extraction methods (Shouqin and others 2004). Due to limited energy, covalent bonds are not broken, insinuating that the structural components of the sample should not be changed (Shouqin and others 2004).

4.5. Baking

4.5.1. Introduction

The use of pea flour fractions in crackers, cookies, pita bread, muffins, and cake have been documented. These applications were focused on fortification or partial substitution (Singh and others 2015). Baking with wheat flour works well due to the gluten-forming proteins, glutenin and gliadin (Mahsa and others 2012). When gluten is formed, elastic and viscous properties are added to dough, which produce porous and spongy textures of baked goods. Peas do not possess glutenin and gliadin, which means the viscous and elastic properties are not present when utilizing pea flour (Wang and others 2016). Therefore, applications with pea flours must be targeted to specific applications such as partial fortification of gluten free foods.

4.5.2. Gluten-free baking

Wheat flour forms gluten when water is mixed with the glutenin and gliadin proteins. Glutenin contributes elasticity and strength to gluten, while gliadin contributes viscosity and extensibility to the gluten (Majzoobi and others 2012). Gluten provides the structural components that are essential to producing a sponge-like texture, such as those found in bread and cake. Baking without gluten causes problems such as sticky dough, extremely sensitive to over- and under-mixing, and temperature sensitivity (DiMaggio 2015). The primary proteins in dry peas include albumin and globulin. Albumin comprises of 15-25% of the total protein in peas, globulins, 50-60% of the total protein, and do not possess the gluten forming proteins (Guegen and Barbot 1988).

Other issues arise in baking due to the absence of the viscoelastic properties contributed by the gluten-forming proteins. Addition of various hydrocolloids in baked products was found to help mimic the visco-elastic properties of gluten-forming proteins (Witczak and others 2016, Taylor and others 2016). Hydrocolloids, including xanthan gum, guar gum, locust bean gum, carboxymethylcellulose, carrageenans, and several others, are used in different systems of baking, and can improve the viscoelastic properties and help with air retention (Witczak and others 2016). In gluten-free products such as cake or bread, it is necessary to have gums to help mimic the texture of wheat cakes and breads.

Making gluten-free cake batters or bread is more similar to making short bread, which has very thick batter that does not flow. Much more intense mixing is required to trap air in the batters (Taylor and others 2016). The texture of the dough and batter varies significantly because it is not free flowing after mixing, due to the entrapment of air.

4.6. Volatile compounds and analysis

4.6.1. Volatile flavor causing compounds in peas

The strong green and earthy flavors found in peas are caused by aroma-causing compounds. The volatile compounds in blanched green peas were evaluated and found to contain several compounds that cause different odors. The significant compounds include hexanal and 1-hexanol, which were thought to contribute a hay-like odor in peas (Jakobsen and others 1998). Hexyl acetate was responsible for a sweet perfume-like odor. Octanal was responsible for contributing the sweet orange smell to peas, and 1-octen-3-ol was responsible for the strong mushroom smell (Jakobsen and others 1998). The sour onion-like odor was identified as dipropyl disulfide. Several methoxypyrazines were identified as causing a strong scent of green beans, which included 3-isopropyl-2-methoxypyrazine, 3-sec-butyl-2-methoxypyrazine, and 3-isobutyl-2-methoxypyrazine (Jakobsen and others 1998). Though these are main compounds in peas that cause odor, there are hundreds of compounds that compose the aroma of peas.

4.6.2. Solid-phase microextraction (SPME)

SPME method works by adsorbing the volatiles on a fiber of a filament (Arthur and Pawliszyn 1990). The filament is placed into a gas chromatograph (GC) and the fiber is released into the injection port. Volatiles are desorbed from the fiber, which releases them onto the column where they pass through the column based on temperature changes and interaction with the column. Compounds can be identified by comparing peaks on the chromatograph with those of known standard retention times or by the use of the mass spectrometer (Arthur and Pawliszyn 1990).

SPME method was developed as a solvent-free method of extraction. The volatiles are adsorbed onto a small fiber that is part of a syringe-like piece of equipment. Volatile adsorption

amounts depend upon the type of coating on the fiber, and sample matrix and volatile distribution ratio. The fibers are selected based on “like dissolves like” principle. The most useful coating that should be considered is polydimethylsiloxane (PDMS) because of its durability and versatility with different compounds, i.e. polar or nonpolar (Pawliszyn 2001). Adsorption times for compounds to settle on the fiber are affected by thickness of the coating, and, in general, a thinner coating will provide a faster sample preparation time (Pawliszyn 2001).

4.6.3. Headspace evaluation methods

The analysis of volatile compounds in peas has been previously evaluated. Three different volatile extraction methods evaluated included solid phase micro-extraction (SPME), solvent assisted flavor evaporation (SAFE), and purge and trap extraction (Murat and others 2012; Murat and others 2013). Each method produced different results. The SPME and SAFE methods were much better than the purge and trap extraction method. The SAFE method was chosen as the best option due to the reproducible results and the ability to freeze extracts for reuse, where the SPME method extracts could not be reused (Murat and others 2012). SPME does the best job by producing results for analyzing compounds with low molecular weight and obtained the widest range of volatile compounds.

4.7. Sensory analysis

When products are prepared, consumer sensory testing are completed to evaluate the acceptance of products. Consumer acceptance testing consists of evaluating several attributes of a product for consumer ‘liking’. A 9-point hedonic scale is the traditional way of having panelists evaluate products, which is demonstrated below using descriptors such as ‘like extremely’ to ‘dislike extremely’. A 9-point hedonic scale is used because it helps find smaller significant differences (Villanueva and others 2005). Issues do arise with the 9-point hedonic

scale, such as the contrast effect. This means that panelists evaluate samples after the worst sample better than they would have if they had the worst sample first. This problem is combatted by giving each panelists the samples in random order (Villanueva and others 2005). These scales do have good discriminatory power, which is important for finding significance between samples.

5. PRELIMINARY STUDY

5.1. Introduction

Since limited work has been conducted on volatile extraction in flour and the impact of extraction on flavor and quality, investigation on extraction methods was necessary prior to creating the extracted pea flour. By investigating several methods of volatile extraction, a measure of effectiveness in terms of sensory analysis and quality could be quantified. The purpose of the preliminary study was to identify in the best extraction methods that would remove the most pea flavor and to use this flour in cookies.

5.2. Objectives

The main goal of this study was to determine the effectiveness of extraction methods on deodorization of yellow pea flours and determine the impact of the extraction on flour quality. Other objectives included testing the prepared flours in sugar cookies, analyzing the sensory characteristics of the flours, determining the acceptance of the sugar cookies, and developing a process for the best extraction method(s).

5.3. Materials and methods

5.3.1. Materials

Dry yellow field peas (Specialty Commodities, Fargo, ND) were purchased and milled on an Urshel Mill Model MG 104 (El Paraiso, IN). Milling conditions were not recorded in the preliminary study. High-pressure extraction unit (Supercritical Fluids Timatic Micro Series Extractor, Newark, DE) was utilized for the extraction. The extracted flour was dried in a commercial vacuum oven and milled on a Retsch Z-mill (Ultra Centrifugal ZM 100, Haan, Germany**)** with the 10.5 mm screen. Sugar, eggs, baking powder, baking soda, butter, wheat flour, and cookie cutters were purchased from a local grocery store. Supercritical fluid carbon

dioxide extracted flour was prepared at Thar Technologies (Pittsburgh, PA), but no parameters for the extraction were provided.

5.3.2. Methods

5.3.2.1. High pressure solvent extraction

A high pressure extraction unit from Supercritical Fluids was used to extract the pea flour volatile compounds. Pea flour was weighed (300-350 g) and placed into a mesh bag. The mesh bag was placed in the extraction chamber and filled with solvent (1 L). The solvent chamber had to be filled exactly to the top before the program would start. The program ran for 30 and 60 minutes with different solvent concentrations of ethanol and water. Concentrations utilized were 1:1, 2:1, and 100% ethanol. The flours were dried overnight (12 hours) in a vacuum oven (Buflovak; Buffalo, NY, USA) at 60 °C for at 15 psi vacuum.

5.3.2.2. Supercritical fluid extraction

Untreated pea flour was sent to Thar Technologies (Pittsburgh, PA) to be treated under different conditions involving supercritical carbon dioxide. Although they did not provide the parameters, this method is the least invasive and destructive method used to treat the flour.

5.3.2.3. Hot oven treatment

Peas were soaked in room temperature tap water overnight (12 hours) and dried in a hot convection oven. The dry times were impacted by oven temperature. Temperatures tested ranged from 125-150 °C and times ranged from 90 minutes to 5.5 hours.

5.3.2.4. Distillation treatment

Pea flour (250 g) and water (600 mL) were added to a flask in a distillation unit (Figure 2). The flask containing sample was set on a heating mantle, and the sample heated under a vacuum of 15 inHg. The steam temperatures used were 50, 60, 70, 80, and 85 °C. The slurry of

flour and water was spread in pans and dried in the vacuum oven under the same conditions as the high pressure solvent extraction drying.

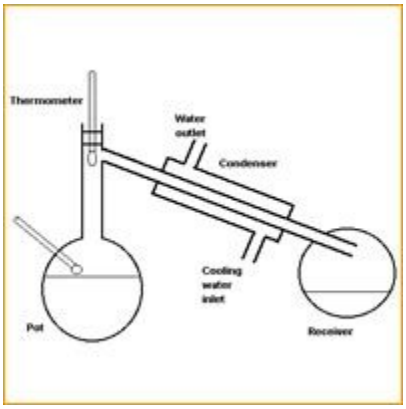

Figure 2. Distillation unit framework.

5.3.2.5. Germination study

Peas were placed in growth chambers on a shelf system with a saturated potassium chloride system to regulate the humidity between 80-85%. The temperature was set to approximately 23°C and samples were collected after 24, 48, and 72 hours. The samples were dried in the vacuum oven following the same procedure as the high pressure extracted flour.

5.3.2.6. Milling

Milling of all samples were handled slightly different, but the same mill and grinders were used for all samples. A Retsch Z-mill (Ultra Centrifugal Mill ZM 100) and a Braun Coffee Grinder (Aschaffenburg, Germany) were used to break up the peas and mill to a fine powder. Whole peas were ground on the Braun coffee grinder on the finest setting, then were ground to a fine powder on the Z-mill with a 10.5 mm screen. For high pressure solvent extraction, the samples were ground in the Z-mill following drying in the vacuum oven. For the distilled samples, the dried gelatinized mixture was broken up in the Braun coffee grinder and then milled on the Z-mill.

5.3.2.7. Trained sensory panel

A trained sensory panel was used to taste test the treated flours and rate them on an unstructured line scale. The panel consisted of six individuals. The control was a raw pea flour, and the blank control was corn starch. The panelists rated the flours for appearance, flavor, and strength of pea flavor and aroma. Eleven treatments were rated by the panel. The results were calculated by measuring the distance from the left start of the scale to the mark on the line, in millimeters. The results were the average of the six panelists ratings. The flour results were then ranked from lowest to highest ratings.

5.3.2.8. Rapid visco analyzer (RVA)

To test the pre-gelatinization and starch profile, the starch profiles were tested on an RVA 4500 (SN 2143306-45A, Hägersten, Sweden). The pasting properties, viscosity, and pasting temperatures were measured with the RVA, and the data was compared to an untreated pea flour. The temperature profile started at 50 °C and was raised to 95 °C at 4 minutes and 42 seconds. The temperature was held until 7 minutes and 12 seconds, where it was then dropped to 50 °C at 11 minutes. The temperature remained at 50 °C until the end of the 23 minute profile. The speed of the paddle rotation was 960 rpm for the first ten seconds and then lowered to 160 rpm for the remainder of the profile. Parameters evaluated included the peak, trough, breakdown, final viscosity, setback value, peak time, and pasting temperature.

5.3.2.9. Sugar cookie trial baking

Baking of gluten-free sugar cookies with the treated pea flours and the untreated pea flour was done and a formula was developed (Table 4). The room temperature butter and sugar were creamed together in a KitchenAid Commercial mixer (Benton Harbor, MI) at speed 2 for 30 seconds. The egg and vanilla were then added and mixed for another 30 seconds. The flour,

baking powder, and baking soda were finally added and mixed for 1 minute or until the dough formed a hard ball. These cookies were baked in a Baxter of oven for 8-10 minutes (until slight browning appeared while in the oven) at 350°C. Cookies were cooled on cooling racks for 60-90 minutes before being placed in bags.

Table 4. Preliminary gluten-free sugar cookie formulation.

Ingredient	Amount Added (g)
Butter	115
Sugar	110
Flour	185
Egg	25
Vanilla	7
Baking Powder	1.5
Baking Soda	0.7
Total	444.2

5.3.2.10. Sugar cookie consumer sensory panels

Sensory evaluation was completed on flours rated best by the trained panel. Additional flours were used due to their unique preparation method. A consumer acceptance test was run with a minimum of 50 panelists rating the appearance, flavor, texture, and overall acceptance of the cookies using a nine-point hedonic scale were evaluated. A single factor analysis of variance (ANOVA) was completed on each of the attributes tested with an alpha level of 0.05. The panelists tasted the cookies in a random order and each sample was delivered one at a time.

5.4. Results and Discussion

5.4.1. High pressure solvent extraction

High pressure solvent extraction was completed with pressures ranging from 0-7 psi over 30 and 60 minute periods. When only water was used, the flour turned into a gelatinized mass, which overheated the mill when grinding was attempted. Mixtures of ethanol and water were tested. A 1:1 mixture of ethanol and water resulted in a product that was easy to break apart and

grind to a fine powder. The same was observed for the 2:1 mixture of ethanol to water and the 100% ethanol treated flours.

5.4.2. Supercritical fluid extraction

Two pea flours were received from Thar Technologies. Parameters of extraction were not provided, but the flours appeared to be less yellow in color, in comparison to a control pea flour. The particle size appeared similar to the control pea flour as well. There did not appear to be noticeable differences in the moisture content of the flour.

5.4.3. Hot oven treatment

The hot oven method was done and a number of samples were produced. The aroma smelled like roasted nuts in a few samples. Furthermore, samples browned significantly in the oven. The samples at lower temperatures still had a strong pea aroma and flavor.

5.4.4. Distillation

Distillation seems to be a good method in theory due to the ability to remove aroma without using corrosive chemicals. However, the gelatinization of the starch during the heating periods was problematic in the distillation method. The heating caused the starch in the flour to gelatinize, creating a large mass of thick and viscous material. This material, when dried, was not millable without being broken up, as it resembled pasta made from semolina. Due to the issues producing a quality product and the complications, these flours were not considered for sensory.

5.4.5. Germination Study

Peas that were germinated for 1, 2, and 3 days were dried and ground to a fine powder. The peas at day two had a rootlet that was visible and established, where at day three had long rootlets, that began growing small visible leaves. Peas at day one had small rootlets. These samples were ground to a fine powder without issues following drying in the vacuum oven.

5.4.6. Milling

Milling on the Z-mill was completed on all the samples to determine ease of reduction and for purposes of sensory and quality testing. The whole pea samples treated in the hot oven and the germinated were very easy to break down using the Braun grinder prior to the Z-mill. The high pressure solvent extracted samples with ethanol were very easy to remill to a fine powder. The sample extracted with water caused the mill to get very hot and did not reduce the particles sufficiently. The distilled samples were very hard to mill and were not completely ground due to the potential of breaking the mill.

5.4.7. Trained sensory panel

A trained sensory panel of six individuals rated the flour for appearance, flavor acceptability, and pea flavor intensity. The panelist scores were averaged. The strongest pea flavor intensity was in the untreated pea sample, followed by the supercritical test 3, supercritical test 2, supercritical test 1, and the sixty-minute soak and vacuum dry. This is where a clear break was observed between pea flavor intensity. The least intense sample was the 1:1 ethanol water high pressure extraction for 60 minutes, followed by the 2:1 ethanol:water soak and vacuum dry, 60 minute whole pea soak in water and hot oven dry, 2:1 ethanol:water high pressure solvent extraction for 60 minutes, 1:1 ethanol:water high pressure solvent extraction for 30 minutes, and finally the 2:1 ethanol:water high pressure solvent extraction for 30 minutes (Figure 2).

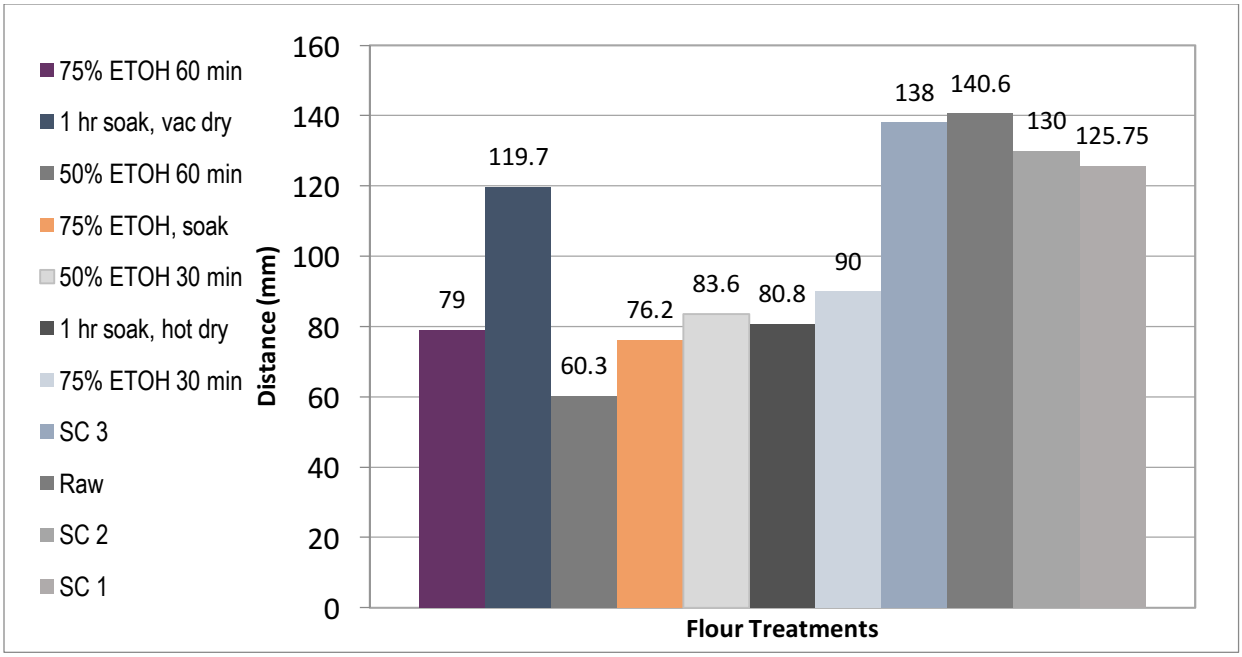

Figure 3. Intensity of pea flavor in treated pea flours as determined by a trained sensory panel.

5.4.8. Rapid visco-analyzer (RVA)

The samples were run on the RVA to determine how much damage had been done to the starch during the treatment and drying. The data collected was used to select the flour samples to move into baking trials. No statistical analysis was completed, but by looking over the RVA output, it was apparent that the starch profiles of treated peas were very different from the untreated pea flour. The extracted flours showed lower final viscosities, but the pasting temperatures were close to untreated flour (Table 5). The supercritical fluid extracted flours were also very similar for final viscosity.

Table 5. RVA parameters of treated and untreated pea flour.

Flour Treatment	Final Viscosity (cP)	Pasting Temperature (°C)	Peak Time (minutes)
Untreated Pea Flour	3790	76.7	7.0
Supercritical Fluid 1	3147	72.6	5.27
Supercritical Fluid 2	3375	71.8	7.0
Supercritical Fluid 3	3320	72.7	7.0
Distilled 50°C	841	92.9	7.0
Distilled 60°C	718	Error	7.0
Distilled 70°C	640	Error	7.0
Distilled 80°C	428	Error	5.87
1 hour soak, 30 minute dry	966	83.0	7.0
30 minute soak, 15 minute dry	1297	76.7	6.93
50:50 Extract	1971	76.3	7.0
75:25 Extract	2678	72.7	6.2

5.4.9. Consumer sensory testing

Sugar cookies following an at home formula were baked and evaluated in a consumer sensory evaluation. Both 30% and 100% flour substitutions were done on separate days. Panels of 50-75 individuals were recruited and tasted four cookies, supercritical fluid 1 extracted flour, 50:50 ethanol and water high pressure solvent extraction, 75:25 ethanol and water high pressure solvent extraction, and untreated pea flour. For both overall acceptance and flavor, cookies made from the raw pea flour was significantly liked less than the cookies made from treated flours (Table 6). The sensory data supported the further evaluation of a few flours.

Table 6. Average sensory scoring for gluten-free sugar cookie sensory evaluation using a 9-point hedonic scale.

Treatment**	Flavor Average Score	Overall Acceptance Score	Appearance Average Score	Texture Overall Average Score
Control Flour	5.87b*	5.97b	7.00a	6.41a
1:1 HPSE	6.93a	6.84a	6.93a	6.93a
3:1 HPSE	6.77a	6.78a	6.89a	6.99a
Supercritical Fluid 1	6.92a	6.79a	6.85a	6.54a

*Values followed by different letters indicate significant differences.
**Where control flour is untreated pea flour, 1:1 HPSE is HPSE flour extracted with 1:1 ratio of ethanol to water, and 3:1 HPSE flour was extracted with 3:1 ethanol to water, and supercritical fluid 1 was supercritical treatment with unknown parameters.

Sensory evaluations were completed and data analysis done based on a complete randomized design using an analysis of variance (ANOVA) at an alpha level of 0.05, and Tukey-Kramer test. No significance was observed in the appearance or texture; however, the flavor and overall acceptance of all the treated flours were liked more than the untreated sample. These results directed the research to move into a more focused study on the functionality and acceptance in baked goods using high-pressure solvent extracted flours.

6. MATERIALS AND METHODS

6.1. Materials

Commercial whole yellow peas were obtained from three commercial suppliers: AGT Foods (Bismarck, ND), Great Northern Agriculture Plaza (Plaza, ND), and Specialty Commodities (Fargo, ND). Food grade, non-denatured 95% ethanol was obtained from the NDSU Chemistry Stockroom. Whole eggs, skim milk, butter, shortening, vanilla, baking soda, baking powder (sodium bicarbonate), sugar, xanthan gum, potato starch, and foil pans were purchased from a local grocery store (Fargo, ND).

6.2. Methods

6.2.1. Milling of whole, dry peas

Peas from each supplier were milled on the Northern Crops Institute using a hammer mill model DA506 (Fitzpatrick Company, Elmhurst, IL). Each sample was milled separately. Samples were run through the mill twice, with the screen mesh size of 4,000 micron for the first run and 813 microns for the second. The hammer speed was 7,200 rpm and the feed rate was 15 rpm. Feed rate in weight per unit time was not able to be calculated because the amount moving through each revolution of the hopper was not known. Following milling, samples were stored in sealed bags in a walk-in freezer (-10 to -15 °C) until treatments were done.

6.2.2. High pressure solvent extraction (HPSE)

High pressure solvent extraction was completed on the pea flour using solvent solutions of non-denatured 95% ethanol and distilled water. A 1:1 and 3:1 ratio of ethanol to water were used in this study. The unit used for HPSE was the Timatic Micro Series Extractor (Supercritical Fluid Technologies Inc., Newark, DE; Figure 4). The pressure cycles ranged from 6 psi to 9 psi,

which allowed for solvent flow through the flour. Program parameters were 30 minutes with three-minute pressure cycles.

Flour (150 g) was weighed into a fine mesh bag and sealed. The flour was pre-wetted with about 100 mL ethanol water. The bag containing flour was then placed in the extraction vessel and filled to the top with solvent (1 liter). The pressure chamber was sealed and the program was started. The thirty-minute program went through 10 three-minute pressure cycles. Following the completion of the program, the solvent was drained under pressure, and the flour was spread thinly in foil pans.

Figure 4. High pressure solvent extraction (HPSE) (Supercritical Fluid Technologies Inc.) unit for flour extractions.

6.2.3. Vacuum oven drying

After the flour had been spread thinly in foil pans, the pans were placed in a vacuum drying oven (Buflovak, Buffalo, NY, USA; Figure 5) to remove the solvent and reduce moisture content. The oven held up to 16 pans, or approximately 8 extractions (1200 g after placement in the vacuum oven), a vacuum (13-16 psi) was applied and samples were allowed to dry in the oven for 14 hours. The oven temperature ranged from 65-68°C. After 14 hours, samples were removed from the oven and placed in plastic Zip-Loc bags (gallon sized) The bags were placed in the freezer (-10 to -15 °C) for at least one day prior to milling.

Figure 5. Interior of vacuum oven (Bufloavk Buffalo, NY, USA) used for drying treated flours.

6.2.4. Milling treated pea flour

After the dried flour was frozen, milling was completed to eliminate clumps that formed during drying. The purpose of chilling prior to milling was to prevent over-heating in the mill. The samples were milled on a Retsch Z-Mill (Ultra Centrifugal Mill ZM 100, Haan, Germany) at 14,000 RPM with a screen mesh size of 0.5 mm. There was not a hopper to control feed rate, so feed rate was not controlled, and no value is known. The mill was run until the cover for the flour collection was warm to the touch. This prevented the mill from over-heating and damaging starch in the pea flour. The 1:1 ratio could be milled for about 10 minutes before the mill started to overheat, where the 3:1 ratio could be milled for about 30 minutes before the mill started to overheat. The resulting flours had particle distribution different from the raw pea (Table 7). However, the concern with the oven hardening during milling overrides the concern of different particle size distribution.

Table 7. Representative particle size distribution for each treatment and control at different screen mesh sizes.

Treatment*	40 mm	60 mm	70 mm	80 mm	100 mm	Fines
SC Raw	3%	15%	10%	2%	14%	57%
SC 5050	0%	8%	10%	46%	9%	28%
SC 7525	0%	10%	20%	46%	15%	9%

* SC Raw is Specialty Commodities untreated flour, SC 50:50 is Specialty Commodities 1:1 ethanol and water, SC 75:25 is Specialty Commodities 3:1 ethanol and water

6.2.5. Chemical analysis

6.2.5.1. Moisture content of flour

Moisture content of flour was determined using a modified forced-draft air oven (AACCI 2016a, Official Method 44.15-02). Small metal tins were weighed (g) and 3-4 grams of flour were added to the pan. The weight of the flour and pan was recorded. The pans were placed in

the oven at 130 °C for 2 hours and samples were cooled in desiccators for 1 hour. The final weight was recorded. Moisture content was calculated using a difference equation.

$$M_n = ((W_w - W_d)/W_w) \times 100 \quad (1)$$

Where W_w: initial weight

W_d: dry weight

M_n: moisture content

6.2.5.2. Rapid visco-analyzer (RVA)

Starch profiles were measured using the RVA 4500 (SN 2143306-45A, Hägersten, Sweden). Base weight for flour and water based on a 14% moisture content were 3.5 g flour and 25 g water, which was adjusted for flour moisture content. The temperature profile started at 50 °C and was raised to 95 °C at 4 minutes and 42 seconds. The temperature was held until 7 minutes and 12 seconds, where it was then dropped back to 50 °C at 11 minutes. The temperature remained at 50 °C until the end of the 23 minute profile. The speed of the paddle rotation was 960 rpm for the first ten seconds and then lowered to 160 rpm for the remainder of the profile. Parameters measured included the peak, trough, breakdown, final viscosity, setback value, peak time, and pasting temperature.

6.2.5.3. Protein content of flour

Protein content was measured on a LECO FP628 (LECO, St. Joseph, MI) nitrogen analyzer located at the Northern Crops Institute (Fargo, ND). The method of analysis followed the AACCI official method of analysis 46-30.01 (AACCI 2016)b. The nitrogen conversion factor (NCF) used for the protein calculation was 6.25. Samples (0.500 g) were weighed into small foil pieces and sealed shut. The sample weights were recorded in the Leco software. The samples were dropped into the auto-sampler of the machine. Using the nitrogen (%) and the constant, the total crude protein was determined.

Protein content was measured using the Dumas combustion method to measure nitrogen concentration. Because pulses have higher nitrogen content (approximately 16%), the nitrogen factor used is 6.25 (Hall and Schonfeldt 2013). This number varies for different types of food, such as wheat uses a factor of 5.7 (Hall and Schonfeldt 2013). Protein content is calculated using the following equation, where *NF* stands for nitrogen factor:

$$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times NF \quad (2)$$

6.2.6. Baking

6.2.6.1. Cookie baking

Baking cookies roughly followed the official method 10-54.01 (AACCI 2016c) for cookie quality, with several modifications. Gluten-free cookies were prepared using a recipe from an at home cookbook. The recipe (Table 8) was converted to a percentage formulation, and wheat flour was substituted with pea flour. Cookies were mixed on a KitchenAid Commercial (Benton Charter Township, MI) mixer at speed 4. Sugar and butter were creamed together for 1 minute. Eggs and vanilla were then added and mixed an additional 30 seconds on speed 4. Once the wet ingredients had been mixed, the dry ingredients were added. The mixer started at speed 1 for 30 seconds to prevent loss of dry ingredients, and thereafter was increased to speed 4 for 90 seconds. The cookies were rolled with a rolling pin using end rings (6.35 mm). The cookies were cut using a circular cookie cutter (5.08 cm). The cookies were baked at 350°F for 9 minutes and cooled for 30 minutes prior to bagging. After the cookies were bagged, they were left at room temperature overnight in sealed plastic zip-loc bags. The cookies were then used for sensory and quality testing the following day.

Table 8. Sugar cookie formulation in percentage.

Ingredient	Percent
Granulated Sugar	24.8
Butter	25.9
Vanilla	1.6
Egg	5.6
Pea Flour	41.6
Baking Powder	0.2
Baking Soda	0.3
Total	100

6.2.6.2. Gluten-free cake baking

Cakes were baked and evaluated following a previously used procedure, and modified for pea flour (Levent and Bilgicli 2011)**.** Gluten-free cakes were baked following a formula and procedure (Levent and Bilgicli 2011). In this formula (Table 9) pea flour was substituted for the other forms of flour at a 100% substitution. Cakes were mixed on a KitchenAid Commercial mixer. The eggs and sugar were mixed together on speed 6 for five minutes to form a cream. Following, all of the remaining ingredients were added. The mixer was started at speed 1 for 30 seconds to prevent the loss of powder ingredients, and then the speed was increased to speed 6 for one minute. Cakes were baked in foil pans (20.32 cm x 20.32 cm x 3.81 cm) that were sprayed with vegetable oil. Each pan consisted of 700 g of batter, and the batter was spread using a spatula to facilitate an even distribution throughout the pan, because gluten-free batters are very viscous. Cakes were baked for 27 minutes at 350°F, and cooled to room temperature for two hours prior to covering with aluminum foil.

Table 9. Gluten-free cake formulation in percentage.

Ingredient	Percentage (%)
Corn Starch	14.2
Pea Flour	14.2
Sugar	21.2
Eggs	14.2
Shortening	21.2
Vanilla	0.1
Xanthan Gum	0.3
Baking Powder	0.4
Salt	0.1

6.2.7. Cookie and cake quality

6.2.7.1. Cookie quality

6.2.7.1.1. Cookie physical dimensions

Physical dimensions of the cookies were determined using a clear ruler (cm and mm). The cookie was measured from the 0 mm to the height of the center of the cookie. There was very little difference in the cookie height from the outside to the inside. The height was measured on the cut out dough and the cooled, baked cookie. The spread of the cookies was determined using a clear ruler as well. The diameter of the rolled and cut out cookie dough was measured in mm, and again the diameter was measured after baking. Weight of the cookie dough and the cooled, baked cookie was taken. Three samples per batch were measured to account for differences throughout the rolling or mixing processes.

6.2.7.1.2. Texture analysis of cookies

Hardness of cookies was evaluated on day 1 and day 5. The force needed to break a cookie in half was evaluated on the texture analyzer (TA.XT.Plus SN 41813**)** using AIB Method for Cookies, measuring snapping force and deflection of cookie as a measure of shelf-life. The attachment used for this measurement was the TA-92N at the 2” width. Settings for this method

included a pretest speed of 2.5 mm/s, test speed of 2.0 mm/s, post test speed of 10 mm/s, distance of 6 mm, trigger type of 20 g, tare rate was automatic, and data acquisition rate of 200 pps. This method is a three-point break, which measures the amount of force needed to break the cookie in half. Five cookies from each treatment were evaluated on days 1 and 5 to help account for any issues with inconsistent texture.

6.2.7.2. Cake quality

6.2.7.2.1. Cake physical dimensions

Cakes were baked in disposable foil pans (20.32 cm x 20.32 cm x 3.81 cm). The height was measured with a cake measurement template. Because the cakes were square shaped instead of circular, the height in the center was the only height taken. The height was obtained after baking and cooling to account for any volume loss after being removed from the high heat.

6.2.7.2.2. Texture analysis of cakes

Firmness of gluten-free cake were evaluated on day 1 after baking using the texture analyzer (TA.XT.Plus SN 41813**)** and AIB Method for Cake measuring the firmness of cake by compression. The probe used was a one inch flat ended cylinder (P/25P)**.** Parameters for this method included a pretest speed of pretest speed of 3.0 mm/s, test speed of 1.7 mm/s, post test speed of 10 mm/s, distance of 6 mm, trigger type of 20 g, tare rate automatic, and data acquisition rate of 200 pps. Square pieces of 1”x1” were used rather than a circular 1” diameter piece of cake, typically used in the AIB method. Force (kg) was measured on five pieces from each treatment.

6.2.8. Sensory evaluation

Sensory evaluation on cake and cookies was completed similarly. Each panel included two sources and three treatments. For example, one panel included the three treatments of

Specialty Commodities and AGT flours but not Great Northern Ag Plaza. The set up included three separate panels for each product, six samples per panel. The sensory panels aim for 50-100 consumer panelists. The panels were set up as incomplete blocks, with an alpha value of 0.05 and a randomized complete block design. The IRB Protocol #AG14295, Development of Reduced Flavored Pea Flour was followed for sensory evaluations. Panelists for both cookie and cake sensory panels were a mix of students, staff, faculty, and visitors. Demographic information was not collected, but a relatively diverse population was observed.

Sensory evaluation of the treated flours used in cookies and cake were compared. Panelists were asked to score products based on appearance, flavor, texture, and overall acceptability on a 9-point hedonic scale from 1 (dislike extremely) to 9 (like extremely). The results indicated whether or not a significant difference was detectable between the control (untreated pea flour) and the treatments (1:1 and 3:1 ethanol to water HPSE extracted pea flour) in the parameters evaluated.

6.2.8.1. Sensory evaluation of cookies

Sensory evaluation of cookies occurred the day following preparation. The panel included six samples, cracker, and water were provided to panelists. The panels were held one time per week in Harris Hall Room 11, the IRB protocol followed for this study was AG14295, Development of Reduced Flavored Pea Flours. Panelists were a mix of students, staff, faculty, and visitors. No demographic, dietary restriction, or preference information was collected on panelists. The panels were organized as follows: panel 1 included cookies prepared with flours from AGT and Great Northern Agriculture Plaza (GNAP). Panel 2 included Specialty Commodities (SPC) and GNAP. Panel 3 included SPC and AGT. Cookies were served in randomized sets of three. For panel 1, AGT samples were served first in random orders, followed

by the GNAP samples in random order. This was the same for each of the three panels. Panelists were asked to complete the evaluation using a 9-point hedonic scale for appearance, texture, flavor, and overall acceptance (Figure 6).

Random three-digit codes were assigned to each sample, and to reduce risk of error when combining data, each sample code was kept the same for the treatment for the duration of sensory. AGT samples and codes were as follows; raw – 125, 1:1 – 262, and 3:1 – 343. Great Northern Agriculture Plaza samples and codes were as follows; raw – 471, 1:1 – 589, and 3:1 – 628. Specialty Commodities samples and codes were as follows; raw – 829, 1:1 – 914, and 3:1 – 765.

6.2.8.2. Sensory evaluation of gluten-free cake

Sensory evaluation of cake occurred the day following preparation. Each panel included six samples. The panels were held one time per week in Harris Hall Room 11. Sensory panelists were untrained panelists, a mix of students, staff, faculty, and visitors. There was no demographic, preference, or dietary information collected from panelists. The panels were organized as follows: panel 1 included GNAP and SPC, panel 2 includes SC and AGT, and panel 3 included AGT and GNAP. The set-up of these panels were similar to that of cookies. Cake samples were cut from cakes into 1”x1” squares. They were kept in sealed cake pans to prevent them from hardening prior to serving. Panelists were asked to complete the evaluation using a 9-point hedonic scale for appearance, texture, flavor, and overall acceptance.

Sugar Cookies

Sensory Evaluation of Pea Flour Cookies

SAMPLE NUMBER: _____###_____

Please evaluate the bread sample for the following qualities: Flavor, Texture, Appearance and Overall Acceptability (i.e. liking). Make an X on the appropriate line. Please give comments in the space provided below each quality if desired.

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. Sensory acceptance test evaluation form.

Random three-digit codes were assigned to each sample, and to reduce risk of error when combining data, each sample code was kept the same for the treatment for the duration of sensory. AGT samples and codes were as follows; raw – 125, 1:1 – 262, and 3:1 – 343. Great Northern Agriculture Plaza samples and codes were as follows; raw – 471, 1:1 – 589, and 3:1 – 628. Specialty Commodities samples and codes were as follows; raw – 829, 1:1 – 914, and 3:1 – 765.

6.2.9. Headspace analysis of pea volatiles

Headspace analysis of flavor and aroma volatiles was completed using the solid phase microextraction (SPME) method on the flour, cake, and cookies. The analysis was completed on an Agilent 7820A Gas Chromatography (Santa Clara, CA) system with manual injections. The filament used for adsorption of volatile compounds was a Supelco (Bellefonte, PA) 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane StableFlex fiber.

6.2.9.1. Standards and standard curve development

Standards used for this study determined from previous studies, detailed in section 4.7.1. Standards were obtained from Sigma Aldrich (St. Louis, MO) and kept frozen until use. Hexanal, 1-hexanol, hexyl acetate, octanal, 1-octen-3-ol, dipropyl disulfide, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and 2-sec-butyl-3-methoxypyrazine were the compounds evaluated. Standard curves were produced by diluting the standard in corn starch. The procedure used was a bench-top procedure that was completed by first weighing test tubes and screw caps. Approximately 6 g of corn starch was added to the test tubes, weights were taken again, and placed in a freezer, averaging -13 to -15 °C, for 120 minutes. Cold standard was then pipetted into each tube (10, 20, 30, and 40 μL). The test tubes, including corn starch (6 g), and internal standard, were sonicated in an ultrasound bath for 60 minutes at 60 °C. Test tubes

were dried and were then allowed to cool to room temperature (60 minutes) and weights were taken again. Another 6 g of corn starch was then added to each tube and the samples were sonicated for 30 minutes at 60 °C. Test tubes were dried and allowed to cool to room temperature for 60 minutes. The weights of each test tube and contents were recorded again, and the samples were allowed to equilibrate for three days.

After the equilibration period, composite standards for each concentration were weighed into test tubes. Running composite standards is important to determine how compounds interact with each other on the fiber. For each standard 0.5 g of each standard was measured and added to a test tube, and each of the 9 standards at that concentration were added. The composite samples were then mixed and sonicated for 30 minutes at 60 °C. The composite samples then equilibrated for about three days prior to analysis.

Dilution of the standards was done by adding a set amount of the higher concentration standard to neutral corn starch. These weights were then collected and the sample was added to a test tube. These tubes were sonicated for 60 minutes at 60 °C. The samples were allowed to equilibrate for three days prior to analysis.

6.2.9.2. Solid phase micro-extraction (SPME)

The SPME method used for evaluation of headspace of pea flour, cookies, and cake was modified from a previous student's method (Prasad 2013). Prior to the headspace analysis, 0.5 g of flour or ground cake/cookie were added to a headspace vial (4 mL). Internal standard (99% 2-heptanone, 10 μL) was diluted in 12 g corn starch and allowed to equilibrate. Internal standard (0.10 g) was added along with salt (0.15 g) into the headspace vial. Distilled water (1 mL) was added to help release the volatile compounds. The vial was sealed with a PTFE silicone septa baked for 4 hours at 150 °C prior to use. The samples were then vortexed for 10 seconds on high

speed and heated at 40 °C in a HAAKE L (Vreden, Germany) D* water bath for 10 minutes. Following the heating, the filament was inserted into the headspace of the vial, and the fiber was allowed to adsorb volatiles for 10 minutes in the 40 °C water bath. The filament was then removed from the vial.

6.2.9.3. Headspace volatile evaluation

Once the volatiles were adsorbed, the filament was inserted into the injection port (250 °C) of the the Agilent 7820 GC. The sample was allowed to desorb. The Phenomenex ZB Wax 60 m x 250 μm x 0.25 μm column with temperature restrictions of 40 °C-260 °C was used. The pressure used was 39.918 psi, with a flow of 60 mL/ minute for the first two minutes, and 20 mL/ minute for the remainder of the program for the front inlet. To separate volatiles, the GC oven initial temperature program was set to 35 °C for 6 min, then ramped 12 °C/min to 80 °C for 2 min, followed by a second ramp set at a rate of 12 °C/min ramp to 120 °C, followed by the third ramp with a set rate of 20 °C/min ramp to 250 °C, and followed by a 6 minute hold to the program. The total time of the program was 27.583 minutes. The air flow was set to 400 mL / minute. The hydrogen flow was set to 30 mL / minute. High purity hydrogen was substituted for helium gas due to the significant increase in helium price. This flow rate was set to 25 mL / minute.

6.2.10. Statistical design

The experimental design for this project was split into several different designs to fit the data collected. The laboratory analysis of moisture, protein, and RVA values were analyzed using a two-way analysis of variance (ANOVA). Being the sample sizes were even, Tukey's Multiple Range test was used to control familywise error rate where multiple comparisons were made. For sensory analysis, a split-plot design was used with panel as a random effect, and fixed

effects being treatment and supplier. The Tukey-Kramer Multiple Range test was used as a post hoc test when significance was identified. This controlled experiment-wise error rate. The alpha level used in both instances was 0.05.

7. RESULTS AND DISCUSSION

7.1. Flour treatments

Flour treatments were accomplished using a Timatic Microseries Extraction unit. The program allowed for movement throughout the flour. Flours were treated with a 1:1 and 3:1 solution of ethanol and distilled water. The flour treated with the 1:1 solution appeared to have more key structural changes after vacuum drying. The dried pieces were large and very firm, slightly softer than pasta. This caused problems with milling. Because the pieces were larger and harder to break apart, the mill overheated more, which the combination of two situations, may have influenced structural and functional properties. The changes to structural properties and `baking quality are further discussed in section 7.5 (Starch Pasting Properties).

7.2. Flour moisture content

The native, untreated flour had significantly higher moisture content than the treated flours (Table 10). When the treated flours were dried, they were left in the vacuum oven overnight to remove the ethanol and water, so it was expected that the moisture content would be lower than the untreated ground peas.

7.3. Protein content

There were significant differences between the treated samples and the untreated flours (Table 11). These were analyzed in blocks, where each block was a treatment. All the protein contents for untreated, 1:1, and 3:1 samples were pooled and the data was analyzed for differences between treatments. Each treatment and source followed trends where all of the untreated samples had protein contents below 20.5%, and all of the flour treatments had protein contents above 21.1% (Figure 7). An assumption to understanding this increase is that the total amount of soluble material after the extraction was reduced because soluble carbohydrates and

lipids could be removed by the ethanol and water treatment. This would cause the ratio of protein to total amount of material to increase.

Table 10. Moisture content of treated and untreated pea flours.

Source	Treatment	Moisture Content (%)
Specialty Commodities	3:1[1]	6.2[b]
Specialty Commodities	1:1[2]	6.4[b]
Specialty Commodities	Control[3]	11.2[a]
AGT	3:1[1]	6.2[b]
AGT	1:1[2]	6.9[b]
AGT	Control[3]	12.2[a]
Great Northern Ag Plaza	3:1[1]	6.0[b]
Great Northern Ag Plaza	1:1[2]	6.4[b]
Great Northern Ag Plaza	Control[3]	13.2[a]

[1] Indicates the 3:1 treatment of ethanol to water HPSE flour. [2] Indicates the 1:1 treatment of ethanol to water HPSE flour. [3] Indicates the control untreated pea flour.

Table 11. Average protein content for treated and untreated flours.

Source	Treatment	Protein Content (%)
Specialty Commodities	3:1[1]	21.3[a]
Specialty Commodities	1:1[2]	21.2[a]
Specialty Commodities	Control[3]	19.9[b]
AGT	3:1[1]	21.9[a]
AGT	1:1[2]	21.7[a]
AGT	Control[3]	20.4[b]
Great Northern Ag Plaza	3:1[1]	21.6[a]
Great Northern Ag Plaza	1:1[2]	21.2[a]
Great Northern Ag Plaza	Control[3]	20.4[b]

[1] Indicates the 3:1 treatment of ethanol to water HPSE flour. [2] Indicates the 1:1 treatment of ethanol to water HPSE flour. [3] Indicates the control untreated pea flour.

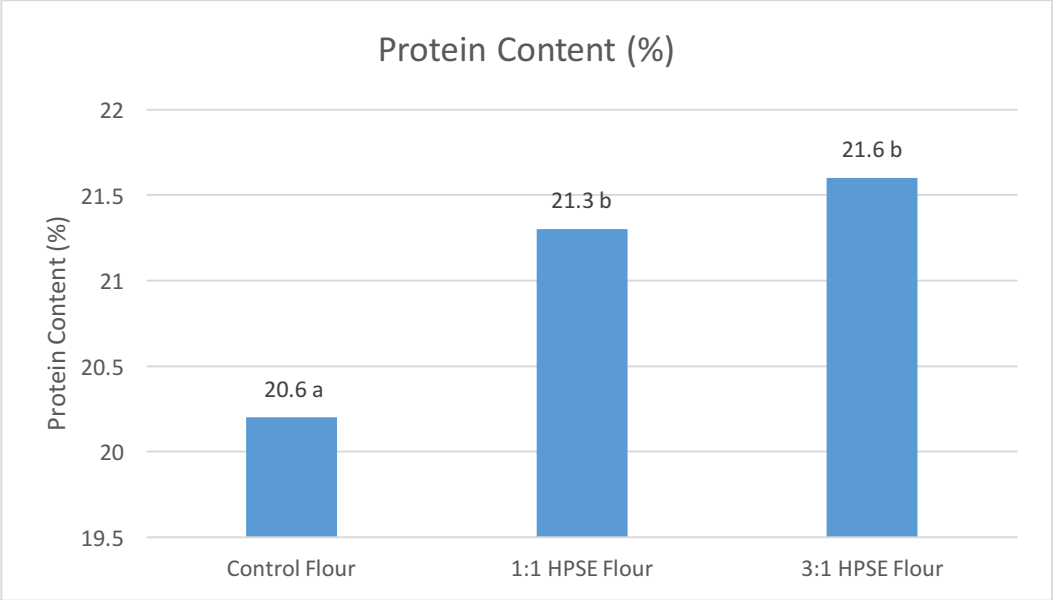

Figure 7. Protein content averages by treatment, including data from all three suppliers.

Certain carbohydrates are considered water or ethanol soluble, and several are soluble in 80% ethanol, which would be a solution of 80% ethanol and 20% water. Monosaccharides, disaccharides, and fructans are considered soluble in 80% ethanol (Ranwala and Miller 2008). This indicates the potential loss of sugar molecules to the solution used during the HPSE. Carotenoids are also ethanol soluble, which means that they are removed during HPSE as well (Araus and others 2011). The extraction of different materials was likely the reason for the increased protein contents in the treated flours.

7.4. Starch pasting properties

The RVA profile measures the changes that occur in the starch as it is heated with agitation by a turning paddle. The program heats flour and water with a constant turning of the paddle. As the mixture heats, the starch gelatinizes, meaning the crystalline structure of the starch is disrupted and broken, allowing the chains of amylose and amylopectin, to absorb water. Starch retrogradation occurs when the disaggregated chains of amylose and amylopectin begin to reorganize themselves into ordered structures (Cozzolino 2016). The peak viscosity and pasting

temperatures of the 1:1 ethanol to water HPSE flour were significantly lower than the control and the 3:1 ethanol to water HPSE flour (Table 12). The final viscosities for each treatment were significantly different from one another. This means that each treatment impacted the final viscosity differently, with the most significant difference between the 1:1 treatment and the control.

Table 12. RVA data for treated and untreated pea flours.

Treatment**	Peak Viscosity (cP)	Final Viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting Temperature °C
Control	1632b*	2821a	60.8a	1254a	74.5a
1:1	1426a	1941c	90.8a	1056b	78.7b
3:1	1658b	2501b	78.7a	582c	74.1a

*Values followed by same letters indicate no significance differences among values.
**Where: 3:1 treatment is the 3:1 ethanol water HPSE flour, 1:1 treatment is the 1:1 ethanol water HPSE flour, and the control is the control pea flour.

The curves for the control, 1:1, and 3:1 HPSE treated flours were compared and similar peak viscosities were observed for the 3:1 and the control flours. The breakdown was more for the 3:1 than the control flour. The final viscosity of the control was higher than both the 1:1 and 3:1, but the 3:1 was also higher than the 1:1.

The 1:1 treatment was significantly different from the control and the 3:1 treatment. This could be due to the starch damage that occurred during the treatment with ethanol and water. Being this treatment has a higher concentration of water used, there was more water removed in the vacuum oven. When exposed to heat and water, starch is susceptible to gelatinization, which in this case would cause pre-gelatinized starch. This starch would possess different baking properties. Though there were significant differences in the final viscosity, the peak viscosity and pasting temperatures between the control and the 3:1 treatment were very close, and could be suitable replacements for one another. A goal of this research was to determine if the treatment

impacted raw flour characteristics. Based on the RVA profile, small changes supports the minimal impact of the 3:1 (ethanol to water) treatment on starch properties.

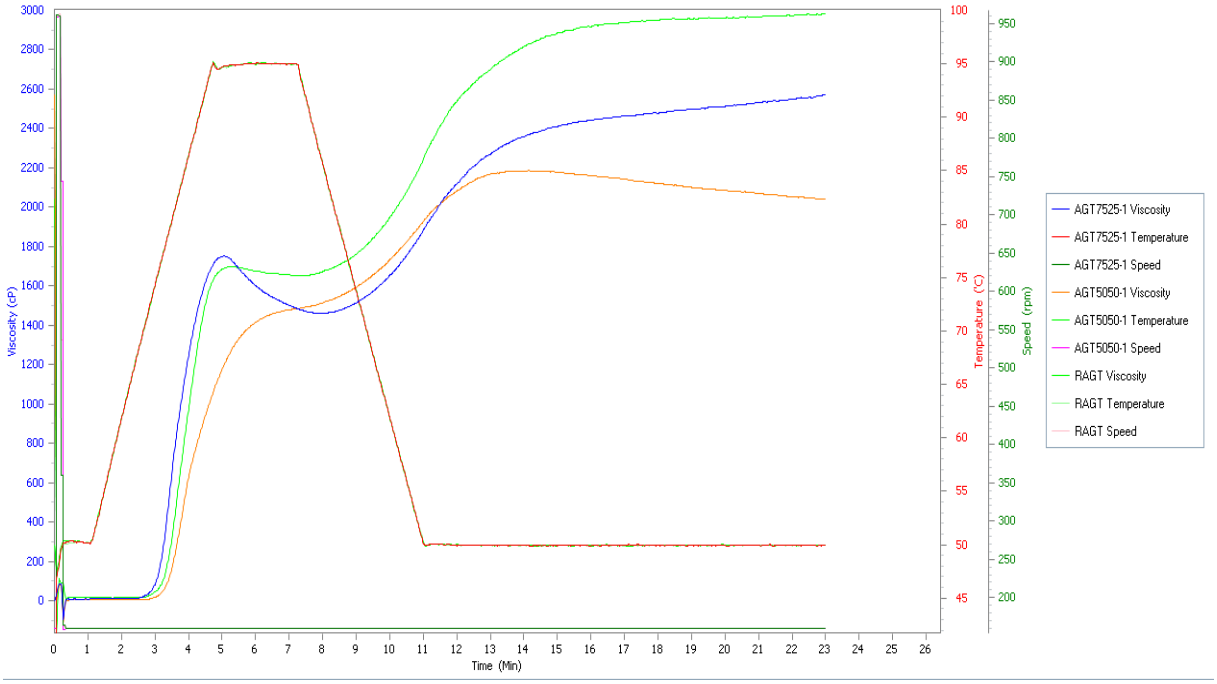

Figure 8. RVA profiles of AGT 1:1, 3:1, and control flours.

7.5. Sensory and quality characteristics of cookies

7.5.1. Baking properties of cookies

To determine if baking properties were altered by treating flour to HPSE, the samples were measured before and after baking. The same samples were selected at random before and after baking. Three samples were measured from each treatment (Table 13). The appearance of the cookies was similar to the appearance of wheat flour sugar cookies (Figure 9). The diameter and height of the cookies were significantly different for the treated flours and the control flour. The height of the cookies from treated pea flours was significantly higher, but had significantly less diameters than the control. The cookie weight increased for the 1:1 t (i.e. 1:1 cookie) reatment and 3:1 (i.e. 3:1 cookie) treatment. The 1:1 and control cookies were not significantly

different, but the 3:1 cookie was significantly heavier than the control. However, there was no significant differences in cookie weight between the 3:1 and 1:1 treatments.

Figure 9. An example sugar cookie made with pea flour

Several parameters of cookies were measured before and after baking, including the weight, height, and diameter. The cookie weight difference after baking was not significantly different among the treatments, with average weights for the control, 1:1, and 3:1 being 17.17 g, 17.61 g, and 18.22 g, respectively. This suggests that the rate of moisture loss was similar among the cookies. The differences in diameter and height were consistent, where the control treatment was significantly different from HPSE treatments.

Overall cookie spread was reduced for the cookies made with the HPSE treated flour from the control flour. There are several possible explanations for this. First, the slight increase in total protein may have impacted the spread, being the spread ratio is influenced by protein interactions. Flour particle size and damaged starch both play an important role on quality. When the flour has lower water absorbance, the sugar can absorb more water, which allows for a reduction in dough viscosity, increasing spread. The treated flours had lower moisture contents,

thus the flour took more residual moisture to hydrate, meaning less water was available for the sugar interactions to reduce dough viscosity (Barak and others 2014).

Table 13. Weight, diameter, and height for cookies baked with treated and untreated pea flour.

Treatment**	Cookie Weight (g)*	Cookie Diameter (cm)*	Cookie Height (mm)*
Control	17.2a	6.63a	4.6a
1:1	17.6ab	5.93b	6.6b
3:1	18.2b	6.13b	6.3b

*Values followed by same letters indicate no significance differences among values.
**Where: 3:1 treatment is the 3:1 ethanol water HPSE flour, 1:1 treatment is the 1:1 ethanol water HPSE flour, and the control is the control pea flour.

7.5.2. Cookie sensory

7.5.2.1. Cookie appearance

Cookie appearance was evaluated in each sensory panel for three treatments and three suppliers. Combining the data from all three sensory panels, using an alpha of 0.05, there were no significant differences observed between suppliers. However, differences were observed between the treatments (Table 14). The control was significantly different than the two treatments, meaning that the panelists noticed a significant difference in appearance between the treatments and the control. The average acceptance scores for the cookies made with 3:1 treated flour were determined the best.

The results of the cookie appearance acceptance show that the control and the 1:1 cookies treatment were not significantly different, but the control was different from the 3:1 cookie. The 1:1 cookies had significantly lower appearance scores from the 3:1 cookies. This means there were differences in acceptance of the appearance, with the 3:1 treatment being rated the best.

Table 14. Acceptability ratings for cookies made with treated and untreated pea flour.

Treatment**	Appearance Score*	Flavor Score*	Texture Score*	Overall Acceptance Score
Control	6.7a	4.3a	5.1a	4.5a
1:1	6.6a	6.7b	6.6b	6.6b
3:1	7.1 b	6.5b	6.7b	6.5b

*Values followed by same letters indicate no significance differences among values.
**Where: 3:1 treatment is the 3:1 ethanol water HPSE flour, 1:1 treatment is the 1:1 ethanol water HPSE flour, and the control is the control pea flour.

7.5.2.2. Cookie flavor

The cookie flavor was evaluated for all treatments and suppliers with an alpha value of 0.05. There were significant differences observed at this level, where the control was liked significantly less than the treated flours, but there was no significance between the cookies made with treated flours (Table 14). The difference was over 2 points on a 9-point scale, which is a very drastic improvement in rating. There were no significant interactions between supplier and treatment, and supplier and panel after adjustment with Tukey-Kramer. This adjustment is needed to prevent potential for finding false significance when there is no significance, due to the fact that t-test does not make multiple comparison corrections. There were no significant differences between supplier or panel.

This indicates that with treating the flour with high pressure solvent extraction, the flour flavor was improved significantly. There was no significant difference between the two treatments, thus suggesting that those treatments improved flavor of the flour, equally.

7.5.2.3. Cookie texture

The cookie texture was evaluated for all treatments and suppliers with an alpha value of 0.05. There were significant differences observed at this level. There were no significant differences seen between the suppliers and the combination of supplier and treatment, but there

were significant differences observed between the treatments (Table 14). The control was liked significantly less than the cookies from treated flours, but there were no significant differences between the two treatments was observed.

7.5.2.4. Cookie overall acceptance

The overall acceptance rating of cookies indicates the panels' acceptance of that product overall, factoring in the parameters evaluated and any other parameters they thought impacted the eating experience of the cookie. There were no significant interactions for overall acceptance, but again, there were significant differences between treatments (Table 14). The significant differences were between the control and the cookies made from treated flour. No significance was detected between the cookies made with the two HPSE flours.

The treated flours were rated much higher than the control, indicating that eating properties of these cookies were improved from the control flour. This indicates that sensory properties are improved by treating the flour with either HPSE treatment prior to use in baking.

7.5.2.5. Cookie sensory conclusions

Compiling the results of all four attributes of the cookie sensory evaluation, results for the flavor, texture, and overall acceptance were consistent. The control was liked significantly less than the cookies made with the treated flours, but there was no significance between the treatments. The cookie appearance results did show that the 1:1 treatment and control were not significantly different, but there was significant differences between the 3:1 treatment and the control as well as the 1:1 treatment. The goal was to produce flour that had improved flavor without impacting the appearance. Therefore, both the 1:1 and 3:1 treatments resulted in cookies with improved sensory characteristics compared to the raw flour.

Cookies made with other treated flours were investigated in sensory in previous research. Lupine flours were tested in cookies in sensory, and similar trends in scoring were observed in this study (Maghaydah and others 2013). Different extraction processes have shown to improve the sensory characteristics of food products prepared with the flour. Further discussion is provided in section 7.7. sensory conclusions.

7.6. Sensory and quality characteristics of cake

The texture (Table 15) showed no significant differences on day one for any of the cakes made with treated flours. The cake height was significantly lower for the control than the treated flour. Starch gelatinization impacts the formation of bubbles, which create the texture and volume of the cake. The cakes made with HPSE treated flour had several large air cells in the cake, which reduced the texture, similar results have been observed in gluten-free bread (Defloor and others 1991). The appearance of the pea flour cake crumb was inconsistent throughout the whole crumb, with some places having large air bubbles, some spots appeared gummy, and others had normal distribution (Figure 9). The areas that appeared gummy, may be the result of not mixing the flour in well enough before adding the wet ingredients when making the batter, or poor air movement through the baking process (Majzoobi and others 2016).

Figure 10. Gluten-free pea flour cake crumb appearance.

Treating pea flour by HPSE and vacuum drying likely caused pre-gelatinization of starch, due to hydrating and drying with heat under vacuum. The increase in height of the cakes made with treated flour were higher than the control, which may be due to the pre-gelatinization of starch during HPSE. Pre-gelatinized starch can facilitate trapping of air during mixing, and thus aiding in increased volume during baking due to release of air while cake is baked.

Table 15. Texture and height measurement for cake made with treated and untreated flours.

Treatment**	Height (mm)*	Texture (g)*
Control	34.33a	0.706a
1:1	38.83b	0.424a
3:1	37.75b	0.427a

*Values followed by same letters indicate no significance differences among values.
**Where: 3:1 treatment is the 3:1 ethanol water HPSE flour, 1:1 treatment is the 1:1 ethanol water HPSE flour, and the control is the control pea flour.

Particle size also impacts volume and viscosity. Finer flour produces denser products with lower specific volume with more uniform bubble distribution (de la Hera and others 2013). Though cake volume was not measured, taller cakes imply larger specific volumes. The bubble distribution and uniformity was not measured using a c-cell or visual imaging software, but through visual observation, there were more uniform bubbles in the cake made with the control flour. The control flour had finer particle size than the HPSE flour treatments.

7.6.1. Cake sensory

7.6.1.1. Cake appearance

There were no significant differences in the appearance of the cake made with different treated pea flour (Table 16). There was a significant interaction between the supplier and treatment, but with the Tukey-Kramer adjustment, the interaction no longer was significant. The Tukey-Kramer adjustment was used to help prevent detection of significant differences that were

not significant. With the adjustment, there were no significant differences detected, indicating that the treatments had minimal impact on the appearance of cake (Table 16).

There were no significant differences indicated by the sensory analysis of cake appearance. The appearance was not impacted by different HPSE treatments to the pea flour based on similar appearances sensory scores between cakes made from treated and untreated flour. One of the important factors was that the products keep an appealing appearance, which was maintained with the cakes.

Table 16. Average acceptance scores of cake made with treated and untreated pea flours.

Treatment**	Appearance Score*	Flavor Score*	Texture Score*	Overall Acceptance Score*
Control	5.9a	3.8a	5.3a	4.2a
1 to 1	6.0a	6.5b	5.9b	6.2b
3 to 1	5.9a	6.4a	5.9b	6.2b

*Values followed by same letters indicate no significance differences among values.
**Where: 3:1 treatment is the 3:1 ethanol water HPSE flour, 1:1 treatment is the 1:1 ethanol water HPSE flour, and the control is the control pea flour.

7.6.1.2. Cake flavor

Significant differences in cake flavor were observed between treatment and supplier (Table 16). These significant differences were observed between the 3:1 cake and the control, as well as the 1:1 cake and the control. No significant differences were observed between the 1:1 and 3:1 treatment. This means that the panelists determined distinct differences between the cake made with raw flour and those made from treated flours, but did not detect differences between the cakes made with treated flour. The cakes made with treated flour were rated higher than the control.

Significant differences between suppliers also existed (Table 17). This means that the supplier of the peas also impacted the rating of each sample. The cakes made with the flour from the AGT were rated highest of the three suppliers consistently. Though there is a significant

difference in the suppliers, the trend for the cake from untreated flour control to be rated significantly lower than the cakes from the treated flour still existed, meaning any significance between suppliers did not impact the panelists' abilities to detect differences between treatments.

Table 17. Acceptability ratings for flavor of cake by supplier.

Source*	Average Acceptance Score by Supplier**
AGT	6.2a
GNAP	5.3b
SC	5.3c

**Values followed by same letters indicate no significance differences among values.
*Where AGT is a supplier of peas, GNAP is Great Northern Ag Plaza peas, and SC is Specialty commodities peas, each are an average for the control, 1:1 and 3:1 treatment scores.

7.6.1.3. Cake texture

Texture of cakes were rated and significant differences were observed between treatments. Significant differences were observed between the cake made with the raw flour and the cakes made from the 3:1 flour and 1:1 flour (Table 16). There were no significant differences between the cakes made with the two treatments. There were no significant interactions or significant differences between suppliers.

The texture was liked significantly better for the cakes made with HPSE flour treatments, which indicates that the cake texture was improved with the HPSE treatment. This parameter was not targeted with the goals of deodorization, but is an advantage of the extraction procedure.

7.6.1.4. Cake overall acceptance

The overall acceptance of cake showed significant differences between treatments (Table 16). No significant differences between suppliers or interactions were observed. The significant differences were observed between the control cake and the 1:1 cake, and the control cake and the 3:1 cake (Table 16). No significance was seen between the cakes made from the two

treatments. This indicates that source of the peas had no significant impact on the consumer rating.

The overall acceptance ratings indicated that the control samples were rated significantly lower than the HPSE treated samples. This implies that the HPSE treated flour cakes were improved in all four sensory attributes, which aligns with the main goal of the study.

7.6.1.5. Cake sensory conclusions

When compiling all the cake sensory data, no significant differences for the appearance between any of the cakes made with different treatments were observed, indicating that the appearances are liked the same between the treatments. For flavor, texture, and overall acceptance, there were significant differences between the cakes made with treated flours and untreated flours, but there were no significant differences between the cakes made from treated flour. This shows that the eating experience of the treated cakes was liked more, but the appearance had not been changed by the HPSE. There was some variance within suppliers, where the data implied that the source of the flour was significantly different, but even so, the results when looking at treatment as a whole, came back consistent, similar to those of the cookies.

These results align with previous studies on different treated flour used in cake formulations. No specific studies on treated pea flour were found, but extruded bean flour was used in baking cakes, and these cakes showed improved sensory characteristics (Gomes and others 2015). Another study focused on heat treated sorghum flour in cake baking, and a similar trend in the sensory scoring was observed (Marston and others 2016).

7.7. Sensory conclusions

The results of the sensory panels were similar throughout both the cookie and cake panels, indicating that the products made with raw flour were liked significantly less in terms of the texture, flavor, and overall acceptance, than the products made from treated flour. This implies that through the HPSE process, flavor and aroma compounds were reduced, thus improving the sensory characteristics. It also implies that other chemical changes occurred, allowing the texture to be improved. This is most likely due to the low moisture content, which creates a drier and crumblier texture. Cake texture is impacted by the protein quality. The potential for denatured proteins can reduce the cake's ability to expand due to early stiffening (Lee and Boonsupthip 2014). It is important to specify that the process for sensory analysis in this study did not include training for panelists. Panelists were consumers and therefore the scoring of the products was based on their opinion and how they compare to products they like and dislike.

The potential for negative baking quality is possible due to the reduced moisture. Dry texture, which would result in firming, is expected due to the significantly lower moisture content. This would cause a firmer product, which is the result of moisture migration (Luyts and others 2013). As the moisture moves from the center of the system toward the exterior, water is lost, which causes a firming sensation. This reduces the shelf-life of products, and causes an unacceptable eating experience.

Gluten-free cake made with heat treated sorghum flour showed improved sensory qualities from control flours, with similar ratings on a 9-point hedonic scale (Marston and others 2016). Extruded bean flour used for baking cake showed improved sensory acceptance, with scores averaging between 7 and 8 on a 9-point hedonic scale (Gomes and others 2015). Cookies

made with lupine flour were rated the highest along with a control in a study with lupine flour and corn starch, and rice flour, and wheat flour (Maghaydah and others 2013). The results determined in this study matched those of other studies where treated flour was tested in sensory.

Being this panel was composed of consumers that may or may not have been celiac, those that can eat gluten might have expectations for the products compared to wheat based cookies and cake, which could account for lower scores (McCarthy and others 2005). Being these products would be marketed toward panelists with CD, a panel of CD patients would be ideal for determining the true organoleptic properties of these products compared to what they currently have available (McCarthy and others 2005). Finding a panel of entirely CD patients is not feasible at this level, due to the limited population of people with CD.

Gluten-free foods on the market are typically low quality and lack essential nutrients such as fiber. They are also prone to being higher calorie and have higher impact on the glycemic index. These factors can cause people who follow strict GF diets to be at higher risk of weight gain. Other nutrients that these individuals need to get from other sources include calcium, folic acid, and B vitamins (Cross 2013).

7.8. Headspace analysis of volatiles

Previous researchers identified eight compounds that were identified to cause significant flavors or aromas in peas (Jakobsen and others 1998). These include: hexanal, octanal, 1-octen-3-ol, 1-hexanol, 2-sec-butyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 1-hexanol, and dipropyl disulfide. Standard compounds were run against samples to identify the presence of these compounds in flour, cake, and cookie samples.

7.8.1. Volatile identification in flour

Table 18. Volatiles identified in pea flour based on comparison to pure standards.

Sample	Hexanal	Octanal	1-octen-3-ol	1-hexanol	2-sec-butyl-3-methoxypyrazine	2-isopropyl-3-methoxypyrazine	2-isobutyl-3-methoxypyrazine	Dipropyl Disulfide
AGT Control**	++*	--	++	++	+-	--	--	+-
AGT 1:1	++	++	+-	++	++	--	--	++
AGT 3:1	++	--	+-	++	+-	--	--	+-
GNAP Control	+-	--	++	++	++	--	--	--
GNAP 1:1	++	--	++	+-	++	--	--	++
GNAP 3:1	++	+-	+-	++	++	--	+-	++
SC Control	+-	--	++	++	++	+-	--	--
SC 3:1	++	+-	+-	+-	+-	--	--	+-
SC 1:1	++	--	++	+-	++	+-	+-	+-

*Plus signs indicate the standard was identified in the sample, a minus sign indicates it was absent from the sample, a +- indicates that the standard was identified in only one of the two replicates**.**

** Where AGT, GNAP, and SC are suppliers, the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

The standards that were identified most frequently in flour samples were the hexanal, 1-octen-3-ol, 2-sec-butyl-3-methoxypyrazine, and 1-hexanol (Table 18). Each sample was done in duplicate. The compounds 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine were detected in only a few flour samples. Octanal was detected only in a couple of samples. Dipropyl disulfide was found in about half the samples. In treated pea flour, hexanal was the only quantified compound that was significantly different from the raw pea flour (Table 19). A significant increase in hexanal occurred during treatment of flours. This could be due to the drying process utilizing heat. Heat accelerates the speed of lipid oxidation (Monahan 2000). Other compounds did not decrease significantly with the treatment by HPSE.

Table 19. The concentration (μg/g) of volatiles in treated and untreated pea flour.

Treatment **	Hexanal		1-octen-3-ol		1-hexanol		2-sec-butyl-3-methoxypyrazine	
	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance
Control	3.64a*	0.35	2.76a	0.01	2.52a	0.04	34.24a	0.03
1:1	10.69b	0.37	2.67a	0.03	2.46a	0.02	35.68a	0.01
3:1	7.94b	0.22	2.66a	0.03	2.47a	0.01	35.55a	0.01

*Values followed by the same number indicates no significant differences.
**Where the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

7.8.2. Volatile identification in cookies

Similar to the flours, 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine were not detected in all cookie samples. Octanal was detected in only one sample, (AGT 3:1; Table 20), which was not associated with a flour sample that tested positive for octanal. Dipropyl disulfide was found in about half the samples.

There were no significant differences in 1-octen-3-ol or 2-sec-butyl-3-methoxypyrazine concentration among cookies (Table 21). Significant differences were identified in 1-hexanol and hexanal concentrations among cookies. There was a significantly lower concentration of hexanal in the 3:1 treated sample compared to the control, but there were no significant differences between the cookies from the 1:1 treatment and the control. There were significantly higher 1-hexanol concentrations in cookies made from treated flours compound to cookies made from the untreated flour.

Table 20. Volatiles identified in pea flour cookies based on comparison to pure standards.

Sample	Hexanal	Octanal	1-octen-3-ol	1-hexanol	2-sec-butyl-3-methoxypyrazine	2-isopropyl-3-methoxypyrazine	2-isobutyl-3-methoxypyrazine	Dipropyl Disulfide
GNAP 1:1**	++*	--	++	--	++	--	--	--
GNAP 3:1	++	--	+-	--	++	--	--	+-
AGT 1:1	++	--	++	--	++	--	--	++
AGT Control	++	--	++	++	++	++	--	++
AGT 3:1	++	+-	+-	+-	++	--	--	--
SC 1:1	++	--	++	+-	++	++	--	++
GNAP Control	++	--	++	++	++	++	+-	++
SC Control	++	--	+-	++	++	+-	+-	--
SC 3:1	++		+-	--	++	--	--	--

*Plus signs indicate the standard was identified in the sample, a minus sign indicates it was absent from the sample, a +- indicates that the standard was identified in only one of the two replicates.
** Where AGT, GNAP, and SC are suppliers, the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

Table 21. The concentration (μg/g) of volatiles in cookies made with treated and untreated pea flour.

Treatment*	Hexanal***		1-octen-3-ol		1-hexanol****		2-sec-butyl-3-methoxypyrazine	
	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance
Control	5.60a*	0.10	2.63a	0.01	2.15a	0.01	35.43a	0.01
1:1	5.69a	0.12	2.63a	0.01	2.42b	0.01	35.75a	0.01
3:1	4.42b	0.07	2.62a	0.01	2.44b	0.01	35.65a	0.01

*Values followed by the same number indicates no significant differences.
**Where the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

7.8.3. Volatile identification in cake

The standards that were identified most frequently in flour samples were the hexanal, 1-octen-3-ol, 2-sec-butyl-3-methoxypyrazine, and 1-hexanol. Similar to the flours, 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine were not detected in all samples. Octanal was detected in only one sample (AGT 1:1; Table 22), which was not associated with a flour sample that tested positive for octanal. Dipropyl disulfide was found in about half the samples.

There were no significant differences in the concentration of 1-hexanol, 1-octen-3-ol, or 2-sec-butyl-3-methoxypyrazine in cake (Table 23). There were significant differences in the concentration of hexanal, where the concentration of the control was significantly higher than the two treatments. The concentration dropped significantly from the control to the treatments. There was no significant difference between the two treatments.

Table 22. Volatiles identified in pea flour cake based on comparison to pure standards.

Sample	Hexanal	Octanal	1-octen-3-ol	1-hexanol	2-sec-butyl-3-methoxypyrazine	2-isopropyl-3-methoxypyrazine	2-isobutyl-3-methoxypyrazine	Dipropyl Disulfide
GNAP Control	++	--	++	++	++	++	--	++
GNAP 3:1	++	--	--	+-	++	--	--	+-
AGT 1:1	++	+-	++	++	++	--	--	++
AGT 3:1	++	--	++	++	++	--	--	--
GNAP 1:1	++	--	++	++	++	++	--	--
AGT Control	++	--	++	++	++	+-	--	--
SC 3:1	++	--	++	--	++	+-	--	+-
SC Control	++	--	++	++	++	--	+-	--
SC 1:1	++	--	++	++	++	--	--	+-

*Plus signs indicate the standard was identified in the sample, a minus sign indicates it was absent from the sample, a +- indicates that the standard was identified in only one of the two replicates**.**

** Where AGT, GNAP, and SC are suppliers, the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

Table 23. Volatile concentration (μg/g) in cakes made with treated and untreated pea flour.

Treatment *	Hexanal***		1-octen-3-ol		1-hexanol		2-sec-butyl-3-methoxypyrazine	
	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance	Concentration (μg/g)	Coefficient of Variance
Control	7.30a**	0.14	2.74a	0.02	2.52a	0.01	36.04a	0.03
1:1	5.79b	0.06	2.69a	0.02	2.48a	0.02	35.77a	0.01
3:1	5.88b	0.32	2.65a	0.03	2.46a	0.03	35.58a	0.01

*Values followed by the same number indicates no significant differences.
**Where the control is untreated pea flour, 1:1 is HPSE flour treated with 1:1 ratio of ethanol to water, and 3:1 is HPSE flour treated with 3:1 ratio of ethanol to water.

7.8.4. Headspace conclusions

Significant differences in volatile concentration was only observed in hexanal and 1-hexanol. The significance observed in the cake was that the control had significantly higher hexanal and 1-hexanol than the cookies made with the 1:1 and 3:1 treatments. The hexanal concentration in cookies was significantly less in only the 3:1 treatment, but the 1:1 treatment and control were not significantly different. The hexanal concentration in the control flour was significantly lower than the 1:1 and 3:1 treatments. This is unexpected, being the concentration in baked goods is lower. The reason for this could be due to the reduced concentration of antioxidants such as carotenoids during the extraction and heating during the drying process (Monahan 2000).

The concentration of 1-hexanol was only significantly different in the cookies. The 1:1 and 3:1 HPSE flours had significantly higher levels of 1-hexanol than the control. There were no significant differences in the flour or the cakes. This could be due to similar reasons as the hexanol. Being it is a product of oxidation, and the likely reduction in antioxidants through HPSE extraction with ethanol, there are fewer free electrons to donate during lipid oxidation, thus resulting in higher concentrations of lipid oxidation products (Monahan 2000). There were

no significant differences in the concentration of either 2-sec-butyl-3-methoxypyrazine or 1-octen-3-ol. 1-Octen-3-ol is an oxidation product of linoleic acid (Inamdar and others, 2013).

The reduction of hexanal and 1-hexanol concentrations during the baking process in the treated flours likely explains the results. The potential that these compounds were created during the drying process of the HPSE is high, but then during the baking at high temperatures, the volatiles were released (Brauss and others 1999). Being the contents of these compounds was higher in the baked products than the flours, there is also a chance that different ingredients used to bake the cakes contributed to the higher concentration, such as oil or shortening.

Other compounds that were not investigated but known to cause aroma in peas include octanol, pent-1-en-3-ol, nonanal, nonan-2-one, (Z)-hex-3-en-1-ol, octan-2-ol, 2-methylheptan-3-one, and (E)-hex-2-enal. Pent-1-en-3-ol and octanol present green and vegetal flavors. Nonanal presents a green floral aroma (Murat and others 2012). Nonan-2-one presents a green fruity aroma. (Z)-hex-3-en-1-ol presents a fresh cut grassy aroma. Octan-2-ol is thought to present a woody, green, herbal note. 2-methylheptan-3-one and (E)-hex-2-enal provide leafy green flavors (Murat and others 2012).

7.9. General conclusions

The expected results from the sensory analysis and headspace analysis were not what the results produced. The volatile compounds that were quantified were not reduced. The sensory results indicated that flavor and overall acceptance of baked products containing these flours were improved, which suggests that volatile compound concentrations were reduced. The concentrations were not significantly different or they were increased depending on the compound. This insinuates that the compounds quantified were not the compounds that caused

strong off-flavors. The chance that other compounds that were not listed in literature may be the compounds responsible for flavor.

Cake height was significantly higher for the treated flours, and the diameter of cookies made with the HPSE flours were significantly less than the control. This indicates that pre-gelatinization of starch impacted the baking quality. Pre-gelatinized starch has a higher binding capacity, which means less water is available to interact with sugar. The sugar-water complexes are what decrease dough viscosity and reduces cookie spread. The expectation was that HPSE treated pea flour products would have greater height, and less spread for cookies (Seyhun and others 2005). Particle size also impacts volume and gas bubble distribution. The particle size of the control flour was smaller than that of the 1:1 and 3:1 treated flour, the finer particle size produced a cake with less height, indicating a lower volume than those with the higher cake height.

8. CONCLUSIONS

A gluten-free flour that has improved sensory attributes and retained baking quality was created. The results of this study supported that by deodorizing pea flour with HPSE, the sensory attributes were improved, baking quality was retained, and flour quality was retained. Even though the headspace analysis had results that did not seem to follow the sensory results, the sensory results proved that the flavor was improved significantly.

HPSE treated pea flour had the greatest impact on pea flavor. Cake flavor was improved from an average of 3.8 for the control to 6.5 and 6.4 for the 1:1 and 3:1 HPSE treated flours, respectively. The flavor of sugar cookies made with pea flour was improved from 4.3 for the control to 6.7 and 6.5 for the 1:1 and 3:1 treatment, respectively.

9. FUTURE DIRECTION

This study achieved improving the flavor of pea flour with HPSE ethanol:water solvent. The flour had improved flavor, texture, and overall acceptance when tested in sensory studies. The two HPSE treatments did significantly improve the flavor, but more investigation into supercritical fluid carbon dioxide should be investigated. If this was an interest in other applications, different commodities could be investigated including other pulses, ancient grains, and pseudo-cereals with undesirable sensory attributes.

Further flour analysis would be needed to determine the quality of the nutrients present in the treated pea flour. Starch characterization to determine the degree of starch damage would be important for baking quality. Fiber content would also be something to determine, being water soluble fiber could potentially be removed through HPSE. Protein quality should be evaluated due to the fact that heat and solvent could have an impact on protein quality.

The intent of the study was to determine the difference in flavor, texture, overall acceptance, and appearance of the different treatments. The optimization of cake and cookie formulas would be necessary for further sensory analysis. A sensory test with a panel of individuals who follow GF diets or are celiac and understand the differences in GF foods. This would provide more accurate and reasonable results for sensory analysis. This could be accomplished by working with the Celiac support group in the Fargo area.

Finally, further evaluation of the headspace analysis should be completed. Results from the headspace analysis contradicted the results from consumer sensory testing, indicating that there may have been compounds missed through the process. Further investigation into the compounds that were not quantified and those that were not investigated at all in this study could solve that contradiction.

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APPENDIX

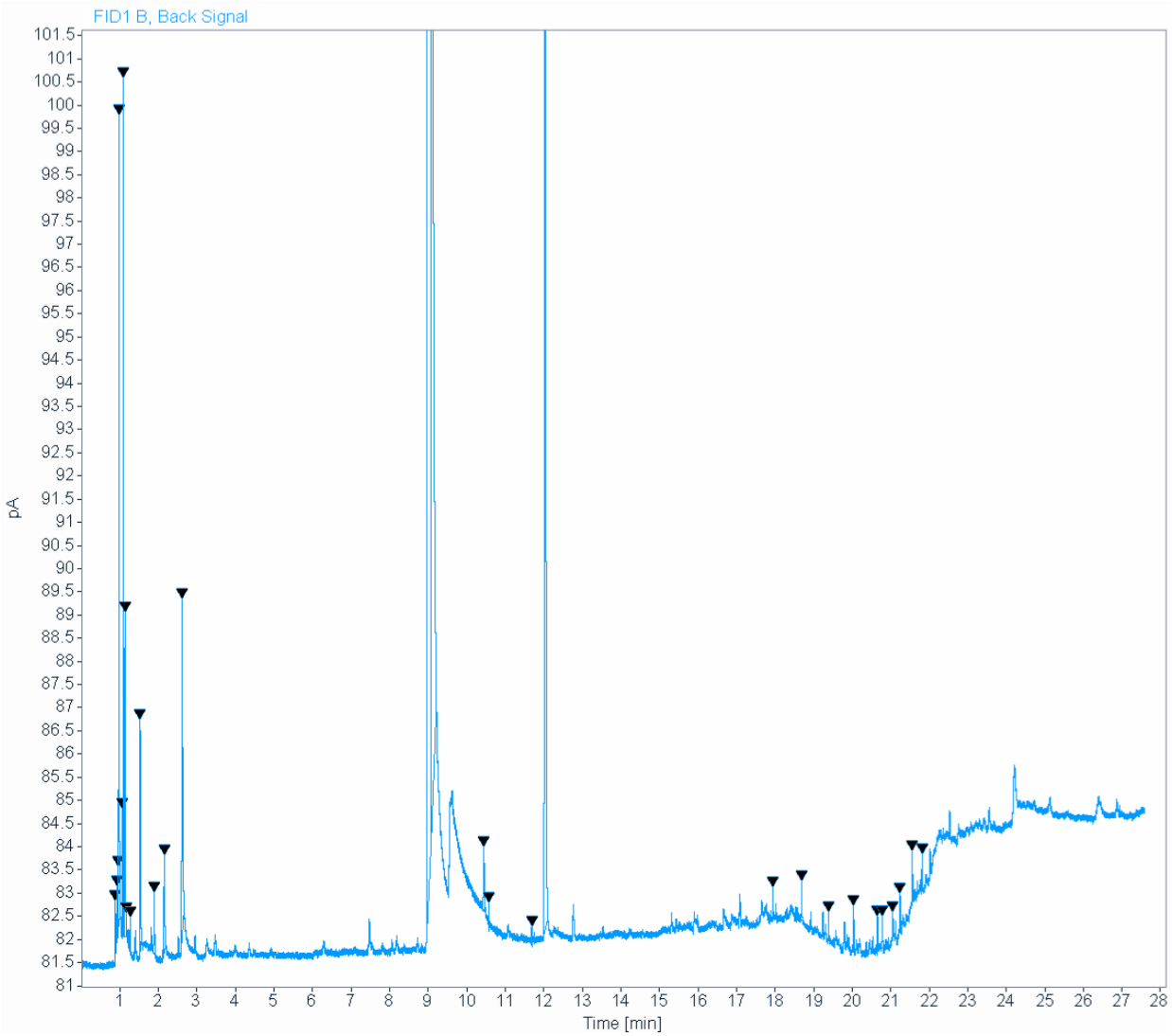

Figure A1. GNAP Control flour headspace chromatogram.

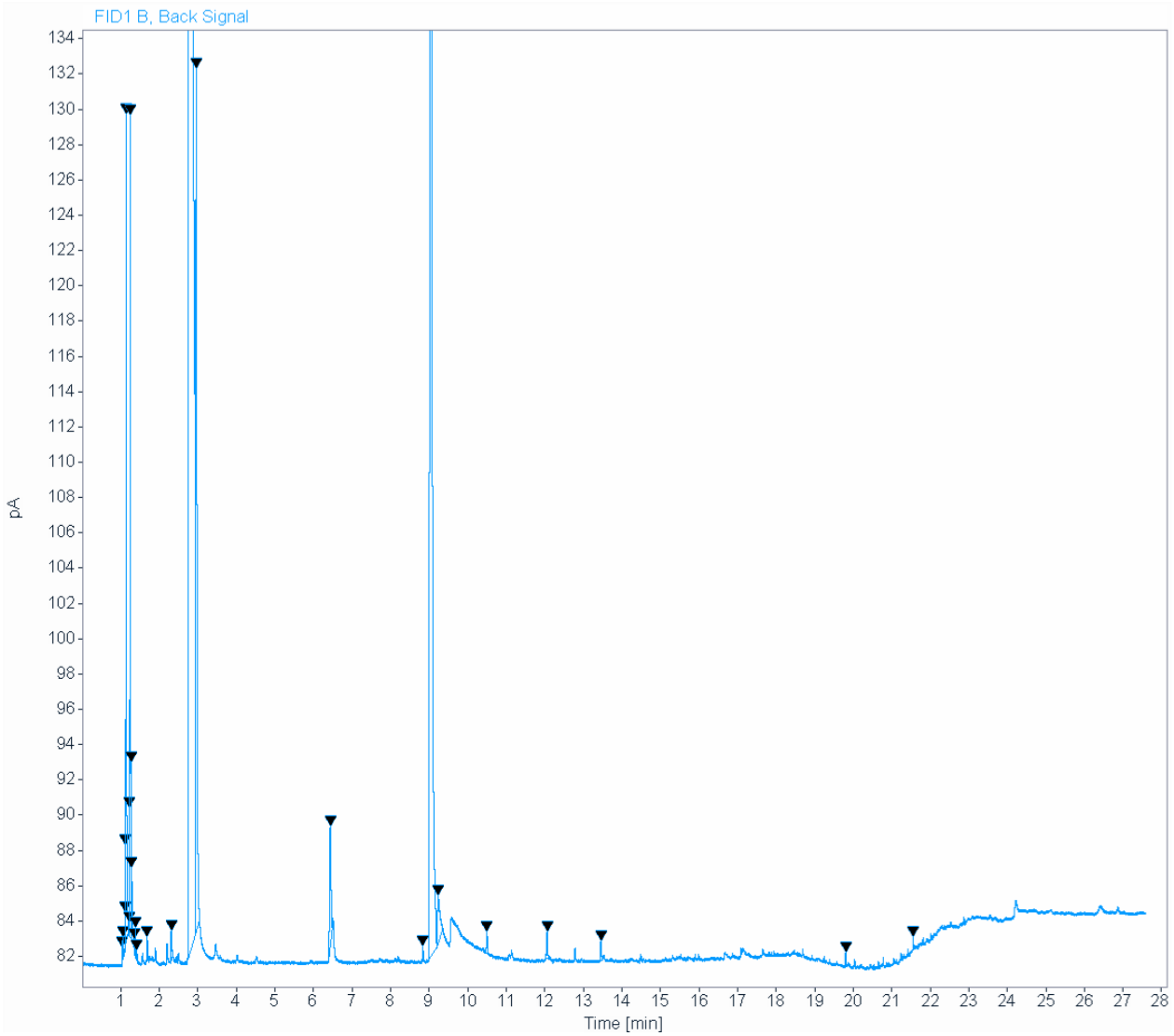

Figure A2. GNAP 1:1 flour headspace chromatogram.

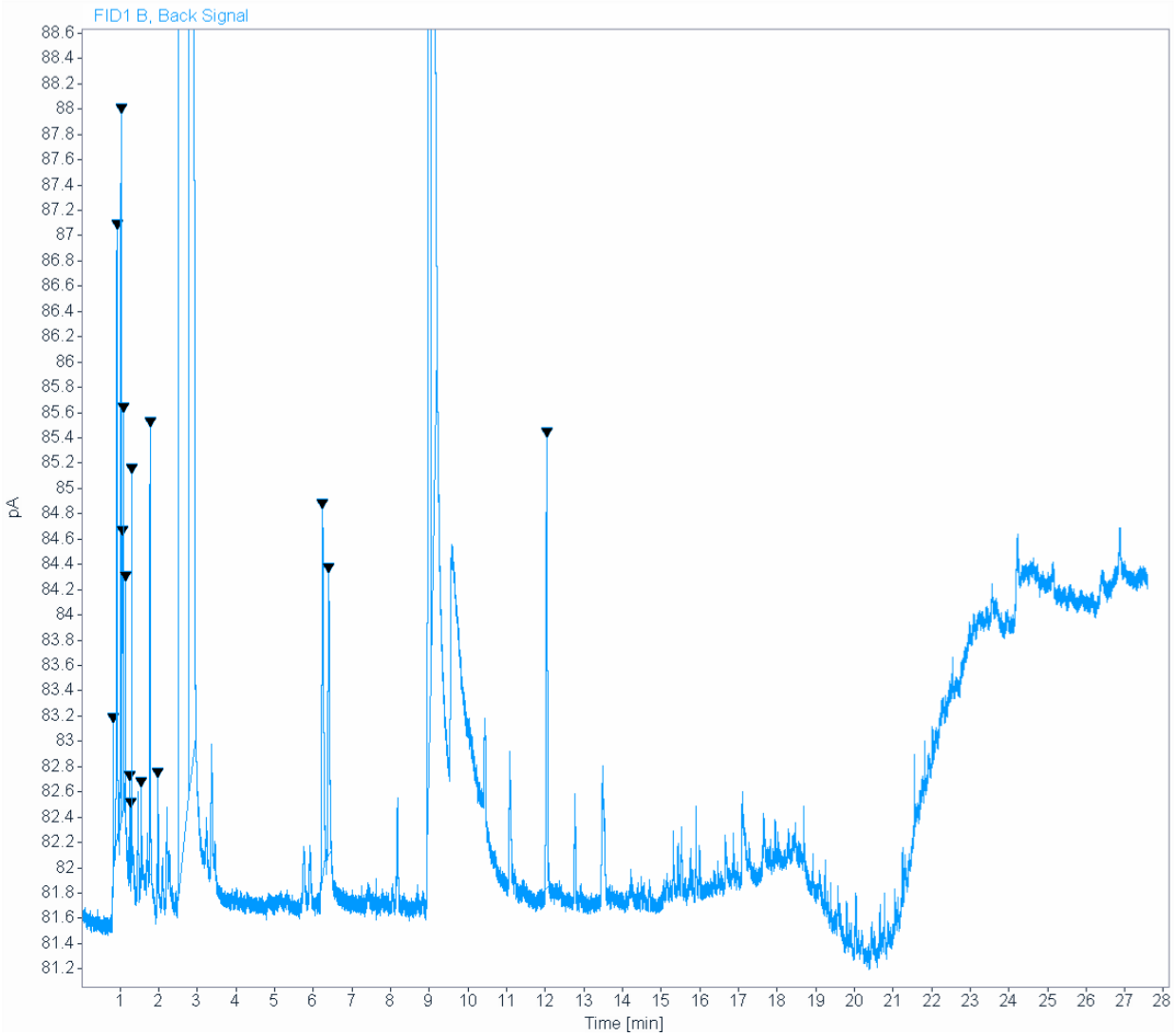

Figure A3. GNAP 3:1 flour headspace chromatogram.

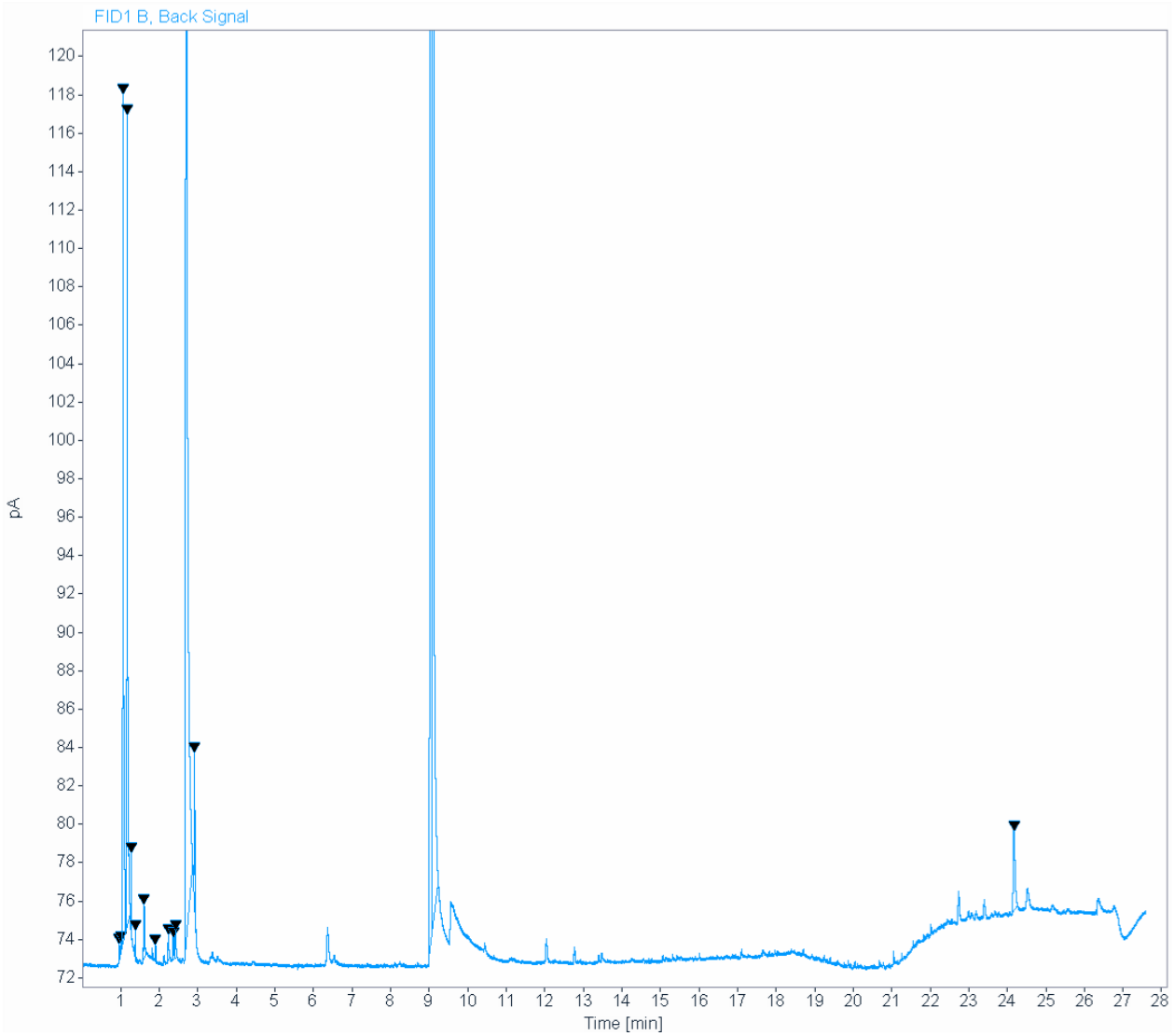

Figure A4. SC Control cookie headspace chromatogram.

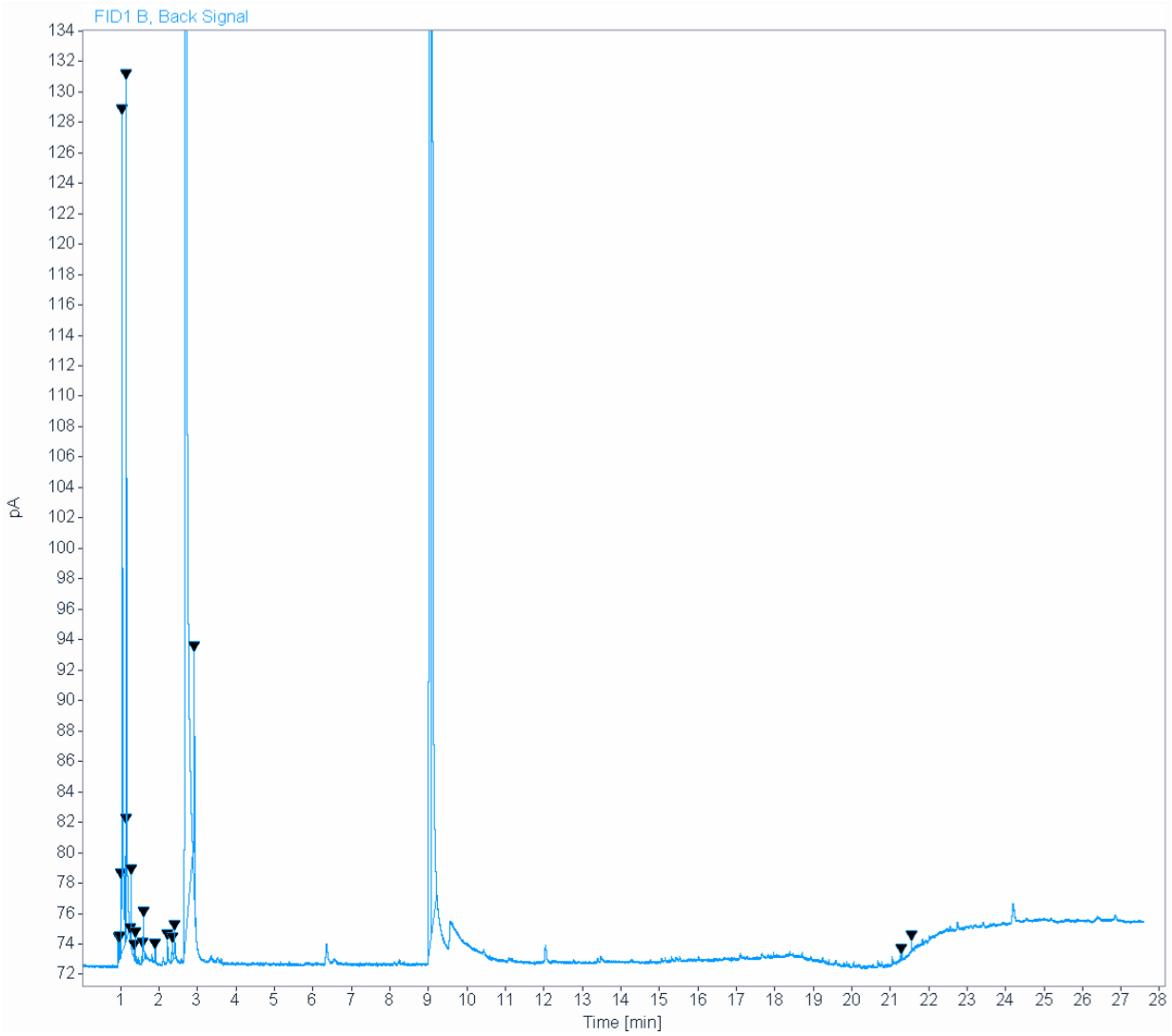

Figure A5. SC 3:1 cookie headspace chromatogram.

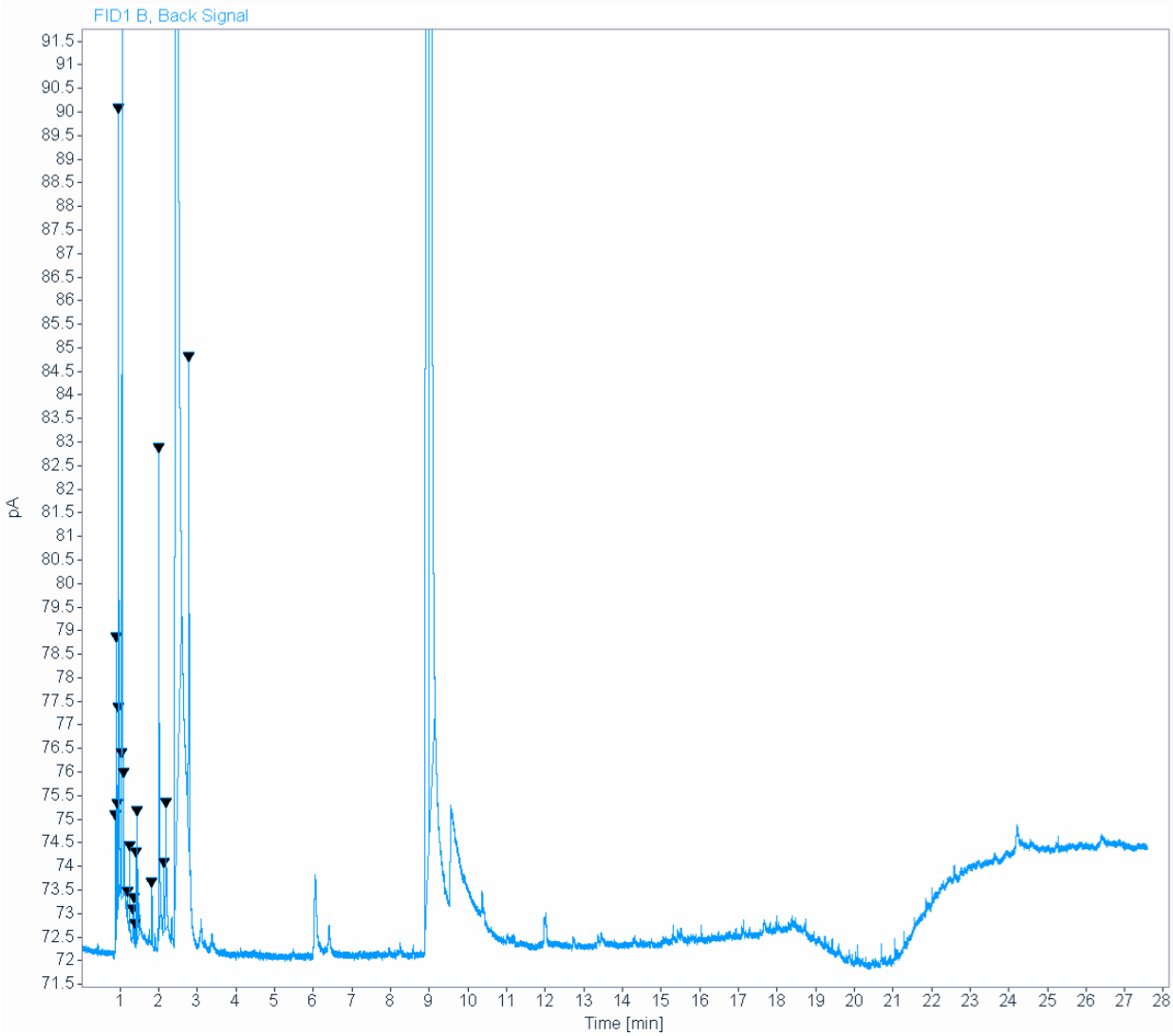

Figure A6. SC 1:1 cookie headspace chromatogram.

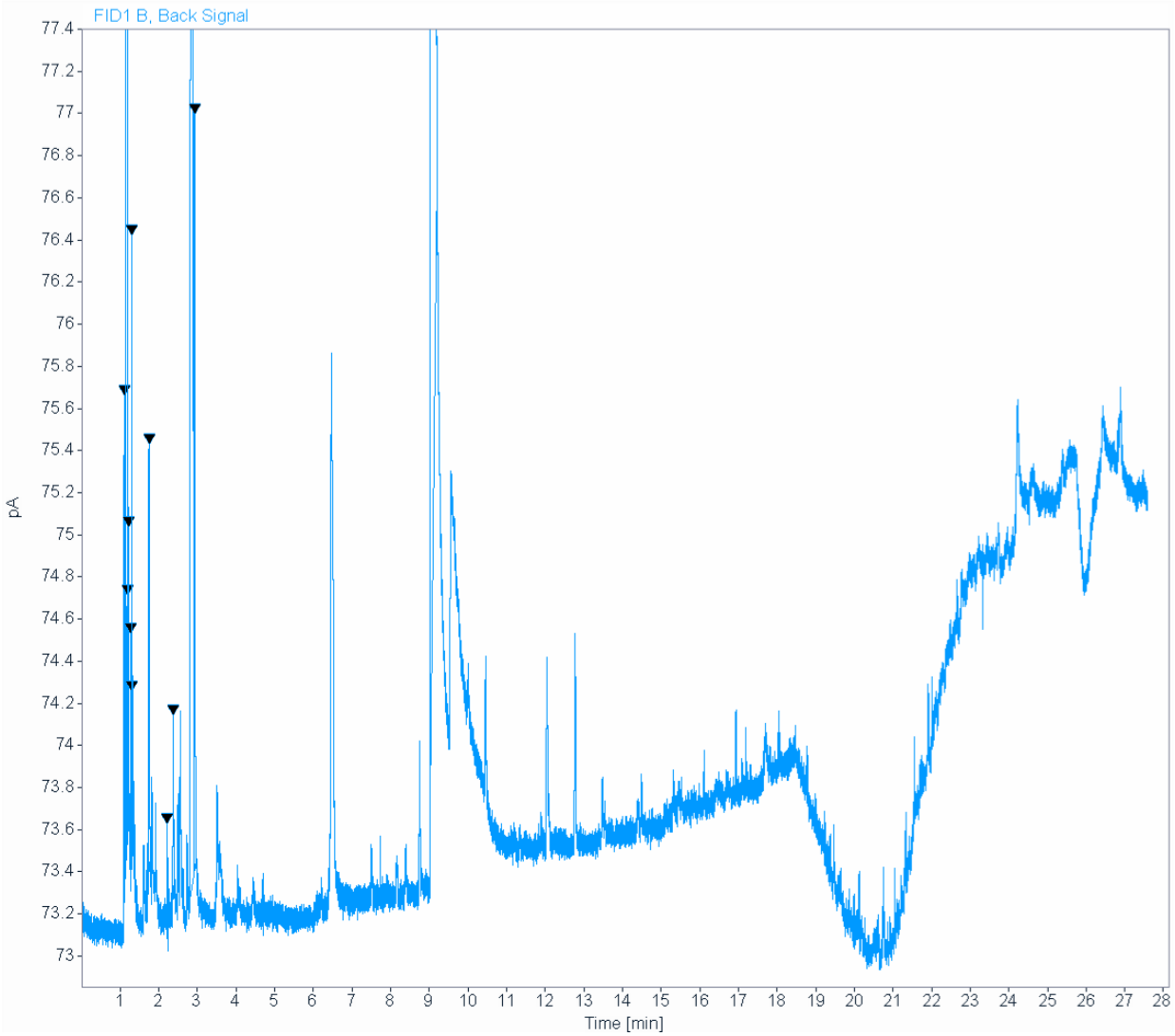

Figure A7. SC Control cake headspace chromatogram.

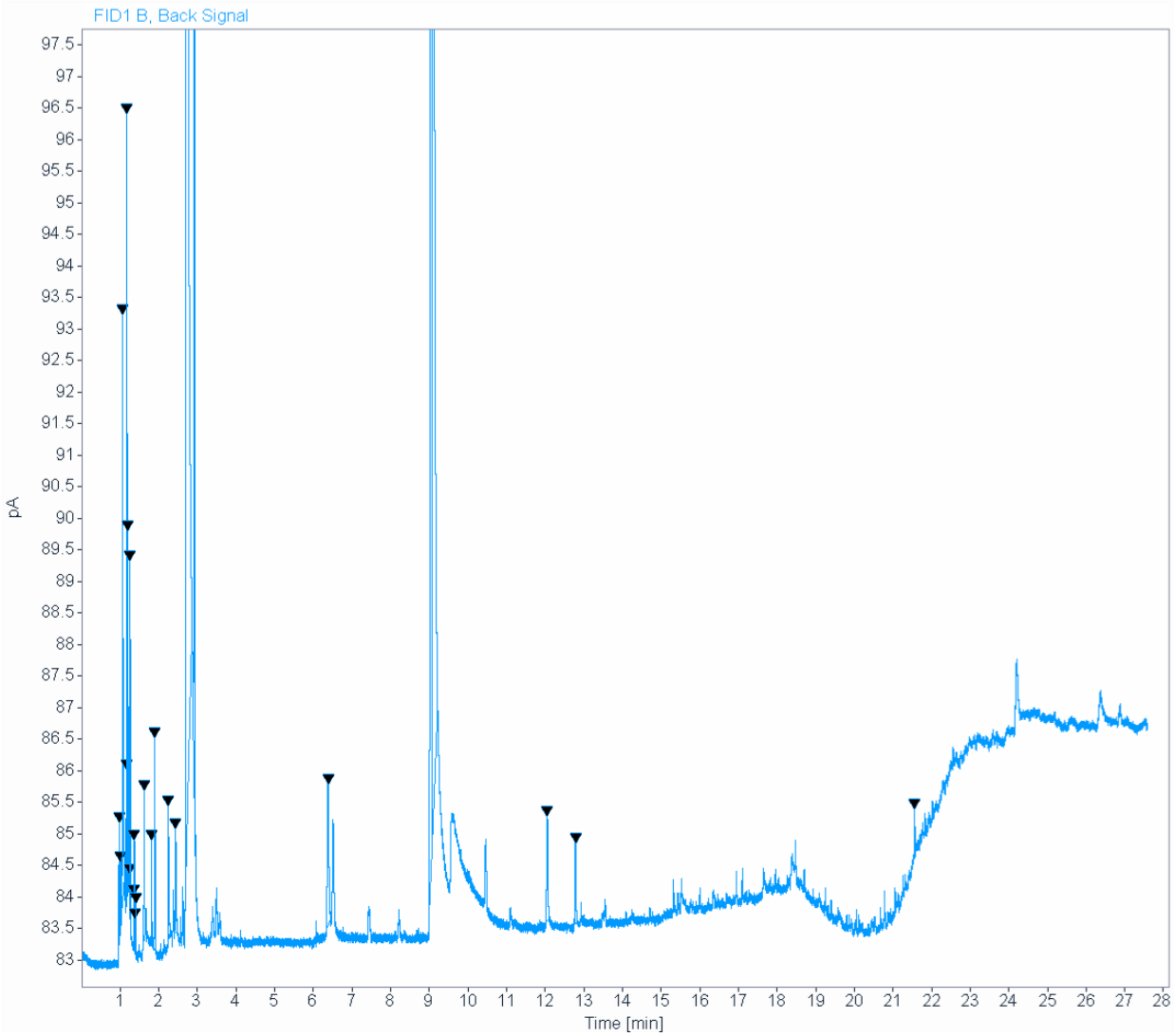

Figure A8. SC 1:1 cake headspace chromatogram.

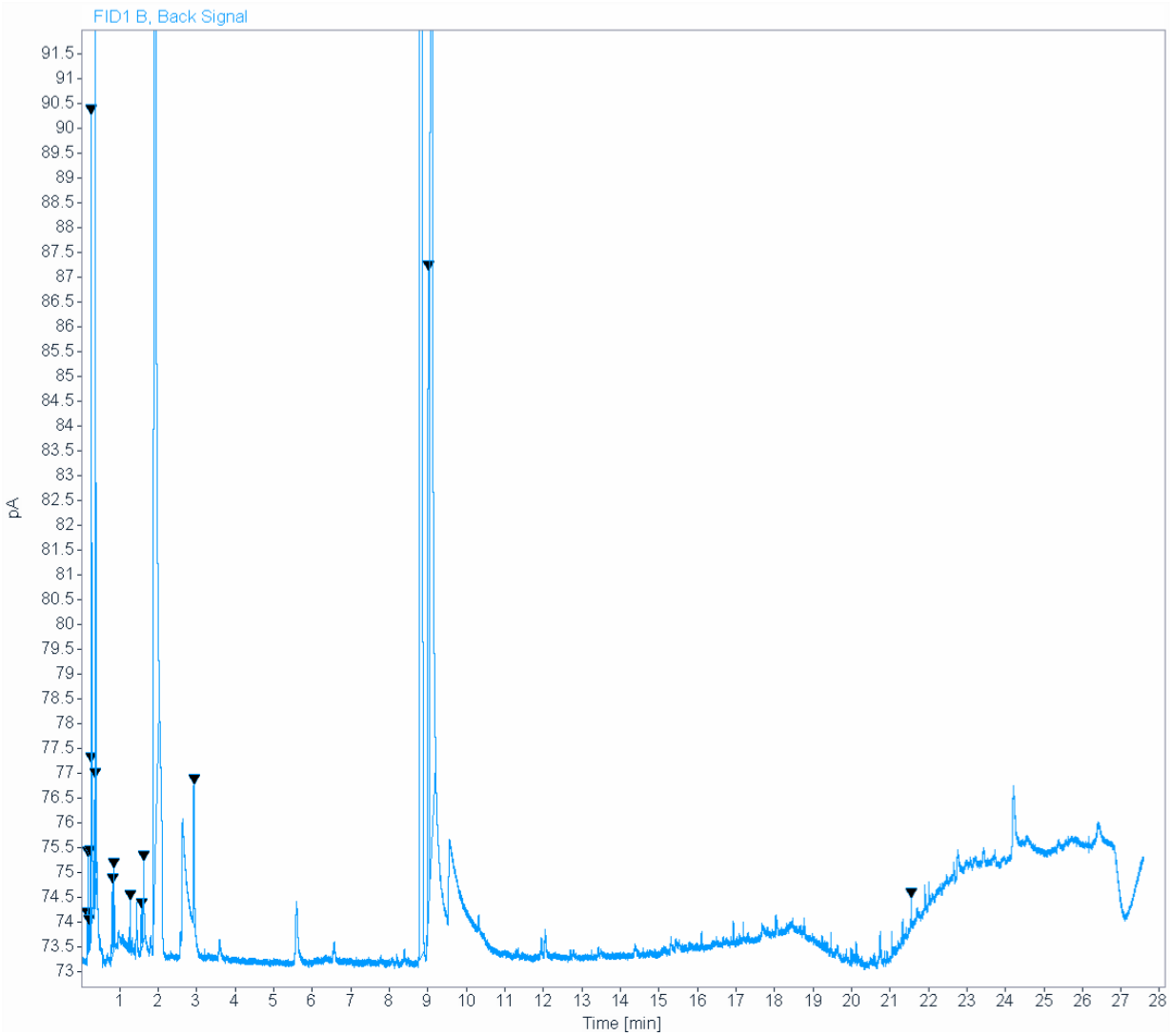

Figure A9. SC 3:1 cake headspace chromatogram.