

AROMA CHEMISTRY AND CONSUMER ACCEPTANCE OF NAVY BEAN POWDER AS
PREPARED BY COMMERCIAL OR EXTRUSION PROCESSING

By

Edward Szczygiel

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ABSTRACT

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Bean powder has been gaining in popularity among consumers due to its reported health benefits including reduced risk of heart disease, obesity and diabetes. Extruded Navy bean (*Phaseolus vulgaris* L.) powder can be considered as a cost-effective alternative to resource intensive traditional methods of processing. The objectives of the study presented in this manuscript were 1) to profile the volatile compounds derived from extruded and commercial Navy bean powders using headspace solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry; 2) to determine which of the volatile compounds are odor-active with gas chromatography-olfactometry; and 3) to investigate consumer acceptance of products formulated with extruded or commercial bean powder. The odor-activity was highest from aldehydes in the extruded and commercial samples, representing 55% and 47.5% of the total response, respectively. Commercial bean powder had approximately 12.5 times more peak area from aldehydes. Furthermore, the commercial powder contained 20 times more hexanal than the extruded sample. Aliphatic aldehydes are most often formed during the degradation of unsaturated fatty acids such as linoleic and linolenic, which are the primary fatty acids found in Navy bean. This finding indicates that the commercial powder may have undergone severe lipid oxidation prior to the experiment. Consumer acceptance data showed a significant preference for baked Navy bean crackers made with the extruded bean powder over the crackers made with the commercial bean powder.

This thesis is dedicated to my wife Lauren Szczygiel and my parents David and Margaret Szczygiel for their vigilant moral support.

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KEY TO ABBREVIATIONS

HS	Headspace
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GC-O	Gas Chromatography Olfactory
SPME	Solid Phase Microextraction
DVB	Divinylbenzene
CAR	Carboxen
PDMS	Polydimethylsiloxane
PCA	Principle Component Analysis
DFA	Detection Frequency Analysis
RI	Retention Index
OAV	Odor Activity Value

Chapter 1: Introduction

Dry beans are a staple crop in Michigan and represent an important part of the state's agricultural economy. Michigan is the second largest producer of dry common beans in the US, producing 58% of all black beans and 29% of all Navy beans grown in the states (Wells 2011). However, only 14% of households consume dry beans on a given day; of these, only 4% of those are Navy beans, and less than 1% are black beans. While about 43% of the US population reside in the Midwest and northeast census regions, they only account for 24% of nationwide dry bean consumption (Lucier and others 2000). The other 76% are consumed in the western and southern states, largely due to the high percentage of Hispanics, who make up 11% of the total population and 33% of the nation's bean consumption (Lucier and others 2000). Growth in consumption has largely been stagnant at 1.6-2.7lbs per person per year (Wells 2011). Thus, there is substantial room for growth both through increasing consumption in regions such as the Midwest and improving appeal to under-consuming demographics such as Black and Asian consumers.

To meet the needs of these new markets, new strategies must be developed in order to limit barriers to consumption, such as preparation difficulty and off beany flavor, and create novel options for wary consumers who typically do not seek out dry beans. Incorporating new strategies to increase bean consumption will not only be beneficial to the global economy, but will also benefit consumers in a wide variety of specific regions where beans are a primary agricultural output, such as Michigan (Lucier and others 2000). Dry beans are good sources of protein, dietary fiber, many vitamins and minerals and unsaturated fats. More specifically, they are composed of 20-35% protein, are free of cholesterol, and low in fat and sodium, making them an ideal food to promote to a population battling diabetes, chronic cardiovascular diseases

and colon diseases (Siddiq and others 2011). Dry beans (*Phaselous vulgaris L.*) are also higher in resistant starch content than corn or potato, even after being cooked (Shuang-Kui and others 2014). This indigestibility increases fecal bulk and butyrate production which are considered markers for good colon health (Tharanathan and Mahadevamma 2003)

One strategy being employed to increase the amount of beans consumed is the development of new bean-based ingredients, such as bean powder, that can be used as a supplement or replacement of current corn and wheat ingredients in many products. The popularity of gluten-free products has created a need for healthy alternatives to powder that also taste good, and thus providing an opportunity for increased dry bean powder production. Soybean has had much success with this strategy; however, dangerous allergic reactions and concerns about estrogenic compounds have made consumers increasingly wary in the past decade. Similar health benefits can be achieved with dry beans without the allergy concerns (Geil and Anderson 1994). By positioning novel bean ingredients under the health halo and within the gluten-free trend, dry beans could also reach these low consumption demographics in the Midwest and Northeast. Current gluten-free options, such as rice starch, have been noted by experts to be nutritionally lacking in fiber, protein, and vitamins and minerals, which bean powders have in abundance (Siddiq and others 2011, Geil and Anderson 1994). Bean powders are also of interest to vegetarian consumers as they can replace protein and B vitamins that are abundant in meat (Craig and Mangels 2009).

While dry bean powders may meet many consumer needs, they have one major barrier to consumption that must be addressed before consumers will embrace such products: off flavor or “beany” flavor. Many studies acknowledge the existence of off beany flavor and its contribution to consumer dislike of bean-based products (Simons 2013, Han and others 2012, Oomah and

others 2007, Sathe and Salunke 1984, Vara-Ubol 2004, Van Ruth and others 1995). Off flavors may develop during processing, which typically involves soaking, steam-cooking (Mishra and others 2017, Hall 2008). In order to reduce or eliminate these off flavors, new processing strategies such as extrusion are being explored (Simons 2013). Extrusion processing, a novel strategy for bean powder processing that is more efficient, may have advantages over steam-cooking, such as cost, consistency, improved digestibility, improved functional properties and potentially minimizing the development of off flavor (Simons 2013)

The manuscript presented in this thesis will be submitted a journals focusing on agricultural sensory and food chemistry. The objectives of the study presented in this manuscript were 1) to profile the volatile compounds derived from extruded and commercial Navy bean powders using headspace solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry; 2) to determine which of the volatile compounds are odor-active with gas chromatography-olfactometry; and 3) to investigate consumer acceptance of products formulated with extruded or commercial bean powder. This research will help to improve and diversify potential applications for dry bean powder and help guide future breeding practices by qualifying off flavor components that could hinder dry bean usage for novel ingredients.

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Chapter 2: Literature Review

2.1. Common Dry Bean- Production, Consumption and Utilization

Beans, or pulse, are the seeds from the edible pods of plants from the Leguminosae family, and are commonly referred to as “legumes”. Legumes are listed as ninth among the top vegetable crops grown in the United States in the most recent USDA Dry Bean Consumer Report (Wells 2011). Seeds from legumes are often dried (known as dry beans) and recooked for consumption. While there are thousands of species of legumes, only about 20 are normally consumed by humans (De Almeida Costa and others 2006). *Phaseolus vulgaris* (common bean) is one of the most commonly consumed dry bean species in the Leguminosae family and is the most widely cultivated globally (Lucier and others 2000, Akibode and Maredia 2011). Common beans are a major crop worldwide and are produced and consumed in a myriad of regions including North America, Africa, Asia and Europe (Akibode and Maredia 2011).

2.1.1 Production

The Food and Agriculture Organization of the United Nations (FAO) reported that in 2006-2008, over 21 million metric tons of dry beans were produced across the globe (Akibode and Maredia 2011). Dry common beans are an important crop for not only Michigan, but the entire United States. The U.S is the 4th largest producer of dry beans in the world, at 12.26% of the worldwide production (Lucier and others 2000). Within the U.S., North Dakota (38% of output), Michigan (14%) and Nebraska (11%) are the leading producers of dry beans (Wells 2011). In the U.S., the three top classes of common dry bean are pinto (42% of production), Navy (17%) and black (11%) (Wells 2011). Michigan is the leading producer of black beans and the second largest producer of Navy beans (Lucier 2000). Between 1980 and 2008, improvement

in technology has promoted a 35.55% boost in yield/unit area in the United States (Siddiq and others 2011).

2.1.2 Utilization

Dry beans are most commonly consumed as a canned, heat treated product at home by consumers, although this varies by bean variety (Lucier and others 2000). Approximately 90% of all U.S domestic Navy beans are sent to canning facilities (Lucier and others 2000). Heat processing, such as canning, is helpful to improve the palatability, nutrient availability and deactivate enzymes. There are large varieties of canned products that include beans, such as soups, chilis, baked beans or refried beans. Grinding and milling of dry beans and subsequent separation of starch and seed coats is common. High starch bean powders are also used in baked goods. Typically, white beans such as Navy beans are used for powder as the coloring is similar to wheat powder traditionally used in baked goods. Bean powder and isolated bean protein have been used for in a variety of foods including meat analogs (Salunkhe and Kadam 1983). Dry bean usage is dependent on cultural factors and ethnicity, with taste and variety preference varying significantly from country to country (Ensminger and Ensminger 1993; Tharanathan and Mahadevamma 2003). Beyond canning, other uses for beans include salads, fresh soups, or serving them freshly boiled (Geil 1994).

2.1.3 Consumption

The consumption of dry beans in the U.S. is low compared to other countries, particularly in countries where the population is less wealthy (Barampama and Simard 1993). Dry beans represent a low cost source of protein and vitamins for many countries where malnutrition is a common problem (Siddiq and others 2011). Drewnowski (2010) found that dry beans have a

favorable energy and nutrient density to cost ratio, which explains the economic value of beans relative to both meat and other more perishable vegetables.

However, in the U.S. only 14% of households consume dry beans on a given day; of these, only 4% of those are Navy beans, and less than 1% are black beans (Lucier and others 2000, Akibode and Maredia 2011). Growth in consumption has been largely stagnant at 1.6-2.7 lbs per person per year, after declining consumption each decade from the 1960s (Wells 2011). While about 43% of the US population resides in the Midwest and Northeast census regions, they only account for 24% of nationwide dry bean consumption (Lucier and others 2000). The other 76% are consumed in the western and southern states, largely due to the high percentage of Hispanics, who make up 11% of the total population and 33% of the nation's bean consumption (Lucier and others 2000). Thus, there is substantial room for growth both through increasing consumption in regions such as the Midwest and improving appeal to under-consuming demographics such as Black and Asian consumers.

There have been several explanations for the low consumptions of dry beans in the U.S. Three key factors seem to contribute to this problem primarily. First, the presence of anti-nutritional factors such as trypsin inhibitor, lectins and phytic acid make processing of beans to remove these factors a necessity (Vanderpoel 1990). Secondly, oligosacharides, which are can be reduced by long soaking times, can cause flatulence and gastrointestinal discomfort (McPhee and others 2002). Third, undesirable beany flavor, which can be present in processed and packaged products containing bean ingredients (Sathe and Salunkhe 1984).

2.2 Dry Bean Nutrition

2.2.1 Nutritional Factors

Dry beans are one of the most nutritionally complete foods available and thus have been recommended by many experts for their overall contributions to good health (Venter and van Eyssen 2001). They are perhaps most important in the diets of citizens of developing countries where animal protein sources are not reliable due to cost (Broughton and other 2003). Dry common beans (*Phaseolus vulgaris* L.) are a good source of a myriad of vitamins and minerals and contain 2-3 times the amount of protein found in cereal grains (Costa and others 2006; Siddiq and others 2011). Additionally, dry beans are a rich source of dietary fiber and contain non-nutrient components such as antioxidants (Wu and others 2004; Siddiq and others 2011). A variety of health benefits are provided by dry bean consumption, including prevention of diabetes mellitus (Hutchins and others 2012), obesity (Anderson and Major 2002), cardiovascular diseases (Bouchenak and Lamri-Senhadjji 2013), stroke (Flight and Clifton 2006) and colon diseases (Tharanathan and Mahadevamma 2003).

Amylose in dry bean starch is present in high quantities (25-60%) and exhibits especially strong interactions between chains, which in combination with an inherently high dietary soluble fiber content from the coat of the bean, make dry beans highly resistant to digestion (Hoover and Zhou 2003). This indigestibility increases fecal bulk and butyrate production which are considered as markers for good colon health (Tharanathan and Mahadevamma 2003). The high amylose content has also been shown to increase the production of resistant starch upon processing (Hoover and Zhou 2003). Hayat and others (2004) proposed that consumption of dry beans could decrease the risk of developing diabetes and obesity due to dry beans' high resistant starch content which can lower postprandial glucose levels and increase satiety (Reddy and

others 1984). Dry beans have more resistant starch after being cooked than corn or potato (Shuang-kui 2014).

2.2.2 Anti-nutritional Factors

While there are many dietary benefits associated with dry bean consumption, there are also several factors that limit the bioavailability and digestibility of dry beans. Trypsin inhibitors, hemagglutinins and flatulence causing oligosaccharides are the most relevant to processors and consumers (Siddiq and others 2011)

Protease inhibitors reduce human's ability to digest certain proteins, thus preventing use of amino acids for nutritive purposes. In dry beans, the main protease inhibitor of concern is trypsin inhibitor, which can be destroyed or inactivated by sufficient heat or mechanical force. A 70% reduction in antitrypsin activity in beans is adequate to ensure nutritive quality (Balandran-Quintana and others 1998). A heating step is generally used in the preparation of dry beans, and due to the long cooking times required for the beans to become edible, trypsin inhibitor is usually not a concern in dry beans. However, in the case of dry bean powder, where a heating step may or may not be used, mechanical force, such as the high pressure exerted by extrusion, can be used. Extrusion cooking has been shown eliminate trypsin inhibitors (Alonso and others 2000).

Hemagglutinins, also known as lectins, are heat labile glycoproteins that can impair nutrient absorption (Siddiq and others 2011). Extrusion processing can significantly reduce lectin content due to thermal degradation of these molecules (Alonso and others 2000).

Oligosaccharides are medium chain carbohydrates that include digestible sugars such as maltodextrin and fermentable sugars such as raffinose. In dry beans, raffinose and stachyose content are a major limiting factor in dry bean consumption due to the flatulence caused by the

fermentation of these sugars by gut flora (Barampama and Simard 1993). This can be reduced by a bean pre-soaking step that is a very effective method of removing raffinose and stachyose from dry beans (Alonso and others 2001). Cooking and extrusion can also reduce oligosaccharide levels in *Phaseolus vulgaris* (Siddiq and other 2011, Alonso and others 2001).

2.3 Dry Bean Sensory Characteristics

Due to the large variety of species found in the Leguminosae family and the wide variety of uses for beans, including canning, salads, fresh soups, or serving them freshly boiled it is difficult to generalize any sensory challenges reported in research (Geil and Anderson 1994).

Within the *Phaseolus vulgaris* species, differences in sensory attributes of cultivars, particularly between market classes (black, red, white etc.) have been reported using trained panels (Simons 2013). Even with these variations, there are some issues that have been found to be present in many bean cultivars and applications. Mkanda and others (2007) correlated physiochemical, descriptive sensory, and consumer preferences of dry beans and found that factors that contributed to dislike of boiled dry beans were bitter taste and soapy and metallic aftertastes (as high as 3.5 and 2.6 on a 9-point intensity scale, respectively) and used principle component analysis (PCA_ analysis with consumer sensory tests to identify sources of variation.

Additionally, the researchers found that beans with good hydration properties that softened quickly during cooking appeared to be correlated with absence of these negative attributes.

Bitter, soapy and metallic tastes can likely be contributed by phytochemicals such as saponins which can be present in dry beans in high quantities (Heng and others 2006). Saponins are thermal sensitive and water soluble and thus are often destroyed or removed during soaking, boiling and cooking used in the preparation of beans for consumption (Shi and others 2004).

Siddiq and others (2006) used cooked red kidney beans to make sugar-coated bean snacks and

found them to be acceptable in all attributes. The sugar-coated kidney beans received a 5.59 for flavor at the lowest syrup level on a 9 point hedonic scale. Processing methods appear to be a major driver in determining the level of undesired flavors in dry beans.

Health attributes of dry beans, such as their high protein content, are being leveraged by fortifying existing foods with dry bean powders. When using bean powders for fortification purposes, sensory challenges shift towards texture and flavor challenges imparted by removal of a functional ingredient (such as wheat powder replacement and thus gluten in baked goods causing texture changes) or addition of unwanted flavor (beany flavor in an inappropriate product such as cake) (Dryer and others 1982). Many off-flavor phytochemicals are reduced, removed or altered during processing; thus, remaining cooked beany flavors become the primary concern for sensory issues related to flavor (Shi and others 2004). Flavor is often described as a combination of gustatory taste and olfaction, where olfaction plays a very dominant role (Spence 2015). While not easily quantified, it is often reported that between 75-90% of what we think of as taste originates from olfaction (Spence 2015). Thus, beany flavor may be best defined by the aroma volatiles that contribute to overall beany flavor.

2.4 Bean Powder Production and Consumption

While dry bean consumption remains stagnant, there has been a five-fold increase in the introduction of gluten-free products from 135 in 2003 to 832 in 2008 due to consumer's interest in healthful non-cereal based products (Clemens and Dubost 2008). However, current gluten-free options, such as rice powder, are comparatively nutritionally lacking in fiber, protein, vitamins and minerals and (Siddiq and others 2011). Thus, there has been growing interest in developing

new strategies to incorporate beans in gluten-free products to increase the health attributes of these products.

One strategy being employed to increase the amount of beans consumed is the development of new bean-based ingredients, such as bean powder, that can be used as a supplement or replacement of current corn and wheat ingredients in many products. The popularity of gluten-free products has created a need for healthy alternatives to powder that also taste good, and thus increased use of dry bean powder has potential. The soybean industry has had much success with this strategy; however, dangerous allergic reactions and concerns about estrogenic compounds have made consumers increasingly wary in the past decade. Similar health benefits can be achieved with dry beans without the allergy concerns (Geil and Anderson 1994). By positioning novel bean ingredients under the health halo and within the gluten-free trend, dry beans could also reach these low consumption demographics in the Midwest and Northeast. Current gluten-free options, such as rice starch, have been noted by experts to be nutritionally lacking in fiber, protein, and vitamins and minerals, which bean powders have in abundance (Geil and Anderson 1994; Siddiq and others 2011). Bean powder is gaining in popularity as a wheat substitute because it is not only gluten-free, high in protein and dietary fiber, but also has the profile of essential amino acids, which is complementary to that in most cereals (Steinke and Hopkins 1983, Ugwuona 2009, Gupta and others 2006). Bean powders can be used as a texturing ingredient in tortilla chips, baked products, and pasta but also processed into breakfast and snack food products (Hall 2008).

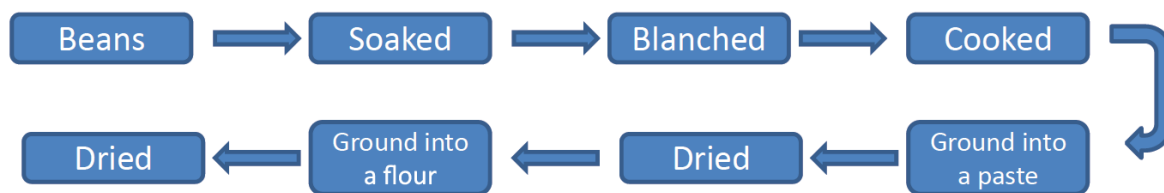
Navy beans, pea-sized white legumes, are characterized by a creamy texture and mild but dense flavor. Navy beans are one of the most popular dry bean varieties on the market in the U.S. (Lucier and others 2000). They are particularly advantageous when being used to produce

powder, as the creamy white color results in a powder that is visually similar to powders made from traditional cereal grains. The flavor of Navy beans, however, might be considered a barrier to consumption, as it is thought to be relatively strong compared to other cultivars of *Phaseolus vulgaris* (Dreher and others 1982)

2.4.1 Bean Powder Production Methods

2.4.1.1 Typical Commercial Bean Powder Processing

Bean powders can be produced using several different methods. A heating step is necessary to remove anti-nutritional factors and a soaking step is desirable to reduce flatulence-causing oligosaccharides (Sathe and Salunkhe 1984). Raw bean powders are generally not used, although some raw powders are available in grocery stores (Hall 2008). Raw bean powder may be problematic for consumers due to anti-nutrition concerns that may be a health concern of consumers do not properly treat the raw powder (Section 2.2.2). Currently in industry, the processing method most commonly used is the pre-cook method. In brief, the beans are soaked, blanched, cooked, dried and ground into a powder (Hall 2008). The less common alternative to the pre-cook method is to grind the beans prior to heating.



Preparation of bean flours using a pre-cook method.



Preparation of bean flours using a grinding step prior to heating.

Figure 2.1 Typical Commercial Bean Powder Processing Steps (Hall 2008)

2.4.1.2 Extrusion Processing of Bean Powders

The process of continuous-use shear force under high pressure and low heat to force product through a barrel is known as extrusion cooking. Extrusion processing may have advantages over the standard bean powder processing techniques as only a small amount of water and heat is used while eliminating the majority of anti-nutritional components (Nyombaire and others 2011). Nyombaire and others (2011) used bean extrudates to produce porridge and found that a consumer panel could not detect beany flavor. However, the demographic used for the study was composed entirely of Rwandans, who may be more accustomed to beany flavor. Simons (2013) proposed that due to starch-lipid and protein-lipid complexes formed during extrusion, extruded bean powders may have lipids that are less available to be oxidized and produce off-flavor compared to typical commercial products. Thus, bean powder produced by extrusion may have superior flavor properties compared to those produced by the standard

commercial process. It is unclear how extrusion processing alters aroma chemistry or consumer acceptance of bean powders in application. Balandran-Quintana and others (1998) found that trypsin inhibitors were eliminated at extrusion temperatures as low as 140°C and that in vitro protein digestibility can be significantly improved by extrusion processing at 160°C and 22% feed moisture.

2.4.2 Sensory Barriers to Bean Powder Consumption

Dry beans have a variety of undesirable characteristics, aside from anti-nutritional factors, that have long contributed to a small consumer base in the United States. Long cooking times, “beany” flavor, high amounts of flatulence-causing oligosaccharides such as raffinose and stachyose, and make beans attractive to only a select, persistent consumer group who is willing to work around these barriers (Lucier and others 2000, Sessa 1979). Because bean powder generally is pre-cooked, it eliminates some of these barriers. However, beany flavor remains a major barrier for bean powder consumption, particularly in foods where beany flavor is not complementary or is out of place, such as delicately flavored products or desserts. Bean powder is susceptible to development of flavor by degradation or modification phytochemicals and odor-active chemicals inherently present in the beans. This could range from furans from Maillard reaction products to aliphatic aldehydes from lipid degradation (Simons 2013)

Currently, there are a handful of published research papers that have studied the impact of pulse powder substitution in grain-based foods such as crackers, porridge, cookies, bread, pasta, meat (as filler) and extruded snacks (Han 2010, Dreher and Patek 1984, Dryer and others 1982, Cady and others 1987, Borsuk and others 2012, Gallegos-Infante and others 2010,

Nicholson 2013, Nyombiare and others 2011). In general, most studies find that at higher levels of substitution (beyond 25-35% of original powder weight) of the traditional grain of the food, the more adverse the impact on functional and sensory properties. Borsuk and others (2012) found that pitas made with Navy bean powder were rated 2.7 on a 9-point hedonic scale for flavor and 2.4 in overall liking. Dry and others (1982) found that at the 50% substitution of Navy bean powder in pumpkin bread, average flavor score on a 9-point hedonic scale was 5.3. Baking using legume powders is challenging due to the lack of elasticity characteristics typically imparted by gluten. Kohajdova and others (2013) recently found that above 10% substitution of wheat powder mass in baked bread rolls, sensory attributes such as shape crust color, crumb elasticity and hardness were all adversely affected. The researchers did not specify the recipe for the baked bread rolls.

Instances where high percentage bean powder products are highly acceptable are nearly missing. One instance was found by Nyombaire and others (2011) in Rwanda, where the researchers were studying bean porridge's similarity to a traditional dish called sosuma. This may have been related to the demographic used. Simons (2013) found that extruded 100% bean snacks were able to exceed the minimum flavor value for acceptability, but also found that many panelists noted an undesirable "beany" flavor. Dreher and Patek (1984) found that shortbread cookie scores on a hedonic scale of 1-9 were inversely related to the level of whole Navy bean powder used. Han and others (2010) developed gluten-free pulse-based crackers and found that while some legumes, such as chickpea, made highly acceptable crackers, Navy bean did not, due to a strong "beany" flavor. Hans and others (2010) also noted that flavor is likely to be the largest challenge in producing bean-based products.

Few studies have studied the sensory attributes of extruded bean-based products. Siddiq and others (2013) used low temperature extrusion and steam cooking (typical commercial process) to produce Navy and pinto bean powders. Sensory tests of these powders in cookies have shown that extruded powders were more acceptable than steam cooked powders and only a small fraction noted any beany flavor. This is likely due to cookies inherently having many ingredients, especially sugar, that can hide off characteristics.

2.5 Food Volatile and Aroma Analysis Techniques

2.5.1 Non-headspace Volatile and Aroma Extraction Techniques

Extraction and separation of volatile aroma compounds from food matrices is a complex process that is complicated by a variety of challenges. Extracting the volatiles in a way that will still provide a profile relevant to form of the food actually consumed can be especially challenging. The low concentrations of these compounds, interactions of compound with macronutrient components of the food, large number of distinct volatiles with a wide variety of volatilities and instability of aroma compounds especially when exposed to air, heat or extreme pH are the main challenges associated with extraction of aroma compounds (Parliament 1996). Modern non-headspace volatile techniques are generally aimed at preparing sample for direct injection into gas-chromatography mass spectrometry for identification of compounds.

Direct solvent extraction is the most straightforward extraction techniques available for volatile analysis. Ethers, hydrocarbon mixture and other mid-polarity and non-polar solvents are generally used in direct solvent extraction. However, direct solvent extraction is generally only used for liquid foods, such carbonated drinks or fruit juices, where sample matrix will not interfere with extraction. A more common volatile extraction technique is steam distillation,

which involves extracting water soluble volatiles by capturing steam that is distilled off the product or an aqueous solution containing product. Compounds that are not water soluble or that have a boiling point less than 100°C will be lost using this technique. Direct steam distillation involves using the water inherent to the product as a steam source. For products that are dry, indirect steam distillation, where steam is generated externally, can be used to achieve a similar effect. If sample decomposition is a concern either technique can be performed under a vacuum (Parliament 1996).

2.5.2 Headspace-Gas Chromatography Extraction Techniques

Headspace extractions utilize the atmosphere next to or surrounding a food (described as the “headspace”), and thus do not require any modification of sampling of food itself. Headspace extraction is the preferred methods for volatile aroma analysis as it allows for sampling to occur without modifying the food matrix (as aroma compounds inherently must be volatile) or introducing liquid solvents which could interaction with compounds. Headspace techniques can be organized into two distinct styles: static and dynamic.

2.5.2.1 Static Headspace Techniques

Sampling the gaseous space above samples in a sealed container after the system has reached equilibrium between the food and headspace is known as static headspace sampling. For example, a food might be placed in a sealed vial and be left to stand for a period of time while volatiles from the sample equilibrate between the food phase and the headspace phase. The amount of volatile entering the headspace depend primarily on the temperature of the system, how long the sample has been allowed to equilibrate and the solubility of the volatiles in both food sample and the air (Wampler 1996). Special care needs to be taken in static headspace that

equilibrium time and temperature is constant across replicates, as the quantity of volatiles in the headspace area rapidly change as equilibrium takes place, which can occur for up to an hour at 50°C. This lengthy time requirement is a major disadvantage to using static headspace techniques.

2.5.2.2 Solid Phase Microextraction

Another form of static headspace, solid phase microextraction (SPME), involves a three phase equilibrium consisting of the sample, the headspace and an adsorbent material that will later be removed and used to transfer compounds to a GC system. Pawilczyn and coworkers developed SPME in 1990 that has become a commonly used tool for food, environmental and aroma analysis (Snow and Slack 2002). There are many commercial devices designed to execute this process. Devices generally consist of an assembly housing a coated fiber that is housed inside a septum piercing needle which can be mounted onto a sample container (Figures 2.2). The fiber can be retracted and moved to a gas chromatography set-up where the fiber is exposed for desorption onto the column. This process is advantageous in that requires no sample clean up and no solvent and can improve detection limits drastically (Pawilczyn 1997). Sample enrichment is particularly important when analyzing compounds present in trace amounts, such as aroma volatiles (Klob and others 1998). Greater selectivity can be gained by using different trap materials on the fiber itself.

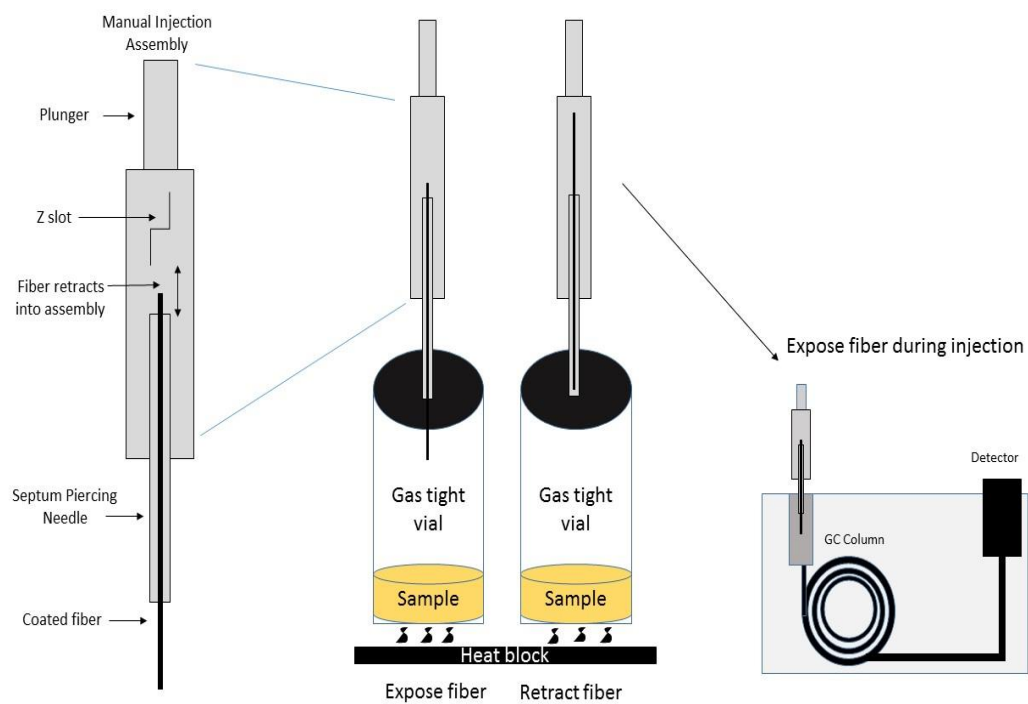


Figure 2.2 SPME Assembly for Manual Extraction and Generalized Injection Procedure

2.5.2.3 Dynamic Headspace Techniques

Dynamic head space techniques consist of using some carrier gas to “collect” volatiles in the headspace as the carrier move through the headspace. The obvious advantage to this technique is that instead of extracting approximately half of the aroma compounds from an equilibrated headspace, a much higher percentage can be extracted from the food and the quantity is not restricted by the volume of the headspace. As carrier gas passes through the headspace, volatiles are transferred to a “trap” where they will remain until re-volatilized for GC-analysis. “Traps” consist of high-surface area adsorbent material that are either cooled to condense volatiles or reactive in some manner (Wampler 1996). The carrier gas in dynamic headspace could be considered a type of purge, hence the designation “purge and trap.” However, purge and trap also generally refers to bubbling air through a liquid to concentrate flavor compounds. During dynamic headspace, samples might be heated to volatilize the maximum amount of compounds. This may help mimic the volatilization of compounds that occurs in the mouth during consumption of the food (Drumm 1988)

2.5.2.4 Trap and Coating Materials

A variety of trap materials have been utilized to collect volatiles from the headspace volume. Most commonly used is the Tenax® (poly-2,6-diphenyl-p-phenylene oxide), particularly with dynamic systems. It has a wide range of adsorbance and an affinity for aromatics. Its common usage makes it a “tried and true” material that has an abundance of information regarding its characteristics. However, in the case of aroma “finger printing”, or characterizing aroma profiles for food analysis, a wider range of volatile affinity is often required (Wampler 1996)

SPME coating materials are essentially traps in that they are often polymers designed to capture and hold certain classes of volatile chemicals. There are three main coatings that are offered for SPME fiber coating in various combinations representing polar and non-polar options. Poly dimethyl siloxane (PDMS) is the most commonly used non-polar option that absorbs non-polar volatiles effectively (Camara and others 2007). Carboxen (CAR) is a polar coating that is often offered commercially in combination with PDMS to create a bipolar fiber for a wider range of volatile collection. Divinylbenze (DVB) is a polar coating often used in combination with PDMS as it functions through adsorption instead of absorption. All three can be used to coat a single fiber known as a DVB/CAR/PDMS fiber. This combination is optimal for flavor analysis of volatiles and semi-volatiles (Camara and others 2007)

2.5.3 Gas Chromatography-Mass Spectrometry

Gas Chromatography (GC) is a staple in many disciplines of science and a go-to tool for precise, repeatable separations of volatile compounds. GC comes in many forms, headspace chromatography and liquid chromatography to name a few of the more common usages (Grob 2004). Generally, GC consists of moving a sample of volatile analyte through a coated column with specific adsorptive or absorptive properties so that analytes of interest are separated by polarity. With dynamic temperature programs, an additional level of separation is gained in the form of boiling point. The mobile phase in GC is carrier gas, usually inert gas such as hydrogen, nitrogen or helium, does not generally interact with the sample, thus selection of stationary phase is particularly important. Stationary phases are most commonly made from polysiloxanes due to their good coating characteristics, wide range of operating temperatures and non-reactivity with carrier gas (Grob 2004). Gas chromatography combined with mass spectrometry detectors has been widely used in aroma research to identify odorants, which are inherently volatile.

GC procedures can be optimized by understanding plate theory, developed by Martin and Synge (1941), and the van Deemter equation developed by van Deemter and others (1956). Plate theory provided a means to evaluate the efficiency of a separation—that is, the more theoretical plates a separation could achieve, the more resolution could be achieved in the resulting chromatogram. Van Deemter further explained the optimization process by accounting for longitudinal band diffusion, mass transfer and pathing that occurs during separation. Modern GC is always operated using open tube columns, thus pathing is not relevant. However, longitudinal band diffusion, the kinetic movement of mass from high concentration areas to low, which is inversely related to linear flow rate, is hugely impactful on GC chromatogram resolution. As mass moves through the column, diffusion will quickly cause what was originally a “plug” or band of separated analyte to diffuse, resulting in low peaks with a lot of background noise. Thus, high flow rates are desirable in GC. Once analytes are separated, there are a variety of detectors that can be used to identify compounds. Most commonly used is mass spectroscopy.

Optimizing resolution of as many analytes as possible is particularly important when screening for odorants using olfactory detectors. When sampling from the headspace, splitless injection is used, which can cause large, overlapping bands and low resolutions due to the large volume needed. SPME reduces this somewhat, but introduces a separate problem in the form of long sample desorption time from the fiber. Desorption of analytes from a SPME fiber can take up to three minutes, providing additional time for diffusion of compounds and also potentially producing double peaks as compounds may desorb at different rates depending on the context. However, these issues can easily be addressed by leveraging cryofocusing. Cryofocusing refers to the technique of cooling either the GC oven or the inlet tubing prior to the column to a sub-ambient temperature at which volatiles will condense. The column is then rapidly reheated,

allowing analytes to boil off during temperature programming for optimum separation (Klob and others 1998).

2.5.4 Gas Chromatography-Olfactory

Odor research is centered on the purpose of relating human perception of odors to compounds which we can identify. These compounds often times play a dominant role in what humans perceive as flavor, so the information gained from identifying relevant compounds is invaluable to defining the flavor of a specific foods (Spence 2015). While gas chromatography alone is an excellent identification method which can be combined with descriptive sensory and threshold analysis to characterize odorants in food, complex odorants present at low thresholds and no direct human perception can lead to incorrect conclusions regarding the contributions of particular compounds. Variation in human perception further complicates these correlations. That being said, the human nose is often times a more sensitive detector than physical detectors (Pollien and others 1997). Gas chromatography-olfactory (GC-O) is using the human nose as a detector which describes analytes as they elute from the column. In modern GC, the olfactory is almost always utilized in combination with a detector that can identify the compound simultaneously. This is achieved using micro-split wafers or other specialized connectors that can divide the carrier gas stream as it elutes.

Band diffusion and resolution are of particular importance in GC-O analysis. As bands diffuse, the smell that is ultimately eluted will be less intense and persist for a longer time, potentially interacting with other smells if full resolution is not achieved. Cryogenic trapping, as discussed above, is commonly used along with SPME extraction to improve resolution in GC-O analysis (Klob and others 1998).

There are a variety of techniques used today to quantitatively measure the impact of an odorant on a food. GC-O techniques can largely be divided into dilution methods and intensity methods. Dilution methods, largely based on successive dilutions of an aroma until no aroma is perceived by the detector, are not as popular as they were a decade ago, but have proven their efficiency for qualitatively assessing impact odor contributors in foods (Pollien and others 1998). These methods have issues with repeatability and have not been shown to be highly representative of the foods from which the aromas are extracted (Abbot and others 1993).

However, quantitative methods have been developed in the form of intensity methods in order to collect repeatable and reproducible data that is representative of the aroma which is truly perceived by the human olfactory system. The odor activity value (OAV) which is equal to the concentration divided by the odor threshold, makes the assumption that the perceived intensity is proportional to the aroma concentration and has been shown to be inaccurate in practice (Abbot and others 1993). This concept has been the source of great confusion, as intensity models gravitated towards peak area and size being directly correlated to odor intensity. Rather, Steven's Law, which accounts for differences in thresholds as a result of properties of the compounds, has been verified in the case of GC-olfactory experiments (Da Silva and others 1994). With these limitations in mind, the most repeatable method today for GC-O analysis utilizes a panel of 6-10 sniffers who report detection as a binary over the course of GC-O run (Pollien 1997). In this method, intensity is correlated with number of assessors. Using a panel of 6-10 panelists, detection noise can also be eliminated by labeling odors with only a few assessors as "background noise". This is known as detection frequency analysis (DFA). Independent panels of 6-10 are able to reliably reproduce aromagrams which have useful correlations to intensity of the odorant in the original sample (Pollien 1997).

2.6 Aroma Chemistry of Dry Beans

2.6.1 General Overview of Aroma Compounds Found in Dry Beans

Buttery and others (1975) used steam distillation continuous extraction under a vacuum to extract volatiles from raw *Phaseolus vulgaris* and used GC-MS to characterize major components and identify potential sources of off flavor. Buttery and others (1975) identified several key classes of compounds that likely contribute the most to overall beany flavor as well as other off flavors. At the time of this study, aroma analysis methods were extremely limited. Pyridines, pyrroles and thiazoles and other sulfur containing compounds were suspected to be major contributors to overall bean aroma. Similarly, Drumm (1988) found the majority of volatile compounds from direct vapor analysis (headspace in dry beans) to have Kovats indices between 600-1200 and 900-2200 from total vapor analysis and be comprised mainly of pyrazines and sulfur containing compounds. However, no sensory was performed to assess odor impact of these compounds.

Lovegren and others (1979) analyzed dried beans using direct transfer gas chromatography and found significant differences compared to the work of Buttery and others (1975). The researchers found that the technique used was the primary source of these differences, and that there is much similarity between the volatile chemistry of dried beans, lima beans and southern peas. One difference noted was that dry beans have slightly higher amounts of enones and enols, indicating that the technique used Buttery and others (1975) included soaking which provided ample opportunity for lipid oxidation and thus production of these chemical classes. Key aroma compounds identified included naphthalene, toluene, benzaldehyde, o-xylene, styrene, and acetophenone.

Variety, growing condition and time of harvest have all yielded small but significant differences in the aroma profiles of dried beans. Mishra and others (2017) found differences among three varieties of red kidney beans but did not conclude that there would be any major impact on overall aroma. Oomah and others (2007) found significant differences in both quality and quantity of aroma compounds between six varieties of dried beans and concluded that the main concern regarding aroma would be differences in quantity, as there was a distinct common set of compounds seen across all varieties.

2.6.2 Changes in Aroma Chemistry of Dry Beans During Processing

Drumm (1988), Lovegren (1979) and Buttery (1975) all researched aroma chemistry of raw dry beans, which has limited applicability to the aroma chemistry in the cooked beans. Soaking and cooking processes can introduce new aroma compounds and eliminate dominating compounds. Maillard reaction products, lipid oxidation products, sugar degradation products and amino acid degradation products are all potentially created upon processing.

Drumm (1988) showed that thermal processing (116°C) can have a significant impact on the degradation or formation of volatile compounds in dry beans, including the development of pyrazines and sulfur compounds. Drumm found that raw bean volatiles profiles are dominated by low-boiling point compounds which are likely boiled off during canning or volatilized during soaking. Mishra and others (2017) recently investigated the changes in aroma chemistry that occur during cooking of beans. The researchers used GC-MS and descriptive sensory to correlate aroma compounds with dominant descriptors. There were significant differences between the volatile profiles of red kidney beans in a raw state and cooked state. Mainly, a reduction of aldehydes and alcohols was observed. Hexanal was reduced by 96%, for example. Conversely,

sulfur compounds and pyrazines increased dramatically. The researchers concluded that sulfur compounds were formed during cooking and possessed low odor thresholds, and thus likely contributed greatly to the odor of cooked red kidney beans. There are no published papers regarding aroma chemistry of cooked bean powders, which likely have significantly different volatile profiles due to extensive drying steps and different processes involved.

Aroma compounds can be formed during thermal processing through non-enzymatic browning process, such as the Maillard reaction, of which sugars and amino acids are the precursors, and degradative processes involving only sugars or only amino acids (caramelization and Strecker degradation, respectively). Depending on the inherent composition of amino acids and sugars in the food and on interactivities between products from early in the reaction, the final aroma compounds generated by this reaction can vary wildly (Mottram 2007). In regards to the aroma chemistry of beans, alkylpyrazines and furans described by Buttery (1975) and Lovegren (1979) are the main products that are likely to contribute to overall aroma of cooked beans and cooked bean ingredients. Further degradation through other mechanisms (Strecker) can produce other compounds, such as aldehydes. Buttery and others (1975) found 2,3-dimethylpyrazine and 2,6 dimethylpyrazine in vacuum steam distillation oils from kidney beans. Alkylpyrazines are generally thought to impart nutty, smoky aroma notes (Vara-Ubol and others 2004).

The Strecker degradation is described as the oxidation of amino acids by dicarbonyl compounds and can yield aldehydes, ketones and sulfur containing compounds. Strecker degradation of sulfur-containing amino acids can result in a variety of sulfur containing aroma compounds such as methional, which can be further oxidized into a variety of potent aroma compounds (methanethiol, dimethyl sulfide). Lovegren and others (1979) reported methanethiol, methylated sulfides, and 2-methylthiophene in dried kidney beans. Mishra and others (2017)

found a variety of sulfur-containing compounds developed in red kidney beans during the cooking process (boiling). Sulfur compounds are generally thought to impart eggy, cooked vegetable type of aroma notes.

Aromatic hydrocarbons have also been implicated as a potential source of off aroma in dry beans and have been found to be formed by heat treatment in certain foods (Cao and others 2012, Buttery and others 1975). Buttery and other (1975) found naphthalene to comprise approximately 2% of vacuum steam distillation oil.

2.6.3 Aroma Compounds in Dry Beans from Lipid Degradation

During processing of dry bean there are two main mechanisms by which unsaturated fatty acids might be degraded to form volatile aroma compounds. First, lipases which act on fatty acids are found in most legume tissues and can cleave fatty acids from triglycerides. Hydroperoxides form when lipolytic enzymes such as lipoxygenase, act on fatty acids and can further degrade peroxides to produce aliphatic aldehydes and ketones. Aldehydes and ketones from lipid oxidation have been identified as major contributors to off flavor in soy products, such as soymilk (Feng and others 2001). Lipoxygenase, an oxygen-catalyzed lipase, is found in nearly all species of legumes (Sessa 1979, Oomah and others 2011). Secondly, autoxidation of unsaturated lipids can occur when free radicals initiate a chain reaction that results in decomposition of unsaturated fatty acids to hydroperoxides. The types of hydroperoxides formed in either mechanism are dependent on degree of saturation and site (Frankel 1991). Aliphatic aldehydes produced by hydroperoxides have been described generally as grassy, beany, musty, stale and astringent aromas (Drumm 1988).

Sessa (1979) found that soybean lipoxygenase acts on linoleic and linolenic acids to product pentylfuran and hexanals which can contribute to green, grassy and beany flavors. Dry beans are primarily composed of unsaturated fatty acids (83-87%) and of these above 50% are generally linoleate and linolenate (Siddiq and others 2011, Simons 2013, Oomah and others 2011). Several researchers have postulated that by effectively inhibiting the lipoxygenase enzyme, which catalyzes the oxidation reaction, off flavor development in legumes can be reduced or eliminated (Sessa 1979). Achieving this has been proposed in a varieties of ways, including processing in acidic conditions (Okaka and Potter 1979) and blanching (Drumm 1988). However, most techniques used are resource- and time-intensive, particularly when considering these options in regard to producing bean powders or powders (Oomah and others 2011). Blagden and Gilliland (2005) have even suggested using microbial fermentation to eliminate volatiles from lipids such as hexanal from soymilk. To be able to grind whole beans without cooking and drying and maintain acceptability would make bean powder a more appealing option for powder producers from an economical and environmental standpoint.

Autoxidation can be catalyzed by metals, light (photoautoxidation), or heat. Metals can be introduced by processing equipment used to produce edible bean ingredients and initiate off odor and flavor development. Bean ingredients are often times not considered light-susceptible and are not packaged with light protection in mind to prevent these types of reactions. Heat is necessary during processing to eliminate anti-nutrients and deactivate lipases and cannot be eliminated. Finally, linoleic and linolenic acids are particularly susceptible to autoxidation and can produce a wide variety of aldehydes, many of which have been considered to possess off-flavors (Drumm 1988).

Buttery and others (1975) found that a 16-26% of vacuum steam distillation oil from soaked raw bean was comprised of the aliphatic aldehyde oct-1-en-3-ol and another 2-8% to the aliphatic ketone to octa-3,5-dien-2-one. While these compounds were initially thought to be inherent to the raw beans, it was more likely that they developed from enzymatic oxidation of linolenic acid while soaked overnight (Lovegren and others 1979). Buttery also found benzaldehyde, which can be formed via lipid degradation or through Strecker degradation of phenylalanine, present in raw dry beans using vacuum steam distillation (Adamiec and others 2001). Mishra and others (2017) found a significant increase in benzaldehyde between raw and cooked red kidney beans. The researchers concluded that it formed during processing due to heat application, but it was also possible that it formed through lipid degradation.

Although the total lipid content in Navy beans is low (~1.0 g/100 g) this small percentage of lipids impacts the flavor quality of bean powder over time due to lipid oxidation (Siddiq and others 2011). Lipid fractions in dry beans are mainly comprised of only unsaturated fatty acids, and lipid oxidation occurs at varying rates among different types of oils: if oxidation in oleic acid occurs at specific rate (1 time), oxidation in linoleic or linolenic acids occurs at 10 to 100 times that rate (Singh and Tiwari 2012). The major fatty acid composition of Navy bean consists of linoleic and linolenic acids, meaning that potential for lipid oxidation is high, possibly preventing Navy bean powder from being used as a functional ingredient in food products (Oomah and others 2011).

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Chapter 3: Manuscript- AROMA CHEMISTRY AND CONSUMER ACCEPTANCE OF NAVY BEAN (*Phaseolus vulgaris*) POWDER PREPARED BY EXTRUSION OR CONVENTIONAL PROCESSING METHODS

3.1 Abstract

Bean powder has been gaining in popularity among consumers due to its reported health benefits including reduced risk of heart disease, obesity and diabetes. Extruded Navy bean (*Phaseolus vulgaris* L.) powder can be considered as a cost-effective alternative to resource intensive traditional methods of processing. The objectives of the study presented in this manuscript were 1) to profile the volatile compounds derived from extruded and commercial Navy bean powders using headspace solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry; 2) to determine which of the volatile compounds are odor-active with gas chromatography-olfactometry; and 3) to investigate consumer acceptance of products formulated with extruded or commercial bean powder. The odor-activity was highest from aldehydes in the extruded and commercial samples, representing 55% and 47.5% of the total response, respectively. Commercial bean powder had approximately 12.5 times more peak area from aldehydes. Furthermore, the commercial powder contained 20 times more hexanal than the extruded sample. Aliphatic aldehydes are most often formed during the degradation of unsaturated fatty acids such as linoleic and linolenic, which are the primary fatty acids found in Navy bean. Peroxide values collected using the ferric thiocyanate method also showed an approximate two-fold difference in apparent level of oxidation. This finding indicates that the commercial powder may have undergone severe lipid oxidation prior to the experiment. Consumer acceptance data showed a significant preference for baked, Navy bean crackers made with the extruded bean powder over the crackers made with the commercial bean powder.

3.2 Introduction

Dry common beans (*Phaseolus vulgaris* L.) are a good source of a myriad of vitamins and minerals and contain two to three times the amount of protein found in cereal grains (Costa and others 2006; Siddiq and others 2011). Additionally, dry beans are a rich source of dietary fiber and contain beneficial non-nutrient components such as polyphenol antioxidants (Wu and others 2004; Siddiq and others 2011). Consumption of dry beans has the potential to impart a variety of health benefits, including reduction of the risk for diabetes mellitus (Hutchins and others 2012), obesity (Anderson and Major 2002), cardiovascular diseases (Anderson and others 1990) and colon diseases (Tharanathan and Mahadevamma 2003). Dry beans are particularly useful food for promoting overall health in developing countries (Venter and van Eyssen 2001)

Amylose in dry bean starch is present in high quantities (25-60%) and exhibits especially strong interactions between chains, which in combination with an inherently high dietary soluble fiber content from the coat of the bean, make dry beans highly resistant to digestion (Hoover and Zhou 2003). This indigestibility increases fecal bulk and butyrate production which are considered as markers for good colon health (Tharanathan and Mahadevamma 2003). The high amylose content also increases the production of resistant starch upon processing (Hoover and Zhou 2003). Hayat and others (2004) proposed that consumption of dry beans could decrease the risk of developing diabetes and obesity due to dry beans' high resistant starch content which can lower postprandial glucose levels and increase satiety (Reddy and others 1984).

Despite these benefits, the consumption of beans has remained stagnant in the United States at 1.6-2.3 lb per person per year (Lucier and others 2000). Consumption is also very regionally concentrated, with 76% of consumption occurring in the southern and western U.S., largely due to these regions' high populations of Hispanics who disproportionately consume

beans at 33% of total consumption in the U.S. (Lucier and others 2000). While dry bean consumption remains stagnant, there has been a five-fold increase in the introduction of gluten-free products from 135 in 2003 to 832 in 2008 due to consumers' interest in healthful non-cereal based products (Clemens and Dubost 2008).

Rice powder is a popular and highly suitable gluten-free option for many products. However, rice powders are comparatively lacking in fiber, protein, vitamins and minerals (Siddiq and others 2011). Beans are also an important part of the vegetarian diet as they can replace protein and B vitamins that are abundant in meat (Craig and Mangels 2009). In order to increase the health attributes of gluten-free and vegetarian options, there has been growing interest in incorporating beans powders into food products.

Incorporating bean powder into gluten-free products, however, still remains challenging due to an unacceptable impact on flavor and texture of the products. In general, the greater the level of substitution (beyond 25-35% of original powder weight) of the traditional grain of the food, the more adverse the impact on texture and flavor (Cady and others 1987, Gallegos-infante and others 2010, Dreher and Patek 1984). Currently, only a few published research papers have studied the sensory impact of dry bean powder substitution in grain-based foods such as crackers, porridge, cookies, bread, pasta, meat (as filler) and extruded snacks (Dryer and others 1982; Dreher and Patek 1984; Cady and others 1987; Gallegos-Infante and others 2010; Han and others 2010; Nyombiare and others 2011; Borsuk and others 2012; Nicholson 2013). Producing baked goods with acceptable textures using dry bean powders is challenging due to the lack of elasticity characteristics typically imparted by gluten. Kohajdova and others (2013) recently found that above 10% substitution of wheat powder mass in yeast raised baked bread rolls, sensory attributes such as shape, crust color, crumb elasticity, and hardness are all adversely

affected. Han and others (2010) concluded that beany flavor is likely to be the largest challenge in producing bean-based products. They developed gluten-free pulse-based crackers and found that while some legumes made highly acceptable crackers, Navy bean did not, due to a strong “beany” flavor. Dreher and Patek (1984) found that shortbread cookies’ sensory scores were inversely related to the level of whole Navy bean powder used. Navy beans are considered to be one of the most promising beans for use in gluten-free products by growers in the State of Michigan, the second largest producer of dry beans in the U.S., and thus were used as a model bean in the current study (Lucier and others 2000; Kelkar and others 2012).

Processing dry beans into value-added food ingredients is one strategy being employed to increase incorporation of healthful beans into new food products. Bean powder, a value-added bean ingredient, can be positioned as a gluten-free wheat powder alternative that provides added health benefits to the consumer. Commercial bean powders are generally produced via soaking, blanching and steam-cooking beans followed by drying and particle size reduction. This process eliminates flatulence-causing oligosaccharides and anti-nutrients such as phytohemagglutinins and lectins (Siddiq and others 2011). However, the entire standard commercial process uses a significant amount of water and energy.

The process of continuous-use shear force under high pressure and low heat to force product through a barrel is known as extrusion cooking. Extrusion processing may have advantages over the standard bean powder processing techniques as only a small amount of water and heat is used while eliminating the majority of anti-nutritional components (Nyombaire and others 2011). Simons (2013) proposed that due to starch-lipid and protein-lipid complexes formed during extrusion, extruded bean powders may have lipids that are less available to be oxidized which could result in production of off-flavor compared to typical commercial

products. Thus, bean powder produced by extrusion may have improved flavor properties compared to those produced by the standard commercial process. There is little information in the literature to clarify how extrusion processing alters aroma chemistry or consumer acceptance of bean powders in application.

The objectives of this study were 1) to profile the volatile compounds derived from extruded and commercial Navy bean powders using headspace solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry; 2) to determine which of the volatile compounds are odor-active with gas chromatography-olfactometry; and 3) to investigate consumer acceptance of products formulated with extruded or commercial bean powder.

3.3 Materials and Methods

3.3.1 Sample Preparation

3.3.1.1 Commercial Bean Powder

A shipment of 18 kg of commercial Navy bean powder produced by the Standard Method was delivered at ambient temperature from a commercial supplier and was stored at -20°C until analysis (Figure 3.1). Three replicates were immediately sampled randomly from the 18 kg multi-ply paper bag and stored separately in glass.

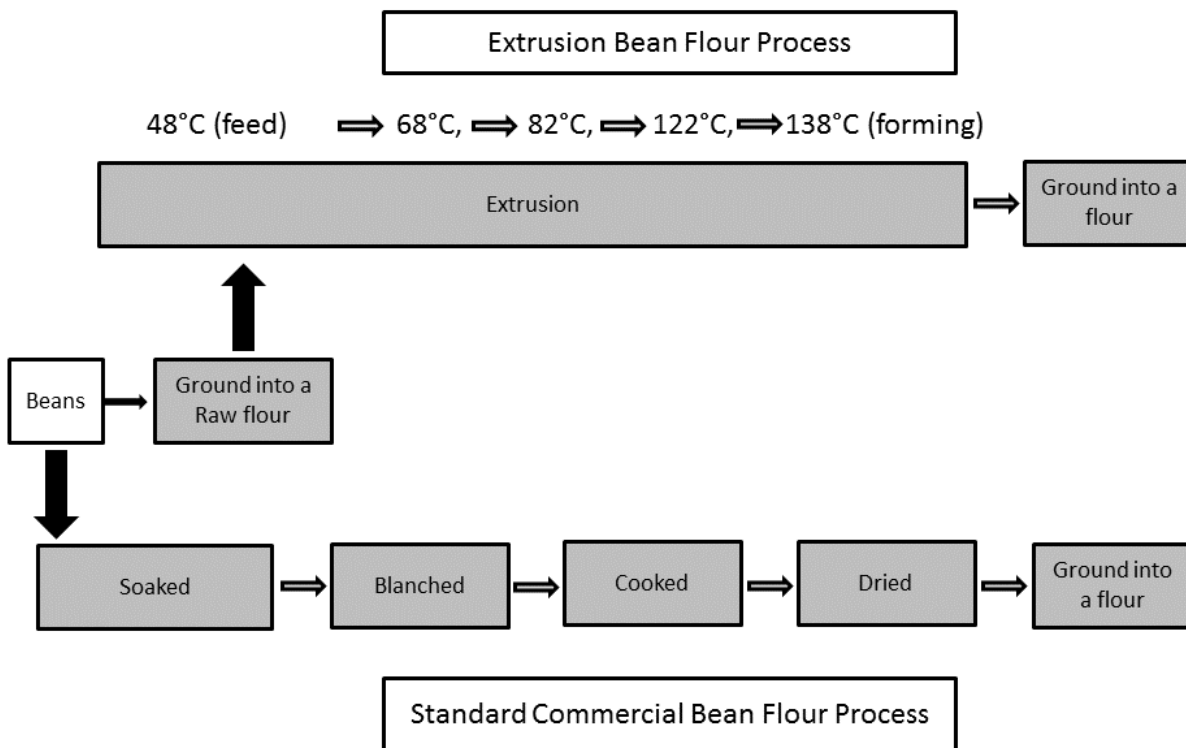


Figure 3.1. Bean Powder Processing Flow Comparison.

3.3.1.2 Extruded Bean Powder

Dry Edible Medalist Navy beans were obtained from the Michigan Bean Commission (Frankenmuth, MI., U.S.A.). The raw dry beans were ground using a Thomas Wiley® Mill (Model 4, Thomas Scientific Inc., Swedesboro, NJ., U.S.A.) to pass through a screen with 0.5-mm pore size to prepare ground raw bean powders (particle size ≤ 0.5 mm) which were then stored at -20°C until further processing.

A co-rotating twin-screw extruder (Model MP19T2-25, APV Baker, Grand Rapids, MI., U.S.A.) with a 19 mm barrel diameter and a 3 mm diameter exit die was used to extrude raw

bean powder samples (Figure 3.1). Extrusion conditions were selected based on preliminary research and previously published literature (Ai and others 2016). The set temperature of the barrel of the extruder was held at 40, 60, 80, 120, and 140 °C for zones 1, 2, 3, 4 and 5, respectively, with a screw speed of 200 rpm. Moisture in the extruder was controlled using a water injector (Brook Crompton E2 Metripump, Hudders Field, England) and was kept at 30% throughout each run. The raw ground bean was fed into the extruder at a rate of 2 kg/hr. Three replicates were sampled at random time points throughout the run and stored separately. The bean powder extrudate was manually cut into strings approximately 2-4 cm in length and dried on aluminum trays at 50°C for 24 hr or until moisture content was approximately 6%. The dried extrudates were then ground using the same mill and 0.5-mm sieve to prepare extruded bean powders with the exception of randomly selected replicates for aroma analysis by gas chromatography-mass spectrometry (GC-MS). The separate samples for aroma chemistry analysis were held frozen in glass at -20°C under the same conditions as the commercial bean powder until analysis.

3.3.2 Chemicals

Analytical standards for propanal, pentanal, hexanal, 2-hexanal, heptanal, octanal, 2-octenal, nonanal, 2-nonenal, 2,3-butanedione, 2,3-pentanedione, 2,4-hexadienal, 2,4-heptadienal, 2,4-nonadienal, benzaldehyde, 6-methyl-5-hepten-2-one were obtained from Sigma Aldrich (St. Louis MO., U.S.A). C₇-C₃₀ saturated alkanes used for Kovats Index determination were from Supleco Inc. Bellfonte, PA. Ammonium thiocyanate, acetic acid, chloroform and ferric chloride for peroxide determination were from Sigma Aldrich.

3.3.3 Peroxide Value Determination

Peroxide values were measured in triplicate upon removal of bean powder samples from storage at -20°C. Raw powder was sampled from pre-extruded supply, and was subject to the same conditions until that point. Peroxide value was determined using the ferric thiocyanate method as described in Hornero-Mendez and others (2001).

3.3.4 Solid Phase Microextraction (SPME) absorption of aroma compounds

Solid phase micro-extraction (SPME) was used to concentrate analytes from the headspace of the bean powders. Frozen bean extrudates were ground into a powder immediately before analysis using a blade grinder (106854, General Electric, Fairfield, CT., U.S.A.), and 5 g of resulting bean powder was equilibrated at 50°C for 20 min in a 50-mL gas-tight vial. Using a manual SPME fiber holder, a carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) 2-cm fiber assembly (Supelco, Sigma Aldrich, St. Louis, MO., U.S.A.) was exposed to the headspace of bean powder for 60 min to load the fiber. The SPME fiber was desorbed for 2 min in the injection port at 250°C and conditioned for 20 min at 250°C after each run.

3.3.5 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

A Clarus 680 gas chromatography system (Perkin Elmer, Waltham MA., U.S.A.) mass spectrometer (Clarus 600S, Perkin Elmer, Waltham MA., U.S.A.) attached for detection was used to separate and detect the headspace aroma compounds. A Restek (Bellforte PA., U.S.A.) RTX-1301 60 m column with a 1.5 µm film thickness and 0.32 mm ID column was used with 99.999% purity helium (Airgas, Radnor PA., U.S.A) as the carrier gas for all analyses.

A block of dry ice approximately 2.5 cm x 2.5 cm x 10 cm was placed on the bottom of the GC oven away from the column to bring the entire GC oven to sub-ambient temperature in order to condense analytes on the column head. This technique has been described as a cost-sensitive, yet reliable, method for cryofocusing in aroma analysis (Elmore 2014). A temperature gradient program was used as follows: 7°C hold for 2.0 min, 1°C/min to 40°C, 2°C/min to 200°C, 50°C/min to 240°C, hold for 5 min. The total run time was approximately 80 min. For detection with mass spectrometry, ion source was kept at 230°C with electron energy at 70 eV and a scan range of 35-350 mass units. Flow rate throughout the run was maintained at 1.8 mL/min at the exit port. Each of the triplicate samples from the preparation stage was run in duplicate on a GC-MS as sub-replicates.

Spectra obtained from mass spectrometry were compared to NIST 02, NIST 08 and Massbank spectral libraries to identify separated analytes. The Kovats retention index (RI) was used to further confirm identities using a series of hydrocarbons run under identical conditions. Additionally, available authentic standards were used to confirm identities of some key compounds. Areas under the peaks were integrated using Turbomass software (Perkin Elmer, Waltham MA., U.S.A.) and presented as peak area/10⁶.

3.3.6 Gas Chromatography-Olfactory (GC-O)

GC-Olfactory port (SNFR™ Olfactory port, Perkin Elmer, Waltham MA., U.S.A.) integrated to the GC-MS was used to collect intensity and frequency data on separated aroma compounds. An S-Swafer Perkin-Elmer split wafer was used to split at a 1/7 ratio of the separated sample towards the sniff port. GC-MS conditions were the same as described above. GC-O effluent was combined with vaporized distilled water to enhance odor perception.

Seven panelists for GC-O analysis (3 male, 4 female) ranging from 20-27 years of age were selected who had some familiarity with GC-O and who could smell and describe a series of 5 different diluted aroma compound standards (hexanal, 1-hexanol, heptanal, benzaldehyde and (E)-2-nonenal). Panelists were asked to sniff continuously and briefly describe any eluting odors. Prior to sniffing, several 2-min time periods in the separation with no perceivable odors were identified to allow for panelists to take short breaks from sniffing. Each panelist provided written informed consent, and the study was approved by the Institutional Review Board of Michigan State University (East Lansing, MI., U.S.A.).

3.3.7 Consumer Sensory Evaluation

Consumer testing was conducted using the sensory evaluation lab in the Department of Food Science and Human Nutrition at Michigan State University. The study protocol was approved by the Institutional Review Board of Michigan State University (East Lansing, MI., U.S.A.). Written informed consent was obtained from all participants.

3.3.7.1 Navy Bean Powder Product Samples

Among a myriad of potential bean powder applications, three representative products were selected based on the results of preliminary tests: white shortened cakes, seasoned crackers, and vegetarian chicken nuggets. Baked, seasoned Navy bean crackers were developed to gauge consumer liking in a gluten-free product formulated primarily with bean powder (84.5% w/w). Gluten-free white shortened cakes were used to represent a value-added baked good product that contained bean powder as a wheat powder replacement. Vegetarian “chicken nugget” analogs were selected to gain understanding of potential impact on flavor in vegetarian products. (Table

3.1). Due to the inherent differences in the three product applications, controls were selected that represented competing products where applicable.

Table 3.1 Ingredient Percentages for Three Bean Powder Products Used in Consumer Testing

Seasoned Crackers		White Cake		Chicken Nugget Analog	
Ingredient	%	Ingredient	%	Ingredient	%
Navy bean powder	46.5	White sugar	26.1	Water	46.9
Water	43.4	Navy bean powder	22.5	Vital wheat gluten	39.3
Shortening	7.8	Butter	17.3	Navy bean powder	13.1
Salt	1.3	2% Milk	17.0	Monosodium glutamate	0.4
Whey protein isolate	1.0	Large egg	15.0	Baking powder	0.3
		Vanilla extract	1.5		
		Baking powder	0.5		
		Xanthan gum	0.1		

3.3.7.1.1 Seasoned Navy Bean Crackers

All dry ingredients were sifted together into a mixer bowl with a paddle attachment (Kitchen Aid, KSM500, St. Joseph, MI., U.S.A.) (Table 3.1). Whey protein was used to improve the texture of the crackers. The wet ingredients (Crisco shortening, reverse osmosis filtered water) were added to the bowl and mixed on low speed (2/5) for 3 min until a dough formed. Dough was then rolled to 2 mm thickness and cut into 20 discs, 2.5 cm in diameter. Discs were baked for 7 min at 190.5°C on a non-stick cookie sheet in a convection oven (Whirlpool Corp, Benton Harbor, MI., U.S.A.). After cooling, the crackers were lightly sprayed with cooking spray (PAM brand) and 10 g seasoning blend (Fuchs tomato basil seasoning) was distributed as evenly as possible between the 20 crackers using a shaker. No control was used as the product was specifically designed as a bean powder application.

3.3.7.1.2 Navy Bean Shortened Cakes

All dry ingredients were sifted together (Table 3.1). Butter and sugar were mixed at high speed (setting 10) together in a mixer (Kitchen Aid, KSM500, St. Joseph, MI., U.S.A.). Eggs and vanilla were added to the mixture followed by dry ingredients. Milk was then added and the ingredients were mixed for 1 min. The batter was distributed amongst six 3-in diameter muffin top pans and was baked for 20 min at 176.6°C in a convection oven (Whirlpool Corp, Benton Harbor, MI., U.S.A.). A control containing a gluten-free powder blend (Betty Crocker™) was used because it represented an established competitor to bean powder. In addition, it produced a cake with similar texture and appearance using identical methods and a 1 to 1 powder substitution.

3.3.7.1.3 Navy Bean-substituted Vegetarian Chicken Nugget Analogs

All dry ingredients were sifted together (Table 3.1). The wet ingredient (reverse osmosis filtered water) was added until a dough was formed. The dough was kneaded on a hard surface by hand and left to rest for 15 min. Dough was then rolled into a 2.5 cm diameter cylinder and cut into 2 cm thick slices. All slices were then boiled in 1.2 L of vegetarian chicken-flavored broth for 30 min. Treatment Navy bean powder was sifted with 0.5 g black pepper and 1 large egg to coat each boiled slice and then fried in vegetable oil at 180°C for 3 min and immediately frozen and held at -20°C until sensory evaluation was conducted. The nuggets were thawed to room temperature and reheated for 10 s in a 1000 W microwave oven (General Electric, JE15506W -002, Fairfield, CT., U.S. A.) to 50-60°C immediately prior to being served to panelists. A control containing no bean powder was studied by substituting the bean powder for additional vital wheat gluten.

3.3.8 Participants and Procedure

A 9-point hedonic scale, where 1=dislike extremely and 9=like extremely, was used to measure consumer acceptability of various sensory attributes, such as aroma and flavor, of each product. For the cake and crackers, 104 consumers between the ages 18-55 were recruited using emails and flyers. For the chicken nugget analogs, 60 consumers of vegetarian meat alternatives were recruited via emails and flyers.

3.3.9 Data Analysis

Consumer sensory evaluation data was collected using SIMS (vs. 6.0) sensory software (SIMS, Berkely Heights NJ., U.S.A.). SAS version 9.3 (SAS institute, Cary, NC., U.S.A.) was used to conduct analysis of variance, and means were compared using Tukey's test at $\alpha=0.05$ confidence level. ANOVA for peroxide values was performed using XLstat (Addinsoft, New York, NY., U.S.A.) at the 95% confidence level and mean comparison utilizing Tukey's test.

3.4 Results and Discussion

3.4.1 Odor-Active Compounds

Headspace SPME-GC-O analysis based on a detection frequency (DF) method was conducted to detect, identify, and characterize odor-active compounds in extruded Navy bean powder and commercial bean powder using 7 panelists. The DF method uses a number of panelists to detect and quantify an odor-active compound at the olfactory detector outlet as a measure for the odor intensity of the compound (van Ruth 2001). The main advantage of this method is repeatability and the results reflect the differences in sensitivity between the panelists (minimizing the impact of specific anosmia) (Brattoli and others 2013). Thus, the method has

been shown to be a reliable technique for estimating intensity of odor-active compounds using untrained panelists (Pollien and others 1997).

A total of 26 and 35 odor-active compounds were identified and characterized in extruded and commercial Navy bean powder, respectively (Table 3.2). The GC-O panelists also detected and characterized four odors in either or both samples, which did not have corresponding peaks that were distinguishable from background noise. The two largest chemical classes in both samples were aldehydes and ketones, accounting more than 70% of the odor-active compounds present; aldehydes (48%) and ketones (22%) in extruded Navy bean powder and aldehydes (50%) and ketones (25%) in commercial Navy bean powder.

Table 3.2. Aroma-active Volatile Compounds Identified in Navy Bean Powders Using GC-O

RI ^a	Identity Assigned ^b	Compound	Extruded		Commercial		Descriptors
			Peak area ^c	DF ^d	Peak area ^c	DF ^d	
Aldehydes							
422	MS, NIST, RI, STD	Propanal	NR	NR	5.9±2.5	4	chemical/fruity
711	MS, NIST, RI	(E)-2-butanal	4.0±0.8	2	5.4±1.4	3	nutty/toasted
744	MS, NIST, RI, STD	Pentanal	0.3±0.1	3	5.6±1.2	3	fruity/toasted
818	MS, NIST, RI	(E)-2-Pentenal	NR	NR	7.4±1.5	3	Wheat/musty
851	MS, NIST, RI, STD	Hexanal	11.5±1.9	7	231.2±36.7	7	grassy
919	MS, NIST, RI, STD	(E)-2-Hexenal	0.7±0.2	4	9.5±1.9	5	musty/earthy
956	MS, NIST, RI, STD	Heptanal	0.8±0.3	7	5.3±0.8	7	grass/green/meaty/rotting/musty
993	MS, NIST, RI, STD	(E,E)-2,4-Hexadienal	0.2±0.1	7	6.8±1.4	7	savory/green/cheesy/nutty/dusty
1040	MS, NIST, RI, STD	Benzaldehyde	2.6±1.5	5	27.0±2.9	7	nutty almond/bitter
1058	MS, NIST, RI, STD	Octanal	0.5±0.1	NR	13.0±2.2	3	fruity/nutty
1076	MS, NIST, RI, STD	(E,E)-2,4-Heptadienal	0.2±0.0	6	11.0±1.9	6	nutty/dirt/grassy/musty
1115	MS, NIST, RI, STD	2-Octenal	0.2±0.1	5	2.1±0.4	7	fatty/grass/soap/beany/soy/nutty
1130	MS, NIST, RI, STD	Nonanal	7.3±0.9	6	20.9±3.9	5	dusty/nutty/cleaner
1168	MS, NIST, RI, STD	(E)-2-Nonenal	0.2±0.1	7	2.2±0.6	7	cucumber
1189	MS, NIST, RI, STD	(E,E)-2,4-Nonadienal	4.5±1.2	4	7.4±1.3	7	nutty/beany/soy/wet clothes
1203	MS, NIST, RI	Decanal	NR	NR	1.0±0.2	4	earthy/caramel/nutty/sweet
Ketones							
639	MS, NIST, RI, STD	2,3-Butanedione	NR	NR	0.2±0.0	5	buttery
748	MS, NIST, RI, STD	2,3-Pentanedione	1.3±0.5	4	2.3±0.3	5	buttery/cheesy
949	MS, NIST, RI	2-Heptanone	0.3±0.1	NR	2.4±0.4	5	sour/brown/fruity

Table 3.2 (cont'd)

976	MS, NIST, RI	1-Octen-3-one	NR	NR	11.4±1.7	5	green/mushroom/metallic
1048	MS, NIST, RI, STD	6-Methyl-5-Hepten-2-one	1.7±0.4	6	3.0±0.3	6	sour candy/old/citrus/rain
1103	MS, NIST, RI	3-Octen-2-one	NR	6	60.0±5.9	6	earthy/floral/nail-polish/garbage/moldy
1123	MS, NIST, RI	(E,E)-3,5 Octendian-2-one	6.1±1.5	3	645.2±60.0	6	green/pine/nutty/fatty
1138	MS, NIST, RI	Acetophenone	4.5±1.2	3	209.2±25.7	7	mushroom/ brown/burnt/spicy/cherry/vanilla
Alkane/alkenes							
754	MS, NIST, RI	1,1-Diethoxyethane	2.8±1.0	5	0.5±0.3	2	citrus/sweet/soap
Alcohols							
835	MS, NIST, RI	1-Pentanol	0.2±0.1	2	4.7±0.6	3	green
1159	MS, NIST, RI	Maltol	0.2±0.1	4	3.9±0.9	4	spicy/burnt sugar
Aromatic Hydrocarbons							
898	MS, NIST, RI	o-Xylene	1.1±0.4	4	0.4±0.2	4	buttery/licorice/sweet/candy
932	MS, NIST, RI	Styrene	2.4±0.7	6	2.3±0.1	6	chemical
Furans							
772	MS, NIST, RI	2-Ethylfuran	0.3±0.1	3	1.2±0.3	5	floral/green/sweet/sour
970	MS, NIST, RI	2-Furfurylthiol	0.4±0.1	2	0.9±0.2	3	floral/dairy/mushrooms/coffee
1022	MS, NIST, RI	2-Pentylfuran	0.4±0.2	NR	8.3±1.8	3	earthy/licorice/leaves
Other/Unknowns							
746	MS, NIST, RI	Pyridine	NR	NR	1.4±.2	6	rotten/sulfur
870	MS, NIST, RI	Unknown	NR	NR	NR	4	Peanuts/potato chips
1238	MS, NIST, RI	Napthalene	NR	4	1.8±0.6	6	mothballs/musty
1257	MS, NIST, RI	Nonanoic acid	NR	3	1.4±0.4	6	chicken/bread/bad
1347	MS, NIST, RI	Unknown	NR	4	NR	2	yeasty/wheat
1373	MS, NIST, RI	Unknown	NR	NR	NR	4	rotten fish/meat

Table 3.2 (cont'd)

1390 MS, NIST, RI	Unknown	NR	2	NR	6	sweet/dusty/dirt
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^a Retention index based on RT-1301 column using a series of n-hydrocarbons.

^b Method of identification: comparisons of mass spectrum with NIST and Massbank mass spectral libraries, RI by comparison of RI with those from the literature and of retention time and spectrum of identified compound with those of an authentic compound

^c values (Mean \pm standard deviation) acquired from GC-MS peak responses of bean powders using six replicates and expressed as peak area/10⁶, NR: No reading

^d Detection frequency (DF) indicated the total number of panelists (n=7) that detected an odor that corresponded with this peak

Oomah and others (2007) proposed an eighteen-compound profile based on volatile headspace GC-MS across three common uncooked *Phaseolus vulgaris* varieties (black, pinto and red kidney). While nearly all of these compounds were present in the headspace of the bean powder used in the current study using high temperature heated bean powders, only seven of these compounds (hexanal, o-xylene, styrene, 6-methyl-5-hepten-2-one, benzaldehyde, nonanal, and 3,5-octadien-2-one) had odor activity in the current study (Table 3.2). (E,E)-3,5-octadien-2-one has been identified as a major volatile constituent in dry red beans (*Phaseolous vulgaris*) using steam distillation (Buttery and others 1975). Oomah and others (2007) also predicted overall bean flavor using PCA analysis, and concluded that hexanal, 3,5-octadien-2-one, and benzaldehyde were likely the authentic volatile markers for dry beans. These same volatiles appear to also contribute greatly to the characteristic beany aroma in extruded and commercial Navy bean powders as evidenced by the high GC-O panelist detection frequency for each of these compounds (Table 3.2). Thus, differences in the quantities of these compounds may be good indicators for the intensity of overall beany flavor (Gallegos-Infante and others 2010; Han and others 2010; Figure 3.2). Styrene has been reported in SPME headspace analysis of common dry beans previously, but it has never been implicated as a major odor-active compound in legumes (Oomah and others 2007). In both samples, styrene was detected by 6 GC-O panelists, and was present at similar quantities.

There were several compounds identified that are derived from heat treatment of bean powders. These were generally present in relatively higher concentrations in the commercial powder compared to the extruded powder. Maltol was present in high concentrations in the commercial sample, although this did not affect olfactory detection frequency. Maltol has been

reported as a component of cooked soy flavor (Cowan and others 1973). One unknown compound with a retention index of 870 was detected by 4 panelists in the commercial sample while it remained undetected in the extruded samples and had an odor characteristic of furan compounds (potato chips). 2-furfurylthiol was present in slightly higher concentrations in the commercial sample. Pyridine was a significant odor-active compound with 6 panelists detecting the compound. All of these differences might be explained by the relatively low amount of time that the extruded powders were directly exposed to heat (approximately 90 s from Zone 1 to Zone 5). Naphthalene is formed by heat treatment of certain foods and was odor-active in both samples (Cao and others 2012). Naphthalene was present in higher concentrations and was detected by 2 more panelists in the commercial sample, further indicating that the commercial powders may have been exposed to either more heat or similar heat for a longer period of time. Naphthalene has been reported in vacuum steam distillation oil from *Phaseolus vulgaris* (Buttery and others 1975). The odor imparted by naphthalene was described as “mothball” and “musty” and thus likely contributes to off-flavor (Table 3.2).

The term “beany” is loosely defined in the literature and is often used interchangeably with “off beany flavor” but generally appears to be some combination of green, earthy and vegetative odors (Vara-Ubol and others 2004). Two compounds were found in the current study that were described by panelists as “beany” alone: (E,E)-2,4-nonadienal and 2-octenal. (E,E)-2,4-nonadienal and 2-octenal both had lower DF in extruded bean powder (5 and 4 respectively) than commercial powder (7,7) (Table 3.2). Eight compounds were described as “green” or “grassy” and five compounds were described as “earthy”. Musty and dusty are also dominant descriptors across both bean powder samples.

Short chain aldehydes ranging from 5 to 9 carbons have been implicated as major odor-active flavor compounds imparting grassy and vegetative aromas in rehydrated *Phaseolous vulgaris* (van Ruth and others 1995). In the current study, hexanal, heptanal and 2-nonenal were detected by all panelists (DF=7) in both the extruded and commercial bean powder.

Benzaldehyde, 2-octenal, 2,4-nonadienal and acetophenone were detected by all panelists in the commercial powder and in by most (≥ 4) panelists in the extruded powder. All seven of these odor-active compounds can be lipid-derived and have the highest odor detection frequency in both commercial and extruded Navy bean powders (Table 3.2). Lipid-derived compounds are thought to be one of the major causes of off-flavor in legumes and this may extend to powders produced with legumes such as Navy bean (Mattick and Hand 1969; Sessa 1979).

Vara-Ubol and others (2004) found that compounds commonly associated with off-flavor, such as hexanal, were not considered beany by themselves. However, hexanal appears to be a major part of a synergistic combination of compounds that make up common bean powder aroma. Hexanal is derived from linoleic acid degradation from either legumous lipoxygenase enzyme or autoxidation. Hexanal contributes to beany aroma in combination with an unclear set of other compounds (Buttery and others 1975; Takahashi and others 1979). Hexanal contributes 18% of the aroma active-volatile peak area of Navy bean powder in the current study. The hexanal peak area for extruded Navy bean powder was 11.56 peak units/ 10^6 , which is comparable to peak areas ranging from 8.44-33.26/ 10^6 peak units for hexanal in raw black bean powders and 20.82-21.87/ 10^6 peak units in pinto beans by Oomah and others (2007). The hexanal peak area for commercial Navy bean powder was 231.18/ 10^6 peak units. There was a 20-fold increase in hexanal between the extruded sample and the commercial sample, indicating that there may be substantial oxidation in the commercial powders. Commercial powder, upon

removal from -20°C storage, exhibited high peroxide values suggesting lipid oxidation was already well underway (Table 3.3).

Table 3.3 Peroxide Values After Removal of Bean Powder From -20°C Storage*

Bean powder	mEq/kg sample ± standard deviation
Extruded	0.23 ± 0.06 b
Commercial	1.16 ± 0.05 a
Raw	0.38 ± 0.01 b

*Means with different letters were significantly different from each other (P<0.05).

This is consistent with the SPME-GC-MS findings that showed very a dominant hexanal peak and high levels of other lipid-derived products. The extruded powder, upon removal from -20°C storage, showed significantly lower peroxide values (table 3.3, P<.001). From a broad perspective, oxidation appears to be the major driver of the differences in aroma chemistry between the two powders. The GC-O data suggests that beany aroma as a whole may be largely defined by aroma-active compounds derived from polyunsaturated lipids as a result of lipid oxidation. Dry bean lipid fractions are primarily composed of unsaturated fatty acids (83-87%) and of these, over 50% are generally linoleic and linolenic acids, which are highly susceptible to lipid oxidation (Siddiq and others 2011; Simons 2013). Thus, the lipid makeup of the bean cultivar may have considerable impact on overall beany flavor, and susceptibility to off beany flavor development, of the resulting powder. 3,5-octendien-2-one, the most abundant volatile in the commercial sample, is derived from linolenic acid; hexanal, the second most abundant volatile in the commercial and most abundant in the extruded sample, is derived from linoleic acid (Sessa 1979; Belitz and others 2004; Table 3.2). Changes in the available fatty acid for oxidation would impact the ratio of these compounds which have different aroma descriptors.

This may explain the inability for past research to agree on the nature of off flavor imparted by beans, as fatty acid composition is variable across common bean cultivars (Siddiq and others 2011).

3.4.2 Consumer Sensory

In the food industry, oxidation is avoided, as it is commonly understood that most lipid-derived aroma compounds from oxidation will negatively impact consumer liking. However, in the case of Navy bean powder, this generalization may not apply. The commercial bean powder, which contained more oxidation products (Table 3.2), and the extruded powder performed differently depending on the food application. Significant differences in liking between the tested powders depended on the product being tested (Figure 3.2).

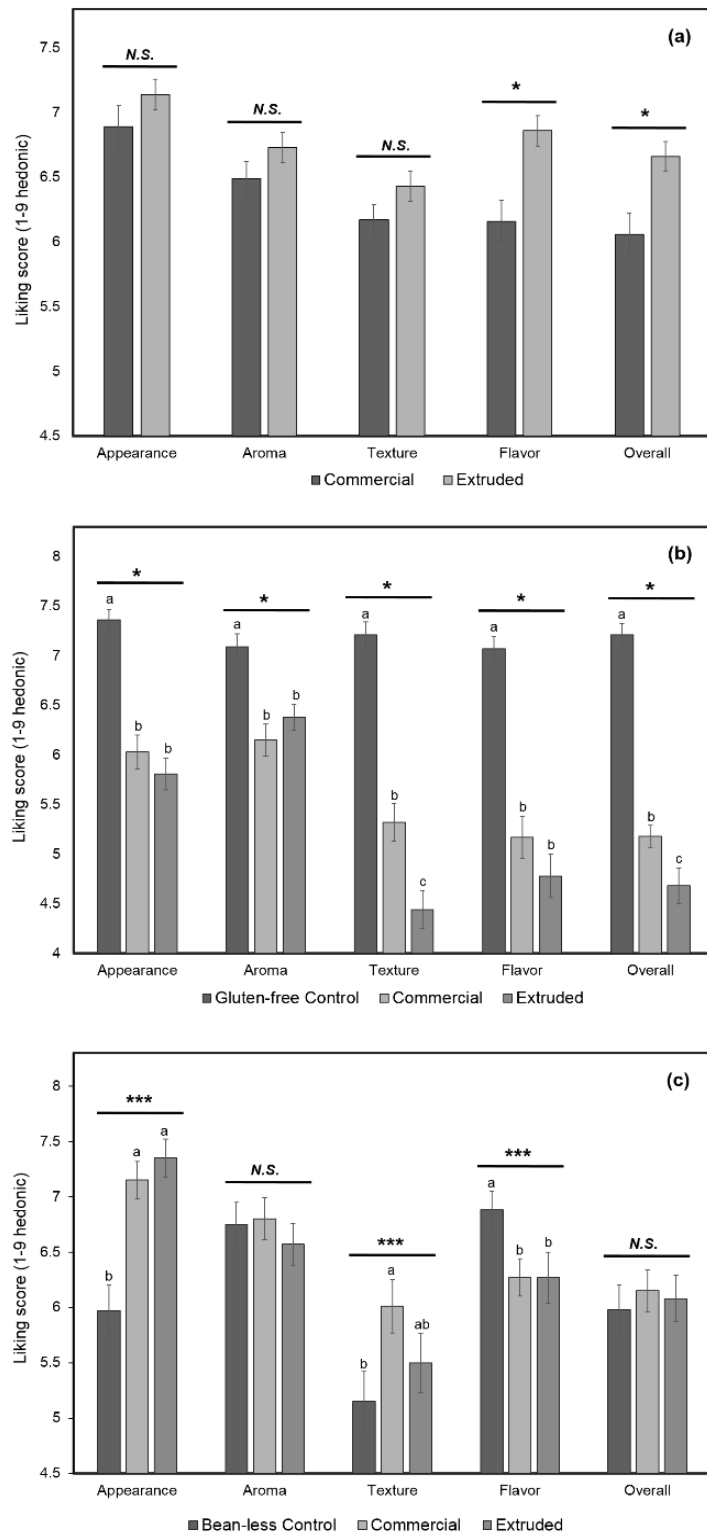


Figure 3.2. Mean Differences in Preference of Appearance, Aroma, Texture, Flavor and Overall Liking

Figure 3.2 (cont'd) of Seasoned Crackers (a), White cakes (b), and Chicken Nugget Analogs (c) on a 1-9 Point Hedonic Scale where 1= Extremely Dislike and 9=Extremely Like. Means with Different Letters were Significantly Different From Each Other (*= $P<0.001$, ***= $P<0.05$). The Error Bars Represent the Standard Error of the Mean

There was no clear trend of bean powder preference across all seasoned crackers, shortened cakes and vegetarian chicken analogs. However, the consumer acceptance data suggested a relationship between the adverse impact of bean oxidation on flavor and the amount of bean powder used in the product.

In the shortened white cakes, the commercial bean powder cake was significantly ($P=0.0001$) more liked overall than the extruded bean powder cake, although this is likely due to significant differences in appearance and texture of the two products (Figure 3.2). Texture in the cake produced with commercial beans was reported as denser, and less airy, whereas appearance was noted to be darker. There is a significant ($P=0.0001$), 1-2 point difference between the gluten-free control and both bean powder treatments across all attributes. There was no significant difference in either flavor or aroma between the two bean powder treatments, although it should be noted that “beany flavor” was noted by approximately one-third of panelists in the optional comments section provided during the panel. These findings were consistent with previously published literature. Cady and others (1987) substituted 35% of wheat powder in a pumpkin spice muffin recipe with Navy bean powder and found that there was a 1.0 point drop on a 5-point hedonic scale compared to a non-substituted muffin.

The seasoned crackers contained the largest amount of bean powder per 100g (46.5%) of all the products tested. In this application, the extruded bean powder was significantly preferred over the commercial sample in flavor and overall liking, with means of 6.89 ($P=0.0003$), and 6.66 ($P=0.0002$), respectively. There were no significant differences with texture, aroma or

appearance. Thus, we attribute the significant difference in overall preference to the increased levels of green, grassy, musty and other oxidized odor-active compounds present at higher concentrations in the commercial sample (Table 3.2). Off-flavors in the bean powder will have more impact in a product where a large amount of the bean powder is used. Han and others (2010) performed acceptability tests on crackers made with pulse powders and found that Navy bean was unacceptable due to strong “beany flavor” reported by panelists (3.9 flavor liking on a 9-point hedonic scale, n=20). The 6.86 score using the 9-point hedonic scale in the current study illustrated that extruded Navy bean powder and the commercial Navy bean powder could be used to produce acceptable snack products.

The chicken nugget-analogs illustrated the impact that beany flavor can have on consumer acceptance in vegetarian products. Despite a significantly lower acceptance for texture ($P=0.0485$), the bean-less control was rated similarly in overall acceptance compared to both bean-substituted samples. The bean-less controls were less uniform and more rubbery. Both bean-substituted samples were rated significantly lower in flavor ($P=0.0231$). At this usage level (25% of dry weight), there was likely not enough bean powder present in the whole product for consumers to discern flavor differences between the two bean powders, but both appear to negatively impact flavor acceptability in this product. In general, substitution of grain powders with dry bean powder above the 25% level imparts negative flavor that significantly affects consumer acceptance (Dryer and others 1982; Dreher and Patek 1984; Gallegos-Infante and others 2010; Borsuk and others 2012) and this is corroborated by the current study.

In conclusion, extrusion can be used to produce bean powders with substantially different aroma chemistry from that of commercial bean powder. Beany flavor compounds from lipid peroxidation appear to have the potential to negatively impact product acceptance if used as a

major ingredient. Commercial bean powder used in this study shows evidence of more oxidation than extruded powders. Aldehydes and ketones derived from lipids are the major source of differences between commercial and extruded powder aroma chemistry. Significant differences in consumer flavor acceptance of bean powder products were observed in crackers with high percentages of bean powders. This work is a valuable initial step in understanding the importance of processing method and product type of bean powder quality and consumer acceptability. Future work should investigate solutions for limiting the development of off-flavors in bean powders to improve consumer acceptance and ultimately increase the consumption of healthful dry beans.

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Chapter 4: Conclusions, Study Limits and Recommendations for Future Work

4.1 Study Limits and Recommendations for Future Work

While the findings and conclusions that can be drawn from the data collected in the presented manuscript are an important first step in understanding the relationship between aroma compounds, beany flavor and consumer acceptability of bean products, there are still several key questions that this research fails to address. There are such a wide variety of factors that appear to influence the aroma profiles of beans and bean products that the direction of future work suggested by researchers investigating similar problems can vary widely. Similarly, the study presented in this manuscript has only approached this problem from a single direction, while excluding some factors which are potentially major contributors to the issue.

Sensory consumer acceptance testing can be used to address the acceptability of food products either alone or in relation to other food products, and some scaling methods can hint at the nature of the dislike. However, only a very limited set of information was collected during the sensory portion of the study due to constraints. Consumer acceptance studies, while providing very applicable information that can be directly related back to important factors such as product market viability, do not provide a wealth of information regarding the nature of acceptability. Future consumer studies related to bean product acceptance should leverage alternative scales to the 9-point hedonic, such as the just-about-right scale, to get more detailed information on which aspects of the products are performing poorly.

Descriptive sensory tests utilizing trained panels, humans who have been rigorously trained for “instrument-like” analysis of specific aspects of food, such as its color or texture, can provide information about the nature of the difference between foods. The current study does not

leverage descriptive analysis for several reasons. One major issue that prevented this type of experiment was identifying a form in which the bean powder could be reasonably assessed for differences in flavor without adding a host of new variables. Future work should consider in what form bean powder can be subject to descriptive analysis, and how the information will relate to the real end-product use. Bean powder is not consumed in its raw form, so a medium for analysis is necessary. For instance, if a “porridge” of bean powder were to be made with different bean powders for descriptive analysis, would the results be generalizable to all bean products? Still a novel product, bean powder is inherently subject to a significant amount of variation, whether it be from the cultivar of bean used or changes in the growing conditions for the year, where significant differences have been shown in many physical properties (Simons 2013).

Descriptive analysis has worked well in the past to evaluate cooked beans and identify beany flavor compounds, but no descriptive sensory has been performed on bean powder itself (Van Ruth and others 1995, Vara-Ubol and others 2004). The current study found that the differences in aroma compounds aligned with some changes in consumer preference, but without verifying that these changes were because of perceived changes in aroma and flavor, the researchers cannot conclude that the changes in aroma compound profile is the primary factor in determining acceptability of these products. GC-olfactory is generally not performed in tandem with consumer acceptance studies, as the two sets of data are extremely hard to correlate. However, by excluding acceptance data, differences in GC-O aroma profiles, while statistically different, may have no impact on consumer end-use perception, which is ultimately the primary factor that the food industry is interested in impacting. Future work should aim at a holistic

approach that uses descriptive, acceptance and analytical methods in tandem to clarify where the problem actually lies.

Furthermore, greater attention should be given to the demographics of the panel and its effect on hedonic evaluation of bean products. In the current study, the panelists were primarily white Americans (80%) who may not have been accustomed to the taste of bean products due to low consumption (Lucier and others 2000). Studies have shown that consumers of Asian origin are more likely to accept products with bean flavor, are less selective and no drivers of dislike could be identified in soymilk among the Asian population. This may be because bovine milk is less common in many regions in Asia. Beany off flavors were a major driver of dislike in white and African America clusters (Keast and Lau 2006, Lawrence 2015). While beany flavors in food has been cited by many researchers to be a barrier to consumption in both ordinary bean consumption and products containing bean ingredients for Americans, it is not clear if the adversity is due to lack of familiarity, association with products which have gone rancid, or simply different genetic sensitivities to bitter phytochemicals found in beans, such as saponins (Siddiq and others 2011) The latter example is another limitation to the study, as these phytochemical factors may have impacted overall liking and flavor scores, although there were very low numbers of comments collected from consumer acceptance tests related to bitterness and relatively high mentions of beany flavor.

While “aroma profiles” can often be estimated using quantitative GC-MS and odor thresholds (OAV), these methods have been widely shown to have problems with repeatability (Abbot and others 1993). GC-MS/Olfactory with detection frequency using an untrained panel offers more applicable and repeatable information on which to base conclusions about the overall aroma of a food. However, reliable methods in GC-O techniques are still evolving today. With

more time and resources, a trained panel may offer more repeatable results. Future work should investigate options for GC-O methods when analyzing bean aroma.

Perhaps the single biggest constraint to the presented manuscript is the limited amount of information regarding the commercially produced bean powder samples. Due to the proprietary nature of the samples, only basic information could be obtained regarding the processing conditions of these beans. Future work should investigate beans produced using commercial methods, potentially even at a larger scale, but without sourcing beans from commercial sources. Several variables, which have been shown to possibly impact aroma profiles of dry beans, were not possible to consider in this study, such as growing conditions (Simons 2013).

4.2 Conclusions

In conclusion, extrusion can be used to produce bean powders with substantially different aroma chemistry from that of commercial bean powder. These powders may be preferable to powders produced by typical commercial processes, as they exhibit significantly higher flavor and overall acceptance scores based on consumer sensory data. Beany flavor compounds from lipid peroxidation appear to have the potential to negatively impact product acceptance if used as a major ingredient. Commercial bean powder used in this study shows evidence of more oxidation than extruded powders. There is ample opportunity for lipid oxidation to occur during commercial process, but less during extrusion processing. Aldehydes and ketones derived from lipids are the major source of differences between commercial and extruded powder aroma chemistry. Significant differences in consumer flavor acceptance of bean powder products were observed in crackers with high percentages of bean powders (84.5% w/w). The high percentage of bean powder used likely increased the impact of beany off flavor in the product. This is a

likely explanation for the low flavor and overall acceptability scores, as texture and appearance were not greatly different between the two treatments. This work is a valuable initial step in understanding the importance of impact that processing method and product type on the quality and consumer acceptability of bean powder in end-use products.

APPENDIX



Figure A1. Bean Crackers Prepared with Extruded Bean Flour

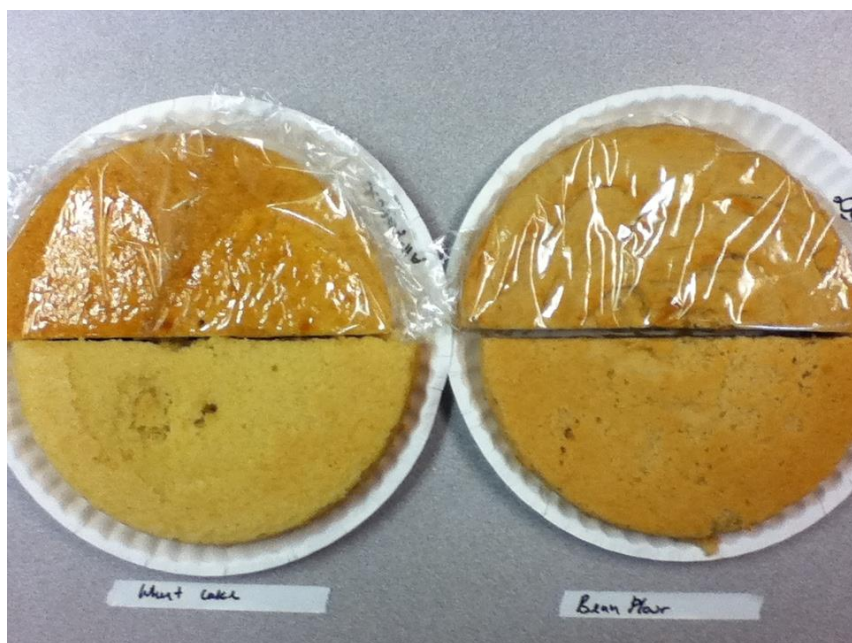


Figure A2. All Purpose Wheat Cake (left) and Bean Cake Prepared with Extruded Bean Flour (right)

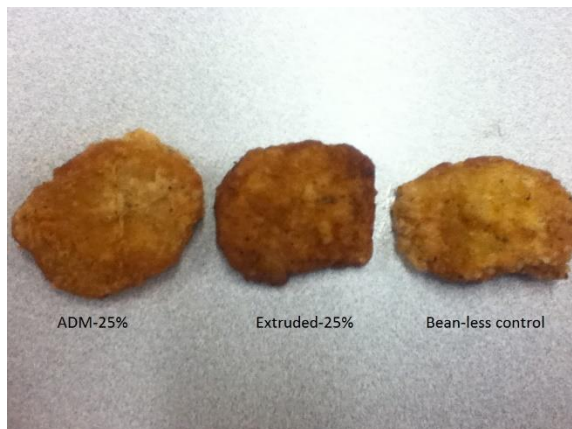


Figure A3. Chicken Nugget Analogs Prepared with Commercial Bean Powder (left), Extruded Bean Powder (middle) and No Bean Powder (right)



Figure A4. Bean Powder Extrudates

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REFERENCES

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