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Kemboi, Jacinta C. ***Utilization of Cranberry Seed Meal in the Development of a Diabetic Snack Product***

Abstract

By 2030, the World Health Organization (WHO) predicts diabetes to be the 7th leading cause of death. Although Type 2 diabetes accounts for 90% of all the diabetes in the world, it can be prevented through physical exercise and diet. The goal of this study was to develop an extruded snack product for diabetic individuals utilizing cranberry seed meal for its phenolic and dietary fiber content linked to the management of Type 2 diabetes. Cranberry seed meal is a byproduct from cranberry containing 24.4% protein and 50% dietary fiber. Accordingly 0, 24, 30 and 36% cranberry seed meal was included in the formulation along with millet, tapioca starch, corn meal that resulted in 4, 16, 19 and 22% total dietary fiber. Anthocyanins survival upon extrusion was a high of 18%. With the tart flavor of cranberry, sensory evaluation was conducted among 103 participants resulting in control followed by 24% cranberry seed meal samples most preferred on appearance, color, texture-crispiness, flavor, aftertaste, and overall acceptability. Statistical analyses revealed the existence of significant differences in various extrudate properties such as color, pH, moisture content, water activity, unit density, hardness, water solubility index, water absorption index, expansion ratio, and pellet durability index.

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Table of Contents

List of Tables

List of Figures

Chapter I: Introduction

"According to the American Diabetes Association (ADA), 18.8 million people in the United States have been diagnosed with diabetes, and another 7 million are thought to have the disease but have not yet been diagnosed" (Hilaire & Woods, 2013, p. 55). Diabetes is a disorder in which the level of blood glucose is persistently raised above the normal range (Oakley et al, 1978). There are two major types of diabetes, Type 1(destruction of B-cell leading to absolute insulin deficiency) and Type 2 diabetes (progression of insulin secretory defects with insulin resistance). Although Type 1 diabetes can occur at any age, "Type 2 diabetes is by far the most prevalent form in the United States, accounting for more than 90% of adult diabetes cases" (Hilaire & Woods, 2013, p. 55).

The ADA released new research in 2013 estimating the rise in the total cost of diagnosed diabetes from $ 174 billion in 2007 to $245 billion in 2012. This figure represents a 41 % increase over a five year period. The existing treatment options for individuals with diabetes are: weight loss is overweight, exercise, oral medication and /or insulin is needed, and education (Milchovich & Dunn-Long, 2003). In addition to the above treatment options, diet plays a huge part in controlling blood sugar. Recent studies have shown the beneficial relationship of cranberry in the treatment of diabetes due to the phenolic compounds found in cranberry, specifically anthocyanins (Apostolidis, Kwon, & Shetty, 2006; Lee, Chan, Lin, Lee, & Sheu, 2008; T Wilson, Meyers, Singh, Limburg, & Vorsa, 2008; Ted Wilson et al., 2010). Cranberries are one of the few fruits containing a large proportion of free phenolics (Mckay & Blumberg, 2007).

Native to Northern America, Cranberries have long been part of the American culture (Berlin, 2002). The United States produces 85 % of the world's total cranberry crop; the State of

Wisconsin produces 60 % of it (Association, 2013; Berlin, 2002; Mckay & Blumberg, 2007). Cranberry is a widely consumed fruit in the United States containing a wide range of phenolic phytochemicals, and has been historically associated with positive health benefits (Apostolidis et al., 2006). In a placebo-controlled study conducted by Chambers and Camire (2003), cranberry-supplemented subjects had significant lower insulin levels compared to the placebo group. Heeg and Lager (2004) developed a special process for the production of cranberry oils and cranberry seed flour that leaves the anythocyanins intact. Cranberry seed oil is the primary byproduct from seed oil production. In a study conducted by Parry et al. (2006), the total anthocyanin content value of 13.8mg/100g was found in cranberry seed flour. Cranberry seed meal is the secondary byproduct from seed oil production. Apart from the seed meal being ploughed back to land, utilized as a supplement or utilized by the cosmetic industry, the food industry has not found much use for it (M. Mueller, personal communication, April 18, 2013).

Due to the presence of anthocyanins in cranberry seed flour with health benefits associated with the management of diabetes, it is hypothesized that cranberry seed meal upon oil extraction using cold press process contains anthocyanins that can be utilized in the development of a diabetic product.

Statement of the Problem

Diabetes is a public health concern, mainly because it causes secondary complications such as cardiovascular disease; the number one killer in the United States according to Center for Disease Control (CDC). With diabetes being projected to increase from 14% to 33% by 2050 in adults, action needs to be taken to ensure the metabolic syndrome does not continue affecting the economic growth of the country (Caspersen, Thomas, Boseman, Beckles, & Albright, 2012).

With the expensive nature of the treatment of diabetes, less expensive solutions for the management of diabetes are desired.

Purpose of the Study

The purpose of this study was to identify the quality and quantity of the anthocyanins in cranberry seed meal, develop a diabetic extruded product based on the available components including dietary fiber and protein, analyze the chemical and physical properties of the end product and determine the consumer acceptability of the developed product through a sensory analysis.

The specific objectives of this study were as follows:

1. Quantify the anthocyanins present in cranberry seed meal.
2. Determine the percent survival of anthocyanins after extrusion process utilized in the development of the end product.
3. Determine the quantity of dietary fiber in cranberry seed meal and the end product.
4. Analyze the chemical and physical properties of the extruded product.
5. Determine the consumer acceptability of the developed snack product through a sensory analysis in regards to percentage inclusion of the cranberry seed meal in the product.

Assumptions of the Study

The only assumption in this study was that the responses submitted by the subjects were honest.

Definition of Terms

Type 1 diabetes. According to ADA (2013), Type 1 diabetes is as a result of the destruction of β-cells leading to absolute insulin deficiency. It is also known as insulin-dependent or juvenile diabetes.

Type 2 diabetes. According to ADA (2013), Type 2 diabetes is as a result of progression of insulin secretory defects with insulin resistance. It is also known as adult-onset diabetes which has been known to occur after age 40.

Cardiovascular disease. Disease of the heart that includes but not limited to hypertension, stroke, coronary heart disease and rheumatic heart disease (Basson, 2008).

Glycemic index. According to Merriam-Webster (2014), glycemic index is a measure of the rate at which an ingested food causes the level of glucose in the blood to rise.

Pytochemicals. According to Groff, Groupper and Smith (2005), phytochemicals are a large group of non-nutrient compounds derived from plants that contain disease-preventing capabilities.

Anthocyanins. According to Lee, Durst and Wrolstad (2005), anthocyanins are responsible for the red, purple, and blue hues present in fruits, vegetables, and grains.

Limitations of the Study

The only limitation in this study was that the cranberry seed meal utilized in the development of the cranberry puffs was organic in nature and may not be directly comparable with the nutritional analysis conducted on the convectional cranberry seed meal.

Chapter II: Literature Review

The literature review covers Type 2 diabetes definition and its symptoms, existing treatments and medications, diet and exercise and cost and the population affected by the disease. This chapter also looks at the history of cranberry, processing, antioxidant properties, phytochemical constituents available including anthocyanins, dietary fiber composition of the cranberry seed meal, and finally sensory analysis.

Type 2 Diabetes

The subsequent paragraphs covers the definition and symptoms, existing treatments and medication, diet and exercise, cost and the population affected by Type 2 diabetes.

Definition and symptoms. Type 2 diabetes, also known as diabetes mellitus, is as a result of progression of insulin secretory defects with insulin resistance (Hilaire & Woods, 2013). The insulin hormone produced in the pancreas by the beta cells control the glucose levels in blood. In an onset of Type 2 diabetes, the insulin produced no longer has the ability to regulate the blood glucose in the blood stream resulting to high blood glucose also known as hyperglycemia. Type 2 diabetes can occur in two different ways: when the body is not able to produce enough insulin and when the insulin being produced ends up not being recognized by the cells; referred to as insulin resistance. The main concern with individuals with Type 2 diabetes is the high blood glucose, which can lead to damage and failure of organs, including: the eyes, kidneys, nerves, heart, and blood vessels (Apostolidis et al., 2006). Type 2 diabetes has also been associated with cardiovascular diseases responsible for 75% of the deaths among people with Type 2 diabetes (Solano & Goldberg, 2005). Unusual thirst, frequent urination, fatigue; extreme tiredness, and persistent infections are some of the symptoms of hyperglycemia associated with Type 2 diabetes (Milchovich & Dunn-Long, 2003).

Treatment and medications. The oral glucose-lowering medications available for Type 2 diabetics address the two main defects of insulin hormone. The two defects are: insulin secretion and the action of insulin with nutritional implications (Janine, 2005). Five classes of oral diabetes medications available are: sulfonylureas, meglitinides, biguanides, thiazolidinediones (TZD), and alpa-glucosidase inhibitors. Biguanides and TZD address insulin resistance while meglitinides and sulfonylureas target insulin deficiency. Sulfonylureas enhance the secretion of insulin from the pancreas but are only effective when the pancreatic beta cells are available. A second generation of sulfobylureas (glyburide, glipizide, glipizide GITS, and glimepiride) are available and have an increased effectiveness in comparison to the first generation. Meglitinides (nateglinide and repaglinide) stimulate the rapid release of insulin from the pancreas following an increase of blood glucose after meals. Meglitinides are rendered inactive in the absence of pancreatic beta cells (Janine, 2005). Biguanides (metformin) decreases the production of glucose made from non-glucose sources in a process called gluconeogenesis (Oakley, Pyke, & Taylor, 1978). Another effect of metformin is to enhance the sensitiveness of insulin in the muscles. TZDs (rosiglitazone and pioglitazone) increase glucose syntheses by skeletal muscles reducing insulin resistance. Alpha-Glucosidase inhibitors (acarbose and miglitol) reduce the rate of digestion of starches and disaccharides lowering the blood glucose hence they are taken with meals (Janine, 2005). Type 2 diabetics take the above medications to help them manage their blood glucose and its effects on the damage of their organs.

Exercise and diet. Over the years, weight gain has been associated with Type 2 diabetes and hence the recommendation of life style changes from sedentary to an active lifestyle involving physical activity (Milchovich & Dunn-Long, 2003). Although all the available oral medications for Type 2 diabetics alter the absorption of glucose after meals or consumed before

meals, some food components like cranberries have been found to improve glucose management (Wilson et al., 2008; Ted Wilson et al., 2010). With prediction of an increase of 45% U.S., population diagnosed with diabetes by 2050, the cost of the management of the disease is also anticipated to increase (Yang et al., 2013). To reduce the cost associated with medication, the option of consuming cranberry products to manage Type 2 diabetes can be a cost effective option if research can be conducted with cranberry to understand its mechanism in the management of Type 2 diabetes.

Cost and population. According to the ADA, the total diagnosed cost of diabetes as of 2012 was $245 billion, 41 % increase in cost since 2007 with $176 billion in direct medical cost. Table 1 below represents the cost of indirect medical burden related to diabetes totaling $61 billion in reduced productivity. With 22.3 million people in the United States in 2012 estimated to be diagnosed with diabetes and half the number thought to have the disease, something needs to be done to reduce the number of new cases being diagnosed with the disease every year (Yang et al., 2013). In a study conducted by Boyle, Thompson, Gregg, Barker, and Williamson (2010), projected the rise in the percentage of the population diagnosed with diabetes in 2050 to one in three U.S. adults (Boyle, Thompson, Gregg, Barker, & Williamson, 2010; Yang et al., 2013). Table 1 below breaks down the indirect cost associated with diabetes in terms of productivity loss (reprinted from “Economic costs of diabetes in the U.S. in 2012”, Yang, Dall, Gallo, Kowal, and Hogan, 2013). Reduced performance at work is responsible for 30% of the proportion of indirect costs precided by reduced labor force participation due to disability with 31%. It is also estimated that diabetes is responsible for 1.8-7 % excess absenteeism from work (Yang et al., 2013). Reduced productivity as a result of diabetes affects the economy of the country as a result of the reduced number of people participating in the work force.

Table 1

The Cost of Indirect Medical Burden Related to Diabetes

Cost component	Productivity loss	Total cost attributable to diabetes ($)	Proportion of indirect costs*
Workdays absent	25 million days	5	7%
Reduced performance at work	113 million days	20.8	30%
Reduced productivity days for those not in labor force	20 million days	2.7	4%
Reduced labor force participation due to disability	130 million days	21.6	31%
Mortality	246,000 deaths	18.5	27%
Total		68.6	100%

Note. Indirect burden of diabetes in the U.S., 2013 (in billions of dollars) adapted from "Economic costs of diabetes in the U.S. in 2012" by W. Yang, T.Dall, P. Gallo, S. Kowal, and P. Hogan, 2013, American Diabetes Asociation, p.12. Copyright 2013 by the American Diabetes Association. Reprinted with permission.

Cranberry

The following paragraphs will explore the history of cranberry, how it is processed, its antioxidant properties, phytochemical constituents of cranberry including anthocyanins, and available dietary fiber content in cranberry.

History. Cranberries are native to northeastern part of North America, including the states of Wisconsin, Massachusetts, New Jersey, Oregon, and Washington (Berlin, 2002). Since

1900s, Wisconsin has been the leading producer of cranberries in the U.S. The American cranberry (Vaccinium macrocarpon), is a low-growing, trailing, vining, woody evergreen vine requiring acidic soil and large quantities of water (Berlin, 2002; Mckay & Blumberg, 2007; Vine, 2007). Commercial growing of cranberries in Wisconsin began in 1860 (Vine, 2007). In 2013, Wisconsin was estimated to produce 4.9 million barrels of cranberries representing 60 percent of all the cranberry produced in the U.S. (Association, 2013). In 2004, Wisconsin declared cranberry as its official state fruit. In addition to 95% of the cranberries being utilized in juice production and other food products, they are ingredients in more than 1000 food products and beverages (Association, 2013; Mckay & Blumberg, 2007).

Processing of cranberries. Thermal processing, also referred to as hot press in the juice industry, is the use of heat to kill microorganisms; the most common process utilized in cranberry juice extraction. In the cranberry industry, 95% of the products produced from cranberry are: juices, sauces, dried fruit and ingredients, which include cranberry seed meal. The remaining 5% are in the form of fresh fruit. One truck load of cranberry seeds produces 5 gallons of oil, hence the high cost of cranberry seed oil (Cunningham et al., 2004). Cold press process is an extraction technique used in cranberry seed oil extraction; a patent process developed by Heeg and Lager (2004). The cold press process extracts oil from cranberry seeds using temperature not exceeding 100oF. Once the cranberry seed oil is extracted, cranberry seed meal, secondary byproduct is left as demonstrated in figure 1 below (Heeg and Lager, 2004).

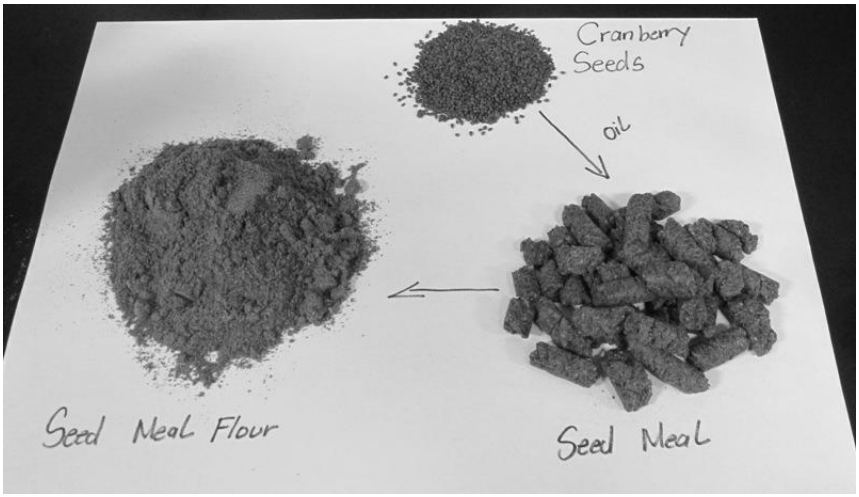

Figure 1. Cranberry seed meal demonstration.

Antioxidant properties of cranberry. According to Halliwell (2001), antioxidants react with chain carriers such as free radicals terminating the oxidative chain reaction (initiation, propagation, and termination). In two studies that analyzed phenolic content in several fruits, both concluded that cranberry fruit had the highest phenolic content (507-709 mg gallic acids equivalents/100g) with the ability to serve as an antioxidant (Mckay & Blumberg, 2007; Sun, Chu, Wu, & Liu, 2002). Due to the association of cardiovascular diseases with Type 2 diabetics, and the relationship of antioxidants in the management of lipid oxidation in cardiovascular diseases, cranberry diets can reduce the risk of cardiovascular diseases (Apostolidis et al., 2006; Chambers, 2002).

Phytochemical constituents of cranberry. The phytochemical profile in cranberry includes flavonoids, catechins, and phenolic acids (Neto, 2007). In cranberry, the two major classes identified are phenolic acids and flavonoids (anthocyanins (cyanidin, peonidin, and resveratrol), and flavonols (quercetin, myricetin, proanthocyanidins, and epicatechin))(Mckay & Blumberg, 2007; Neto, 2007). The six major anthocyanins in cranberries are cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside, peonidin-3-galactoside, peonidin-3-

arabinoside, and peonidin-3-glucoside (Mckay & Blumberg, 2007). Table 2 below shows the content of phenolic compounds in cranberry juice cocktail.

Table 2

The Content of Phenolic Compounds in Cranberry Juice Cocktail

Phenolic compound	**Content (mg/8 Oz serving)**
Proanthocyanidins	90.00
Total phenolics	211.00
Total anthocyanins	1.23
Anthocyanins	
Cyanidin-3-galactoside	0.28
Cyanidin-3-glucoside	0.00
Cyanidin-3-arabinoside	0.13
Peonidin-3-galactoside	0.58
Peonidin-3-gucoside	0.03
Peonidin-3-arabinoside	0.20
Flavonols	
Hyperoside	5.57
Quercetin	3.12
Myricetin	1.27
Quercitrin	1.25
Avicularin	0.43
Phenolic acids	
Benzoic acid	12.00
Chlorogenic acid	2.64
4-Hydroxycinnamic acid	1.06
3,4-Dihydroxybenzoic acid	0.55
Vanillic acid	0.29
Caffeic acid	0.26

Note. Adapted from "Cranberries (Vaccinium macrocarpon) and cardiovascular disease risk factors" by D. Mckay, and J. Blumberg, 2007, Nutrition Reviews, p. 493. Copyright 2007 by International Life Sciences Institute. Reprinted with permission.

Anthocyanins. Galactosides and arabinosides of cyanidin and peonidin are the major anthocyanins present in cranberry (Neto, 2007). Although glucosides of cyanidin and peonidin

are found in cranberry cocktail juice in traces of 0.003 and 0.034 mg/8 Oz respectively as shown in table 2, little is known on its availability in cranberry seed meal after oil extraction. One possible way of managing Type 2 diabetes is inhibiting α-glucosidase, an enzyme found in the small intestine that catalyses the final step of glucose absorption in the small intestine (Apostolidis et al., 2006). Recent studies have shown that phenolic compounds such as anthocyanins can play a role in α-amylase inhibition contributing to the management of diabetes (Matsui et al., 2001; McCue & Shetty, 2004).

Dietary fiber. The recommended daily intake for dietary fiber is 21-38 g in adults, specifically 30-38 g in males and 21-26 g in females (Dietary reference intakes: Macronutrients, n.d.). Dietary fiber exists in two forms as soluble and insoluble. Dietary fiber composed of soluble and insoluble fiber has been associated with the management of Type 2 diabetes through the lowering of glucose in the blood during digestion of carbohydrates (Schulze et al., 2004). The pomace left after oil extraction from the cranberry seeds is very high in fiber, but the specific fiber analysis is not known.

Available Snack Products

Individuals with diabetes are directed to closely watch their simple sugar intakes to control their glycemic index; consumption of dietary fiber has been linked to less blood glucose spikes (Ohr, 2015). In a study conducted by Schulze et al. (2004), lower risk of diabetes was associated with high intakes of fiber. Snack products with high fiber content, specifically extruded snack products are none existent. An example of a common extruded snack product is Cheetos by Frito Lay® which contains dietary fiber <1g per serving size (28 grams). With the rising snacking habits associated with busy life schedules, resulting to less time for lunch, more people are snacking at work (Gill, 2013). More snacks with high fiber content need to be

developed to address the increasing snacking habits and predicted increase in 45% of the U.S. population diagnosed with diabetes by 2050.

Extrusion

According to Harper (1989), extrusion is a continuous rapid process that combines several operations like: mixing, cooking, shearing, puffing, shaping and drying to produce a starchy food product. Some of the food products manufactured through extrusion are: snacks, confectioneries, extruded crisp breads and ready to eat cereals (Suknark, Philips, & Chinnan, 1997; Yagci & Gogus, 2008).

Sensory Analysis

Sensory analysis is a very important part of developing a new food product; it is mainly because one can develop a product, but if no one likes it enough to buy it then the product will not be successful in the market place. Sensory analysis can be conducted by trained and untrained panelists. Sensory analysis utilizes smell, taste and touch human senses in evaluating different attributes of a product by utilizing a 9-point hedonic like scale (1= dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5= neither like nor dislike, 6= like slightly, 7=like moderately, 8=like very much, and 9= like extremely). According to Moskowitz (1997), 40-50 panelists are sufficient when conducting a sensory acceptability test.

Hypothesis

The study hypothesized that the cranberry seed meal after oil extraction from cold press process had anthocyanins and dietary fiber specifically soluble fiber that would be utilized in the development of a diabetic product.

Chapter III: Methodology

The purpose of this research study was to develop and determine consumer acceptability of an extruded diabetic snack product that utilized cranberry seed meal. The research was conducted in five phases: analyzed anthocyanins and dietary fiber in the cranberry seed meal, developed an extruded snack product utilizing cranberry seed meal as the main ingredient, analyzed the nutritionals of the blends and extrudates, analyzed the physical and chemical properties of the extrudates and conducted a sensory analysis that determined the consumer acceptability of the snack product.

Cranberry Seed Meal Analysis

To determine the cranberry seed meal composition before utilizing the meal in a product, nutritional analysis including dietary fiber and anthocyanins was conducted.

Anthocyanins. To determine the concentration of athocyanins available in the cranberry seed meal, a method using high-performance liquid chromatograph (HPLC) was followed in the method described by Brown and Shipley (2011).

Reference standards of five anthocyanins were obtained from Cerilliant Analytical Reference Standards (Round Rock, TX). The five standards were cyanidin 3-glucoside (C3Gl), cyanidin 3-galactoside (C3Ga), cyanidin 3-arabinoside (C3Ar), peonidin 3-galactoside (P3Ga), peonidin 3-glucoside (P3GI). Cyanidin reference standards were all solids while the peonidin standards came in a 500 µg/mL solution. The purchased reference compounds that were solids were used to prepare analytical standards in extraction solvent (33%methanol/2% hydrochloric acid in Milli-Q water). Sub-stocks were prepared from the analytical standards and the sub-stocks were used to create working standards that contained all five anthocyanins. Sub-stocks were analyzed individually to determine the retention time of each of the compounds. The five

working standards were prepared with concentrations of the anthocyanins in the following ranges: C3Gl, 0.72-46.3 mg/L; C3Ga, 0.77-49.0 mg/L; C3Ar, 0.71-45.6 mg/L; P3Ga, 0.65-41.7 mg/L and P3GI, 0.59-37.3 mg/L. Sub-stocks and working standards were prepared in extraction solvent. The working standards were used to prepare analytical calibration curves for each of the four anthocyanins. Upon receipt and when not in use, the reference standards and all standards prepared from them were stored in a freezer.

Analysis was conducted utilizing an Agilent Model 1290 Infinity Ultra High Performance Liquid Chromatograph (UHPLC, Santa Clara, CA) system at the University of Wisconsin-superior. A Phenomenex Kinetex 2.6µ XB-C18 analytical column (100 x 3.0 mm, Santa Clara, CA) was used to perform the separation of compounds present in the standards and sample extracts. Peaks were detected on the diode array detector at a wavelength of 520 nm utilizing a bandpass of 8 nm. Analysis was conducted using gradient elution employing two eluents. Eluent A was 99.5% Milli-Q water/0.5% phosphoric acid (Alfa Aesar, ACS, 85%). Eluent B was 50% Milli-Q water, 48.5% acetonitrile (B&J, HPLC grade), 1.0% glacial acetic acid (Alfa Aesar, ACS, 99.7%) and 0.5% phosphoric acid (Alfa Aesar, ACS, 85%). The gradient program with a mobile phase flow rate of 0.70 mL was: 1.0 min. 90%A/10%B; 13.0 min. 72%A/28%B; 17.0 min. 25%A/75%B; 17.1 min. 90%A/10%B; 20.0 min. 90%A/10%B. A 2.0 minute post-time was also employed. Ten microliter injections of all standards and samples were made.

Samples were prepared for analysis by carrying them through an extraction procedure to remove the anthocyanins from the solid samples. Each sample was carried through the entire extraction and analysis procedure in triplicate. The extraction solvent that was used was 33% methanol (B&J, HPLC grade)/2% hydrochloric acid (Fisher Scientific, Cert. ACS Plus) in Milli-Q water. Approximately 0.25 g samples were weighed into 85 mL polycarbonate centrifuge

tubes and 20 mL of extraction solvent was added to each tube. The samples were mixed on a vortex mixer for 10 seconds followed by sonication for 15 minutes in an ultrasonic bath. Samples were then shaken at 180 rpm for 30 minutes on a platform shaker. Samples were again mixed on the vortex mixer for ten seconds and then centrifuged at 5000 rpm for five minutes at 23°C. After the centrifugation, the samples were carefully decanted into 25 mL volumetric flasks and diluted to volume with extraction solvent. During the decantation, a small amount of the solids were unintentionally transferred to the volumetric flasks. The volumetric flasks were inverted a minimum of ten times were vigorous shaking to ensure the samples were homogeneous. Approximately 1.0 mL of each sample extract was filtered through a 0.45µ nylon syringe filter into an amber autosampler vial. Samples were either immediately placed in the UHPLC autosampler for analysis or stored in a freezer until analysis was conducted. Several standards and one sample were analyzed repeatedly over a several week period to determine the stability of standards and samples stored in a freezer. The results of these analyses indicated that the standards and sample extracts were stable over the period of time that they were analyzed.

Nutritional analysis. Nutritional analysis of the cranberry seed meal was conducted and reported by Commercial Testing Laboratory in Colfax, WI. The nutrients reported were analyzed using carbohydrates (calculated from protein (AOAC reference method 968.06), fat (AOAC reference method 966.06), ash (AOAC reference method 923.03), and moisture (Vacuum oven-AOAC reference method 925.09)), dietary fiber (gravimetric-AOAC reference method 991.43), sugars (HPLC-AOAC reference method 977.20), vitamin A (AOAC reference method 2001.13), Vitamin C (AOAC reference method 967.22), and iron and calcium utilizing AOAC reference method 985.01.

Dietary fiber. The soluble and insoluble fiber analyses in the dietary fiber was conducted and reported by Eurofins Scientific Laboratory in Des Moines, IA and Medallion Labs in Minneapolis, MN following AOAC reference method 991.43.

Development of a Snack Extruded Product

An extruded product was developed using cranberry seed meal as the main ingredient followed by proso millet to increase the protein content in the puffs, tapioca starch, corn meal and salt. The cranberry seed meal utilized in the development of the extruded snack product was purchased from Botanical Innovations Inc, Spooner,WI. The millet used was purchased from St. Charles Trading, Inc, Batavia, IL. The tapioca starch, corn meal and salt ingredients used were provided by Grain Processing Corporation, Muscatine, IA. Anthocyanins present in the extruded snack product were analyzed using UHPLC Agilent Model 1290 was used to determine their survival upon extrusion.

Extrusion process. Extrusion was performed with a co-rotating twin-screw extruder (Wenger TX57, Sabetha, KS) at a pilot plant in Muscatine, IA though Grain Processing Corporation. The extruder had a width of 57 mm with a die diameter of 4 mm and a length/diameter ratio of 25:1. The initial moisture of the blends was 12%. With 30% motor load, the blends were extruded at a feed screw speed of 12 rpm and a shaft speed of 300 rpm with a barrel temperature profile of 30, 57, 90, 124 and121oC and a knife speed of 431 rpm. The extrudates were then dried under twin dryers (Wenger Manufacturing Co., Sabetha, KS) set at 87 and 115oC and cooled. Once cooled the samples were packaged in sealed polyethylene bags into a secondary cardboard barrel and stored in a cool dry place ready for sensory analysis.

Nutritional analysis. Nutritional analyses of the extrusion blends and extrudates were conducted and reported by Commercial Testing Laboratory in Colfax, WI. The dietary fiber

analysis were conducted and reported by Medallion Labs in Minneapolis, MN following AOAC reference method 991.43.

Chemical and Physical Properties of the Extrudates

Properties of the extrudates analyzed were: color, pH, moisture content, water activity, expansion ratio, unit density, water absorption index, water solubility index, and pellet durability index. Below were the steps followed to analyze and quantify the properties:

Color. Color was measured using HunterLab ColorFlex EZ color meter (Reston, VA) by filling the sample cup with sample to 45mm mark and obtaining L*, a*, b* values in triplicates.

pH. pH reading was obtained using Fisher Scientific ABIS plus pH meter (Singapore) upon performing a 2-point calibration of pH buffers 4 and 7. Two grams of each sample in a five ounce plastic cup was dissolved in 20ml distilled water and a pH reading obtained in duplicate.

Moisture content. Moisture content of the extrudates was determine by drying the samples in an oven set at 105oC overnight for 16 hrs and percent calculated through weight reduction.

Water activity (aW). Aqua Lab water activity meter 4TE DUO (Washington, DC) was utilized to obtained the water activity of the ground extrudate samples. 0.500 aW standard for powders was utilized to standardize the meter with a reading of 0.499 within ±0.003 water activity. The water activity readings were measured in duplicates.

Expansion ratio (ER). Expansion ratios of the cylindrical extrudate samples were obtained using a vernier caliper in micrometer (Tokyo, Japan) to measure the diameter. Ten pieces of the extrudates were measured and averaged following the formula below:

ER = Diameter of the extrudate (mm)

Diameter of the die (mm)

Unit density. The actual dimensions of the extrudates were measured to calculate the unit density (Asare, Sefa-Dedeh, Sakyi-Dawson, & Afoakwa, 2004; Thymi, Krokida, Papa, & Maroulis, 2005; Yagci & Gogus, 2008).Vernier caliper was used to measure the diameter and the length of the extrudates. Assuming a cylindrical shape of the extrudates, the unit density was calculated using the formula below:

$\rho_b = 4/\pi d^2 l$

Where ρ_b = unit density (g/cm3)

d = diameter of the extrudate (cm)

l = length per gram of the extrudate (cm/g)

Five pieces of extrudates were randomly selected and an average obtained on diameter and length per gram.

Hardness. Hardness of the extrudates was determined by using a TA-XT2i Texture Analyzer (Surrey, UK). To measure the compression force required for samples breakage, the compression probe with aluminium cylinder and 100 mm diameter was used per ten repetitions at a rate of 100mm/min and mean average obtained.

Water absorption index (WAI). Water absorption index of the extrudates was determined by utilizing a method outlined by Anderson, Conway, Pfeifer, and Griffin (1969). Two and half grams of the ground sample was suspended into 30ml distilled water at room temperature (21-23oC) in a 50 ml tarred centrifuge tube in duplicate. The contents were stirred every 5 minutes over 30 minutes period and centrifuged at 3000x g for 10 minutes using Thermo IEC CENTRA CL2 centrifuge (Porton Down, UK). The supernatant liquid was poured off into a tarred evaporating dish and the remaining sediment weighed and WAI obtained using the formula below:

WAI (g/g) = Weight of sediment

Weight of dry solids

Water solubility index (WSI). Water solubility index of the extrudates was determined from the water absorption index test described above (Anderson, Conway, Pfeifer, & Griffin, 1969) from the amount the dried solids recovered by evaporating the supernatant in an evaporating dish in an oven overnight set at 1050C for 16 hrs. WSI was obtained using the formula below:

WSI (%) =Weight of dissolved solid in supernatant x 100

Weight of dry solids

Pellet durability index (PDI). Seedboro's pellet durability tester (Chicago, IL) was utilized to predict the amount of fines that would exist in the extrudates upon reaching the consumer after transportation. This test was developed by Kansas State University as a standard measure of quality referred to as the pellet durability index. To prepare the sample hand sieve was used to separate broken extrudates. 500 grams of the screened extrudates were weighed, placed in the three compartments of the tester and tumbled for 10 minutes. Extrudates were retrieved from the tester compartment rescreened using sieve number 7 and weighed. PDI was computed using the formula below:

PDI = Weight of rescreened sample (g) x 100

Weight of sample (500g)

Data analysis. Statistical Package for Social Science (SPSS) Statistics 22 software was used to interpret all the data obtained.

Sensory Analysis

In order to determine the consumer acceptability of the developed product, subject selection and description was determined including how the sample presentation.

Subjects selection and description. An email to the University of Wisconsin-Stout students, staff and faculty was circulated with an advertisement (Appendix A) and some copies posted on bulletin boards around campus seeking participants for the sensory study of the extruded snack product that was conducted in the sensory lab room 252 located in Heritage Hall. The study had 103 participants who participated in the sensory study.

Samples. 5-10 bits of each sample of the cranberry puffs were placed into a three digit labeled cup on a tray and provided to each participant with water for evaluation as demonstrated in figure 2 below.

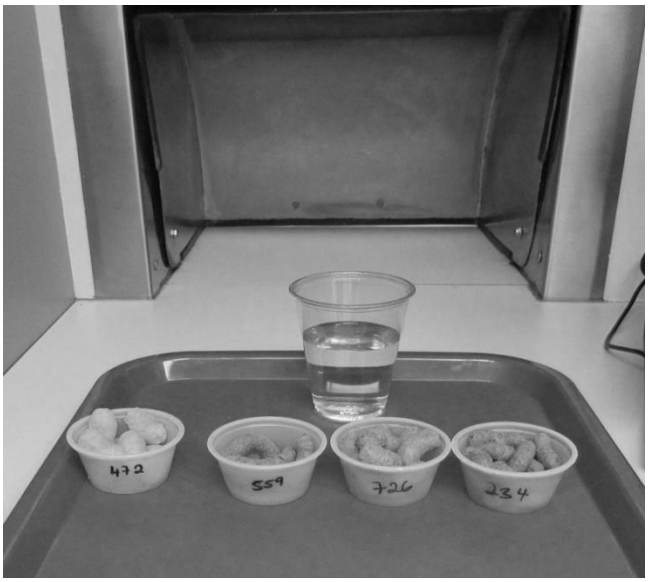

Figure 2. Sensory evaluation sample presentation.

Data Collection Procedures

Permission to conduct the sensory research study was obtained from the University of Wisconsin-Stout Institutional Review Board (Appendix B) before the study was conducted. All

participants were required to sign a consent form (Appendix C) prior to participating in the study. Interested participants were asked to taste and evaluate four samples of extrudates provided to them on appearance, color, texture-crispiness, flavor, tartness, bitterness, aftertaste, and overall acceptability. To determine the influence the amount of protein and fiber in the product had on the consumers' buying power, a survey question was included in the questionnaire. To determine the consumers' preference on flavors to be applied on the cranberry puffs, a poll was conducted on nine possible flavors(Cheddar, Roasted garlic and chilli, Jalapeno, Chipotle, Salt and Pepper, Barbeque, Sweet & Spicy, Cinnamon & Sugar, and Onion & Sour cream).The participants responses to the twenty four questions questionnaire (Appendix D) provided to them during the study was collected using Compusense® computer software when each participant signed into the provided sensory lab computers.

Data analysis. Compusense® Five; a sensory study software was utilized to collect and analyze all the data obtained from the sensory study (Fidlay, 1986).

Chapter IV: Results and Discussion

Nutritional components including dietary fiber, and anthocyanins analyses of the cranberry seed meal were initially determined to maximize its use as an ingredient in the extrusion formulation for a diabetic snack product. With the tart nature of the cranberry fruit, different percentages of cranberry seed meal were included in the formulation to maximize the berry's benefits as well as determine its consumer acceptability through sensory analysis. Accordingly cranberry seed meal (0, 24, 30, and 36%) was included in the formulation along with proso millet, tapioca, corn meal and salt and resulted in 4, 16, 19, and 22% total dietary fiber in 100 g of cranberry puffs. Nutritional components including dietary fiber in the final product were compared across the different cranberry seed meal concentration. Anthocyanins percent survival was also determined in the end product upon extrusion. Physical and chemical properties of the end product were analyzed and significant differences $p \leq 0.005$ found among the different cranberry seed meal concentrations.

Cranberry Seed Meal Preliminary Analysis

Below are discussions on anthocyanins, nutritional analysis including dietary fiber present in cranberry seed meal.

Anthocyanins analysis. Galactosides and arabinosides of cyanidin and peonidin being the major anthocyanins were analyzed along with glucosides in cyanidin and peonidin to determine their availability in cranberry seed meal according to Neto (2007). Table 3 below represents anthocyanins content in cranberry seed meal expressed as the mean and standard deviations of triplicate analysis.

Table 3

Anthocyanins Content in Cranberry Seed Meal and Cranberry Juice Cocktail in mg/ 8 Oz

Anthocyanins	**Concentration (mg/L) Seed Meal**	**Concentration (mg/8 Oz) Seed Meal**	**Concentration (mg/8 Oz) Juice Cocktail (Mckay & Blumberg, 2007)**
Cyanidin- 3-galactoside	1.85 ± 0.04	0.44	0.28
Cyanidin-3-arabinoside	2.68 ± 0.03	0.63	0.00
Cyanidin- 3- glucoside	0.20 ± 0.00	0.05	0.13
Peonidin- 3- galactoside	1.82 ± 0.02	0.43	0.58
Peonidin- 3- glucoside	0.26 ± 0.00	0.06	0.03

The average quantities of the six main anthocyanins (cyanidin-3-galactoside, cyanidin-3arabinoside, cyanidin-3-glucoside, peonidin-3-galatoside, peonidin-3-arabinoside, and peonidin-3-glucoside) in cranberry juice cocktails was reported by Mckay and Blumberg (2007) in mg/8 Oz. as 0.28, 0.003, 0.13, 0.58, 0.034, and 0.20 respectively in mg/8 Oz. serving. In comparison to anthocyanins concentration in the juice cocktail, some of the results from the cranberry seed meal analyzed in this study are considerably higher such as cyanidin-3-galactoside, cyaniding-3-arabinoside and peonidin-3-glucoside by 63, 100, and 50% respectively; more research needs to be done to confirm these findings. Similarly, glucosides of cyanidin and peonidin in the cranberry seed meal as shown in table 3 was found to be 0.05 and

0.06 mg/ 8 Oz considerably higher than 0.03 for peonidin, but lower than 0.13 mg/8 Oz cyanidin in the juice cocktail as shown in table 3.

Nutritional analysis. The nutritional analysis show cranberry seed meal with high protein (24.4%) and fiber (49.2%) contents.

Table 4

Nutrient Content in Cranberry Seed Meal

Nutrient	**Results**
Energy(Calories/100g)	399
Moisture (%)	6.71
Protein (%)	24.4
Carbohydrates (%)	58.4
Fat (%)	7.50
Dietary Fiber (%)	49.2
Sodium (mg/100g)	3.29
Iron (mg/100g)	9.37
Vitamin A (IU/100g	55
Vitamin C (mg/100g)	0.8

Dietary fiber analysis. The table below reports the dietary fiber content in cranberry seed meal; percent soluble an insoluble.

Table 5

Dietary Fiber Content in Cranberry Seed Meal

Nutrient	**Results (%)**
Insoluble Fiber	43.2
Soluble Fiber	6.8
Total Dietary Fiber	50.0

Cranberry Puff Blends and Extrudates Analysis

The following paragraphs report the effect of extrusion on anthocyanins, nutritional analysis including dietary fiber in the cranberry puff blends and extrudates.

Anthocyanins before and after extrusion. Percent survival and loss of the anthocyanins (figure 3 and 4) in the blends upon extrusion was computed from the results of the anthocyanins analyzed in the blends and extrudates as shown in table 6 below.

Table 6

Anthocyanins present in Blends and Extrudates upon Extrusion (mg/L)

% Cranberry Meal	C-3-Ga	C-3-Ar	C-3-GI	P-3-Ga	P-3-GI
24-Before	1.42	1.40	ND	3.05	ND
24-After	0.26	0.23	ND	0.53	ND
30-Before	1.34	1.09	ND	2.60	0.09
30-After	0.17	0.00	ND	0.32	0.00
36-Before	1.69	1.48	ND	3.43	0.14
36-After	0.23	0.20	ND	0.48	0.00

Note. ND= none detected, Before=Blend; After=Extrudate.

C-3-GI was not detected at 24, 30, and 36% while P-3-GI was not detected at 24% cranberry seed meal. To determine significant differences ($p \leq 0.05$) of anthocyanins before and after extrusion, paired t-test was conducted as demonstrated on table 7 below. Significant difference was found among C-3-Ga, C-3-Ar, and P-3-Ga anthocyanins upon extrusion. Except the none detected anthocyanins, an increase in % cranberry seed meal resulted into an increase in the anthocyanins before extrusion.

Table 7

Paired Sample t-test of Anthocyanins Before and After Extrusion

Anthocyanins	**t**	**Df**	**Sig(2-tailed) p-value**
C3Ga	12.842	2	0.006*
C3Ar	21.425	2	0.002*
P3Ga	13.181	2	0.006*
P3GI	1.872	2	0.202

Note. *p<0.05 is significant.

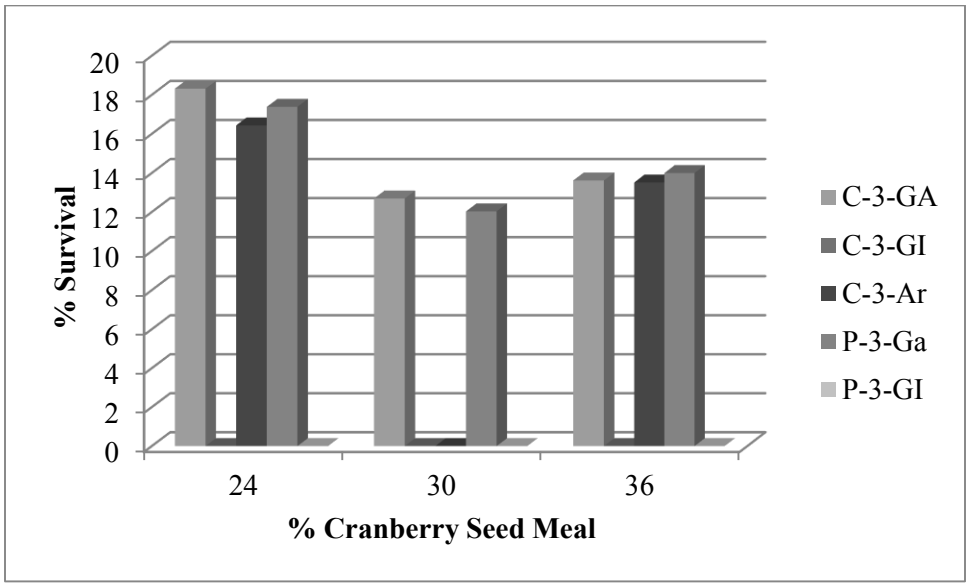

Figure 3. Anthocyanins % survival upon extrusion.

Different studies (Drdak & Daucik, 1990; Patras, Brunton, O'Donnell, & Towari, 2010; Rhim, 2002) have reported anthocyanin destruction increase with increase in temperature. Although this study only varied the concentration of cranberry seed meal, the extrusion process temperature ranged from 30 to 121^{o}C. In a study that looked at polyphenolic composition in extruded cranberry pomace, similar results to seen above in figure 3 and 4 were reported of

anthocyanins having a high survival and lower loss with the least (12%) cranberry seed meal composition (White, Howard, & Ronald, 2010).

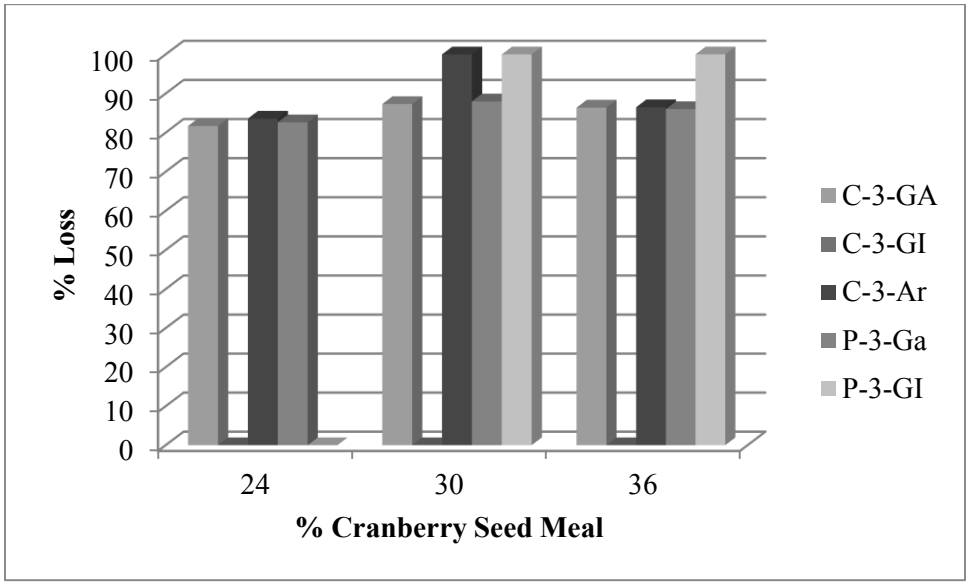

Figure 4. Anthocyanins % loss upon extrusion.

Nutritional analysis before and after extrusion. The figure below demonstrates the effect of extrusion on nutritional components.

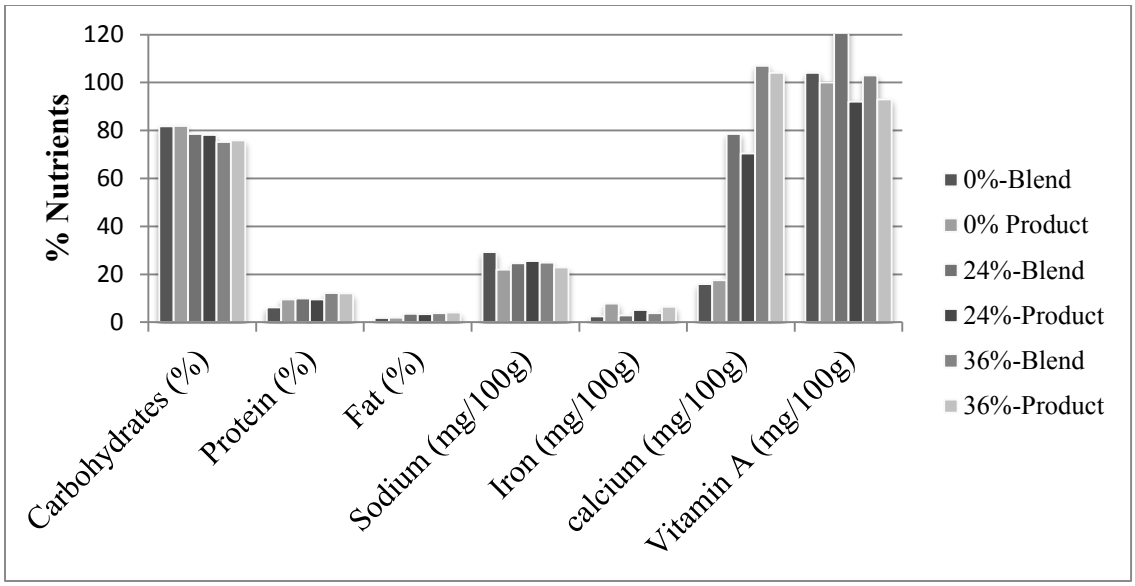

Figure 5. Nutritional results of blends and extrudates with indicated cranberry seed meal concentration.

With an increase in the percent cranberry seed meal in the formulation, a decrease in % carbohydrates together with an increase in protein and fat as well as an increase in iron and calcium (mg/100g) was observed as demonstrated in figure 5. A decrease in carbohydrates is observed with % increase of cranberry seed meal mainly because as cranberry seed meal is increased, corn meal is also reduced as a result of formulation adjustment. Increase in fat and protein with increase in cranberry seed meal can also be attributed to the fat content (7.50%) and protein content (24.4%) in cranberry seed meal. Increase in iron upon extrusion can be speculated to be from the extruder during the extrusion process. Increase in sodium content upon extrusion can be speculated to be from the other ingredients mainly because an equal amount of sodium (0.05%) was added to each treatment.

Dietary fiber analysis before and after extrusion. The figure below demonstrates the effect of extrusion on dietary fiber.

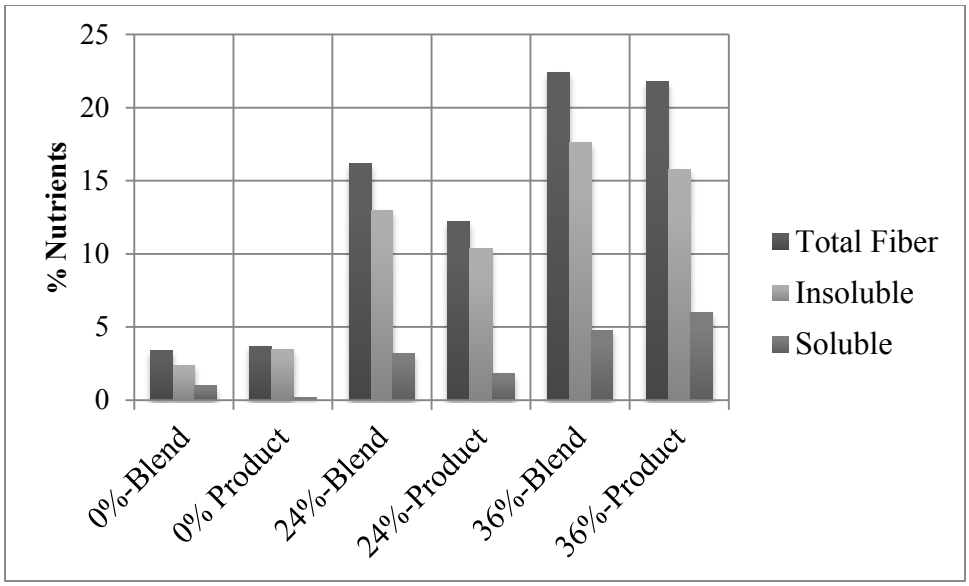

Figure 6. Effect of extrusion on dietary fiber content at indicated cranberry seed meal concentration.

An increase in % cranberry seed meal results into an increase in dietary fiber as demonstrated in figure 6.

Chemical and Physical Analysis of the Extrudates

Below are figures representing the results of the chemical and physical analysis of the extrudates. Significant differences ($p<0.05$) are noted among the extrudates with different concentration of cranberry seed meal in the various chemical and physical properties analyzed using One-way ANOVA.

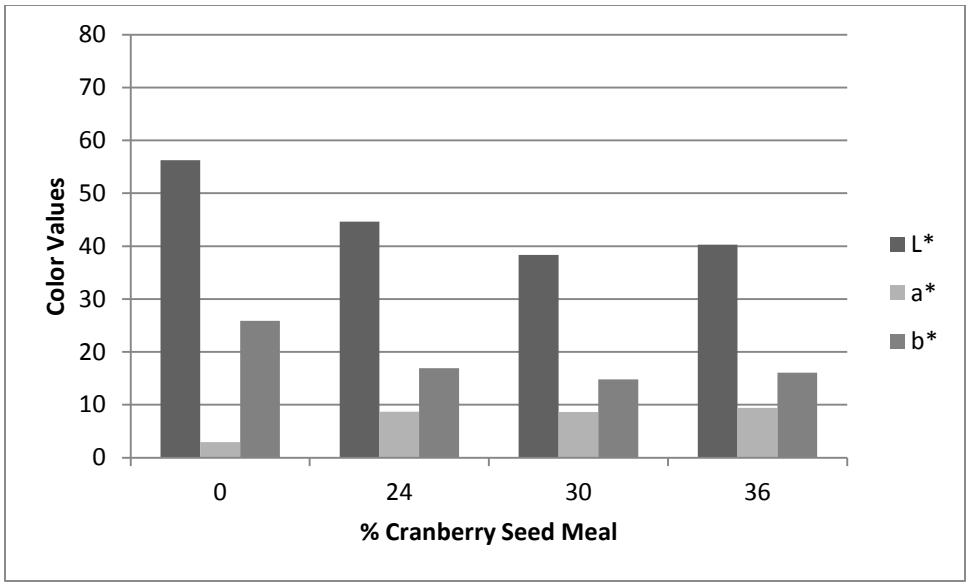

Figure 7. Color values for the extrudates with cranberry seed meal.

There was significant differences ($p \leq 0.05$) in L*, a* and b* values among the different cranberry seed meal concentration except a* value between 24 and 30% using post hoc test of comparison. In addition to the L*, a*, b* values varying with an increase in cranberry seed meal, maillard reactions and protein denaturing has been attributed to color alterations in cooking at high temperatures (Bjorck, Noguchi, Asp, Cheftel, & Dahlqvist, 1983; Rosentrater, 2006).

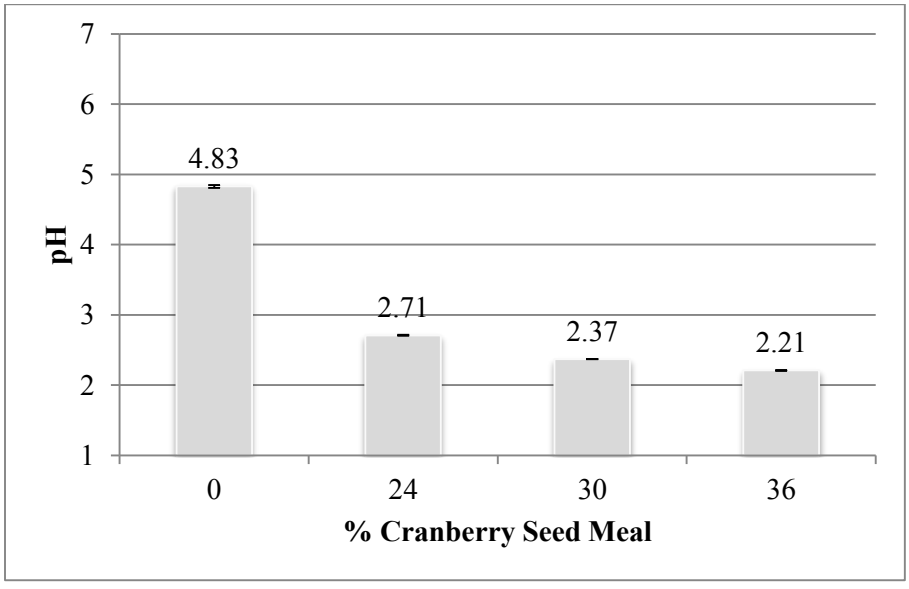

Figure 8. pH of extrudates with cranberry seed meal.

A pH decrease is observed with an increase in the concentration of cranberry seed meal. pH of the cranberry seed meal was found to be 3.96. A pH reduction below 3.96 can be attributed to its interaction with the other ingredients like millet, tapioca and corn meal in the formulation, especially since their initial pH was not determined. pH determination is important because it can be useful in determining the shelf life of the product when microbial growth is concerned.

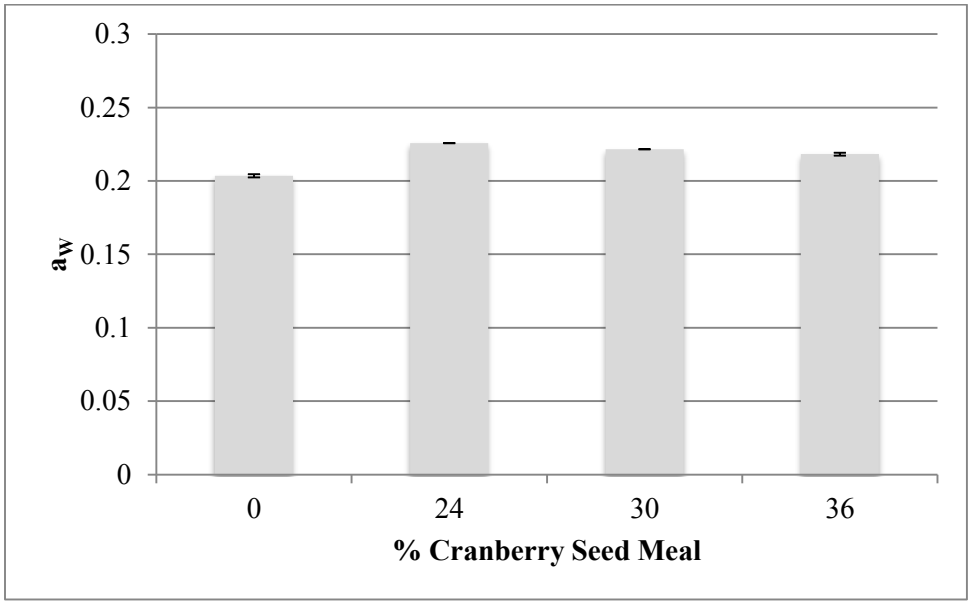

Figure 9. Water activity (a_W) of extrudates with cranberry seed meal.

Water activity reading is important because it gives us the amount of water available for utilization by micro-organisms. According to (Lowe and Kershaw, 1995), water activity < 0.6 will discourage the growth of majority of the micro-organisms hence longer shelf life. In this case, all the various % cranberry seed meal had water activity below 0.25.

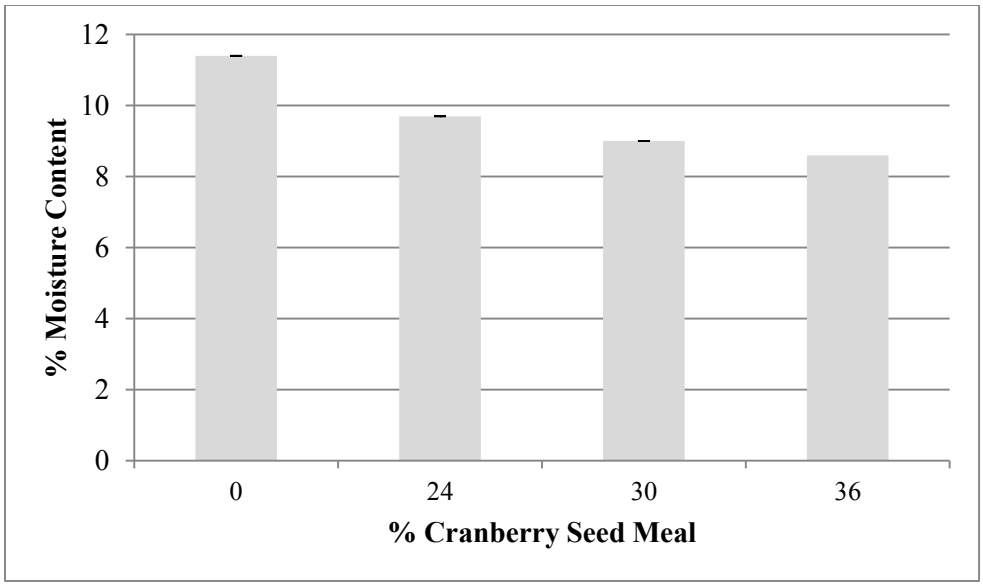

Figure 10. Moisture content (%) of extrudates with cranberry seed meal.

The initial moisture content of the blends was 12%, which a decrease is observed with an increase in the cranberry seed meal concentration as demonstrated in figure 10. The decrease of moisture in the extrudates with increase in cranberry seed meal can be attributed to the low moisture content (6.71%) of cranberry seed meal as reported in table 4.

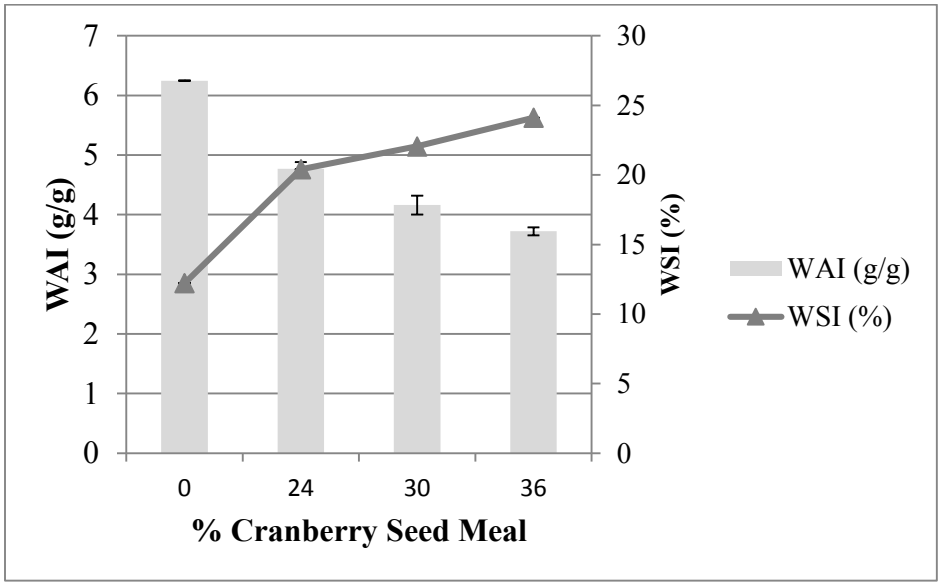

Figure 11. WAI (g/g) and WSI (%) of extrudates with cranberry seed meal.

With an increase in WSI, a decrease in WAI is observed with an increase in cranberry seed meal. Increase in the fiber content with increase in cranberry seed meal as demonstrated in figure 6 can also be attributed to the decrease in WAI as well as increase in WSI. An inverse relationship between WAI and WSI was observed in this study.

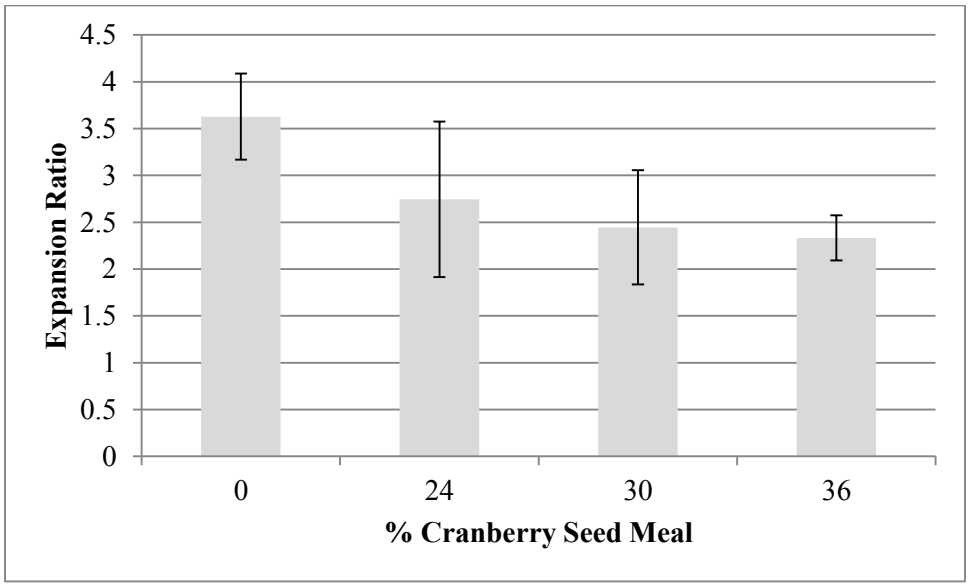

Figure 12. Expansion ratio of extrudates with cranberry seed meal.

A decrease in expansion ratio of the extrudates was observed with an increase in cranberry see meal. A decrease in expansion ratio can be attributed to decrease in starch as a result of corn meal reduction in the formulation with increase in cranberry seed meal together with fiber.

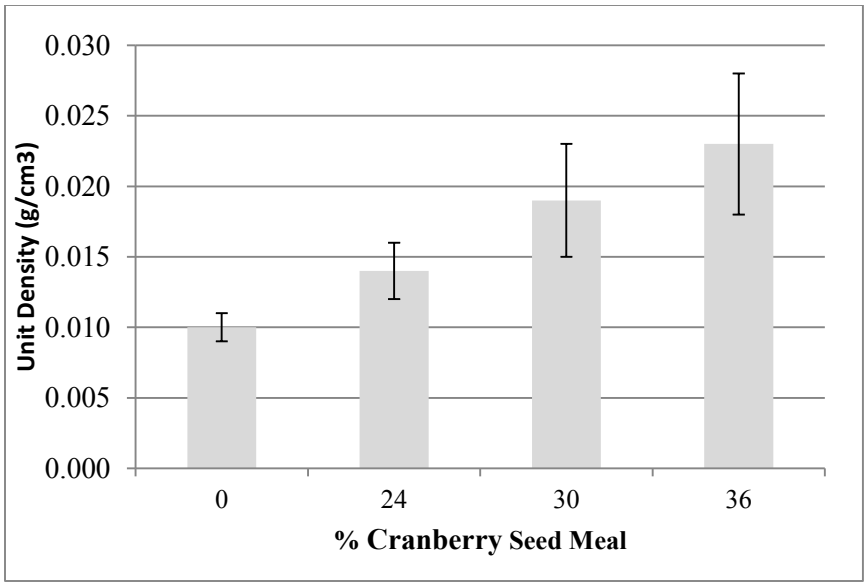

Figure 13. Unit density of extrudates with cranberry seed meal.

Unit density increase is observed with an increase in % cranberry seed meal which can be attributed to the fiber content. Obtaining unit density is important when determining the right packaging to utilize for the cranberry puffs.

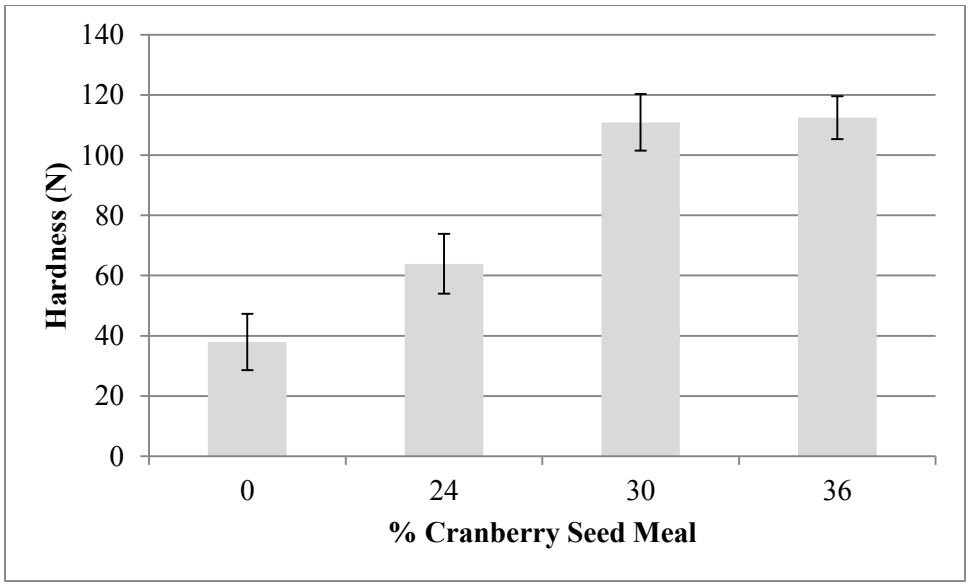

Figure 14. Hardness of extrudates with cranberry seed meal.

Increase in hardness is observed with an increase in cranberry seed meal.

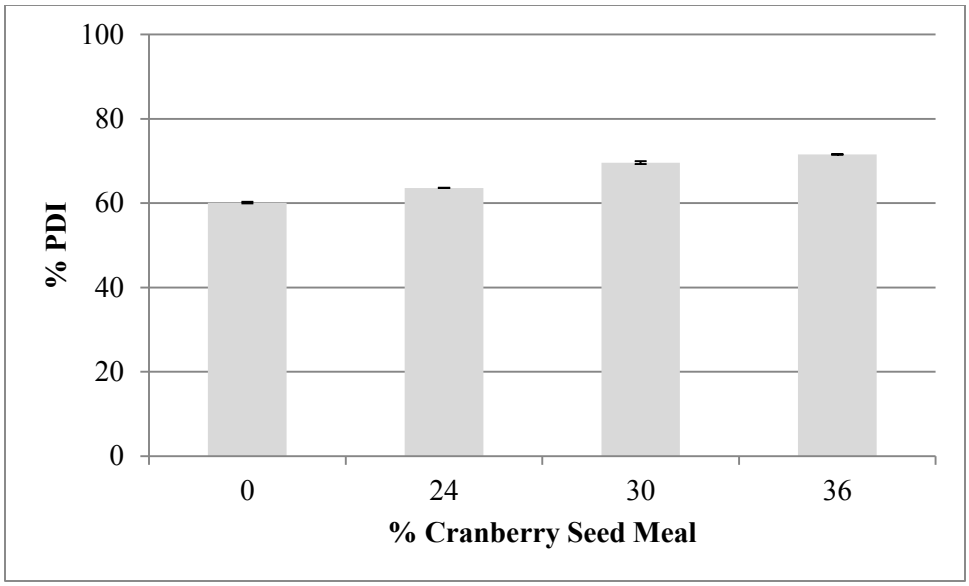

Figure 15. % PDI of extrudates with cranberry seed meal.

In extruded products, mechanical strength is measured and indicated by PDI (Fallahi, Rosentrater, Muthukumarappan, & Tulbek, 2013). The stability of the extrudates during storage and shipping processes is directly proportional to higher PDI. In this study, increase in cranberry seed meal increases PDI with the highest at 60% for 36% cranberry seed meal extrudate samples.

Sensory Analysis of the Cranberry Puffs

Consumer acceptability of cranberry puffs was evaluated by 103 participants (77.7% female and 22.3% male) who analyzed the samples on appearance, color, texture-crispiness, flavor, tartness, bitterness, aftertaste and overall acceptability. The age demographic comprised of 18-20 years of age (16%), 21-23 years (41%), 24-27 years (17%), and 28+ years (26%). Compusense Five, the sensory software was utilized to collect and analyze data. Tukey's HSD mean difference at 5% ($p \leq 0.05$) was used to test the level of significance among the samples in all the various attributes.

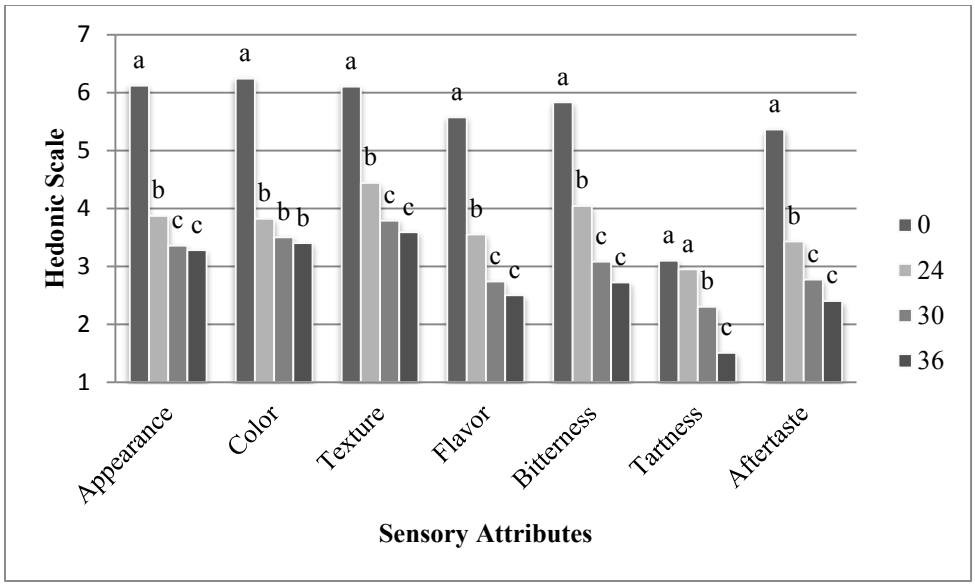

Figure 16. Mean panel score values for appearance, color, texture, flavor, bitterness, tartness and aftertaste of cranberry puff samples.

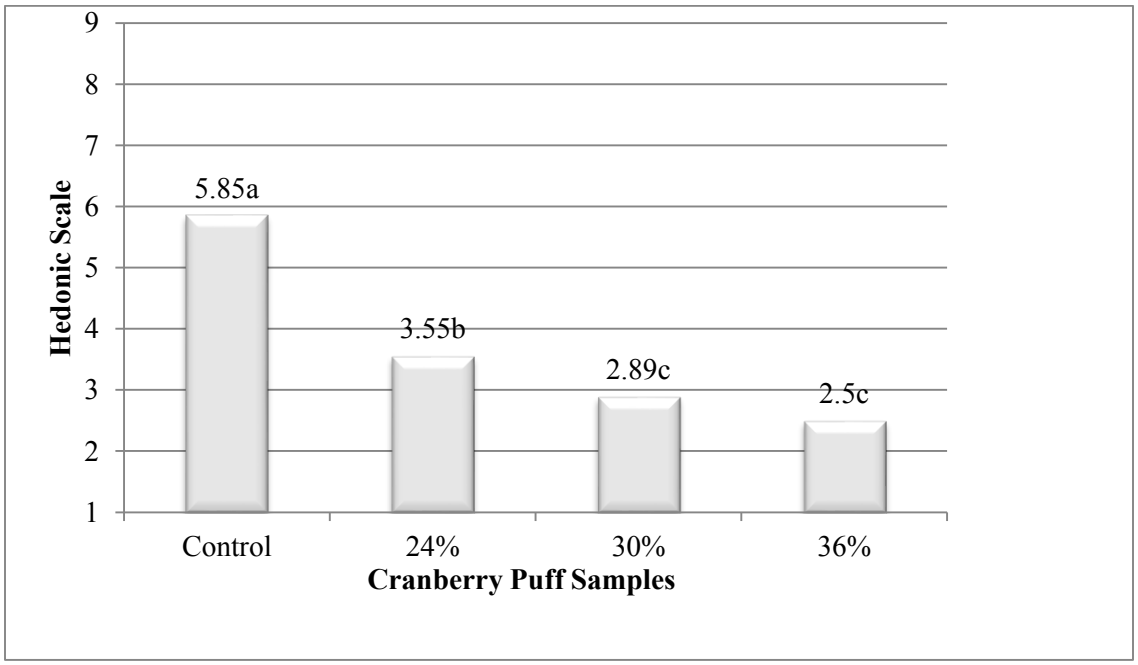

Figure 17. Mean panel score values for overall acceptability of cranberry puff samples. Mean values with different letters are significantly different, n=103, ($p<0.05$)

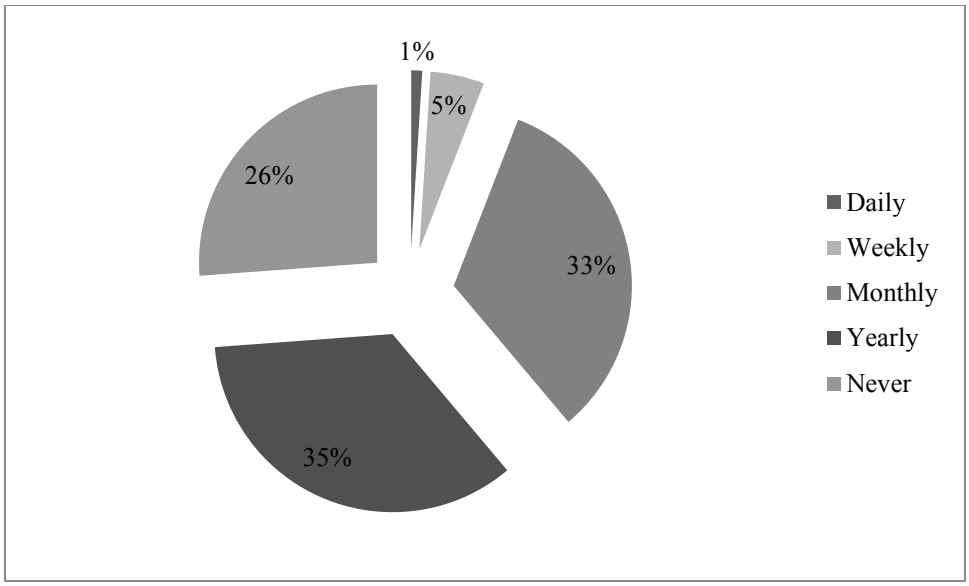

Figure 18. Mean (%) panel scores for intent of purchase of Cheetos.

To determine the intent of purchase of cranberry puffs among the participants, a survey question was asked about the frequency of purchase of Cheetos, snack product with the assumption that participants who purchase Cheetos would also likely purchase cranberry puffs. Majority (35%) of the participants responded yearly, followed by 33% monthly, 26% weekly, 5% weekly, and 1% daily.

To determine the influence protein and fiber content has on consumer buying power, survey questions were asked and the results as illustrated in figure 27 and 28 respectively. In regards to protein, 29% of the participants voted “probably consider”, followed by 22%- definitely consider, 16%- most likely consider, 14%- might or might not consider, 12%- probably not consider, 6%- definitely not consider, and 1%- most likely not consider. In regards to fiber, 22% of the participants voted “most likely consider, followed by 20%- might or might not consider, 18%- Probably consider, 18%-definitely consider, 12%- probably not consider, 6%-definitely not consider and 4% most likely not consider.

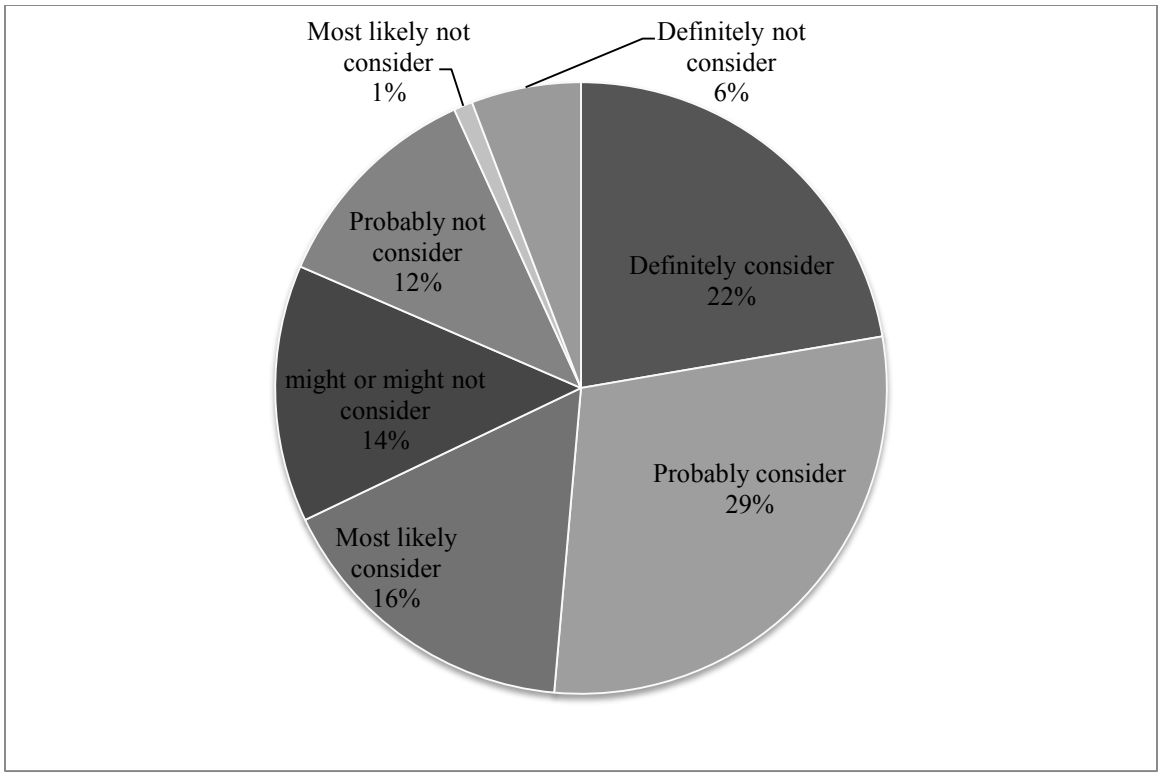

Figure 19. Mean (%) panel scores on the influence of protein content on snack purchase.

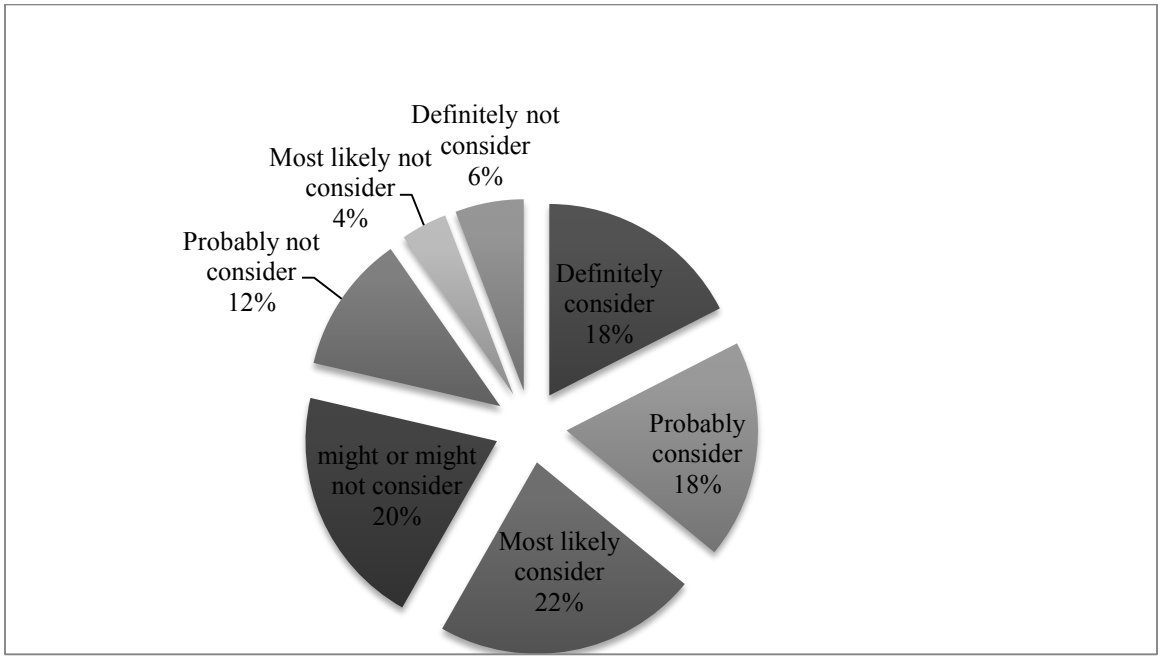

Figure 20. Mean (%) Panel Scores on the influence of fiber content on snack purchase.

Chapter V: Conclusion and Recommendation

This chapter will wrap up this study by summarizing the findings in the conclusion and the recommendation for further research.

Conclusion

Cranberry seed meal, a byproduct of cold press process of oil extraction in cranberry seeds contain 24.4% protein, 50% fiber with 6.8% of it being soluble fiber. This study has demonstrated that the meal has anthocyanins, phenolic compounds, historically associated with positive health benefits including the management of diabetes. With the increasing cost of diabetes medication and the projected rise in individuals with diabetes to one in three U.S adults by 2050 (Yang et al., 2013), cranberry seed meal can be one of the less expensive solutions for the management of diabetes by being incorporated into food as demonstrated by this study. Cranberry seed meal was utilized as an ingredient in an extruded snack product applied in different concentrations at 0%, 24%, 30% and 36%. To evaluate the targeted properties of the meal in a final product upon processing, extrusion was chosen as a test process. The survival of anthocyanins was evaluated upon extrusion which was found to be a high of 19.40% in C-3-Ga with a low of 0% in C-3-Ar and C-3-GI. Statistical analyses among the various properties (color, pH, moisture content, water activity, expansion ratio, hardness, unit density, water absorption index, water solubility index, and pellet durability index) of the extrudates were also conducted with significant differences ($p<0.05$) found among the samples with different cranberry seed meal concentrations.

To determine the consumer acceptability of the snack product, sensory evaluation was conducted where the most preferred extrudates in appearance, color, flavor, texture, bitterness, and aftertaste was 0% followed by 24% cranberry seed meal even with its tart flavor. The top

three preferred flavors were Cheddar (74.8%), Onion and Sour Cream (63.1%), and Cinnamon and Sugar (50%). Apart from the Cinnamon and Sugar flavor, the top two flavors would be ideal for diabetic individuals being that they have less sugar in them. Media influence on protein and fiber on consumers buying power was voted by 29% of the panelists as probably consider for protein and 22% as most likely consider for fiber; this showed that media influence on fiber is more popular than protein which could be helpful in this case because the snack product is high in fiber.

Recommendation

Below are some of the recommendations for further research.

1. Extrusion conditions can be altered to maximize anthocyanins survival upon extrusion.
2. Maximize the benefits of cranberry seed meal as an ingredient by incorporating artificial sweeteners into high percentages of cranberry seed meal in the formulation to improve the flavor and conducting a sensory evaluation to evaluate consumer acceptability.
3. Determine the effectiveness of cranberry puffs consumption in lowering glycemic index of diabetic individuals through a clinical study.

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Appendix A: Advertisement Circulated through Email and Posted on Bulletin Boards Around UW-Stout

Appendix B: UW-Stout Institute of Review Board Approval

October 24, 2014

Jacinta Kemboi

Food and Nutrition

UW-Stout

RE: Sensory Evaluation Study of Cranberry Seeds Meal Snack Extruded Products

Dear Jacinta:

The IRB has determined your project, *"Sensory Evaluation Study of Cranberry Seeds Meal Snack Extruded Products,"* is **Exempt** from review by the Institutional Review Board for the Protection of Human Subjects. The project is exempt under **Category #6** of the Federal Exempt Guidelines and holds for 5 years. Your project is approved from **October 24, 2014** through **October 23, 2019**. Should you need to make modifications to your protocol or informed consent forms that do not fall within the exemption categories, you will need to reapply to the IRB for review of your modified study.

If your project involved administration of a survey, please copy and paste the following message to the top of your survey form before dissemination:

> This project has been reviewed by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46

If you are conducting an **online** survey/interview, please copy and paste the following message to the top of the form:

"This research has been reviewed by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46."

Informed Consent: All UW-Stout faculty, staff, and students conducting human subjects research under an approved "exempt" category are still ethically bound to follow the basic ethical principles of the Belmont Report: 1) respect for persons; 2) beneficence; and 3) justice. These three principles are best reflected in the practice of obtaining informed consent from participants.

If you have questions, please contact Research Services at 715-232-1126, or foxwells@uwstout.edu, and your question will be directed to the appropriate person. I wish you well in completing your study.

Sincerely,

Susan Foxwell

Research Administrator and Human Protections Administrator,

UW-Stout Institutional Review Board for the Protection of Human Subjects in Research (IRB)

CC: Cynthia Rohrer

Appendix C: Consent Form: Sensory Evaluation of Cranberry Puffs

Title: Sensory Evaluation of Cranberry Puffs

Investigator: Jacinta Kemboi, 218-310-7736; Dr. Cynthia Rohrer, x-2088, room 342 HRH.

Description: You will be taking part in the sensory evaluation of a commercially available Cranberry puffs containing cranberry seed meal flour, millet flour, corn meal flour, and tapioca flour ingredients. If you have any dietary restrictions that would make you unable to eat these food items, then you should not take part in the evaluation.

Risks and Benefits: Care has been taken so that all risks associated with food products have been reduced. Cranberry seed meal extruded products have remained sealed until evaluation and have remained in the original container until evaluation.

Time Commitment and Payment: Each subject will receive a candy treat for their participation. Each evaluation should require no more than 15 min.

Confidentiality: Your name will not be included on any documents. We do not believe that you can be identified from any of this information. This informed consent will not be kept with any of the other documents completed with this project.

Right to Withdraw: Your participation in this study is entirely voluntary. You may choose not to participate without any adverse consequences to you. Should you choose to participate and later wish to withdraw from the study, you may discontinue your participation at this time without incurring adverse consequences.

IRB Approval: This study has been reviewed and approved by The University of Wisconsin-Stout's Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University policies. If you have questions or concerns regarding this study please contact the Investigator or Advisor. If you have any questions, concerns, or reports regarding your rights as a research subject, please contact the IRB Administrator.

Investigators: Jacinta Kemboi

218-310-7736

Kemboij0665@my.uwstout.edu Dr. Cynthia Rohrer

715-232-2088.1.1

IRB Administrator:

Sue Foxwell, Director, Research Services

rohrerc@uwstout.edu

152 Vocational Rehabilitation Building

UW-Stout

Menomonie, WI 54751

715-232-2477

foxwells@uwstout.edu

Statement of Consent:

By signing this consent form you agree to participate in the project entitled, "Sensory Evaluation of Cranberry Puffs."

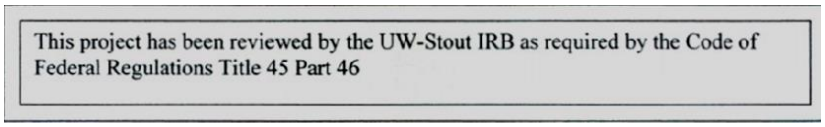
_____

_____

Signature Date

Appendix D: Sensory Analysis Questionnaire

Cranberry Puffs Taste Test



Questionnaire for Sensory Evaluation Sample 1

Instructions: Please taste three bits of the sample in the cup labeled **472** and wait 30 seconds before answering the questions corresponding that sample. Please answer the question below BEFORE tasting the sample.

1. How much do you LIKE or DISLIKE the APPEARANCE of this sample?
    - Dislike extremely
    - Dislike very much
    - Dislike moderately
    - Dislike slightly
    - Neither like nor dislike
    - Like slightly
    - Like moderately
    - Like very much
    - Like extremely
2. How much do you LIKE or DISLIKE the COLOR of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Instructions:

Now you can taste your sample. Please answer the following questions.

3. How much do you LIKE or DISLIKE the TEXTURE (Crispiness) of the sample?
    - Dislike extremely
    - Dislike very much
    - Dislike moderately
    - Dislike slightly
    - Neither like nor dislike
    - Like slightly
    - Like moderately
    - Like very much
    - Like extremely
4. How much do you LIKE or DISLIKE the FLAVOR of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

5. How much do you LIKE or DISLIKE the TARTNESS/BITTERNESS of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

6. How would you rate the INTENSITY of the TARTNESS?

- No tartness
- Weak tartness
- Slight tartness

- Moderate tartness
- Strong tartness
- Very strong tartness
- Extremely strong tartness

Instructions: Please wait 30 seconds after tasting the sample to answer the following question.

7. How much do you LIKE or DISLIKE the AFTERTASTE of this sample?
   - Dislike extremely
   - Dislike very much
   - Dislike moderately
   - Dislike slightly
   - Neither like nor dislike
   - Like slightly
   - Like moderately
   - Like very much
   - Like extremely
8. How much do you LIKE or DISLIKE the OVERALL APPEARANCE of this sample?
   - Dislike extremely
   - Dislike very much
   - Dislike moderately
   - Dislike slightly
   - Neither like nor dislike
   - Like slightly

- Like moderately
- Like very much
- Like extremely

9. Using words, please tell me everything you liked and disliked about the sample

Questionnaire for Sensory Evaluation Sample 2

Instructions: Please taste three bits of the sample in the cup labeled **234** and wait 30 seconds before answering the questions corresponding that sample. Please answer the question below BEFORE tasting the sample.

10. How much do you LIKE or DISLIKE the APPEARANCE of this sample?
    - Dislike extremely
    - Dislike very much
    - Dislike moderately
    - Dislike slightly
    - Neither like nor dislike
    - Like slightly
    - Like moderately
    - Like very much
    - Like extremely
11. How much do you LIKE or DISLIKE the COLOR of this sample?
    - Dislike extremely

- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Instructions: Now you can taste your sample. Please answer the following questions

12. How much do you LIKE or DISLIKE the TEXTURE (Crispiness) of the sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

13. How much do you LIKE or DISLIKE the FLAVOR of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately

- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

14. How much do you LIKE or DISLIKE the TARTNESS/BITTERNESS of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

15. How would you rate the INTENSITY of the TARTNESS?

- No tartness
- Weak tartness
- Slight tartness
- Moderate tartness
- Strong tartness
- Very strong tartness

- Extremely strong tartness

Instructions: Please wait 30 seconds after tasting the sample to answer the following question.

16. How much do you LIKE or DISLIKE the AFTERTASTE of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

17. How much do you LIKE or DISLIKE the OVERALL APPEARANCE of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

18. Using words, please tell me everything you liked and disliked about this sample?

Questionnaire for Sensory Evaluation Sample 3

Instructions: Please taste three bits of the sample in the cup labeled **726** and wait 30 seconds before answering the questions corresponding that sample. Please answer the question below BEFORE tasting the sample.

1. How much do you LIKE or DISLIKE the APPEARANCE of this sample?
    - Dislike extremely
    - Dislike very much
    - Dislike moderately
    - Dislike slightly
    - Neither like nor dislike
    - Like slightly
    - Like moderately
    - Like very much
    - Like extremely
2. How much do you LIKE or DISLIKE the COLOR of this sample?
    - Dislike extremely
    - Dislike very much
    - Dislike moderately
    - Dislike slightly
    - Neither like nor dislike
    - Like slightly
    - Like moderately
    - Like very much

- Like extremely

Instructions: Now you can taste your sample. Please answer the following questions.

3. How much do you LIKE or DISLIKE the TEXTURE (Crispiness) of the sample?
   - Dislike extremely
   - Dislike very much
   - Dislike moderately
   - Dislike slightly
   - Neither like nor dislike
   - Like slightly
   - Like moderately
   - Like very much
   - Like extremely
4. How much do you LIKE or DISLIKE the FLAVOR of this sample?
   - Dislike extremely
   - Dislike very much
   - Dislike moderately
   - Dislike slightly
   - Neither like nor dislike
   - Like slightly
   - Like moderately
   - Like very much
   - Like extremely
5. How much do you LIKE or DISLIKE the TARTNESS/BITTERNESS of this sample?

- o Dislike extremely
- o Dislike very much
- o Dislike moderately
- o Dislike slightly
- o Neither like nor dislike
- o Like slightly
- o Like moderately
- o Like very much
- o Like extremely

6. How would you rate the INTENSITY of the TARTNESS?

- o No tartness
- o Weak tartness
- o Slight tartness
- o Moderate tartness
- o Strong tartness
- o Very strong tartness
- o Extremely strong tartness

Instructions: Please wait 30 seconds after tasting the sample to answer the following question.

7. How much do you LIKE or DISLIKE the AFTERTASTE of this sample?

- o Dislike extremely
- o Dislike very much
- o Dislike moderately
- o Dislike slightly

- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

8. How much do you LIKE or DISLIKE the OVERALL APPEARANCE of this sample?
   - Dislike extremely
   - Like very much
   - Dislike moderately
   - Dislike slightly
   - Neither like nor dislike
   - Like slightly
   - Like moderately
   - Like very much
   - Like extremely
9. Using words, please tell me everything you liked and disliked about this sample

Questionnaire for Sensory Evaluation Sample 4

Instructions: Please taste three bits of the sample in the cup labeled **559** and wait 30 seconds before answering the questions corresponding that sample. Please answer the question below BEFORE tasting the sample.

10. How much do you LIKE or DISLIKE the APPEARANCE of this sample?
    - Dislike extremely

- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

11. How much do you LIKE or DISLIKE the COLOR of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

Instructions: Now you can taste your sample. Please answer the following questions.

12. How much do you LIKE or DISLIKE the TEXTURE (Crispiness) of the sample?

- Dislike extremely
- Dislike very much
- Dislike moderately

- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

13. How much do you LIKE or DISLIKE the FLAVOR of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

14. How much do you LIKE or DISLIKE the TARTNESS/BITTERNESS of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly

- Like moderately
- Like very much
- Like extremely

15. How would you rate the INTENSITY of the TARTNESS?

- No tartness
- Weak tartness
- Slight tartness
- Moderate tartness
- Strong tartness
- Very strong tartness
- Extremely strong tartness

Instructions: Please wait 30 seconds after tasting the sample to answer the following question.

16. How much do you LIKE or DISLIKE the AFTERTASTE of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

17. How much do you LIKE or DISLIKE the OVERALL APPEARANCE of this sample?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

18. Using words, please tell me everything you liked and disliked about this sample?

19. What flavors would like incorporated in this product?

- Cheddar
- Roasted garlic & chili
- Jalapeno
- Chipotle
- Salt & pepper
- BBQ
- Sweet & spicy
- Cinnamon & sugar
- Onion & sour cream

20. How often do you consider the fiber content when selecting a snack product?

- Definitely consider

- Most-likely consider
- Probably consider
- Might or might not consider
- Probably not
- Most-likely not
- Definitely not

21. How often do you consider the protein amounts when selecting a snack product?

- Definitely consider
- Most-likely consider
- Probably consider
- Might or might not consider
- Probably not
- Most-likely not
- Definitely not

22. How often do you purchase Cheetos?

- Daily
- Weekly
- Monthly
- Yearly
- Never

23. Please indicate your gender.

- Female
- Male

24. Please indicate your age.

- 18-20
- 21-23
- 24-27
- 28+