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Enhanced Beta (β) Glucan Beverages – Evaluation of Satiety, Nutrition, and Shelf Stability

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ENHANCED BETA (β) GLUCAN BEVERAGES – EVALUATION OF SATIETY, NUTRITION, AND SHELF STABILITY

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

°C - Degree Centigrade

β- glucan - Beta-glucan

µm – Micrometer

%DV- Percentage Daily Value

AACC - American Association of Cereal Chemists

AACCI - American Association of Cereal Chemists International

AOCS - American Oil Chemists Society

CFR - Code of Federal Regulations

DGAC - Dietary Guidelines for Americans Commission

FAOSTAT - Food and Agriculture Organization Corporate Statistical Database

FDA - Food and Drug Administration

g - gram

GOPOD - Glucose Oxidase Peroxidase

HPSEC- High-performance size-exclusion chromatography

IDFA – International Dairy Foods Association

LFFF – Lactose free Fat free

mg - Milligram

mM - Milli-molar

NHANES – National Health and Nutritional Examination Survey

NIRS - Near Infrared Reflectance Spectroscopy

nkat/ml - catalytic activity per ml

Nm – Nanometer

SD - Standard Deviation

TDF - Total Dietary Fiber

USDA - United States Department of Agriculture

USA - United State of America

VAS – Visual Analogue Scale

GMI423 – General Mills Inc. oat variety

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

ENHANCED BETA (β) GLUCAN BEVERAGES – EVALUATION OF SATIETY, NUTRITION, AND SHELF STABILITY

BEATRICE SERWAA MANU
2020

Oats and barley are unique cereals owing to their nutritional and health benefits. Soluble dietary fiber components, especially β-glucan found in both grains, have been associated with the reduction of cholesterol, normalization of blood sugar levels and suppression of hunger. These nutritional and therapeutic attributes are ideal for production of novel and more diversified food products from these cereals. β-glucan, however, imparts high viscosity in food systems particularly in high moisture foods, thus impairing their sensory characteristics. Reduced viscosity oat-barley beverages, using partial enzyme and acid hydrolysis, may fill a void in the market segment and yet serve as effective vehicles for dietary fiber and protein enrichment.

Reduced-viscosity beverages were developed containing blends of selected cultivars of oats and naked barley that were subjected to enzymatic and acid hydrolysis. Oat cultivar GMI 423 and Natty were combined with commercial barley flour in various oat:barley proportions (100%, 90:10, 80:20, 70:30, & 60:40), and beverages were then formulated using a standard recipe. A factorial analysis involved 20 beverages (2 hydrolysis treatments x 2 cultivars x 5 flour blends). The effects of partial enzyme and partial acid hydrolysis on beverage viscosity were evaluated. Nutritional and dietary fiber content were determined on flour and on beverages made with those flours in order to determine the efficacy of oat and barley enrichment. All analyses were conducted in duplicate.

Sensory evaluation was done on all beverages to determine acceptance and preference of various attributes by untrained panelist (n =16). The most acceptable blend of high β-glucan beverage (80G/20B), low β-glucan beverage (80N/20B), (chosen during the sensory acceptability tests), together with a commercially available hunger suppressing beverage and regular breakfast (controls) were tested to compare their effects on satiety and food intake. These pasteurized selected beverages together with an unpasteurized control beverage, were subjected to shelf-life studies.

Initial viscosities of novel beverages ranged between 149.35 – 184.5cP. Partial enzyme hydrolysis significantly reduced viscosities to 44.9 -57.5cP. Partial acid hydrolysis reduced the beverage viscosities to 130.1 – 170.1cP. Viscosity of the beverages also increased as the proportion of barley in the blend increased. The β-glucan content of GMI beverages, which ranged between 7.3 – 8.1% was reduced to 2.9 – 4.3% after treatment with partial enzyme hydrolysis. The β-glucan content was however not statistically significantly different from the partial acid hydrolyzed beverages which had a β-glucan content range between 4.4 – 5.3%. The initial β-glucan content of Natty beverages which ranged between 4.2 – 5.8%, was decreased to 1.32 – 1.39%, after partial enzyme hydrolysis. No significant difference was observed between the β-glucan content of the partial enzyme hydrolyzed Natty beverage and the partial acid hydrolyzed which had a β-glucan content range between 1.9 – 2.0%. GMI beverages were shown to have a higher protein content (12.5 -15.5%) compared with the beverages formulated with Natty (12.3 – 13.5%). The partial-enzyme hydrolyzed GMI beverage had a total dietary fiber (TDF) content of 7.6g per 240ml serving. This value was not significantly different in comparison to the unhydrolyzed GMI beverage which had a TDF content of 12.6g per

240ml serving. Similarly, the hydrolyzed Natty beverage which had a TDF content of 4.6g per 240ml serving was not significantly different from the unhydrolyzed Natty beverage with a TDF content of 7.3g per 240ml serving. The partial-enzyme hydrolyzed GMI beverages can be classified as high dietary fiber beverages. Paired preference sensory tests showed that, participants significantly preferred the partial-enzyme hydrolyzed samples over partial-acid hydrolyzed samples. QDA showed that partial enzyme hydrolyzed GMI and Natty beverages containing 80% oat and 20% barley were the most accepted with an overall acceptability value of 4.38 ± 0.60 and 4.63 ± 0.48, respectively. Satiety tests showed that reduction in hunger, desire to eat and prospective intake, was significantly greater with consumption of the high β-glucan beverage. The high β-glucan beverage decreased hunger by 48.53%, reduced desire to eat by 45.31%, lowered prospective intake by 29.09% and increased fullness from 2.8cm to 6.4cm, on a 10cm VAS scale, after the 4-hour postprandial period. Additionally, energy intake at lunch was significantly lower following consumption of the high β-glucan beverage (493.7 ± 176.2kcal), compared to the commercial beverage (749.4 ± 171.4kcal), regular breakfast (692.1 ± 195.6kcal) and low β-glucan beverage (640.1 ± 132.0kcal). The 4-week refrigerated shelf life study showed that pasteurized beverages had Aerobic Plate count and Coliform/E. Coli count, significantly below the FDA detection limit of 20,000 and 10cfu/g, respectively.

This study demonstrates that it is possible to formulate an acceptable, functional high β-glucan beverage that is shelf stable under refrigerated conditions using partial enzyme hydrolysis.

CHAPTER 1. INTRODUCTION

1.1 Background

Oat is one of the first cereals cultivated by man. It belong to the Poaceae family. Historically, oat was found to be naturally growing in ancient China as early as 7000 BC. However, the Greeks were the first to make a meal out of oats. In the early years of adventure and migration, Scottish settlers brought oats to North America in 1602 AD, since the climate was similar to the Scandinavia (Small E., 1999), however, the Native Americans rather fed oats to their horses. After the advent of cars and trucks in the 1920s, the popularity of oats grew less because horses were no longer the sole means of transport. Since the 1980s when the nutritional value of oats was realized, farmers in the Mid-western states, Minnesota, Iowa, South and North Dakota have largely cultivated oats for its value. The common oat species (*Avena sativa*) is largely cultivated for human consumption as oatmeal as well as for livestock feed.

Barley, *Hordeum vulgare* L. (Poaceae), belongs to the botanical tribe Triticeae and almost resembles small-grain cereal species like rye and wheat (von Bothmer and Komatsuda 2011). Barley is considered to be among the first domesticated cereals of Old-World of agriculture. It is also an important experimental model due to its attributes, viz, morphology, physiology, genetics, and short life span. Grown in a variety of environments, barley ranks fourth in world cereal crop production, after wheat, rice, and corn with a current world production of 137.47 million metric tons (World Agricultural Production, 2018). Though Barley primarily used as animal fodder and as a source of malt for some alcoholic beverages, it is widely used in the pastry industry and for stews or soups.

Cereals like barley and oat are unique owing to their diverse properties and dietary profiles. Current improvements in food and nutrition has disclosed the significance of their various components (Butt *et al*., 2008). Their rich content of β-glucan and minerals make them important origin of dietary fiber. Dietary fiber, which is primarily found in plant-based foods comprise of lignin polysaccharides and oligosaccharides. It is the digestible section of plants that are resistant to break down and assimilation in the ileum of mammals, thus favoring fermentation in the intestinum crassum. Dietary fibers contribute to some physiological conditions, such as laxation, blood glucose and cholesterol control (AACC, 2018).

β-glucans are a group of non-starch polysaccharides whose major components are D-glucose monomeric units joined by β-glycosidic bonds. In barley, β-glucan is in the sub aleurone as well as in cell walls of the endosperm and in oat it is located in the aleurone and sub aleurone (Sikora *et al*., 2013). β-glucan content of 2 to 20 g and 3 to 8 g (per 100 g dry weight) have been reported for barley and oats, respectively. Positive health-effects of β-glucan are particularly linked to its cholesterol-lowering effects, increasing satiety and inducing weight loss, as well as the reduction of postprandial glucose and insulin response (Schlörmann and Glei, 2017). Due to scientific reports linking β-glucan to reduction of blood glucose and cholesterol, the overall consumption of oats and barley has increased in the United States of America (Daou and Zhang, 2012). Therefore, it is not surprising that the food industry has largely capitalized on the nutritional value β-glucan to develop new products which possess more health benefits. The inclusion of β-glucan into baking products, dairy products, beverages, and meat products has been found to enhance their nutritional, sensory and gustatory attributes. Studies report that viscosities of the

solutions containing β-glucan increases during the preliminary dissolution of the β-glucans, and there has been no detectable decline in viscosity observed thereafter (Ahmad et al., 2012; Faraj *et al*., 2006). This has made research on the stability and viscosity of β-glucan in food matrices, especially high moisture foods like beverages, a complicated one. Integrating substantial amounts of β-glucan into consumer products remains a technological challenge as this may impact the textural quality, which could change the sensory property of foods and secondly, due to its typical slimy mouthfeel texture (Hilliam M., 2003).

1.2 Hypothesis for study

The hypothesis tested in the present study are as follows:

1. H0: There is no statistical difference between the nutritional composition of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages.

H1: There is a statistical difference between the nutritional composition of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages. (Nutrient Composition Treatment A versus Nutrient Composition Treatment B)

2. H0: There is no statistical difference between the viscosity of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages.

H1: There is a statistical difference between the viscosity of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages. (Viscosity Treatment A versus Viscosity Treatment B)

3. H0: There is no statistical difference between the viscosity of treated β-glucan beverages and unhydrolyzed β-glucan beverages.

H1: There is a statistical difference between the viscosity of treated β-glucan beverages and unhydrolyzed β-glucan beverages. (Viscosity Treatment versus Untreated Control)

4. H0: There is no statistical difference between the sensory acceptability of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages.

H1: There is a statistical difference between the sensory acceptability of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages. (Treatment A versus Treatment B)

5. H0: There is no statistical difference between the satiety response of partially hydrolyzed GMI β-glucan beverages and partially hydrolyzed Natty β-glucan beverages.

H1: There is a statistical difference between the satiety response of human subjects who consume partially hydrolyzed GMI β-glucan beverages and partially hydrolyzed Natty β-glucan beverages. (Treated variety A versus Treated variety B)

6. H0: There is no statistical difference between the satiety response of human subjects who consume formulated β-glucan beverages and commercial hunger suppressing beverages.

H1: There is a statistical difference between the satiety response of human subjects who consume formulated β-glucan beverages and commercial hunger suppressing beverages. (Treatment versus commercial standard)

7. H0: There is no statistical difference between the shelf stability of pasteurized and unpasteurized beverages.

H1: There is a statistical difference between the shelf stability of pasteurized and unpasteurized beverages. (Treatment versus control).

1.3 Problem statement

Although oat and barley have been grown in South Dakota and generally, the United States for many generations, their use have been restricted, because a limited variety of products have been made from oats and barley. Various reports have documented that dietary fibers provide an array of well-being advantages including improvement of pancreatic and bowel functions (Wrick *et al*., 1983). Similar to most individuals, dietary fiber is a shortfall nutrient and as such, a nutrient of public health concern in older adults. The American Heart Association recommends a total dietary fiber intake of approximately 25 to 30 grams a day from consumed food, not supplements. Currently, the average adult dietary fiber of 15 grams/day forms only half of the recommended daily intake in the United States. As a dietary fiber, β-glucan obtained from oat and barley, has been considered as a significant chemical active principle for functional food applications (Wood *et al*., 1994). It is well known that oat and barley β-glucan reduce post-prandial blood glucose (Wood *et al*.,1994), blood cholesterol levels (Rimm *et al*., 1996) and increases satiety (Rebello *et al*., 2016). These positive findings have challenged the food industry to create new functional foods containing oat and barley β-glucan, however, the potential products are still limited to cereal-based baked products. Products made from oat and barley are found predominantly in the breakfast cereal aisle in grocery stores, although their nutritional and health benefits can be carried over into more diversified food products such as soups, meat entrees, beverages, etc. The most popular and thriving oat products on the market are the granola bars, instant oatmeal, oat cookies, etc. This limitation has been due to the unique rheological properties of oats and barley such as high viscosity in high moisture conditions. This high viscosity in high moisture foods is mostly unpleasant and

unappealing to consumers. Khoury *et al*., (2012) reported that the addition of β-glucans to beverages seemed to impair the overall perception of products even when other rheological properties were improved. Therefore, developing β-glucan-fortified foods is still a challenge as consumers choose to decline them based on sensory characteristics even though they offer greater health benefits (Khoury *et al*., 2012).

1.4 Justification for work

Individuals who suffer obesity are at elevated risk of developing illnesses like cardiovascular diseases, diabetes, and cancer. A longitudinal research suggested that a diet made of nutritional components such as dietary fiber could ultimately influence appetite of consumers thereby leading to a reduction in body weight when coupled with other lifestyle modifications (Hopkins *et al*., 2016). A recent National Health and Nutrition Examination Survey (NHANES) report also indicated that grains including oats and barley have a better chance of promoting better appetite and satiety regulation, improved dietary nutrient intake as a positive contribution to obesity-related metrics (Ahluwalia *et al*., 2019). Also, a randomized cross-over trial carried out by Rebello *et al*., (2013), revealed that oatmeal consumption reduced hunger ($p = 0.005$), improved satiety ($p = 0.001$) and prospective intake ($p = 0.006$) as well as increased fullness ($p = 0.001$) when compared to ready-to-eat breakfast cereal. Also, consumption of oat and barley β-glucan has been found to increase levels of the hunger-suppressing hormone cholecystokinin, leading to a suppressed meal intake (Beck *et al*., 2009). This project seeks to develop a high β-glucan beverage to help extend the nutritional and functional benefits of oat and barley products into more diversified food products. This will increase the number of consumers who could profit from the health attributes of the two cereals. The unappealing problem of high viscosity of

β-glucan in high moisture foods will be overcome through partial hydrolysis of β-glucan. This is linked to the study by Sibakov *et al*., 2013 in which the aim was to create the hydrolysis conditions that allows the production of stable β-glucan dispersions to be incorporated in foods like beverages (contain high-moisture).

1.5 Aim and Specific Objectives

Aim

To expand the range of products made with oats and barley, a high nutritive value, healthy beverage from specialty oats and barley grown in South Dakota, that may also serve as a base for fortifying with bioactive enrichment ingredients will be developed.

Specific objectives

i. To develop a high β-glucan, high protein dairy-based beverage made with partially hydrolyzed β-D-glucan from selected oat cultivars grown in South Dakota and barley.

ii. To determine the effects of partial enzyme and acid hydrolysis treatment on the nutritional composition, and rheological characteristics (viscosity) of enhanced beta glucan beverages

iii. To conduct shelf stability analysis and consumer acceptability analysis of the pasteurized β-glucan beverages.

iv. To measure the satiety response of formulated beverages and compare these to the responses obtained from commercial hunger suppressing beverages and regular breakfast meals.

CHAPTER 2. LITERATURE REVIEW

2.1 Oat structure and cultivation

The structure of oat groats is like that of the seeds of barley and wheat. The oat kernel comprises of oat groats encapsulated in a hull (Figure 1). The hull protects it from the exterior environment. The three major components of the groats are the bran, endosperm, and germ. Commencing from the outer of the groats, the bran layer consists of the epidermis, hyaline layer, and aleurone cells, in that order. The germ consists of embryonic cells and cell wall materials, and the endosperm is made of starch cells. Throughout development, the germ cells catabolic and anabolic activities increase; essential nutrients are carried from the starchy endosperm to the embryonic tissues (Karlberg., 2010).

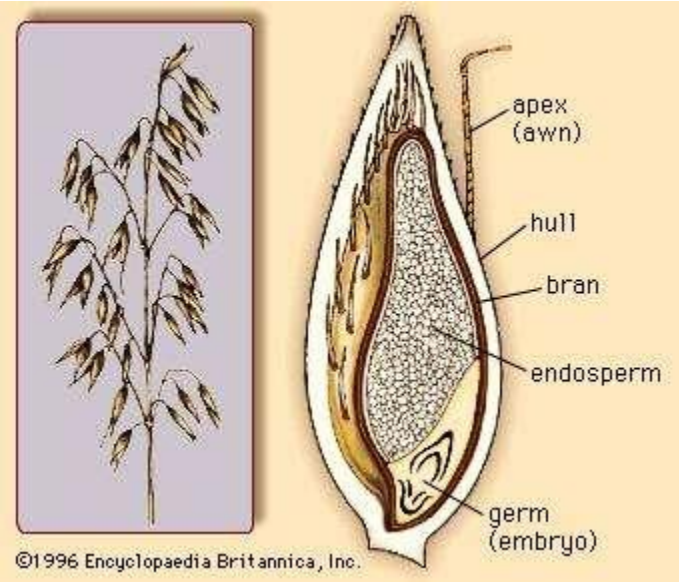

Figure 1. Structure of oat grain (Image source: kellyspantry.blogspot.com)

The oats germ, when examined in a longitudinal section of the kernel, extends roughly one-third of the way up the ventral side of the groat. The germ is larger and narrower than that of wheat. Oats can endure cooler and wetter soils than many other crops, grow quickly, and are able to tolerate mild frosts. They can germinate at soil temperatures as low as 45^{o}F or 7^{o}C. The Northern and Central Plains contribute significantly to oat production, as over half of the grain crop is grown in South Dakota, North Dakota, Wisconsin, Minnesota, and Iowa. Since the production of a whopping 1.5 billion bushels in 1945, there has been a steady decline in the cultivation of oats. Production of oats in the U.S. in the year 2016 amounted to approximately 64.77 million bushels. Oats are at times, planted as a companion crop for the establishment of various grasses and legumes (Winkler *et al*., 2017). Spring oats are often planted in late February through April. Early planting can help to provide production benefits later in the season whilst late planting will push grain fill into warmer weather periods which can reduce yield and test weight. Both hulled and hull-less varieties of oats are available. The US recommended varieties of oats include the Badger, Colt, Excel, Saber, Spurs, Tack, Beta-Gene, Horsepower, Shelby427, Deon, Hayden, Newburg, Rockford and Souris. The *Badger* is an early season, yellow hulled oat developed at the University of Wisconsin. Consistently high grain yields and outstanding test weight have been observed in its cultivation, compared to other early season oats. *Colt* is a white shelled oat developed at South Dakota State University and when compared to the older University of Illinois variety ‘Don,’ has superior grain yield, test weight, protein percentage, and groat percentage (Carlson *et al*., 2017). *Excel* is another oat variety which matures earlier, yields more, possesses medium height and has a strong straw strength. With an ivory yellow seed, it has a good test weight and groat

percentage. *Saber* (yellow-hulled oat) is a variety with exceptional yield, higher test weight and resistance to Barley yellow dwarf disease when compared to other oat varieties. *Spurs* is another oat variety with high yields, good test weight and has a tan to white grain color. *Tack* is a spring oat variety adapted to the Midwestern U.S. It is an early-season oat variety with tan-colored grains, has good yield and high-test weight. Both *Tack* and *Spurs* were released by Ill. Ag. Exp. Station. *Beta-Gene* is a special oat variety possessing high yield potential and established by the University of Wisconsin. It has good straw strength and stature that is similar to the variety Drumlin (Grain Millers, 2016). *Horsepower* is a spring oat variety having high yield potential and robust straw strength. It was developed by the SD Ag. Experiment Station. *Shelby427*, developed at South Dakota State University, yield more, has a high-test weight, and a high groat percentage. It can also be used during oat milling, as a companion crop as well as for forage production (Carlson et al., 2017). *Deon*, is another variety developed by the University of Minnesota, is a high yielding spring yellow oat with overall good agronomic traits. Its kernel has mild yellow color and possess good test weight. *Hayden* is a white-kernelled oat variety developed by South Dakota State University. It has high yield potential and high-test weight. *Newburg* is an oat category notable to the North Central States and prairie provinces of Canada, and has excellent yield potential (Stevens *et al*., 2004). *Rockford* is a high producing species that offers very good lodging resistance. It is a white-hulled spring grain oat, developed by North Dakota State University. *Souris* is a medium-tall, medium maturity oat from North Dakota State University. It is a cultivar with high test weight, high yield potential and white-colored hulled grain. The high-test

weight is consistent with the recommendations of the premium oat markets (Grain Millers., 2016).

2.2 Oat Nutrition and Utilization

Among the various cereals, oat is unique because it has a higher fat content than most cereal grains. Its attributes include high antioxidant, carbohydrate, total protein, mineral and vitamin levels (Sangwan *et al*., 2014). The nutritional composition of raw oats is detailed in Table 2.0 as provided by the United States Department of Agriculture. Avenanthramides are low molecular-weight polyphenols that exist exclusively in oats, where they act as phytoalexins (antimicrobials) produced in response to pathogens. Oat bran is an exceptional origin of dietary fiber β-glucans, B-vitamins, protein, fatty acids and minerals (Kumar 2012, Butt *et al*. 2008). The carbohydrate portion in oats consists about 11% total dietary fiber and 73 - 75% starch (Beloshapka *et al*., 2016). Oats contain high levels of soluble dietary fibers. Dietary fiber as an essential part of the human diet, comprises many substances of plant origin that are resistant to the human digestive system. According to American Association of Cereal Chemists (AACC), dietary fiber is defined as “the edible part of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine”. Dietary fiber comprises of polysaccharides, oligosaccharides, lignin and related plant-based components which are either classified as soluble or insoluble fiber (DeVries *et al*., 2001). Oatmeal and oat bran have soluble fibers that are efficacious to minimizing blood cholesterol levels and stabilizing blood sugar levels (Butt *et al*., 2008). β-D-glucan is an important soluble dietary fiber found in oats.

Table 2.0 Nutritional content of 100g of raw oats

Nutrient	Quantity Available (g)
Water	8
Protein	16.9
Carbohydrates	66.3
Sugar	~
Fiber	10.6
Total Fat	6.9
Saturated	1.22
Monounsaturated	2.18
Polyunsaturated	2.54
Omega-3	0.11
Omega-6	2.42

(United States Department of Agriculture, 2019. Nutrient Database, SR Legacy, 169705)

Since the declaration by the United States FDA in January 1997 that oats contribute to healthy heart, there has been increased usage in food products due to its nutritional value. Oat products comprise of hot cereal, rolled oats, steel-cut groats, quick oats, and oat flour or oatmeal, just to mention but a few. Hot cereal is the product obtained from oats. Rolled oats, thickest of the typical oat-flakes, are processed by flaking whole groats. Steel-cut groats are manufactured through fragmenting groats. The sectioned groats are used in producing flakes, flour and other ingredients. Quick oats are rather made from steel-cut groats which are cut into few (three to four) pieces to be steamed and flaked. Oat flour/oatmeal is processed by milling groats into flour. The flour is further used as an ingredient in different food products (Beloshapka *et al*., 2016). Most commercial oat processing into various products requires the use of superheated steam processing, where oat groats are conditioned with saturated (wet) steam followed by kiln drying. This process aims at deactivating lipid-hydrolyzing enzymes, developing the distinctive oat taste and destroying microorganisms (Rasane *et al*., 2015). One familiar use of oats is as a ready to eat cereal product, which is mostly achieved through extrusion cooking where high heat, high pressure and shear forces are applied to an uncooked cereal mass (Kim *et al*., 2006).

2.3 Barley structure and cultivation

A mature barley grain is made of an embryo and an endosperm, which is a major store of carbohydrates (mainly starch) and protein. The starchy endosperm is protected by a cellular layer called aleurone and an outer husk (Young, B., 2001). The structure of barley is shown in Figure 2.

Barley is cultivated under different climatic conditions ranging from arctic to tropical. It survives well in the temperate regions (with moist climates) and areas of high altitudes. However, barley rarely thrives in dry hot climates. The world barley production has been steadily increasing since the 1980s. Russia, France, Germany, Australia and Ukraine are the high producers of barley with an average quantity of 144 million tons produced annually in 2014 (FAOSTAT 2017).

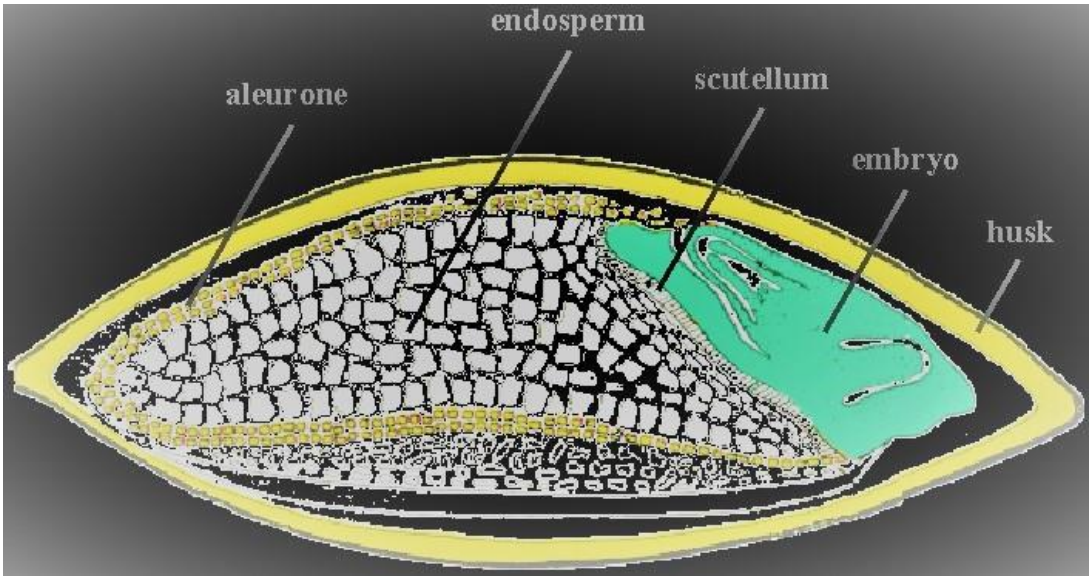

Figure 2. Structure of barley grain (Image source: kellyspantry.blogspot.com)

In 2016, an estimated 2.56 million acres of barley produced in U.S amounted to an average yield of 77.9 bushels/acre, leading to a gross production of approximately 199.9 million bushels. In 2016, the price of Barley averaged $5.17/bushel, resulting in the crop's value at $942.2 million (NASS, 2017). The physical layout of the seeds on the barley plant allows for variety classification. Thus, based on this identification system, it is categorized as either 6-row or 2-row. Barley is also labeled as hulled (husked) by the presence of beards, or awns, surrounding the kernels (Stanca *et al*., 2016). Six-row barley is cultivated

predominantly in some Mid-Western States (Idaho, South Dakota, Minnesota, North Dakota). Two-row barley is cultivated in the Mid-Western and Western States of America (Montana, Idaho, Colorado, Wyoming, Washington, Oregon, and California) (Taylor *et al*., 2017). Few brewing and malting industries develop unique barley categories suited for their work. Also, these companies engage specialist breeders for distinct barley varieties. Industries that specializes in breeding offers these companies a constant supply of high-quality barley including specific species. An estimated 150 categories of barley are produced on a minor scale in the US. The heterogeneity is always changing, new ones are produced and tested but others go into extinction. The barley varieties are categorized into four subdivision as follows:

The Manchuria - OAC 21 - Aderbrucker group, originating from Asia, are 6-rowed, awned, spring-type varieties with medium-sized kernels. These plants are tall with open or lax nodding heads but fail to thrive in dry climates. They are mainly used for malting and cultivated extensively in the upper Mississippi Valley.

The barley species cultivated in California, Arizona and Inter-Mountain Region uncover their ancestry from Northern Africa. They are 6-rowed, awned, with large kernels, and short to medium-length stems and possess medium to short spikes. They have quick growth rate and are not susceptible to breakdown. They mature well in the spring but in Arizona and California (having mild winters) they may be fall or winter grains.

Barley categories found at Tennessee winter group (originated from Caucasus and Korea (North and South) are 6-rowed, awned, with mid-long lax spikes which tend to nod. The plants are medium in height and can withstand the winter climate. These varieties are grown mainly on the southeastern belt of the United States.

The 2-rowed type encompasses sets hailing from Turkey and Europe; the Turkish category is well suited to regions with minimal rainfall. Varieties in this group are geographically cultivated in the Pacific and Intermountain States and mildly in the Northern Great Plains. The sets usually thrive in the spring seasons although two-rowed winter categories exist. Some varieties are used mainly for malting, others for feed (New Crop., 1999).

2.4 Barley Nutrition and Utilization

Barley is rich in nutrients and its utilization in food/meals present the consumer with health benefits that prevents lifestyle diseases. Table 2.1 presents the nutritional composition of barley grains. Whole barley kernel is estimated to comprise of 4-9%, 2-3%, 10-17%, 65-68% and 2-3% of β-glucans, free lipids, protein, starch, and minerals, respectively. Total dietary fiber is estimated to be less than 30%; contains soluble dietary fiber less than 20%. β-glucans, arabinoxylans, and cellulose are non-starch polysaccharide in barley however the major component which contributes to its energy value is β-glucan. The β-glucans content of barley grains is influenced mainly by genetic factors and very little environmental factors. Dehusked barley seed is made up of total dietary fiber (11 to 20%)-consist of insoluble dietary fiber (11 to 14%) and soluble dietary fiber (3 to 10%). Waxy naked barley grains also contain higher β-glucans content compared to those with hulled kernels; but no variation is seen between 2-row and 6-row varieties. Also, the endosperm of barley contain protein with medium nutritional amount, protein efficiency ratio measuring to 2.04. Moreover, barley protein amino acid composition is almost identical to other cereal grains; glutamine, proline and cysteine are high while lysine, threonine, methionine and tryptophan are limiting (Ulrich S. E., 2002). Barley contains

fatty acids such as palmitic acid, oleic acid, linoleic acid and linolenic acid; linolenic acid is the highly produced fatty acid in barley (Pitzer S., 2009).

Table 2.1 Nutritional content of 100g of raw pearled barley

Nutrient Available	Quantity
Protein	9.91 g
Total lipid (fat)	1.16 g
Carbohydrate, by difference	77.72 g
Fiber, total dietary	15.6 g
Sugars, total	0.80 g
Calcium, Ca	29 mg
Iron, Fe	2.50 mg
Magnesium, Mg	79 mg
Phosphorus, P	221 mg
Potassium, K	280 mg
Sodium, Na	9 mg
Zinc, Zn	2.13 mg
Thiamin	0.191 mg
Riboflavin	0.114 mg
Niacin	4.604 mg
Vitamin B-6	0.260 mg
Folate, DFE	23 µg
Vitamin A, RAE	1 µg
Vitamin A, IU	22 IU
Vitamin E (alpha-tocopherol)	0.02 mg
Vitamin D (D2 + D3)	0.0 µg
Vitamin K (phylloquinone)	2.2 µg
Fatty acids, total saturated	0.244 g
Fatty acids, total monounsaturated	0.149 g
Fatty acids, total polyunsaturated	0.560 g
Cholesterol	0

(United States Department of Agriculture, 2019. Nutrient Database, SR Legacy, 169705)

Phosphorus and potassium remain highly abundant elements in barley, followed by low levels of calcium, magnesium, zinc, iron and sodium. The barley grain is classified as having medium oxalate levels which is important to controlling kidney stones. Barley also contain phytochemicals (biologically active compounds produced by plant and microbes) like tocotrienols, sterols, flavanols, and phenolic acids; phenolic compounds are present at higher levels, averaging 0.2 to 0.4%. The flavanols identified in barley includes prodelphinidin B_3, procyanidin B_3 and catechins. (Das M. *et al*., 2016).

Due to the nutritional value of barley there is an increasing interest in using certain barley varieties. Dehusked barley grain is desired for consumption due to easier and faster post-harvest processing which make the final product very appealing and palatable. Also, husked barley can be dehusked, grinded, and sieved to take off the bran layers to develop rice-like products (Slavin J. L., 2000). In addition, barley grains can also be polished to separate the outer layers of the kernel and embryo; grains are refined to obtain small rounded endosperm pieces. Different studies have predicted that gritted barley can be substituted for rice integrated into porridges and soups or used as flour thickener in a variety of different food products including, yoghurts, muffins, flour snacks and extruded cereal products, just to mention a few (Edney M. J., 2002; Das M. *et al*., 2016). However, barley flour when used alone in bread or as a mixture possesses a poor baking quality (Newton et al., 2011). This is in conformity with the report by Stenca *et al*., (2019) that high levels of barley in bread reduces bread loaf volume, storage time and overall consumer acceptability.

2.5 β-D-glucan and its importance in human nutrition

β-D-glucan is a straight, nonbranched polysaccharide comprising of 70% 1-4-*O*-chained and 30% of 1-3-*O*-chained β-D-glucopyranosyl units (Figure 3). The β linkages in the polymer causes the β-glucan to be indigestible. However, β-glucans are highly fermentable in the caecum and colon. Size exclusion chromatography (SEC) represents a unique approach to quantify β-glucan and polysaccharides alike molecular weight and size (Lazaridou et al., 2003). SEC uses multiple detectors including refractive index detection (HPSEC-RI), multi-angle laser light scattering (MALLS) just to mention a few (Wei et al., 2006; Irakli et al., 2004). The various detectors can be used individually or in tandem. The molecular weight of β-glucan sourced from oats ranges from 3.5×10^4 to 2.5×10^5 g/mol and barley ranges from 6.3×10^4 to 1.3×10^6 g/mol.

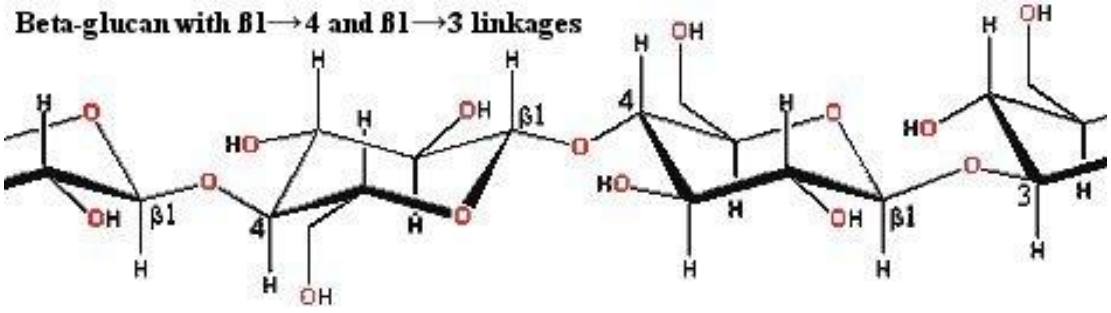

Figure 3. Structure of β-glucan

Table 2.2. Ranking of oat cultivars based on beta-glucan content in 2015 and 2016 South Dakota samples[a]

Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
GMI423	6.93 a	GMI423	6.86 a	GMI423	6.60 a
Newburg	5.35 b	Newburg	5.37 b	Newburg	5.35 b
Jury	5.20 bc	Horsepower	5.24 bc	Jury	5.30 b
Horsepower	5.14 c	Jury	5.14 cd	Horsepower	5.14 c
Rockford	5.13 c	Rockford	4.98 de	Rockford	5.06 c
Goliath	4.92 d	Goliath	4.93 e	Souris	4.87 d
Souris	4.89 de	Souris	4.88 e	Hayden	4.87 d
Hayden	4.75 ef	Hayden	4.86 e	Goliath	4.77 de
Deon	4.65 fg	Deon	4.62 f	Deon	4.68 e
Shelby427	4.53 gh	Shelby427	4.49 fg	Streaker	4.46 f
Streaker	4.38 hi	Streaker	4.36 gh	Shelby427	4.42 f
Stallion	4.32 ij	Stallion	4.31 hi	Stallion	4.35 fg
Jerry	4.18 j	Jerry	4.24 hi	Jerry	4.25 gh
SD110466	4.17 jk	SD110466	4.16 ij	SD110466	4.17 h
Colt	4.00 kl	Colt	4.02 jk	Colt	4.13 h
Natty	3.90 l	Natty	3.99 k	Natty	3.97 i

a Means with the same letter within each column is not statistically different from each other ($P<0.05$).

b NIRS: Near infrared-reflectance spectroscopy

(Paudel D., 2018. Rapid and Simultaneous Determination of Nutritional Constituents of United States Grown Oats Using Near-Infrared Reflectance Spectroscopy).

The starchy endosperm of barley, oat and wheat stores the majority of glucans, precisely in the aleurone and sub-aleurone layer of these cereals. Oats and barley are currently the richest cereal sources of β-glucan with oats containing 3.4g per 100g and 1 cup of cooked pearl barley containing approximately 2.5 g of β-glucans (Webb D., 2014).

Some oat varieties grown in South Dakota have been found to have high levels of β-D-glucan content. GMI 423 has a β-D-glucan content ranging from 6.60 – 6.93 %, Newburg ranges from 5.35 – 5.37 %, Jury ranges from 5.20 – 5.30 %, Horsepower has an average β-D-glucan content of 5.14% and Rockford ranges from 4.98 – 5.13, as shown in Table 2.2. Barley varieties grown in North America have been found to have a β-D-glucan content ranging from 4.38 – 7.49 % (Izydorczyk *et al*., 2000). Animal and human studies have shown a correlation between barley, oat and health benefits. Beneficial characteristics include the ability to lower blood cholesterol levels and improve insulin response, thereby reducing the risk of type-2 diabetes. The high levels of β-glucans in barley and oat results in high intestinal viscosity and slow absorption of food, leading to an efficient control of blood glucose level. β-glucan has exceptional functional and nutritional properties including its ability to maintain high viscosity at relatively low concentrations.

Viscosity is an important rheologic (defines the flow and deformation of matter) attribute of β-glucan. It is related to favorable physiologic responses that adjust appetite modulation including lagged gastric passing and detained intestinal movement. This property of oat and barley β-glucan helps to promote satiation/satiety and there is evidence to indicate that this has a modest long-term effect on weight loss, hence reducing the prevalence of obesity (Rebello *et al*., 2014). β-glucan products have also been shown to have cholesterol-lowering effects in humans (Immerstrand, T. 2010). An elevated level of blood

cholesterol, total and low-density lipoprotein (LDL) cholesterol, are major risk factors for atherosclerosis hence a desirable therapeutic alternative using dietary regimes is being sought to reduce this disease (Jenkins *et al*. 2005). Also, β-glucan has been employed as a therapeutic in different clinical studies to reduce blood glucose level. β-glucans are beneficial in managing diabetes and causes the lowering of blood glucose levels in many ways (Sangwan *et al*., 2014). In addition, β-glucan is known to function as a prebiotic, which stimulates growth of beneficial colon bacteria, such as bifidobacteria.

2.6 Effects of β-glucan on satiety and appetite regulation

Appetite denotes a complex association among four traits: behavioral profile, external environment, stored and metabolism energies, and subjective states. Satiation and satiety are two processes that occur after adequate food intake limits hunger and prevents further intake. Satiation mainly builds up in the period of eating and automatically stops meal intake. Contrarily, satiety is the period that additional eating is prevented, and it happens prior to eating episode. Satiety does not occur instantaneously but occurs over a period of time (Blundell *et al*., 1996). The consumption of high dietary fiber food results in prolonged oral exposure, a condition which is ripe for releasing signals that mediate satiety. Dietary fiber increases the volume of food but reduces the energy needed to metabolize food (Baer *et al*., 1997). Meals that have substantial fiber content positively influence the rate of gastric emptying (frequently steady gastric emptying) due to increased energy required. As a result of increased viscosity of gastrointestinal contents caused by high β-glucans, nutrients take a longer time in intestines before they are absorbed. The prolonged presence of nutrients in the GI tract prolongs the interaction between nutrients and the intestinal mucosa thereby facilitating the release of satiety

promoting hormones, such as peptide YY and glucagon-like peptide 1 (Juvonen *et al*., 2009; Rebello *et al* 2016).

2.7 β-glucan food products

Aside from the nutritional benefits of β-glucan, there also known functional properties such as its usage as a thickener, stabilizer, emulsifier, and to gelatinize food products. These properties determine the suitability of incorporating β-glucan into various food products. All these properties qualify β-glucans to be used as a replacement thickener for xanthan gum, pectin, alginates and gum Arabic. β-glucan of barley origin significantly improved bread loaf volume when inculcated into bread flour and also elevated the soluble fiber content (Trogh *et al*., 2004; Ahmad *et al*., 2008) as well as the stiffness of the bread crumb (Lazaridou *et al*., 2007). Krishnan *et al*., (1987) reported on the increase in farinograph and baking absorption with increasing levels of oat bran in bread formulations. β-glucan of barley and oat origins when added to cake batter improved the rheological and physical properties (Kalinga and Mishra, 2010). The addition of β-glucan into pasta products yielded a reduced glycemic index (Yokoyama *et al*., 1997). Also, Jenkins *et al*., 2002 reported lowered glycemic response in β-glucan supplemented breakfast bar. β-glucan also finds its application in beverages (Lyly *et al*., 2003; Temelli *et al*., 2004) and dairy based products (Konuklar *et al*., 2004). Rinaldi and colleagues, 2015, discovered that yogurts enriched with β-glucan and pectin demonstrated quicker proteolysis-faster release of free amino acids and slower breakdown of large peptides and vice versa. In addition, β-glucan combined with other soluble dietary fiber improve gelation and rheologic properties when added to low-fat dairy products and low-fat cheese curds (Tudorica *et al*., 2004). When the Food and Drugs Administration (FDA) analyzed

researches that studied the consumption of muffins, bread, shakes, etc., it concluded that the incorporation of at least 3 g of β-glucan to decreased serum total cholesterol in clinical studies (FDA, 2003).

2.8 Development of new functional foods

It is the duty of science and industry to educate consumers concerning well-being and the role of foods in improving quality of life during new functional foods development. Functional foods are natural or processed foods that contain known active chemical agents which when taken in required proportions, offers clinical health benefit. Therefore, it is a key source in the prevention, management, and treatment of medical conditions. Foods for weight control and health management fit into the category of functional foods (Krishnan P., 2016). The valuable physiological and nutritional attributes of oat and barley by β-glucans and other dietary fiber components, tocopherol, and antioxidant level position oat and barley in the category of functional foods and hence has generated an increased demand for oats and barley in human nutrition.

Developing new food products is a multi-step process that requires generation of innovative ideas and concepts with an in-depth knowledge about the product and the consumer market. Furthermore, feedback from academic, commercial, and regulatory sources help to refine the product. The consumer market is dynamic and fast changing, and demand for new food products is consistently changing with respect to shifting of needs, wants and technology. Scanning the market can be either a thorough search to see whether a current commercial product can cover an identified need or a firm can keep abreast of new innovative products and even be inspired by it (Learning L., 2015). Food is increasingly being associated with human health in that it offers an alternate option that

can help prevent, manage and cure several ailments. For some customers, this inclination comes from personal health challenges or adaptation to desired taste. Other people are driven by increasing quality of life and preventing illness; for majority of the populace it is about well-being. These market tilt now present unique challenges for the food and beverage companies to satisfy consumer demands.

Over the last decades, lack of vital nutrients has significantly decreased; many American citizens can now anticipate increased quality of life (Befort *et al*., 2012). Nevertheless, the rates of chronic diseases have increased due to low quality diet and less physical activity. Almost fifty percent (50%) of American adults suffer from diet-related noncommunicable diseases, viz, cardiovascular disease, type II diabetes and obesity. The 2015–2020 Dietary Guidelines for Americans policy identified that various enriched grain-based food products are great sources for several deficient nutrients. These nutrients encompassed dietary fiber, folate, iron, and magnesium. Eat habits based on the idea of lowering sodium, total fat, sugar but encourage nutrient-dense grain foods, could aid the shift of food consumption in children and adults toward required intake of nutrient levels identified by 2015 DGAC.

2.9 Beverage Development Process

The behavior of β-glucan in beverages is an attractive and less-studied field (Lyly *et al*., 2003). The elevated viscosity of β-glucan is thought to affect its sensory properties and functional qualities. The assessment of β-glucan viscosity is mainly dependent on its concentration and molecular weight (Wood *et al*., 2000). The increase in β-glucan molecular weight or concentration directly increases its viscosity. Even though there is scanty of information relating to effects of molecular weight on β-glucan efficacy to

increasing satiety and reducing blood glucose, larger molecular weight β-glucan has been shown to reduce blood glucose level compared to low molecular weight β-glucan (Wolever *et al*., 2010). However, Biörklund *et al*., (2005); Naumann *et al*., (2006) demonstrated that low Mw β-glucan (70-80 kg/mol) have the ability to reduce the low-density lipoprotein cholesterol levels when consumed in beverages. Hence, food matrices into which beta glucan is introduced seems to influence the biological activity of β-glucan. The choice of novel food products is mainly influenced by its sensory qualities (Arvola *et al.*, 1999). Lyly *et al*., (2003) in a research evaluating the influence of oat β-glucan preparations on mouthfeel perceptions, concluded that the viscosity effect of β- glucan, may cause challenges in developing high moisture formulations which possess desirable sensory attributes. To assure the safe intake of foods containing β-glucan, products need to have an overall acceptable sensory quality.

2.10 Hydrolysis of β- glucan

High Mw β-glucans when applied to beverages (example of high-moisture applications) yield aggregated and semi-solid or concentrated dispersions. Critical concentration (cc) is determines the concentration at which β-glucan aptamers begin to interact with one other. At cc, the diluted solution undergoes transitions where it changes from semi-diluted into a final concentrated solution (Sibakov *et al*., 2013). To maintain an elevated amount of β-glucan but prevent its aggregation in foods with high-moisture content (example beverages), β-glucan needs to be in the diluted region (elevated cc and reduced viscosity). To lower viscosity, the Mw of β-glucan must be reduced through controlled acid- or enzyme-catalyzed depolymerization (Kaukovirta-Norja *et al*., 2009; Sibakov *et al*., 2013). Both strategies are applicable in new food product formulations.

2.10.1 Enzymatic hydrolysis of β-glucan

Enzymatic hydrolysis is a specific and controlled procedure for the breaking down of many polysaccharides. One of the simplest techniques to analyze the structure of β-glucan is through hydrolysis using lichenase (a β-D-glucanase enzyme: cleaves β-(1-4)-chains adjacent to α-(1-3)-chains) (Colleoni-Sirghie *et al*., 2003). The polysaccharide unit derived include (1-4)-linkages of β-glucan with (1-3)-linked terminal group. Moreover, enzymes like cellulase that hydrolyze only β-(1-4)-glycosidic linkages (Roubroeks et al., 2001) and β-glucosidase that cleaves β-glucosidic bonds depolymerizes β-glucan samples. To quantitatively determine β-glucan in cereal products, β-glucosidase and lichenase enzymes can be used for this analysis (Johansson *et al*., 2000). Tosh and coworkers (2004) conducted a study to produce partially cleaved oat β-glucan by regulating its molecular weight distribution under distinct enzymatic hydrolysis mechanisms. The viscosity of oat β-glucan appears to depend on molecular weight. However, the efficacy of weight loss of β-glucan as a function of molecular weight is never documented (Doublier & Wood, 1995). In Doublier and colleagues, (1995) studies, oat gum was hydrolyzed to different extents to yield aimed molecular weights of 40,000, 100,000 and 200, 000 g/mol. After the required molecular weight was obtained the digest was heated to inactivate the enzymes to halt the partial enzyme hydrolysis process. Bae and coworkers (2009) researched the impact of partial enzyme hydrolysis on oat β-glucan against weight gain and lipid-profile of mice. In their research, oat β-glucans were partially hydrolyzed by enzyme treatment to yield different molecular weights. Afterwards, effects on weight loss and lipid profile were evaluated in preclinical models. It was concluded that the molecular weight of oat β-glucan had different correlations with weight gain and lipid profile of mice. Partial

enzyme hydrolysis of oat β-glucan was demonstrated to have great potential when applied in different food products with minimal impact on biological functions.

2.11 Non-enzymatic hydrolysis of β-glucan

Cereal β-glucan easily forms highly viscous solutions because of its water solubility and high molar mass. Several methods have been used for β-glucan degradation in other to reduce viscosity (affected by molecular weight of polymer). Non-enzymatic methods are useful in degrading β-glucan. Non-enzymatic processes like oxidation, thermal degradation, acid hydrolysis, alkaline degradation and hydrolysis by mechanical energy are widely used. For this study, the focus for non-enzymatic hydrolysis will be on acid hydrolysis.

Acid treatment is a universally utilized hydrolysis procedure in the breakdown of polysaccharides including oat β-glucan (Tosh *et al*., 2004). Hydronium ions assist in the breakage of glycosidic bonds at elevated temperatures. The proton of the catalyzing acid reacts with the glycosidic oxygen, followed by attraction of water molecules, which leads to breakage of glycosidic bond and formation of stable hydrolysis products. The reaction rate and products of acid hydrolysis are influenced by: acid type, concentration, pH, temperature, pressure, and molecular properties. Under partial hydrolysis conditions, the polysaccharide linkages haphazardly cleaved, resulting in lower molecular weight products. Under stronger conditions and at higher temperatures, hydrolysis yields oligosaccharides and monosaccharides (Kivela, 2011).

Ascorbic acid at a pH range of 4.2 to 11.6 can hydrolyze polysaccharides (Robertson *et al*., 1941; Kertesz, 1943). Ascorbic acid produces hydroxyl radicals through the Fenton reaction, and this helps to degrade water soluble polysaccharides. Kivela and coworkers

(2009), observed that the addition of ascorbic acid (10 mM) in the presence of iron sulphate, yielded a decrease in viscosity (by 50%) of the solution. The Mw of β-glucan was lowered from 520,000 to 35,000 g/mol. The viscosity decrease was inhibited by introducing glucose, that reinforces the function played by hydroxyl radicals in the non-enzymatic breakdown of cereal β-glucan. This highlights the usefulness of glucose in inhibiting further hydrolysis during partial acid hydrolysis. Partial ascorbic acid hydrolysis was chosen as a second treatment (in addition to partial β-glucanase treatment) to reduce oat and barley viscosity in our study.

CHAPTER 3. MATERIALS AND METHODS

In the development of this healthy food product, much care and attention were given to consumers preferences through market surveys and preliminary investigations, taste, diversity, and novelty of products, trends, safety, nutrition, and portion control. The proposed beverage will contain on average, 150-200 calories, 10 to 15 g of protein, up to 12 g of carbohydrates, and a target of up to 5 g of dietary fiber to be effective as a health food product (FDA 21 CFR 101.54). These parameters were set in line with FDA's code of federal regulations Title 21 on specific requirements for health claims of food.

3.1 MATERIALS

Two oats varieties, namely GMI423 and Natty, were selected for their protein content and dietary fiber content. GMI423 and Natty cultivars were obtained from General Mill Inc, and the South Dakota State University Oat Breeding Program, respectively. Hulless barley was purchased from Bob Red Mill. β-glucanase enzyme was procured from Enzyme Innovation (Chino, CA) and ascorbic acid was purchased online through Amazon. Vanilla flavoring and other specialty ingredients were obtained from commercial sources.

3.2 METHODS

3.2.1 Market survey and preliminary investigation

A market survey was carried out at the two major grocery supermarkets in Brookings SD, with these being, Walmart and Hy-Vee Supermarket. Hunger suppressing beverages (potential competing products) which are generally in different aisles of the store but mostly found in the health market section were listed. During the preliminary investigation, product screening was conducted with similar products on the market to

ascertain the preferences of the a 10-member consumer panel, regarding taste, appearance, aroma, and texture (viscosity). This investigation followed the Stage-Gate process, as outlined by Edgett S., (2015).

3.2.2 Prototype Development (Beverage formulation)

Figure 4 provides a flow diagram outlining the major steps in the experimental design of this study. Oat grains were dehulled with the use of the Codema oat dehuller (Maple Grove, MN) in the Seed House of South Dakota State University. A kilning process was then used in pretreating the oat and barley groats (Decker *et al*., 2014). The kilning step involved steaming the grains at 0.24mPa at 105°C for 16 min, followed by application of dry heat at 101°C (215°F) in the laboratory dry oven for 30 min to deactivate lipase, lipoxygenase and peroxidase enzymes (Gates F., 2007; North America Millers Association, 2007). The dried grains were then pulsed in a food processor for 5 seconds to increase the surface area for the toasting step. The grains were toasted separately at 300°C in the dry oven on trays for 10 mins and subsequently ground into flour using the 0.2mm sieve in the Retsch Ultracentrifugal grinding mill (Retsch GmbH, Hann, Germany). Beverage Formulations: Five different levels of oat (O) and barley (B) flour blends from the two oat varieties (GMI423 and Natty) were formulated in bulk as follows: 100% oat, 90%Oat:10%Barley, 80%Oat:20%Barley, 70%Oat:30%Barley and 60%Oat:40%Barley. The V-shaped Cross Flow Laboratory Scale Blender (Patterson-Kelley, Harsco, East Stroudsburg, PA) was used in creating a homogenous mixture of the flour blends.

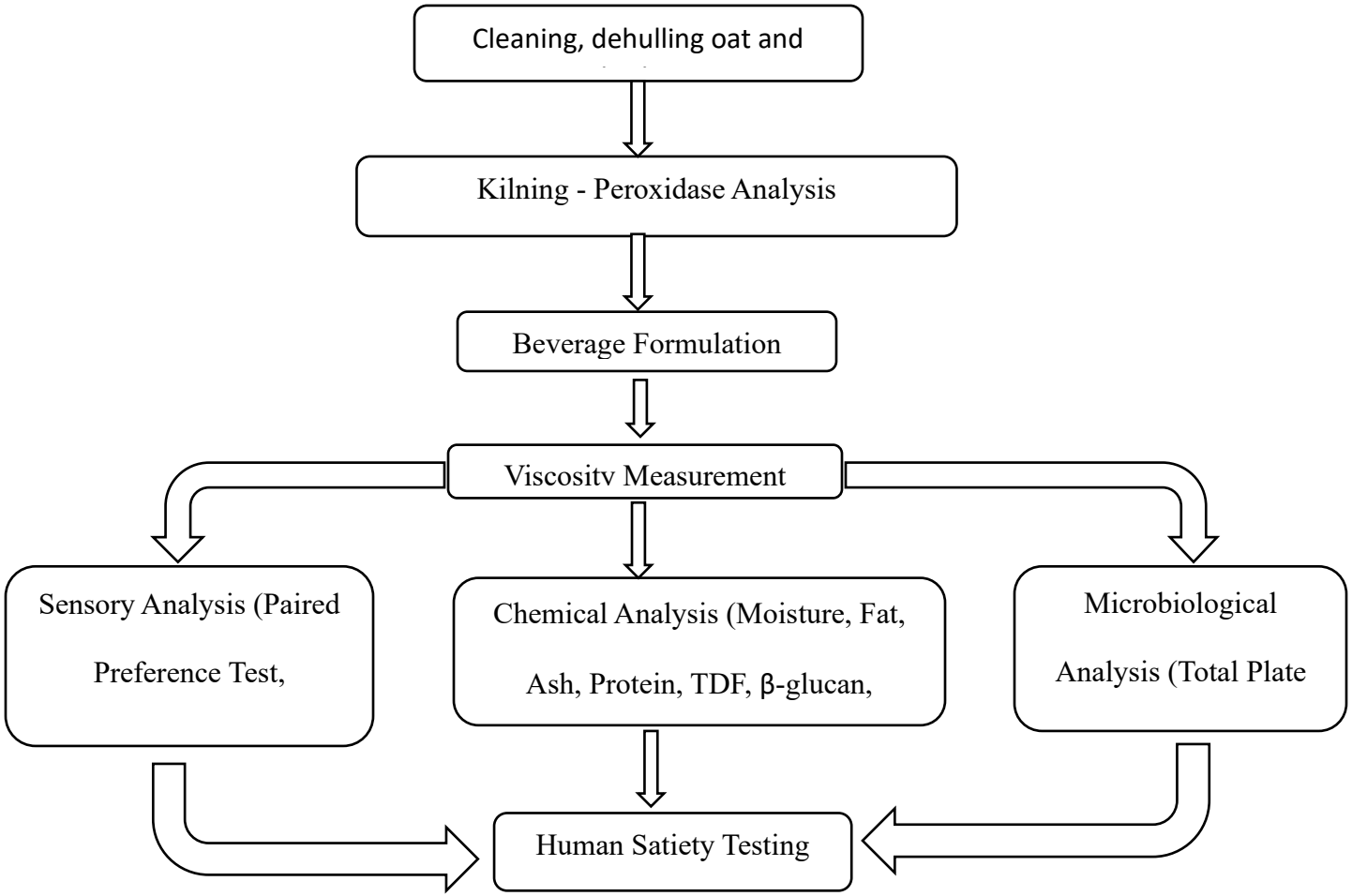

Figure 4. Flow diagram detailing experimental design for development of β-glucan beverages, nutritional analysis, microbiological analysis and satiety response testing.

Ingredients and formulations for the beverages are provided in Table 3.0. To make one liter of the beverage, a slurry mixture comprising 120g of flour in 600ml of water was prepared in 2000ml Erlenmeyer flask. The slurry was passed through a 300-micron mesh hand-held strainer to ensure no grittiness in the beverage. The temperature of the controlled environment was set to 70°C for partial acid-hydrolysis and to 50°C for partial enzyme-hydrolysis with constant stirring using the Thermo Scientific Cimarec Stirring Hot Plate (Thermo Fisher Scientific, United States). Partial enzyme hydrolysis with 2ml β-glucanase (500nkat/ml) was carried out for 3 mins at 50°C with constant stirring and immediately followed with inactivation of β-glucanase enzyme by heating the digest to 80°C (Sibakov *et al*., 2013). Partial acid hydrolysis with 2.64g ascorbic acid and 0.0023g iron sulphate was carried out for 30 mins at 70°C with constant stirring (Mäkelä N., 2017). This reaction was immediately followed with the introduction of 45g glucose in the solution to inhibit further viscosity decrease. For both enzyme and acid hydrolyzed digests, 400ml of lactose-free, fat-free (LFFF) milk, 12g of vanilla flavor and less than 1% of stabilizers (which included xanthan gum and calcium carbonate) were added. To the acid hydrolyzed digest, 15g of no-calorie sweetener was added to make the beverage. To the enzyme hydrolyzed slurry, 55g of the no-calorie sweetener was added to complete the formulation. The beverages were then pasteurized in a Kleen Flo Batch Pasteurizer (Maysville, MO) at 161°F for 25 seconds followed by rapid cooling (IDFA, 2018). The beverage was then packaged into sterile glass jars ready for use.

Table 3.0. Beverage formulation for 120ml batches showing proportion of oat and barley fractions and ingredients used in beverage production

Ingredient	100%O	90%O:10%B	80%O:20%B	70%O:30%B	60%O:40%B
Oats(g)	12	10.8	9.6	8.4	7.2
Barley(g)	0	1.2	2.4	3.6	4.8
LFFF milk(ml)	40	40	40	40	40
Sweetener(g)	5.5	5.5	5.5	5.5	5.5
Stabilizers(g)	0.6	0.6	0.6	0.6	0.6
Flavoring(g)	1.2	1.2	1.2	1.2	1.2
Water	60ml	60ml	60ml	60ml	60ml

LFFF milk = Lactose free Fat free milk, Sweetener = Erythritol and stevia leaf extract, Stabilizers = Xanthan gum and calcium carbonate, Flavoring = Vanilla Extract.

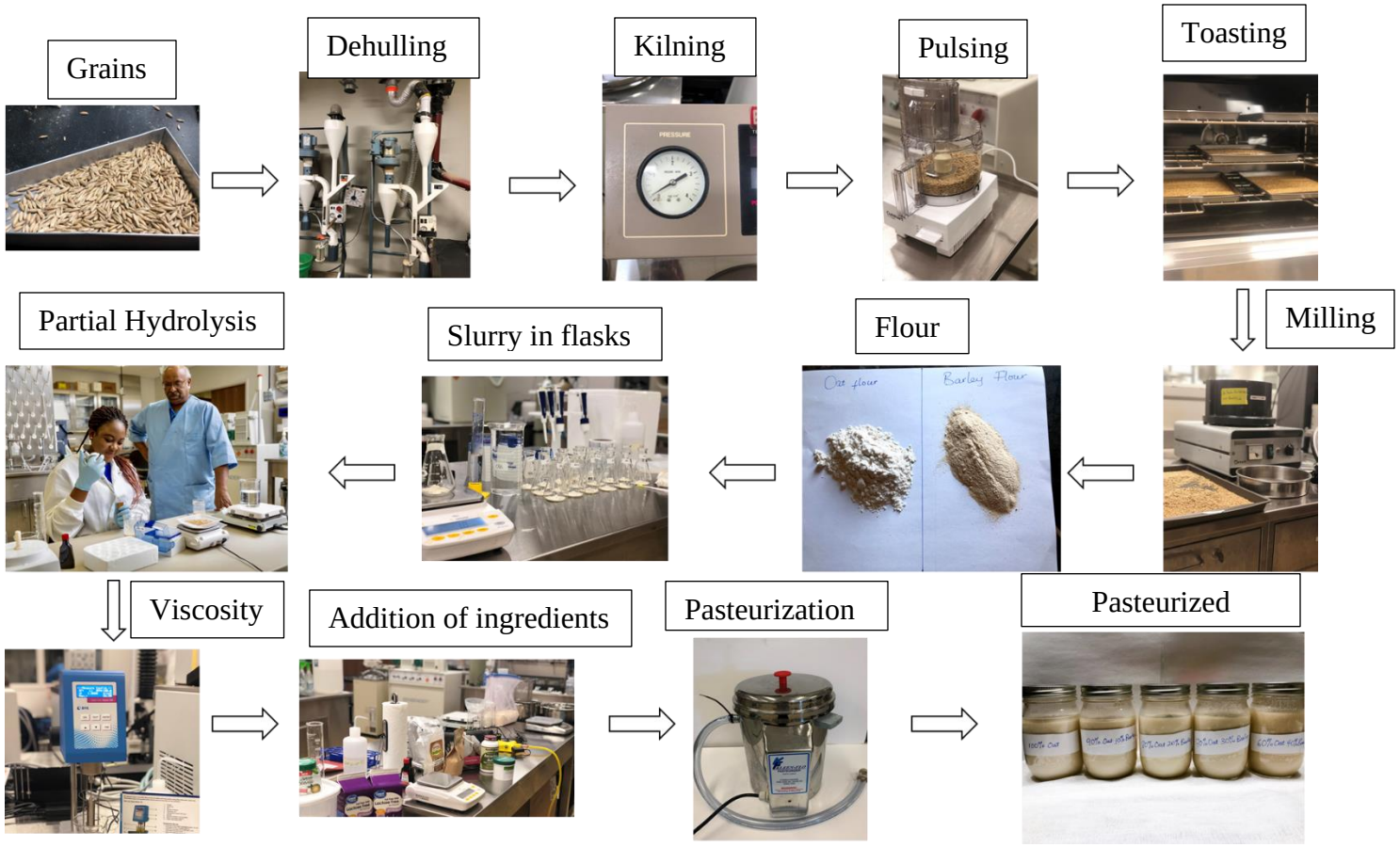

Figure 5. Flowchart of the beverage formulation process.

3.2.3 Number of treatments

Forty-four beverages were formulated during this study. Forty of the beverages were the experimental samples which were developed from two oat varieties (Natty and GMI423). These two oat flour varieties were individually combined with barley at the previously stated blend levels to create five (5) varying blends of oat and barley from each variety. At the beverage phase, two partial hydrolysis procedures (enzymatic and acid hydrolysis) were used on each of the blends and each beverage had two replicates. Hence:

Treatment = 2 oat varieties X 5 blends X 2 hydrolysis treatments X 2 replicates

Table 3.1 shows a summary of the number of beverage treatments produced. The other four beverages were the unhydrolyzed 100% GMI and unhydrolyzed 100% Natty (control samples) which were also prepared in duplicate.

Table 3.1. List of samples and treatments (n = 44) for production of oat/barley beverages

	Hydrolysis Treatment		
Varieties	Partial Enzyme Hydrolysis	Partial Acid Hydrolysis	Unhydrolyzed (controls)
GMI423	100G***a***	100G***a***	100G***a***
	100G***b***	100G***b***	100G***b***
	90G10B***a***	90G10B***a***	
	90G10B***b***	90G10B***b***	
	80G20B***a***	80G20B***a***	
	80G20B***b***	80G20B***b***	
	70G30B***a***	70G30B***a***	
	70G30B***b***	70G30B***b***	
	60G40B***a***	60G40B***a***	
	60G40B***b***	60G40B***b***	
Natty	100N***a***	100N***a***	100N***a***
	100N***b***	100N***b***	100N***b***
	90N10B***a***	90N10B***a***	
	90N10B***b***	90N10B***b***	
	80N20B***a***	80N20B***a***	
	80N20B***b***	80N20B***b***	
	70N30B***a***	70N30B***a***	
	70N30B***b***	70N30B***b***	
	60N40B***a***	60N40B***a***	
	60N40B***b***	60N40B***b***	

G = GMI423 oat variety, N = Natty oat variety, B=Barley, ***a*** = first replicate, b = second replicate.

3.2.4 Physicochemical and Microbiological analysis

At various stages in the prototype development, physicochemical and microbiological analyses were carried. All sample analyses were carried out in duplicate. Peroxidase analysis was carried out on pretreated grains to verify inactivation of lipase, lipoxygenase and peroxidase enzymes. The other compositional analysis included moisture, total fat, protein, amino acid profile, minerals, element, viscosity, pH, total dietary fiber, β-glucan, carbohydrate and caloric content.

3.2.5 Peroxidase Analysis

Oat and barley grains contain lipid-hydrolyzing enzymes namely, lipase, lipoxygenase and peroxidase. These enzymes, when not controlled, convert triacylglycerols and unsaturated fatty acids into non-esterified fatty acids and hydroperoxides, respectively. This reaction produces off flavors and renders the product more susceptible to developing oxidative rancidity (Decker E. *et al*., 2014). The effectiveness of the kilning step, which is intended for enzyme inactivation is assessed by measuring peroxidase activity since peroxidase is more thermostable compared to lipase and lipoxygenase. We followed the AACC method (22-80) for the qualitative analysis of peroxidase activity. We used a coffee grinder in grinding approximately 10g of micronized groats for 30s, after which the groats were passed through a No. 20 sieve to achieve a fine consistency. Any residue with a mass greater than 10% the original size was ground for a third time. We subjected 1g of all sifted samples to enzymatic testing in an Erlenmeyer flask, containing fifty milliliters of water at room temperature. We added two milliliters of 0.1 mL of hydrogen peroxide (4 mL of 30% H2O plus 96 mL of water), 3 mL of sodium 2,6-dichloro-indophenol solution (0.1 g in 500 mL of water) and ascorbic acid solution (0.5 g in 500 mL of water), under

vigorous mixing. The flask was warmed 38°Cfor 5 min, swirled and re-warmed for an extra 5 min. A negative peroxidase result was recorded after 10 minutes of no visible color change, whereas a definite blue color was considered as peroxidase positive, indicating the presence of active peroxidase enzymes.

3.2.6 Particle Size Analysis

The particle size of the flours was determined using the Ro Tap sieve shaker prescribed by the official AACC Standard 55-60.01 (AACC, 2011). In this method, a stack of sieves arranged in order of #40, #60, #80, #100, #200, pan with #40 being on the top, were placed securely on the Ro-Tap machine and run for 5 minutes. The fractions obtained on each sieve were then weighed. We calculated the geometric mean diameter (dgw) for each sieving replicate based on the formula documented in the ASAE Standards (2003).

3.2.7 Viscosity Analysis

The rheological characteristics of the beverage samples were determined by using a Byko -visc basic EX rotational viscometer (BYK - Gardner USA, Columbia, MD) together with a Sper Scientific Immersion thermometer. The measurements were performed by transferring 500 ml of each sample into a 600ml beaker, making sure the sample was free of air bubbles. At a temperature of 25°C and rotating speed of 30rpm, the viscosity of the samples was assessed by immersing the spindle laterally into the center of the sample. All measurements were carried out in duplicate and viscosity was reported as centipoises (cP).

3.2.8 pH Analysis

The pH of the beverage was determined using the official standard method AACCI 02-52.01.

This method measures the pH of aqueous samples where the aqueous phase constitutes at least 20% of the total volume of the sample.

3.2.9 β-glucan content

The β-glucan content in our flour blends, beverage samples and standards (oat and barley), were determined by following the AACCI method 32-23.01 (AACCI, 1999) by using a mixed β-glucan linkage kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Samples of flour blends (80 - 120 mg) were individually dissolved under constant stirring in 0.2 mL of 50% (v/v) ethanol and 4.0 mL of 20 mM sodium phosphate buffer (pH 6.5). The resulting solutions were boiled for 3 min and later equilibrated to 50°C. Beverage samples (3 mL) were then warmed at 100°C in a water bath for 5 min followed by subsequent cooling at room temperature and treated with 8 mL of ethanol (95%). This process was done to remove residual sugars in the beverage. After centrifugation at 3000 × g for 10 min, the supernatant was discarded and the pellets suspended in 8 mL of aqueous ethanol (95%), again centrifuged, reconstituted in 20 mM sodium phosphate buffer (pH 6.5) to total volume of 4mL and incubated at 50°C for 5 min. After incubation, all samples were treated with 0.2ml of lichenase enzyme and incubated at 50°C for 1 hour under constant agitation to ensure complete enzymatic digestion. 5 mL of 200 mM sodium phosphate buffer (pH 4.0) was added to the mixture and centrifuged at 1000 × g for 10 min. 0.1mL aliquots of supernatants from the mixture was carefully pipetted into three 12ml test tubes. 0.1 ml β-glucosidase enzyme diluted in a 50mM sodium acetate buffer was dispensed into two reaction tubes whereas to the third tube, we pipetted 0.1ml of 50mM sodium acetate buffer without any enzyme. All three tubes were warmed at 50ºC for 10 minutes, after which 3ml of GOPOD (glucose oxidase peroxidase) was dispensed

into each tube followed by incubation at 50ºC for 20 minutes. We measured the absorbance of the GOPOD reaction at a frequency of 510nm on a spectrophotometer, within 1 hour of incubation, using one and half milliliter (1.5ml) cuvettes. The measurements were imported to Mega Calc. software (Megazyme Inc.) and the amount of β-glucan was expressed as dry weight through moisture correction. For each set of GOPOD measurement, we calculated an F-factor using a reagent blank and D-glucose standard of 50µg and/or 100µg. The reagent blank comprised of a mixture of 0.1ml of distilled water, 0.1ml of sodium acetate buffer (50mM) and 3ml of GOPOD reagent whereas the glucose standards was formulated as 0.1ml of D-glucose standard (50µg/0.1ml or 100µg/0.1ml) diluted in 3ml of GOPOD reagent and 0.1ml sodium acetate buffer (50mM) (McCleary and Codd, 1991).

3.2.10 Sample Preparation for Chemical Analysis

The beverage samples were poured into trays and placed in a freezer until they were fully frozen. The trays were then placed in the Harvest Right Scientific freeze dryer and an initial freezing was carried out to -30°F. During the drying process, the temperature increased to a maximum of 60°F, in order to preserve all the nutrients. A vacuum pressure of 600mTorr was maintained throughout the freeze-drying process. Figure 6 shows a picture of the freeze-dried beverage process.

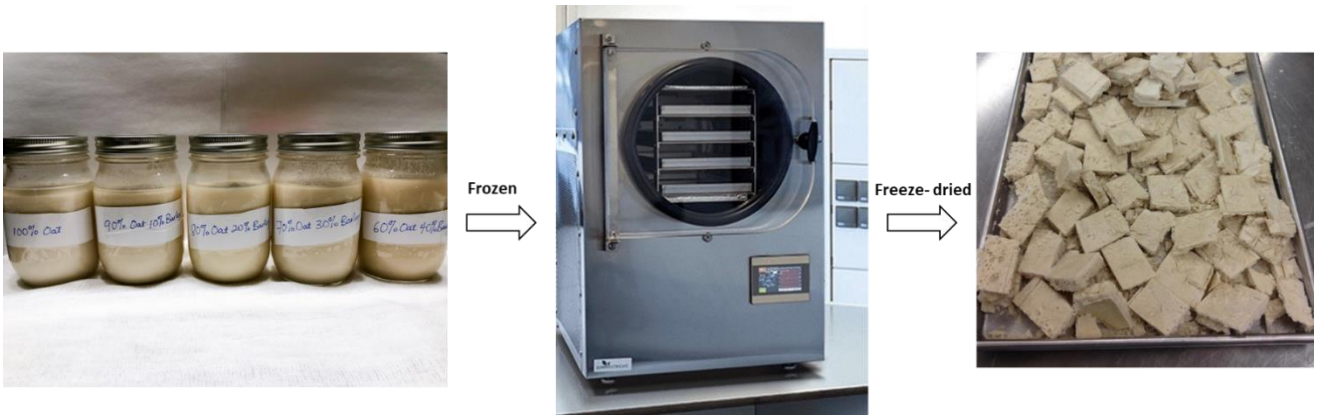

Figure 6. Freeze-drying of oat-barley beverages for compositional analysis.

3.2.11 Moisture Content

The moisture content in each flour and beverage sample was measured in a forced air convection oven heated to 130°C for 1 hour. The loss of water was used to calculate the moisture content according to the American Association of Cereal Chemistry (AACCI) oven drying method 44-15.02.

Moisture (%) = 100 x (W2-W3) W1
Where:

W1= original weight of the sample
W2= Initial weight of the aluminum dish + sample
W3= Final weight of the aluminum dish + sample

3.2.12 Fat Content

The fat content was determined according to the American Oil Chemists' Society (AOCS), Am 5-04 method using an Ankom^{XT15} Crude Fat extractor (ANKOM Technology, Macedon, New York, USA). Fat extraction was achieved by first recording the weight of empty filter bags (W1) and weighing approximately 1.5g to 2g of sample into the bags. The sample weight was recorded and the mouth of the filter bags with sample were sealed shut with a heat sealer. Samples were pre-dried before fat extraction in

a forced air convection oven heated to103°C for 3 hours. The hot samples were cooled in a desiccator for 10 minutes at room temperature. The weight of the cooled filter bags were recorded as (W2). By using a fat extractor, fat content was extracted from the filter bags at 90°C for 60 minutes with continuous solvent recycling. The solvents' high temperature (twice its boiling point) and elevated pressure in the sealed chamber accelerated the kinetic extraction. Finally, thefat content was determined by measuring the change in mass after fat extraction from the sample in the filter bag.

$$\text{Crude Fat (\%)} = \frac{W2-W3}{W1} \text{ X } 100$$

Where:

W1= Original weight of sample

W2= Weight of pre-extraction dried sample and filter bag

W3 = Weight of dried sample and filter bag after extraction.

3.2.13 Protein Content

The estimate protein content of the flours and beverages, we applied the enhanced Dumas combustion method 46-30.01 (AACCI, 2000) using the CE Elantech Flash EA 1112 (Lakewood, NJ). Here, we incinerated 75mg of samples at a high temperature of 900˚C in the presence of oxygen, leading to the loss of N_2, CO_2 and H_2O. H_2O and CO_2 gases released as a result of the combustion are absorbed by a special column which contains magnesium perchlorate and soda lime.. N_2 gas is measured and converted into $N_2$with a thermal conductivity detector (TCD) column . Using a conversion factor of 6.38 for beverage and 6.25 for flour we derived the percentage protein content from percentage nitrogen content. All protein values were expressed on a dry weight basis.

3.2.14 Amino Acid Profile

Amino acids analysis was carried out at the University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL) by employing the AOAC SMPR 2014.013 method, based on the principle of cation-exchange chromatography (cIEC-HPLC) and post-column ninhydrin derivatization as well as quantitation.

3.2.15 Ash Content

The ash content of the various samples was determined by incinerating samples at 525°C for 12 hours in a muffle furnace (Box Furnace, 51800 series). The dry oxidation method according to the AACC 08-03 method was used to estimate the total inorganic mineral content.

$$\text{Ash (\%)} = \frac{(\text{Crucible weight after ash} - \text{Weight of empty crucible})}{(\text{Original sample weight})} \times 100$$

3.2.16 Element Analysis

This analysis was carried out at the University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL) using the Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) instrument where samples are introduced into the ICP instrument in a liquid form. In this procedure, soluble samples are first wet-digested under microwave-assisted combustion by using nitric acid and hydrogen peroxide. Plasma energy in the form of ionized argon gas is introduced into the sample to excite the component elements (atoms). Excitation of the combusted atoms result in specific spectra of emitted rays whose photon wavelength are recorded. Determination of the elements

present in a sample is based on the position of the photon rays whereas the intensity of the element is based on the photon ray intensity (Ghosh *et al*., 2013).

3.2.17 Total Dietary Fiber (TDF)

The non-digestible fibers in the samples were estimated by the automated ANKOM TDF Dietary Fiber analyzer. We used a filter bag technology to determine the TDF present in our samples based on the weight of the recovered TDF residue corrected for ash and protein content according to the AOAC 991.43 method. In this procedure, samples were cooked at ~ 95 -100°C in the presence of heat stable α-amylase to facilitate gelatinization, hydrolyzation and depolymerization of the starch content in the samples. Samples are then incubated at 60°C with a protease (to hydrolyze proteins) and amyloglucosidase (to breakdown starch to glucose). The depolymerized protein and starch were precipitated using four parts of ethanol. Diatomaceous earth (celite) is also used in this procedure to enhance filtration efficiency. Concentrations of α-Amylase (1.25 ml), protease (2.5 ml), and amyloglucosidase (5.0 ml), diluted in 25 mL volumetric flasks before addition to designated sample holders. MES-TRIS buffer, 78% ethanol, deionized water, 90% ethanol and 6N HCL were also added to designated containers on the instrument. Crucibles were conditioned for 3 hours in a muffle furnace at a temperature of 600° C. The crucibles were cooled and stored in a desiccator. SDF filter bags and IDF Flow-Thru bags were installed on Ankom™ Dietary Fiber Analyzer. Celite and the samples were rinsed with 3ml of deionized water into SDF bags. Using clamps, the bags were sealed followed by instrumentation to measure TDF content. Samples bags were agitated automatically throughout the extraction process. The instrument was also programmed to automatically add reagents at the appropriate steps in the process. pH was manually adjusted prior to

addition of amyloglucosidase to achieve a pH ranging between 4.3 and 4.9. Afterwards, the IDF bags were filtered and rinsed after which SDF filter bags were removed. We rinsed the sample bags thrice in acetone in order to dissolve any residual fat. Each IDF bag was sealed in continuity with the filter, by using a heat sealer. The IDF bags were dried in an oven at 105° C ± 3° C for 90 minutes, placed in desiccant pouches and weighed after a complete cooling state was achieved . We assayed for protein content in one[art of the sample using the Dumas method while another replicate was burnt into ashes in a conditioned crucible at 600° C for 3 hours. Percentage dietary fiber was calculated as follows:

$$\%TDF = \frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \times 100$$

$$= \frac{[((f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2))/2] - P - (A_2 - D_2) - B}{(M_1 + M_2)/2} \times 100$$

Where:

M_1, M_2 = Original weight for duplicate samples (g)

R_1, R_2 = Residue for duplicate samples (g)

f_F = Final Filter Bag (g)

f_S = Initial Filter Bag (g)

D = Original weight of Diatomaceous Earth (g)

P = Protein of residue and bag (g)

A = Ash of residue and bag (g)

B = Blank (g)

f_{BF} = Final Blank Filter Bag (g)

f_{BS} = Initial Blank Filter Bag (g)

P_B = Protein of Blank Filter Bag (g)

A_B = Ash of Blank Filter Bag (g)

D_B = Original weight of Diatomaceous Earth in Blank Filter Bag (g)

3.2.18 Total Carbohydrate Content

Using nutrition labeling instructions from the US Food and Drug Administration , we measured the total carbohydrate content in our samples by subtracting differences of other constituents in the food (protein, fat, moisture, ash) from the total weight of the food instead of direct measurement, as described below.

100 - (weight in grams [protein + fat + water + ash] in 100 g of food)

3.2.19 Caloric content

The caloric content per 100grams of the beverage was calculated using the Atwater conversion factor (FAO) method.

{Energy (kcal/100g EP) = protein (g/100g EP) × 4 + fat (g/100g EP) × 9 + available carbohydrates (g/100g EP) × 4 + dietary fiber (g/100g EP) × 2 + alcohol (g/100g EP) × 7.

3.2.20 Sensory Analysis and Satiety Testing

A series of paired comparison tests (AACC Method 33-50.02) and a five-point hedonic test were used to evaluate the beverage by sixteen untrained panelists. The samples were served at a temperature of ~ 5 ± 1°C, in polystyrene cups coded with 3-digit numbers that were randomly generated. The samples were presented in a monadic sequence, following the sample presentation design in balanced complete blocks, aimed at decreasing the carryover and first-order effects (Castro *et al*., 2013). Participants were served with 30-ml of each sample and were instructed to eat a cracker and drink water between samples to

cleanse the palate. Sensory analysis was carried out on two separate test days. On the first day, the paired preference test was conducted with the aim of assessing consumers' preference between the two partial hydrolysis methods. Panelists were given two samples (enzyme and acid hydrolyzed beverage) from each beverage formulation blend and asked to identify which they preferred. The most preferred hydrolysis method was computed, and those beverages were used in the next stage of the sensory analysis. Separated by two days, participants returned and were asked to rate the acceptability of the beverage from each formulation, based on appearance, aroma, taste, texture, color, and overall preference using the five-point hedonic scale (ranging from 1 - dislike extremely to 5 - like extremely). The untrained panelists included students and staff members at South Dakota State University.

3.2.21 Satiety Testing

After performing sensory analysis, the most preferred beverages from each oat variety (experimental samples), together with a commercially available hunger suppressing beverage and regular breakfast (controls), were tested on human subjects to ascertain their effect on appetite, satiety and food intake. For this study, we recruited twenty subjects, ranging from 18 years and above in a randomized trial. We determined the body weight, height, waist circumference and vital signs (blood pressure, pulse rate) for each subject. Even though body mass index (BMI) was not a strict exclusion criterion we chose relatively healthy subjects. We administered a medical screening questionnaire in order to access the basal health status of the participants. To exclude participants from our study, we used the following exclusion criteria: (1) intake of medications other than birth control or hormone replacement therapy; (2) pregnant or nursing women; (3) weight gain or loss ≥

4 kg in the past 3 months; (4) fasting blood glucose >126 mg/dL; and (5) allergy or intolerance to barley, oats or milk. Both studies (sensory and satiety testing) were approved by the Institutional Review Board of the South Dakota State University.

Each of the test meals were served to subjects as breakfast every week, separated by at least seven days, until all the meals had been tested for. On the first test day, the low beta-glucan beverage was served as breakfast, on the second test day, the high beta-glucan beverage was served. The commercially available control beverage and Regular American breakfast (RAB) were served on the 3rd and 4th test days, respectively. The two (low and high) β glucan beverage contained 90kcal each, while the on-market control beverage contained 130kcal. The RAB ranged between 400kcal to 1000kcal. At the onset of breakfast on all four test days, participants arrived after a 10-hour overnight fast (they were not restricted to the intake of water). Participants were strictly required to refrain from alcohol, smoking and strenuous exercise for 24 hours prior to the test meal. Prior to serving the test meal, we administered visual analog scales (VAS) and scored each participant based on a scale of 0 to 10 (Forde C., 2018). Four variables namely, hunger, fullness, desire to eat, and prospective intake were assessed throughout the study by using the VAS. After scoring the VAS, subjects were presented with a 8 fl oz breakfast sample and allowed 20 minutes to consume the meal. We monitored the participants to ensure meal compliance. Another VAS based on the same parameters as before was administered immediately after breakfast for scoring. Participants were allowed to go about their normal duties and instructed to return to the test center exactly four hours after the breakfast meal. They were also required to take note of the time they start to feel hunger pangs. Once subjects returned to the testing center, they were administered the last VAS of the day, and

after scoring, they were presented with a lunch meal. Participants selected from among turkey, ham, roast beef, or vegetable sandwiches served with a side of potato fries or onion, condiments, as well as a calorie-free or calorie-containing beverage. Each participant was also served with 250g of salad. This selection was previously made at the screening visit. The same preselected sandwich, side, salad, condiments and beverage were presented to the participants on all four test days, in quantities greater than they could reasonably consume. The meals were pre-weighed, including the beverage, and participants were instructed to eat to satisfaction for 20 minutes, after which we determined the weight of the unconsumed meal and beverages. To determine the quantity of food consumed at lunch, we subtracted the weight of consumed meal from the total portion. The caloric intakes were calculated using the U.S. Department of Agriculture's Food and Nutrition Database for Dietary Studies 4.1 and product information. Subjects returned daily in the next one week to repeat all four meal (breakfast and lunch) trials (Rebello *et al*., 2016; Matte R., 2005).

3.2.22 Shelf-life Analysis

Shelf-life has been defined by the International Dairy Federation as "the length of time that a food can be held under recommended or practical storage conditions while maintaining its freshness or acceptable quality" (International Dairy Federation, 2012). The ultimate shelf-life of a dairy product is determined by its microbiological deterioration on storage, while the quality should be measured in terms of the sensory characteristics of the food (Wilbey, 2007). The shelf life of the β-glucan beverages developed for our study was defined as the period of refrigerated storage (4–6 °C), mimicking supermarket conditions i.e. frequent opening and closing of refrigerator door,

during which the pH, microbiological assay and sensory assay remains within specifications. Refrigerated storage was carried out for 4 weeks with periodical (weekly) observations of pH, microbiological content and sensory assay as outlined below;

pH Analysis

The weekly pH analysis of the beverage was determined using the official standard method AACCI 02-52.01. The Mettler Toledo benchtop pH meter was used for this analysis. This AACCI method measures the pH of aqueous samples, where the aqueous phase constitutes at least 20% of the total volume of the sample.

Microbiological Analysis

The microbiological analysis included: Aerobic plate count, Coliform and E. Coli. This analysis was carried out at the South Dakota State University, Dairy and Food Microbiology Laboratory. Phosphate Buffered Saline (Fisher Bioreagents, New Jersey) with a pH of 7.4 was used as the dilution buffer in all the parameters assessed.

Aerobic Plate Count

The aerobic plate count (APC) indicates the level of microorganism in the beverage. Pasteurized samples and the unpasteurized control sample were serially diluted up to 10^{-2} and a volume of 10 ml sample, diluted in 90ml buffered saline phosphate was plated from 10^{0} to 10^{-2}. The 3M Petrifilm (3M Co., St. Paul, MN). which conforms to AOAC Official Methods of Analysis 990.12 was used in this study. It contains nutrients to support microbial and 2,3,5-triphenyltetrazolium chloride as an indicator of bacterial growth. Reduction of triphenyl tetrazolium by bacteria resulted in red colored colonies, helping in easier enumeration of microbes. We determined colony forming units (cfu) using a criteria adapted from the FDA's bacteriological analytical manual, as written below:

$$\mathbf{N} = \frac{\sum C}{(n_1 + 0.1n_2 + 0.01n_3)\, d}$$

Where,

$\sum C$ is the sum of colonies counted on the dishes retained

n_1 is the number of dishes retained in the first dilution resulting in between 10 and 250 colonies

n_2 is the number of dishes retained in the second dilution resulting in between 10 and 250 colonies

n_3 is the number of dishes retained in the third dilution resulting in between 10 and 250 colonies

d is the dilution factor corresponding to the first dilution

E. Coli/Coliform

E. Coli enumeration was measured as an indicator of fecal contamination. Pasteurized samples and the unpasteurized control sample were serially diluted up to 10^{-1} and a volume of 10 ml sample, diluted in 90ml buffered saline phosphate was plated from 10^{0} to 10^{-1}. We employed the 3M Petrifilm E. coli/Coliform Count Plate (3M Co., St. Paul, MN) culture medium system for coliform determination based on AOAC Official Methods of Analysis 991.14. It contains Violet Red Bile (VRB) nutrients, an indicator of glucuronidase activity (BCIG), and a tetrazolium indicator that facilitated colony enumeration in the beverage samples.

Sensory Assay

For sensory analysis we employed a five-point hedonic scale to measure sensory characteristics of the beverage over the 4- week shelf life period by using ten untrained volunteers. Each week, participants were asked to rate the acceptability of the beverage from each formulation, based on appearance, aroma, taste, texture, color, and overall

preference using the five-point hedonic scale (ranging from 1 - dislike extremely to 5 - like extremely).

3.3 Statistical Analysis

All data were analyzed using GraphPad Prism version 5.01(San Diego, USA), VassarStat Computation Web (Lowry, 2017) and Microsoft Excel (version 2016) software. For the human satiety testing, we used a mixed model analysis of variance to analyze total energy intake (kcal) and the weight of food consumed. The model included factors with fixed effects (residual treatment characteristics that are consistent from test day 1 to test day 4 [this is hypothesized to be the same on test day 1 to day 4]), test day main effects, and treatment main effects. The secondary outcomes were changes in VAS ratings from time before breakfast, after breakfast and before lunch. These were analyzed using a mixed model analysis of variance. Regression analysis was applied to test for relationships between recorded hunger time over 4h postprandial (breakfast) period and mean ad libitum (lunch) intake. A 2-tailed binomial analysis using the Vassarstat binomial probability calculator for the paired comparison sensory test, was applied to determine the significant difference between means at a 95% confidence interval ($P < 0.05$). A mixed model analysis of variance was used to determine the differences between five-point hedonic acceptability ratings of experimental treatments and unhydrolyzed control samples.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Preliminary Work

The Stage-Gate protocol for new food product development was employed during the preliminary investigation (Edgett S., 2015). The two most predominant flavors (vanilla and chocolate) were identified in products that had ‘a claim of satiety promotion and hunger control’. This preliminary product mapping and sensory evaluation were aimed at determining consumers preference regarding flavor and product consistency. A nine-point hedonic scale as shown in Appendix B was used in the Quantitative Descriptive Analysis (QDA) of the samples. Table 4.0 illustrates the results of the QDA test. No significant difference was observed in the appearance of the ready-to-drink chocolate flavored beverage and vanilla flavored beverage ($p = 0.9958$). When these two ready-to-drink beverages were compared to the vanilla flavored beverage mix and the chocolate flavored beverage mix using the Tukey multiple comparison test, a significant difference was observed ($p < 0.0001$). The vanilla flavored beverage had the most preferred aroma, taste, texture and overall acceptability with mean scores of 7.4, 6.8, 7.2 and 7.2, respectively. The Tukey multiple comparison test showed a significant difference between the vanilla flavored beverage and the two powered beverage mixes when compared on these attributes ($p < 0.0001$). The beverage mix was in a powder form when purchased, and it had required reconstitution, with water to form a drinkable beverage. The powder matrix was the least accepted within all the attributes accessed. This was possibly due to the incomplete dissolution of the beverage which caused an inconsistent texture in the mouth during consumption. For our study we chose to formulate ready to drink beverages which were entirely vanilla flavored, based on the results of the preliminary study.

Table 4.0. Quantitative Descriptive sensory Analysis of commercially available hunger suppressing beverages.

Sample	Appearance	Aroma	Taste	Texture	Overall
Vanilla Flavored Beverage	7.0 ± 0.0^{a}	7.4 ± 0.8^{a}	6.8 ± 1.7^{a}	7.2 ± 1.5^{a}	7.2 ± 0.7^{a}
Chocolate Flavored Beverage	7.2 ± 0.8^{a}	5.8 ± 1.2^{a}	6.2 ± 1.0^{a}	6.6 ± 1.0^{a}	6.4 ± 0.8^{a}
Vanilla Powder Beverage Mix	3.6 ± 2.2^{b}	4.4 ± 2.4^{ab}	3.0 ± 2.3^{b}	3.2 ± 1.9^{b}	3.2 ± 1.9^{b}
Chocolate Powder Beverage Mix	3.0 ± 1.1^{b}	3.6 ± 1.5^{b}	2.6 ± 0.8^{b}	2.6 ± 0.8^{b}	3.4 ± 1.0^{b}

Values are expressed as means ± SD, $^{a\text{-}c}$ Means within each column with different superscripts are significantly different ($p \leq 0.05$). Scores (Seven-point hedonic scale: 1= Dislike extremely, 2 = Dislike moderately, 3= Dislike slightly, 4= Neither like nor dislike, 5 = Like slightly, 6 = Like moderately, 7 = Like extremely). N= 10 subjects.

4.2 Peroxidase Analysis

As oat and barley food products are susceptible to enzymatic degradation and loss of sensory attributes, enzyme inactivation is employed as a part of the processing technique. This is generally in the form of a heat treatment under specified conditions. The undesirable hydrolysis reaction of acylglycerols can be very rapid once the oat is milled, and enzyme-active oat products develop a characteristic bitter taste and rancid flavor within weeks of storage (Laakso and Lethinen, 2004). For this reason, there was a need for enzyme inactivation. The main targets for enzyme inactivation include lipase, lipoxygenase and peroxidase, however, peroxidase is more heat stable, thus to ensure effective enzyme inactivity, the complete inactivation of peroxidase is an indication that lipase and lipoxygenase have been inactivated as well.

Figure 7 shows the peroxidase analysis results of kilned and unkilned grains. The first four flasks to the left are kilned grains which showed no color change after 10 mins of reaction with sodium 2,6-dichloro-indophenol solution and hydrogen peroxide. These tests were therefore recorded as negative, denoting no enzyme activity. The raw groats however, were observed to have a distinct blue color, which indicated the presence of active lipid hydrolyzing enzymes. Table 4.1. provides observations made during the peroxidase test of flour samples. Kilned barley, GMI oat and Natty oat groats had a negative peroxidase presence, while the raw groats had a positive enzyme presence. Based on these observations, peroxidase enzymes in oat and barley groats can be said to have been successfully inactivated by the kilning step which involved steaming the groats at 0.24mPa at 105°C for 16 min, followed by application of dry heat at 215°F for 30 min, as prescribed by the North America Millers Association, 2007 .

Figure 7. Peroxidase analysis of kilned and raw oats and barley groats. First four samples to the left are kilned groats which showed no color change during the test (negative result). The next four samples are raw groats which had the presence of a definite blue color (positive result). The kilning step involved steaming the grains at 0.24mPa at 105°C for 16 min, followed by application of dry heat at 101°C (215°F).

Table 4.1. Effect of the enzyme deactivation step (kilning) on the peroxidase content of oat and barley groats.

Sample	Groat steaming conditions (Pressure/ Temperature/Time)	Groat drying conditions (Pressure/ Temperature/Time)	Color changed observed?	Peroxidase (+) or (-)
Raw Dehulled Natty	0.24mPa/ 105°C/16min	101°C/30min	Yes (Blue)	(+)
Raw Dehulled GMI	0.24mPa/ 105°C/16min	101°C/30min	Yes (Blue)	(+)
Raw Dehulled Barley	0.24mPa/ 105°C/16min	101°C/30min	Yes (Blue)	(+)
Kilned Natty	0.24mPa/ 105°C/16min	101°C/30min	No	(-)
Kilned GMI	0.24mPa/ 105°C/16min	101°C/30min	No	(-)
Kilned Barley	0.24mPa/ 105°C/16min	101°C/30min	No	(-)

(+) = Positive for active peroxidase, (-) = negative for active peroxidase.

4.3 Particle Size Analysis

Knowledge of particle size is important in food product development because it affects the handling of ingredients, the formulation, processing, and quality control of food and beverage products. In beverage formulation, particle size affects reactivity, solubility, and flowability of ingredients and the texture, mouthfeel of products (Bancarz *et al*., 2008). In this study, the sieve analysis method was used (ASAE Standards, 2003), where ground Natty oat, GMI oat or barley flour was separated using sieves with different pore sizes. The quantification of geometric mean diameter has remained an effective way of statistically comparing particle size distribution (PSD) (ASAE Standards, 2003). Based on this method, the geometric mean diameter (d_{gw}) of particles for the three flour samples had an average value of 0.200 mm and a range of 0.195 to 0.209 mm (Table 4.2). No statistically significant difference was observed between the means of the three flour samples ($p = 0.158$). This is a good reflection of the efficacy of the 0.2mm Restch mill sieve used in grinding all three flour samples. According to Foehse and coworkers (1991), finer oat flour size is mainly made up of endosperm while coarser oat flour is made up of sub aleurone layer and cell wall. The cell wall constituents and bran could be more resistant to milling thus producing coarser particles. The results obtained confirms Mitra's (2015) study showing that most flour samples from oat cultivars have a particle size diameter of about 200µm (0.2mm). It also confirms the finding of Prasopsunwattana *et al.*, (2009) who reported that regular ground whole barley flour has an average particle size of 237.6µm (0.24mm).

Table 4.2. Geometric mean diameter (mm) of flour particle diameter (dgw) for Natty Oat, GMI oat and barley flour after milling in the Retsch Mill.

Samples	**Geometric mean diameter (dgw)**
Natty Oat Flour	0.195±0.07[a]
GMI Oat flour	0.209±0.08 [a]
Barley Flour	0.196±0.08 [a]
Minimum	0.1952
Maximum	0.2093
Mean	0.2000

Values are expressed as means ± SD of two independent determinations. Means with same letter within column are not significantly different ($p < 0.05$).

4.4 Proximate analysis (Moisture, Fat, Protein, Ash and Carbohydrate)

Determination of moisture, fat, protein and ash on flour samples and beverage samples were carried out using standard reference analytical procedures. In carrying out these proximate analysis, appropriate controls were used to increase accuracy and precision of results.

Proximate composition of flour blends

Table 4.3 provides the mean proximate composition of the various dry flour blends used in formulating the beverages. Analysis of Variance (ANOVA) and the Fisher's Least Significance Difference (LSD) tests were used to determine significant differences in the constituents between each flour blend type. Data revealed that GMI oat/barley flour blends ranged from 5.9 % to 6.35% in moisture content which was significantly lower than the moisture content of 100B and most Natty oat flour blends. Affirming the results reported by Paudel D., 2018, Natty oat blends had a significantly low-fat content compared to GMI oat blends and barley flour, with 100G having the highest percentage fat of 7.58%. Barley was shown to have a total mineral content of 4.07%. This was a much higher value than the 1.5 – 2.5% reported by Das and Kaur, 2016. 100% GMI oat flour had the highest percentage protein content of 16.52%. The protein content of other GMI oat flour blends decreased as the proportion of barley flour content increased in the blend. A similar trend was observed in the Natty flour blend as 100% barley flour had a protein content of 13.77%. With the exception of 60N/40B protein content was seen to have a tendency to decrease with greater proportions of barley in the blend. This trend was consistent with the findings by Fišteš and coworkers, (2014) that showed that, oat flour (16.9 -17.5%)

contained a higher amount of protein than barley flour (14.5 -15.3%). 60N40B flour blend had a significantly higher protein content than the other Natty oat flour blends ($p < 0.0001$). This result did not change even after a confirmatory analysis was carried out. Total carbohydrate content was calculated by difference using the formula 100 - (weight in grams [protein + fat + water + ash] in 100 g of sample). Generally, GMI oat flour blends were found to have a significantly lower total carbohydrate content than barley flour and Natty oat flour blends.

Table 4.3. Effects of varied oat (GMI & Natty) and barley flour proportions on proximate composition of flour blends.

Formulation Blend	Moisture (%)	Fat (%) db	Ash (%) db	Protein (%) db	Total CHO (%) db
GMI/Barley					
100G	5.96 ± 0.154^{b}	7.58 ± 0.106^{a}	2.06 ± 0.0003^{b}	16.52 ± 0.086^{a}	67.88 ± 0.173^{d}
90G/10B	5.87 ± 0.043^{c}	7.43 ± 0.072^{a}	2.06 ± 0.0007^{b}	15.99 ± 0.081^{a}	68.63 ± 0.053^{d}
80G/20B	5.71 ± 0.058^{c}	6.78 ± 0.153^{b}	2.03 ± 0.007^{b}	15.89 ± 0.096^{a}	69.60 ± 0.198^{c}
70G/30B	5.87 ± 0.005^{c}	6.30 ± 0.125^{b}	2.01 ± 0.029^{b}	15.54 ± 0.186^{b}	70.28 ± 0.037^{c}
60G/40B	6.35 ± 0.063^{b}	6.80 ± 0.059^{b}	1.74 ± 0.004^{c}	13.57 ± 0.035^{c}	71.53 ± 0.091^{b}
Natty/Barley					
100N	6.97 ± 0.197^{b}	5.23 ± 0.040^{b}	1.71 ± 0.005^{d}	13.51 ± 0.099^{c}	72.57 ± 0.143^{b}
90N/10B	6.84 ± 0.044^{b}	5.41 ± 0.118^{c}	1.73 ± 0.008^{c}	13.66 ± 0.078^{c}	72.37 ± 0.161^{b}
80N/20B	6.75 ± 0.092^{b}	4.76 ± 0.018^{d}	1.73 ± 0.009^{c}	13.73 ± 0.036^{c}	73.03 ± 0.119^{a}
70N/30B	6.58 ± 0.032^{b}	5.05 ± 0.031^{c}	1.73 ± 0.0178^{c}	13.66 ± 0.137^{c}	72.98 ± 0.091^{b}
60N/40B	5.89 ± 0.045^{c}	5.68 ± 0.079^{c}	1.97 ± 0.015^{b}	15.53 ± 0.045^{b}	70.93 ± 0.185^{b}
100B	9.65 ± 0.087^{a}	4.36 ± 0.129^{d}	4.07 ± 0.081^{a}	13.78 ± 0.005^{c}	68.14 ± 0.118^{d}

Total CHO; [Total Carbohydrate = 100 – (weight(g) of {Protein + Fat + Moisture + Ash} in 100g of beverage)]. G = GMI423 oat variety, N = Natty, B = Barley. db = dry basis. Values are mean 'proximate parameter' ± standard deviation of two independent determinations. Means with different letters in each column are significantly different ($p < 0.05$).

Proximate composition of oat/barley beverages prepared with select oat cultivars (GMI & Natty)

Table 4.4 and 4.5 provide comparisons between the proximate composition of beverages, treated with two types of partial hydrolysis methods (enzymatic and acid) from two select oat cultivars (GMI & Natty).

Moisture Content: No significant differences were observed between the moisture content of GMI/Natty partial-enzyme hydrolyzed beverages and its corresponding partial-acid hydrolyzed beverages ($p = 0.9984$). However, the moisture content of the unhydrolyzed GMI control (64.09%) was significantly lower than the treated GMI blend beverages, which ranged between 68.21 to 70.11%. There was no significant difference between the moisture content of unhydrolyzed Natty control and most of the partially hydrolyzed Natty blend beverages ($p= 0.9945$). The highest moisture content was registered in the enzyme hydrolyzed 70G30B beverage (70.11 %) and enzyme hydrolyzed 60N40B beverage (69.35 %) for GMI and Natty blend beverages, respectively. Also, the unhydrolyzed GMI beverage (64.09 %) and acid hydrolyzed 80N20B beverage (67.59 %) had the lowest percentage moisture content in the GMI and Natty blend beverages, respectively. Moisture content remains one of the characteristics that inform a sensory perception of food, from a consumer perspective. Thus, any influence on moisture content can considerably impact on flavor, texture as well the physico-chemical properties based on the premise that water can influence chemical reactions in food.

Fat Content: Statistical analysis showed no significant difference ($p > 0.99$) between the fat content of the unhydrolyzed control beverage (1.22) and partially hydrolyzed samples which ranged between 0.83 to 1.49 %. Similarly, no statistically significant difference ($p >$

0.99) was observed in the fat content of the unhydrolyzed GMI beverage (1.54%) and the partially hydrolyzed GMI blend beverages which ranged between (1.49 to 2.09%). This general low percentage of fat in the beverages indicates that during storage of the beverage, the quality, especially sensorial quality may not be affected. The high fat content in beverages usually enhances the chances for rancidity (peroxidation of polyunsaturated fatty acid) that in turn imparts unpleasant odors and would ultimately reduce intake of food and nutrient (Abdulrahman *et al*., 2016). Prior inactivation of the lipid hydrolyzing enzyme will also help to greatly reduce the occurrence of rancidity.

Ash Content: The ash content, which is an expression of total mineral content, was found to occur in the range of 3.69 – 4.13 % and 2.96 – 4.08 % for partially hydrolyzed Natty and GMI beverages, respectively. Ash determination is important because the amount of minerals present in a food product can determine some physicochemical properties of foods, as well as inhibit growth of microorganisms (Dairy F., 2010). The ash content revealed by this study was similar to the ash content of other oat supplemented products previously reported (Krishnan *et al*., 1987; Sharma *et al*., 2011).

Protein Content: It should be noted that the addition of a lactose free fat free milk (3 % protein), contributed to the overall protein content of the beverages. Generally, the beverages developed in the experiments were proposed to have between 10g to 15g of protein in order to be referred to as a high protein beverage, since FDA asserts that ‘a high claim may be made when a food contains at least 20% of the % DV’. Since the recommended daily intake of proteins is 50g, the developed beverages can be said to be a high protein source (FDA., 2013). Both unhydrolyzed beverages in the Natty and GMI categories were seen to have a significantly higher ($p < 0.05$) protein content than the

acid-treated and enzyme-treated blends. Protein content ranged between 12.32 – 13.49% for Natty blends and 12.55 – 15.54% for the GMI blends. As provided in Table 4.22, a serving of the formulated partially hydrolyzed beverage contains between 11.24 to 11.96g per 240ml of beverage. The protein content of these formulations was found to be higher than most commercially available oat 'milk'/beverages which had a protein content range between 1g and 3g per 240ml. The protein content of our beverage was found to be lower than the commercial satiety-increasing beverage which contains 15g of protein per 240ml. In reviewing the ingredients used in formulating the commercial satiety-increasing beverage, it was found to be largely developed using milk protein concentrate, which likely accounts for its high protein content.

Carbohydrate content of the beverages was shown to have significantly decreased in comparison to its corresponding flour blends ($p < 0.0001$). GMI blend beverages were found to have a carbohydrate content ranging between 10.46 – 13.84% with the unhydrolyzed beverage having the highest percentage carbohydrate (14.96%), compared to the partially hydrolyzed beverages. A different trend was seen in the carbohydrate content of Natty beverages which ranged between 12.84 – 14.47 %. However, the lowest percentage carbohydrate was observed in the unhydrolyzed Natty beverage (12.02%).

Table 4.4. Proximate content of partially hydrolyzed GMI/barley blend beverages.

Formulation blend	Hydrolysis Treatment	Moisture (%)	Fat (%) db	Ash (%) db	Protein (%) db	Carbohydrate (%) db
100G	Partial Enzyme Hydrolysis	68.43 ± 0.44^{b}	1.94 ± 0.08^{a}	3.88 ± 0.01^{a}	13.40 ± 0.10^{b}	12.35 ± 0.29^{c}
90G/10B		69.56 ± 0.18^{a}	2.09 ± 0.08^{a}	4.08 ± 0.02^{a}	13.82 ± 0.02^{b}	10.46 ± 0.06^{d}
80G/20B		69.71 ± 0.05^{a}	2.04 ± 0.06^{a}	4.02 ± 0.03^{a}	13.21 ± 0.04^{b}	11.02 ± 0.18^{cd}
70G/30B		70.11 ± 0.21^{a}	1.94 ± 0.04^{a}	3.90 ± 0.06^{a}	12.74 ± 0.04^{bc}	11.31 ± 0.07^{cd}
60G/40B		69.31 ± 0.49^{a}	1.79 ± 0.05^{a}	3.99 ± 0.00^{a}	12.98 ± 0.10^{b}	11.93 ± 0.34^{c}
100G	Partial Acid Hydrolysis	68.60 ± 0.71^{ab}	1.49 ± 0.02^{a}	2.96 ± 0.03^{b}	13.12 ± 0.13^{b}	13.84 ± 0.58^{b}
90G/10B		68.21 ± 0.42^{b}	1.67 ± 0.02^{a}	3.91 ± 0.01^{a}	12.61 ± 0.18^{bc}	13.60 ± 0.63^{b}
80G/20B		68.99 ± 0.10^{ab}	1.59 ± 0.00^{a}	3.71 ± 0.01^{a}	12.93 ± 0.31^{bc}	12.78 ± 0.40^{bc}
70G/30B		68.66 ± 0.24^{ab}	1.56 ± 0.06^{a}	3.83 ± 0.02^{a}	12.55 ± 0.30^{bc}	13.41 ± 0.15^{b}
60G/40B		69.53 ± 0.41^{a}	1.75 ± 0.06^{a}	3.73 ± 0.01^{a}	13.32 ± 0.37^{b}	11.67 ± 0.02^{c}
Unhydrolyzed GMI	NHT	64.09 ± 0.58^{c}	1.54 ± 0.03^{a}	3.87 ± 0.04^{a}	15.54 ± 0.11^{a}	14.96 ± 0.49^{a}

Comparisons except moisture are made on a dry basis (db). G – GMI423 oat variety; N – Natty oat variety; Total CHO; [Total Carbohydrate = 100 – (weight(g) of {Protein + Fat + Moisture + Ash} x in 100g of beverage)]. N = Natty, B = Barley, NHT= No Hydrolysis Treatment. Values are mean 'proximate parameter' ± standard deviation of two independent determinations. Means with different letters in each column are significantly different ($p < 0.05$). Unhydrolyzed GMI beverage (control) does not contain any proportions of barley.

Table 4.5. Proximate content of partially hydrolyzed Natty/barley blend beverage formulations.

Formulation blend	Hydrolysis Treatment	Moisture (%)	Fat (%) db	Ash (%) db	Protein (%) db	Carbohydrate (%) db
100N	Partial Enzyme Hydrolysis	69.31 ± 0.42^{a}	1.49 ± 0.02^{a}	3.83 ± 0.03^{a}	12.43 ± 0.05^{ab}	12.92 ± 0.21^{ab}
90N/10B		68.85 ± 0.01^{a}	1.25 ± 0.03^{a}	3.87± 0.00^{a}	12.60 ± 0.05^{a}	13.43 ± 0.12^{ab}
80N/20B		68.73 ± 0.55^{a}	1.31 ± 0.04^{a}	4.03 ± 0.06^{a}	12.58 ± 0.04^{a}	13.35 ± 0.52^{ab}
70N/30B		68.77 ± 0.46^{a}	1.45 ± 0.03^{a}	4.01 ± 0.00^{a}	12.93 ± 0.04^{a}	12.84 ± 0.47^{ab}
60N/40B		69.35 ± 0.59^{a}	1.29 ± 0.02^{a}	4.13 ± 0.00^{a}	12.32 ± 0.02^{ab}	12.91 ± 0.61^{ab}
100N	Partial Acid Hydrolysis	68.21 ± 0.17^{ab}	1.31 ± 0.02^{a}	3.69 ± 0.04^{a}	12.62 ± 0.36^{a}	14.17 ± 0.50^{a}
90N/10B		68.32 ± 0.36^{ab}	0.83 ± 0.03^{a}	3.82 ± 0.00^{a}	13.25 ± 0.36^{a}	13.77 ± 0.76^{a}
80N/20B		67.59 ± 0.29^{b}	1.11 ± 0.03^{a}	3.78 ± 0.01^{a}	13.13 ± 0.35^{a}	14.39 ± 0.67^{a}
70N/30B		68.23 ± 0.11^{ab}	1.13 ± 0.01^{a}	3.84 ± 0.02^{a}	13.21 ± 0.24^{a}	13.57 ± 0.12^{a}
60N/40B		67.9 ± 0.55^{ab}	1.47 ± 0.04^{a}	3.77 ± 0.02^{a}	12.39 ± 0.30^{ab}	14.46 ± 0.83^{a}
Unhydrolyzed Natty	NHT	69.32 ± 0.15^{a}	1.22 ± 0.00^{a}	3.94 ± 0.03^{a}	13.49 ± 0.06^{a}	12.02 ± 0.19^{b}

Comparisons except moisture are made on a dry basis (db). G – GMI423 oat variety; N – Natty oat variety; Total CHO; [Total Carbohydrate = 100 – (weight(g) of {Protein + Fat + Moisture + Ash}x in 100g of beverage)]. N = Natty, B = Barley, NHT= No Hydrolysis Treatment. Values are mean 'proximate parameter' ± standard deviation of two independent determinations. Means with different letters in each column are significantly different ($p < 0.05$). Unhydrolyzed Natty beverage (control) does not contain any proportions of barley.

4.5 pH Analysis

Milk and milk-based beverages, pasteurized, canned, or dry are acid-forming foods. Its pH level is below neutral at about 6.5 to 6.9. This is because milk contains lactic acid even when not fermented (Iftikhar N., 2018). Table 4.6 shows the pH values of various beverage blend formulations which were either partially-acid or partially-enzyme hydrolyzed. All partial-enzyme hydrolyzed beverage blend formulations were within the acceptable pH limits of milk-based beverages i.e. 6.5-6.8. Beverages within the GMI variety had a pH between (6.62 to 6.70) and (5.90 to 5.97) for partial enzyme hydrolysis and partial acid hydrolysis, respectively. Within treatments no significant difference was observed between in the pH values of the GMI and Natty beverages ($p = 0.9537$). Natty beverages had a pH range between (6.61 to 6.72) and (5.88 to 5.96) for partial enzyme hydrolysis and partial acid hydrolysis, respectively. Generally, pH was seen to decrease as barley content increased. At the 95% confidence interval, the pH of partial-acid hydrolyzed beverages was observed to be significantly lower than that of partial-enzyme hydrolyzed beverages. This difference in pH can be attributed to the addition of ascorbic acid during the process of partial acid hydrolysis. pH measurement, one of the commonest analytical procedures in industrial food processing, is the direct measurement of acidity (H^+). We did not observe any significant difference between the pH of the GMI and Natty unhydrolyzed samples ($p = 0.168$). In order to maintain regulatory requirements and to meet standard practices, food processing involves pH measurement to ensure formulations that are consistent with well-defined properties (SSI., 2015).

Table 4.6. pH of partial enzyme and acid hydrolyzed samples. pH of unhydrolyzed GMI beverage = 6.67, pH of unhydrolyzed Natty beverage = 6.7

Formulation blend	Partial enzyme hydrolysis treatment	Partial acid hydrolysis treatment	t-ratio	p value
GMI/Barley Blends				
100G	6.70[a]	5.97[b]	22.65	0.001944
90G/10B	6.68[a]	5.96[b]	33.94	0.000867
80G/20B	6.67[a]	5.95[b]	15.18	0.004312
70G/30B	6.65[a]	5.92[b]	23.08	0.001871
60G/40B	6.62[a]	5.90[b]	21.62	0.002133
Natty/Barley Blends				
100N	6.72[a]	5.96[b]	37.11	0.000725
90N/10B	6.69[a]	5.95[b]	104.7	0.000091
80N/20B	6.65[a]	5.93[b]	64.85	0.000238
70N/30B	6.62[a]	5.91[b]	47	0.000452
60N/40B	6.61[a]	5.88[b]	26.93	0.001376

Values are means of two independent determinations. Means with different letters within same row are significantly different ($p < 0.05$). G= GMI423, B= Barley, N = Natty.

4.6 Viscosity

Beta-glucan is composed of linear unbranched β-(1→4)-D-glucopyranose monomeric units, linked by a single β-(1→3)-linked glucose unit every 2–3 units. The (1→3)-linkages influence the high-water binding ability, viscosity as well as the contribute to overall flexibility of beta-glucan (Anttila *et al*., 2004).

Viscosity results were discussed in two steps. The first step involved comparison of the viscosity (cP) of unpasteurized beverage formulations treated with both partial-enzyme hydrolysis and partial-acid hydrolysis. The second step involved comparison of the viscosity (cP) of pasteurized partial-enzyme hydrolyzed beverages and partial-acid hydrolyzed beverages. Table 4.7. shows the viscosity in centipoise (cP) of unpasteurized GMI/barley and Natty/barley blend formulations, before and after treatment with either enzyme hydrolysis or acid hydrolysis. Generally, it was noted that partial enzyme hydrolysis significantly ($p < 0.0001$) reduced the viscosity of the slurry in comparison to partial acid hydrolysis on both GMI and Natty blends. Sibakov and coworkers (2013), while comparing acid and enzymatic hydrolyses of oat bran β-glucan at minimal water content (50% dry matter), observed that enzyme-catalyzed hydrolysis yielded more stable extracts, required very little harsh processing conditions as well as produced no inorganic side stream The partial hydrolysis of β-glucan by the enzyme preparation method was dependent on sample incubation at 50 °C and low concentration of the solution. The results for partial acid hydrolysis disagree with Lee *et al*., 2015, who concluded that acid hydrolysis was shown to be an effective method of reducing viscosity of β-glucan solutions. This difference may have been due to the use of a weak acid i.e. ascorbic acid

(0.04g/ml) in our study, in comparison to hydrochloric acid (0.1 - 0.5N) used in Lee's research.

Table 4.7. Viscosity of un-pasteurized blend formulations at 5g/ml conc. solution before and after partial enzyme (50°C) and partial acid (70°C) hydrolysis treatments.

Formulation blend	Untreated beverage	Partial enzyme hydrolysis	Partial acid hydrolysis
GMI/Barley Blend			
100G	152.90 ± 0.42[a]	45.10 ± 0.14[c]	127.40 ± 0.00[b]
90G/10B	169.75 ± 0.92[a]	45.70 ± 0.85[c]	130.10 ± 0.28[b]
80G/20B	174.45 ± 0.35[a]	50.25 ± 0.92[c]	167.00 ± 5.37[b]
70G/30B	178.50 ± 0.85[a]	54.00 ± 0.57[c]	163.85 ± 2.05[b]
60G/40B	184.50 ± 0.42[a]	57.45 ± 0.35[c]	170.15 ± 2.48[b]
Natty/Barley Blend			
100N	149.35 ± 0.07[a]	44.90 ± 0.28[c]	134.05 ± 3.18[b]
90N/10B	156.35 ± 0.50[a]	46.70 ± 0.00[c]	132.30 ± 1.13[b]
80N/20B	162.05 ± 0.50[a]	49.15 ± 0.92[c]	139.70 ± 0.57[b]
70N/30B	164.65 ± 1.20[a]	56.00 ± 1.84[c]	144.15 ± 0.64[b]
60N/40B	169.55 ± 1.06[a]	55.60 ± 0.85[c]	149.80 ± 1.84[b]

Values are mean ± standard deviation of two independent determinations. Means with same letter within each row are not significantly different ($p < 0.05$). G=GMI oat variety, N=Natty oat variety, B=Barley.

In our study, partial enzyme hydrolysis was shown to reduce viscosity of beverages (65-73%) much higher partial acid hydrolysis which produced a 10-20% reduction in beverage viscosity. Our study also contrasted with the findings reported by Kivela and coworkers (2009), who stated that hydrolysis with 10 mM ascorbic caused an approximately 50% drop in viscosity of the barley beta-glucan solution. Within formulations of same oat varieties, it was realized that viscosity of the slurry increased as the barley content increased, which implied barley flour contributed a higher viscosity in the slurry than oat flour. A study conducted by Mikklesen and coworkers, (2010) concluded that, at equivalent 5% β-glucan concentrations, barley beta glucan was characterized as a low-viscosity β-glucan (0.01 to 1 Pa-s) with Newtonian flow behavior while oat beta glucan was characterized as a high-viscosity β-glucan (1 to 10 Pa-s) with shear thinning flow behavior. Our observations, however, did not confirm the results of that study and this could have been due to differences in variety of oat and barley used in the study. Such differences may also be due to differences in β-glucan content of the respective samples.

4.6.1 Viscosity of pasteurized beverages

Table 4.8 shows the viscosity in centipoises (cP) of the pasteurized beverages. The viscosity of the pasteurized beverages is significantly higher ($p < 0.0001$) when compared to its viscosity immediately after partial hydrolysis treatment. This can be attributed to gelatinization of starch present by the heat introduced during pasteurization and the presence of stabilizers which is known to enhance viscosity (TIC Gums., 2017). Across both partial hydrolysis treatments (enzyme and acid), it was shown that formulations containing the GMI oat variety had a higher viscosity that range between (131.45 – 752.90 cP) compared to formulations with the Natty oat variety (130.20 – 633.15 cP). This was

expected as Paudel D., (2018) established that the GMI423 variety had a significantly high β-glucan content (6.93%) compared to Natty which has a β-glucan content of 3.90%. Also, according to Antilla and coworkers, (2013), viscosity depends directly on the concentration and molecular weight of β-glucan. However, there was no significant difference observed in GMI and Natty for 100%Oat and 90%Oat10B acid-hydrolyzed blend formulations ($p = 0.9991$; 0.9599). Within blend formulations, partial-enzyme hydrolyzed beverages were shown to have a significantly lower viscosity compared to the partial-acid beverages ($p < 0.0001$). Based on the viscosity chart provided in Appendix D, the viscosities of partial-enzyme hydrolyzed beverages can be compared to that of liquid yogurt and chocolate milk which are reported to have viscosities of 152cP and 280cP, respectively. These viscosities are higher than that of regular whole cow milk which has a viscosity of 10cP. Viscosities of partial-acid hydrolyzed beverages on the other hand can be likened to caramel and citrus fruit pulp which have viscosities of 400cP and 600cP, respectively.

Table 4.8. Effects of partial enzyme and partial acid hydrolysis on the viscosity of pasteurized oat/barley beverages made with high beta glucan (GMI) and low beta glucan (Natty) oat varieties.

Proportion of oat and barley	Partial Enzyme Hydrolysis (cP)		Partial Acid Hydrolysis (cP)	
	GMI Beverage	Natty Beverage	GMI Beverage	Natty Beverage
100Oat	131.45 ± 2.47^{c}	130.20 ± 1.13^{c}	280.25 ± 9.55^{a}	197.25 ± 5.73^{b}
90Oat/10Barley	145.05 ± 0.63^{b}	140.50 ± 1.70^{b}	400.25 ± 1.20^{a}	391.45 ± 11.53^{a}
80Oat/20Barley	181.10 ± 5.37^{c}	159.45 ± 5.16^{c}	571.75 ± 14.07^{a}	486.65 ± 17.75^{b}
70Oat/30Barley	198.20 ± 7.21^{c}	177.80 ± 3.39^{c}	609.75 ± 19.59^{a}	555.10 ± 0.57^{b}
60Oat/40Barley	259.55 ± 9.55^{c}	206.85 ± 3.32^{d}	752.90 ± 8.77^{a}	633.15 ± 6.15^{b}

Values are mean ± standard deviation of two independent determinations. Means with same letter within each row are not significantly different ($p < 0.05$). cP = centipoises. Unhydrolyzed GMI and Natty beverages (controls) do not contain any proportion of barley. Viscosity of unhydrolyzed GMI beverage = 457.05 ± 11.24 cP; Viscosity of unhydrolyzed Natty beverage = 392.7 ± 12.02 cP.

4.7 β-Glucan content

Employing the standard AACCI method 32-23.01, different concentrations of β-glucan (g per 100g of sample) were analyzed and recorded. The variety of oats and type of hydrolysis were important independent variables. Barley and oat control flour were provided by Megazyme International to ensure accuracy and precision in the implementation of their assay. Table 4.9. provides a summary of the β-glucan content of 100% barley flour and 100% oat flour (GMI and Natty) used in the beverage formulation. The accuracy and precision are assessed by comparing the mean, standard deviation (SD) and coefficient of variation (CV). This analysis was carried out to determine how each grain flour type (barley, oat) contributed to the final β-glucan content of the developed beverage. The β-glucan content of Megazyme control flours used during each analysis are reported in Table 4.10. Mean values of barley control flour and oat control flour determined by our analysis shows close fit with the values claimed by Megazyme. Low values for standard deviation (0.02 – 0.17) and low coefficient of variation (0.40 – 2.14), attest to good precision achieved in our laboratory assay.

Table 4.11. provides a summary of the analysis of variance (ANOVA) of data obtained for the β-glucan content in analyzed samples. Blend formulation and type of hydrolysis treatment were shown to have statistically significant effects on the β-glucan content of both flour and beverage samples. However, there was no significant difference between interactions of blend formulation and hydrolysis treatment.

Table 4.9. β-glucan content of 100% barley, 100% GMI oat and 100% Natty oat flour samples

Statistical Parameter	Barley	GMI Oat Variety	Natty Oat Variety
Mean (%)	8.49[a]	7.33[a]	4.20[b]
Standard Deviation	0.02	0.07	0.04
Coefficient of Variation (CV)	0.25%	0.96%	0.97%

Values are means of two independent determinations. Means with same letters within row are not significantly different ($p < 0.05$). GMI = High β-glucan oat variety, Natty = Low β-glucan oat variety.

Table 4.10. Summary of β-glucan content of Megazyme control flour samples to determine the accuracy and repeatability of the assay achieved in the lab

	Barley Control			Oat Control		
	Mean	Standard Deviation	Coefficient of Variation (%)	Mean	Standard Deviation	Coefficient of Variation (%)
Day 1	4.07	0.03	0.74	7.94	0.17	2.14
Day 2	3.95	0.02	0.51	7.57	0.03	0.40
Day 3	4.07	0.06	1.47	7.57	0.04	0.53
Day 4	4.11	0.02	0.49	7.87	0.1	1.27
4-day mean	4.05	0.06	1.53	7.74	0.17	2.17

Reported value for Megazyme barley control flour: 4.1%, Megazyme oat control flour: 8%. Values are reported on an as is basis.

Table 4.11. Analysis of variance of β-Glucan content in oat/barley blend flour and partially hydrolyzed beverage samples.

ANOVA table	% of total variation	SS	DF	Mean Square	Significance level
Blend Formulation	2.394	8.231	5	1.646	*
Treatment	94.02	323.3	5	64.66	**
Blend Formulation x Treatment	3.548	12.2	25	0.488	ns
Subject x Blend Formulation	0.00397	0.01365	5	0.00273	-
Subject x Treatment	0.001984	0.006821	5	0.001364	-
Subject	0.000208	0.0007153	1	0.0007153	-
Residual		0.09164	25	0.003665	-

Significant codes: ‘ns’= $p > 0.05$ (not significant) ‘*’= $p \leq 0.05$ ‘**’= $p \leq 0.01$.

β-glucan content of Natty and GMI samples are provided in Tables 4.12 and 4.13, respectively. The β-glucan content of flour and beverages are presented on an as-is basis. The beverages were analyzed using the Megazyme β-glucan content method for beverage and ready-to eat products. Ranging from 4.20 - 8.07%, the β-glucan content of the flour blends was observed to increase as the proportion of barley added into the blends increased. The β-glucan content of the beverage samples was also seen to increase as the barley content of beverages increased. The GMI oat flour variety was shown to have a significantly higher β-glucan content (7.3g/100g) than the Natty oat flour variety (4.2g/100g) ($p = 0.0201$). Our results were in line with the study by Paudel and coworkers (2018), who reported on a variability study of β-glucan content of South Dakota oat cultivars. In their study, GMI oat flour (6.93%) was shown to have a higher β-glucan content than the Natty oat flour (3.90%).

Detailed Tukey multiple comparison tests showed a significant difference in the β-glucan content of flour samples and partially hydrolyzed beverages. The percentage β-glucan content of GMI flour samples ranged between 7.33% - 8.10%, but after partial hydrolysis treatment, the β-glucan content decreased to 2.89% - 4.28% with β-glucanase enzyme treatment and 4.40% - 5.32% with ascorbic acid treatment. A similar hydrolysis effect was observed in the Natty oat variety where flour samples with β-glucan content 4.20% – 5.87% decreased to 1.32% - 1.59% with partial enzyme hydrolysis and 1.90% - 1.99% with partial acid hydrolysis. The multiple comparison test generally showed no significant difference in β-glucan content between the unhydrolyzed beverage samples (control) and partially hydrolyzed beverage samples. No significant difference was observed in the β-glucan content of the two hydrolysis treatments ($p = 0.055$), although partial-enzyme

hydrolysis as previously discussed significantly reduced the viscosity of the beverage. These observations could be explained by previous studies by Nguyen and coworkers, (2020) and Johansson and coworkers, (2005). These studies report that enzyme hydrolysis can produce certain polysaccharide fractions other than the targeted β-glucan polymer hence reducing matrix viscosity. Lee *et al*., 2015, reported a significant reduction in the total β-glucan contents of raw barley slurries (*Saechal* and *Hinchal* varieties) from 7.77% and 8.24%, to 2.19% and 2.24% respectively, based on an acid hydrolysis treatment. The hydrolysis treatment used was however, a complete hydrolysis method.

Currently, no data or food guidelines exist for classifying foods as high or low β-glucan products. However, since β-glucan is a dietary fiber, it can be said to fall under the FDA Code of Federal Regulations Title 21, Section 101.54. This section states that a high dietary fiber food must contain at least 5g of dietary fiber. It also states that if a food product contains between 2.5g to 5g of dietary fiber, that product can be labelled with a "more fiber," "added fiber," or "extra fiber," claim. Based on the FDA specification of a 240ml (8 fl oz) serving size, consumption of the partial-enzyme hydrolyzed GMI beverage is expected to provide between 6.93 – 10.27 g of β-glucan per serving. Consumption of the partial-acid hydrolyzed GMI beverage on the other hand is expected to provide between 10.27 - 12.79 g of β-glucan per serving. A 240ml serving of the partial-enzyme hydrolyzed Natty beverage is expected to provide between 3.20 – 3.27 g of β-glucan per serving whilst the partial-acid hydrolyzed Natty beverage is expected to provide between 4.56 – 4.78 g of β-glucan.

Results found in our study, on β-glucan content and the relationship with viscosity, were compared to viscosity reduction observations reported by Bae *et al*., (2009). Bae and

coworkers (2009) reported that a reduction in the β-glucan content of a β-glucan solution has direct impact on the viscosity and as such, its functionality. The results of our study therefore indicate that, partial enzyme hydrolysis is a better hydrolysis treatment at reducing the viscosity of the beverages, whilst conserving its β-glucan content and subsequently its functionality.

Table 4.12. Percentage β-Glucan content in GMI oat/barley blend flour and partially hydrolyzed beverage samples.

Blend type	Flour (%)	GMI Beverage (Partial Enzyme Hydrolysis) (%)	GMI Beverage (Partial Acid Hydrolysis) (%)
100 Oat	7.33 ± 0.07^{a}	2.89 ± 0.04^{b}	4.40 ± 0.10^{b}
90Oat/10Barley	7.63 ± 0.02^{a}	3.73 ± 0.11^{b}	4.76 ± 0.05^{b}
80Oat/20Barley	7.77 ± 0.02^{a}	3.98 ± 0.00^{b}	4.93 ± 0.11^{b}
70Oat/30Barley	7.97 ± 0.00^{a}	4.17 ± 0.01^{b}	5.09 ± 0.18^{b}
60Oat/40Barley	8.07 ± 0.03^{a}	4.28 ± 0.11^{b}	5.33 ± 0.05^{b}

Values are mean ± standard deviation of two independent determinations. Values are reported on an as is basis. Means with same letters within rows are not significantly different ($p < 0.05$). GMI = High β-glucan oat variety. β-Glucan content of unhydrolyzed GMI beverage = 4.47 ± 0.06%.

Table 4.13. Percentage β-Glucan content in Natty oat/barley blend flour and partially hydrolyzed beverage samples.

Blend type	Natty (Flour) (%)	Natty Beverage (Partial Enzyme Hydrolysis) (%)	Natty Beverage (Partial Acid Hydrolysis) (%)
100 Oat	4.20 ± 0.04[a]	1.32 ± 0.06[b]	1.90 ± 0.01[b]
90Oat/10Barley	4.54± 0.01[a]	1.45 ± 0.01[b]	1.94 ± 0.03[b]
80Oat/20Barley	4.91± 0.00[a]	1.46 ± 0.02[b]	1.94 ± 0.05[b]
70Oat/30Barley	5.33± 0.02[a]	1.49 ± 0.02[b]	1.99 ± 0.01[b]
60Oat/40Barley	5.87± 0.02[a]	1.55 ± 0.03[b]	1.99 ± 0.03[b]

Values are mean ± standard deviation of two independent determinations. Values are reported on an as is basis. Means with same letters within rows are not significantly different ($p < 0.05$). Natty = Low β-glucan oat variety. β-Glucan content of unhydrolyzed Natty beverage = 1.92 ± 0.01%.

4.8 Total Dietary Fiber Content

Total dietary fiber (TDF) content of the partially hydrolyzed and unhydrolyzed beverages are provided in Table 4.14. Of the 80:20 oat-barley beverages, the TDF content was found to be significantly higher in the GMI beverages, compared to the Natty beverages (9.5% TDF in GMI blend versus 7.5% TDF in Natty blend). This was expected as the GMI beverages had a higher β-glucan content (a soluble and fermentable type of dietary fiber). Enzyme-treated beverages containing purely 100% of each two varieties yielded corresponding levels of TDF - Namely, higher TDF for 100%GMI (8.25%TDF) compared to 100%Natty (7.25% TDF). For the effects of enzyme hydrolysis, enzyme treatment yielded mixed results. Enzyme treatment of Natty samples reduced TDF% in the controls (from 8.20% TDF down to 7.25 % TDF). This was a statistically significant reduction with $p = 0.00095$. Enzyme treatment of the 100% purely GMI variety beverage actually increased TDF content from 6.9% TDF (in 100%GMI No Enzyme) to 8.25% TDF in 100% GMI Enzyme Treatment. Perhaps there was a problem with the 100% GMI control (no enzyme). 6.9% seems low for a GMI variety. The effects of enzyme action relative to TDF reduction needs to be further explored. After moisture corrections were carried out, the partial enzyme hydrolyzed 80/20 GMI and Natty beverages was shown to have a TDF content of 8.61 g and 6.70 g per 240ml serving, respectively. According to the FDA Code of Federal Regulations Title 21, Section 101.54, both beverages can be classified as high dietary fiber food products since they contain more than 5g of total dietary fiber on a ready to drink basis as provided in Table 4.22.

Table 4.14. Total Dietary Fiber content of partially hydrolyzed and unhydrolyzed beverages

Parameter	Total Dietary Fiber (%)
80%GMI20%Barley (Enzyme)	9.50 ± 0.14^{a}
100%GMI (Enzyme)	8.25 ± 0.07^{b}
100% GMI (Control- No Enzyme)	6.90 ± 0.14^{cd}
80%Natty20%Barley (Enzyme)	7.50 ± 0.00^{c}
100%Natty (Enzyme)	7.25 ± 0.07^{c}
100% Natty (Control- No Enzyme)	8.20 ± 0.14^{b}

Values are mean ± standard deviation of two independent determinations. Means with different letters within column are significantly different ($p < 0.05$). GMI = High β-glucan oat variety, Natty = Low β-glucan oat variety β-G = β-Glucan. Unhydrolyzed GMI and Natty beverages (controls) do not contain any proportion of barley.

4.9 Sensory Analysis and Preference

Table 4.15 shows a summary of the statistical analysis of the paired preference test. The results were analyzed using a 2-tailed binomial test (Singh-Ackbarali and Maharaj, 2014). Our 16-member taste panel showed that consumer preference seemed to be largely influenced by percentage barley content, hydrolysis method used and apparent viscosity of the beverages. A paired preference test was engaged to predict consumer behavior with regard to preference of one hydrolysis method over the other within the various blend formulations. Measures of consumer acceptance of food are important metrics that influence overall product development, as acceptance of a food product may influence a consumer's choice to consume or purchase a product (Xia *et al*., 2016). The number of judges preferring each sample was totaled and k tested for significance using the Vassarstat binomial probability calculator. The calculator provided the p-value for various combinations of k and n. Since a p-value of 0.05 or less is usually required for the observation to be considered significant, it was realized that the most acceptable hydrolysis method for the prototypes on the overall, was the partial-enzyme hydrolysis, as it was statistically significantly more preferred by the panelists over the acid hydrolyzed beverages ($p = 5.63\times10^{-12}$).

After the first stage of paired preference testing confirmed that partial enzyme hydrolyzed beverages for each formulation were the most preferred, participants used the quantitative descriptive analysis (QDA) method to test attributes of appearance, aroma, taste, texture and acceptability of the partial-enzyme hydrolyzed beverages in the second stage. The test was done by assessing the intensity of above-mentioned parameters on a five-point

hedonic scale and the results observed in both oat blend varieties are illustrated on Tables 4.16 and 4.17.

Table 4.15. Paired preference test between partial-enzyme hydrolysis and partial-acid hydrolysis within the various beverage formulations

Statistical Parameter	100G	90G10B	80G20B	70G30B	60G40B	100N	90N10B	80N20B	70N30B	60N40B
n	16	16	16	16	16	16	16	16	16	16
k - EH	12^a	11^a	16^a	12^a	14^a	14^a	12^a	14^a	13^a	14^a
k - AH	4^b	5^b	0^b	4^b	2^b	2^b	4^b	2^b	3^b	2^b
Binomial z ratio	1.75	1.25	3.75	1.75	2.75	2.75	1.75	2.75	2.25	2.75
p-value	0.028	0.067	0.000015	0.028	0.0018	0.0018	0.028	0.0018	0.085	0.0018

n= the number of panelists engaged in the sensory test, k – EH = the number of panelists preferring the partial-enzyme hydrolysis formulation; k – AH = the number of panelists preferring the partial-acid hydrolysis formulation; p- value about the 95 % confident interval ($p<0.05$). Values with different letters in each column are significantly different ($p < 0.05$).

Table 4.16. Quantitative descriptive sensory analysis of partial enzyme hydrolyzed GMI/Barley blend beverages. n = 16 subjects

Blend	Appearance	Aroma	Taste	Texture	Overall Acceptability
100G	3.81 ± 0.88^{a}	3.13 ± 1.11^{b}	3.50 ± 1.06^{b}	3.38 ± 0.99^{ab}	3.13 ± 0.93^{b}
90G/10B	4.00 ± 0.71^{a}	3.38 ± 0.86^{ab}	3.50 ± 0.94^{b}	3.56 ± 0.93^{ab}	3.44 ± 0.79^{ab}
80G/20B	4.06 ± 0.75^{a}	4.13 ± 0.86^{a}	4.56 ± 0.61^{a}	4.50 ± 0.61^{a}	4.38 ± 0.60^{a}
70G/30B	4.25 ± 0.75^{a}	4.25 ± 0.75^{a}	4.00 ± 0.87^{a}	3.94 ± 0.90^{a}	4.06 ± 0.83^{a}
60G/40B	4.38 ± 0.78^{a}	4.31 ± 0.77^{a}	3.56 ± 1.06^{b}	3.00 ± 0.87^{b}	3.50 ± 0.79^{ab}
Unhydrolyzed GMI	2.56 ± 1.27^{b}	2.94 ± 1.09^{b}	1.88 ± 0.70^{c}	1.25 ± 0.43^{c}	1.88 ± 0.60^{c}

Values are mean ± standard deviation of sixteen independent determinations. Means with different letters in each column are significantly different ($p < 0.05$). G= GMI423 oat variety, B = Barley. Scores (Five-point hedonic scale: 1= Dislike extremely, 2= Dislike slightly, 3 = Neither like nor dislike, 4 = Like slightly, 5 = Like extremely). Unhydrolyzed GMI beverage (control) does not contain any proportion of barley.

Table 4.17. Quantitative descriptive sensory analysis of partial enzyme hydrolyzed Natty/Barley blend beverages. n = 16 subjects

Blend	Appearance	Aroma	Taste	Texture	Overall Acceptability
100N	3.00 ± 1.06^{b}	3.31 ± 1.21^{ab}	3.25 ± 1.48^{ab}	3.13 ± 1.05^{ab}	3.13 ± 1.27^{b}
90N/10B	3.50 ± 1.00^{ab}	3.56 ± 1.06^{ab}	3.50 ± 1.22^{ab}	3.44 ± 1.06^{a}	3.50 ± 1.06^{b}
80N/20B	4.44 ± 0.61^{a}	4.19 ± 0.88^{a}	4.50 ± 0.50^{a}	4.38 ± 0.48^{a}	4.63 ± 0.48^{a}
70N/30B	4.63 ± 0.60^{a}	4.38 ± 0.70^{a}	3.81 ± 1.01^{a}	3.75 ± 0.97^{a}	3.50 ± 1.06^{b}
60N/40B	4.75 ± 0.43^{a}	4.56 ± 0.50^{a}	3.25 ± 1.09^{ab}	2.50 ± 1.00^{ab}	3.25 ± 0.90^{b}
Unhydrolyzed Natty	3.06 ± 1.03^{b}	3.25 ± 1.09^{ab}	2.69 ± 1.26^{b}	1.63 ± 0.60^{b}	2.31 ± 0.92^{bc}

Values are mean ± standard deviation of sixteen independent determinations. Means with different letters in each column are significantly different ($p < 0.05$). N= Natty oat variety, B = Barley. Scores (Five-point hedonic scale: 1= Dislike extremely, 2= Dislike slightly, 3 = Neither like nor dislike, 4 = Like slightly, 5 = Like extremely). Unhydrolyzed Natty beverage (control) does not contain any proportion of barley.

Data analysis was completed using a mixed model analysis of variance for treatment by subject, in replicates. Based on the results of the QDA, participants showed a very similar trend in acceptance between GMI and Natty beverages on all parameters analyzed. The appearance (4.38; 4.75) and aroma (4.31; 4.56) of 60Oat40B for both oat varieties (GMI and Natty) were seen to be much liked compared to the other blend formulations. There was no significant difference between the aroma preference of 60Oat40B, 70Oat30B and 80Oat20B. However, these values were significantly higher compared to 100Oat, 90Oat10B and the unhydrolyzed GMI and Natty control for both oat varieties ($p < 0.0001$). The addition of barley imparted an aroma which was preferred by the panelists and as the barley content increased, the aroma preference for the sample increased also. With regard to appearance, the preference was seen to increase as the barley content increased in both oat variety beverages. However, for the GMI variety no significant difference was observed between the appearance of the hydrolyzed samples which ranged between 3.13 to 4.31, but they differed significantly from the unhydrolyzed control (2.94). Within the Natty variety, the appearance values of 60Oat40B, 70Oat30B and 80Oat20B (4.56; 4.38; 4.19) were seen to differ significantly from the rest of the formulations. The addition of barley increased consumer acceptance of appearance, as barley introduced a desirable yellowish color, which added more brightness to the beverages containing a greater percentage of barley.

Taste test analysis showed 80Oat20B (4.56; 4.50) as the most preferred formulation for both GMI and Natty oat varieties, respectively. In both GMI and Natty oat varieties, 70Oat30B (3.81; 4.90) was not significantly different from 80Oat20B. However, both were significantly different from the other formulations including the unhydrolyzed

control ($p < 0.0001$). No particular trend was observed for this parameter. These observations were in keeping with reports by Matta *et al*., (2006), that increased hydrocolloid content/viscosity contributed to decreased perceived taste in beverages. Beverages 100Oat, 90Oat10B and 80Oat20B were found to have the most acceptable mouthfeel texture ($p<0.05$). The 70Oat30B, 60Oat40B and unhydrolyzed GMI and Natty controls had the least accepted texture. As mentioned earlier, viscosity of the beverages increased as the proportion of barley in the beverage increased. These findings suggest that increase in viscosity is one of the main concerns when developing high protein, high β-glucan beverages. Beverages with high viscosity are difficult to swallow, which might result in a low preference for the product (Vasquez-Orejarena *et al*., 2018). Figure 8 shows the correlation between beverage viscosity and consumer texture (mouthfeel) acceptability. A significant negative correlation between the two was observed ($p < 0.0001$). As the beverage viscosity increased, the less acceptable the beverage became. Beverages with viscosities greater than 180Cp, were less acceptable (hedonic scale < 3). This was especially observed in the unhydrolyzed GMI and Natty controls which had an apple sauce-like consistency (~400cP). With the r squared (R^2) evaluating the scatter of the data points around the fitted regression line, it can be said that about 60 % of the variation in texture acceptability can be accounted for by regression on beverage viscosity. In his book, Regression Analysis, Frost, (2019) surmised certain factors that explain some unexpected variations in research studies. A typical example is the observance of lower R^2 values human behavior, generally less than 50%, owing to the fact that its harder to predict human behavior than naturally occurring physical processes.

As mentioned previously, overall consumer acceptability was to a large extent influenced by percentage barley content, hydrolysis method used and apparent viscosity of the beverages. The most acceptable beverages on the overall were the GMI 80Oat20B beverage and Natty 80Oat20B beverage with overall acceptability values of 4.38 and 4.60, respectively. Within the Natty oat variety this blend was significantly preferred compared to all the other blend formulations and controls ($p < 0.001$). Within the GMI oat variety, there was no significant difference observed in the overall consumer acceptability between 80Oat20B and 70Oat20B ($p = 0.8973$). However, these blends significantly differed from the other blend formulations on the basis of overall acceptability ($p < 0.0001$).

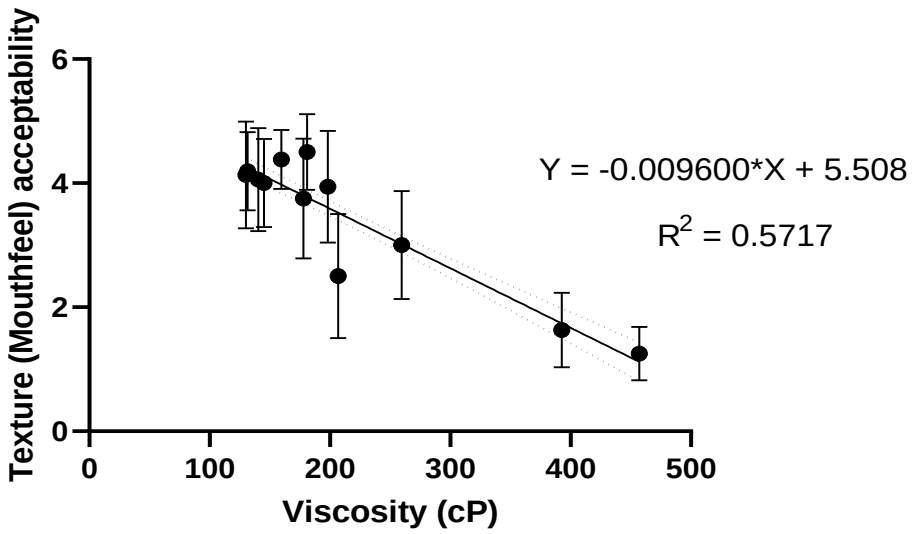

Figure 8. Texture (mouthfeel) acceptability as a function of measured beverage viscosity for the various formulations of enzyme-hydrolyzed β-glucan beverages.

4.10 Human Satiety Testing

In this study, we employed a protocol described by Forde, (2018) to study Satiety. Satiety is a physiological state which contributes to suppression of food ingestion, mainly through the suppression of hunger and a state of feeling full during an inter-meal period. For our study, nineteen participants were recruited including 12 females and 7 males, 28.7 ± 11.0 years of age, with an average BMI of 25.6 ± 4.8 kg/m^2. One male participant was excluded before the start of the study on the basis of an irregularly high fasting blood glucose. Table 4.18 provides a summary of the descriptive characteristics measured at the pre-screening session. The pre-screening was carried out following procedures outlined by Rebello *et al*., (2016).

The weight and caloric content of breakfast meals and lunch meals served are presented in Table 4.19. Calories from fat was not calculated for lunch meals because only the total calories were needed for this particular study. It was seen that breakfast meals served as 'regular' breakfast had a significantly higher caloric content than the other three breakfast options served over the four-week period ($p < 0.05$). During this period, all breakfast meals served were fixed/controlled, in order to enhance monitoring of breakfast intake and consistency with the other studies on gastric emptying. On the other hand, lunch meals were served *ad libitum*, where participants were instructed to help themselves to their pre-selected meal, as much as they wished until they were comfortably full. The amount of food and beverage consumed was determined by weighing the meal before and after consumption.

Table 4.18. Descriptive characteristics measured during pre-screening of 19 participants enrolled in satiety study

	Average	Standard Deviation	Range
BMI (kg/m^2)	25.6	4.8	18.2 - 38.4
AGE (years)	28.7	11.0	18 - 58
Body Weight (kg)	75.4	14.1	48.2 - 108.7
Height (cm)	171.9	10.3	156.5 - 195.0
Waist Circumference (cm)	85.5	11.7	69 - 108.5

BMI = Body Mass Index

Four tests meals were assessed for satiety during the four-week period (one test meal as breakfast per week). Two of these test meals were the developed β-glucan beverages, namely, Natty/Barley (low β-glucan beverage) and GMI/Barley (high β-glucan beverage). The other two breakfast meals, namely, the commercial beverage and regular breakfast (chosen based on popularity) were included as controls. A mixed model analysis of variance was performed to analyze the total energy intake at the *ad libitum* lunch (kcal) and VAS ratings of hunger, fullness, desire to eat, prospective food intake. Ratings for analysis of hunger, fullness, desire to eat and prospective intake before breakfast, as illustrated in Figure 9, showed no significant difference between the four test meals analyzed. All participants came into the testing center somewhat hungry, with a mean VAS rating of 6.51 ± 0.37. After consumption of the breakfast meals, it was observed that the hunger, desire to eat and prospective intake scores for the regular breakfast (1.13 ± 1.17cm; 1.37 ± 1.56cm; 1.36 ± 1.48cm) and commercial beverage (1.38 ± 1.53cm; 1.87 ± 1.77cm; 1.83 ± 1.74cm) were significantly lower than the other two breakfast meals. This could have been due to the large meal size of the regular breakfast, as the effect of meal size in satiation assessed by Holt *et al*., (1995) on thirty-eight isoenergetic foods revealed that food weight or size is the most important factor which affects satiation. Directly after consumption of breakfast meal, the parameter being measured is satiation. Satiation is mostly influences by the serving size of a meal (g or kcal) (Forde C., 2017).The commercial beverage on the other hand is formulated primarily from milk protein isolates which are complete dairy proteins that contain both casein and whey proteins. It is has been reported that have shown that high consumption of casein and whey boost satiety because whey is subjected to quick digestion and a

subsequent increase in circulating amino acids, leading to the release of satiety hormones (Giles-Smith K., 2013).

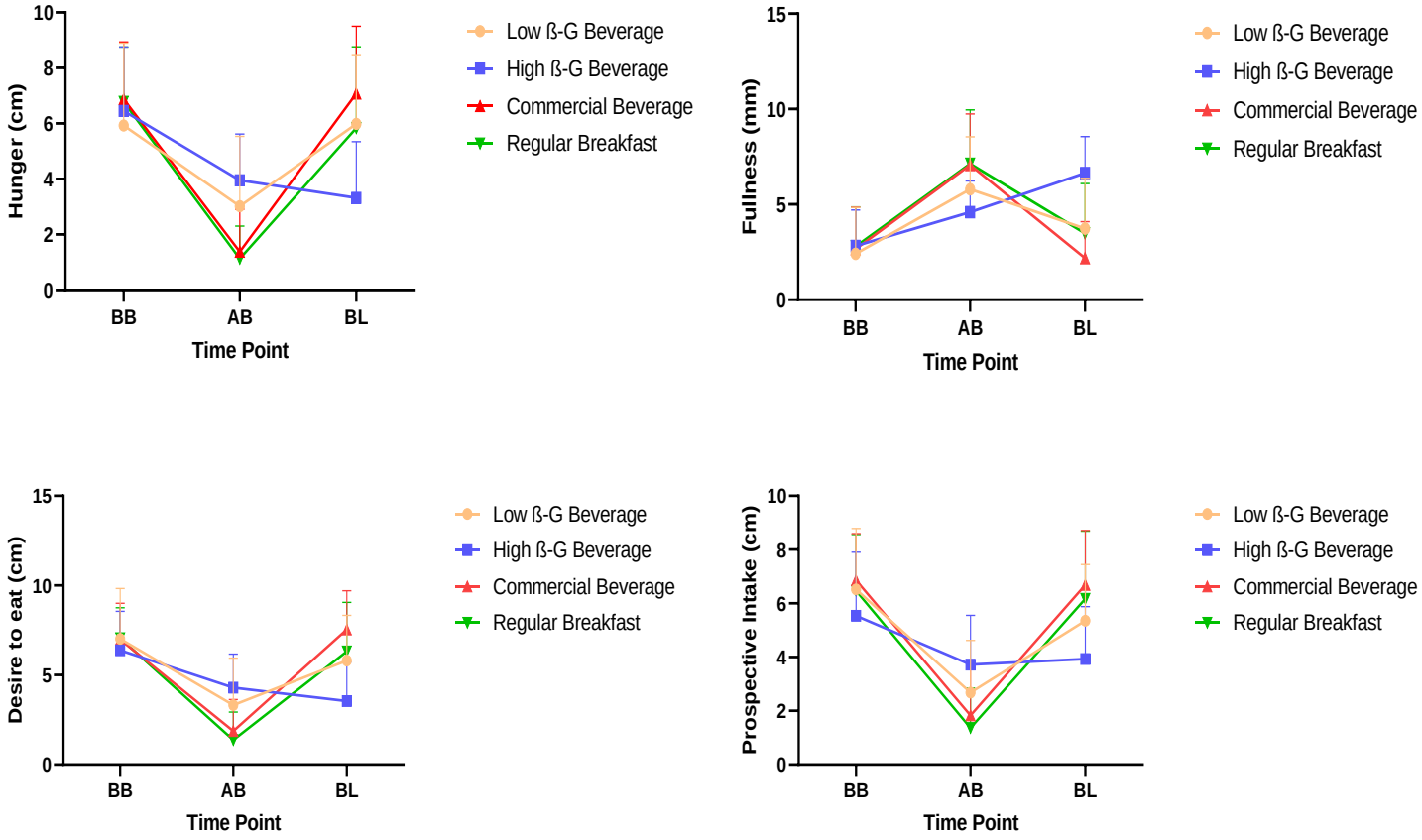

Figure 9. Mean (±SEM) subjective satiety scores using visual analog scale ratings (n = 18) before and after consumption of a low β-glucan beverage, high β-glucan beverage, commercial beverage and regular breakfast. BB – Before Breakfast, AB – After Breakfast, AL – After Lunch. Tukey multiple comparison test used in analyzing data was carried out at the 95% confidence level ($p<0.05$).

Though these factors have been found to improve satiation, their effect on food intake later (satiety) in the day has been found to be inconsistent (Giles-Smith K., 2013). Moreover, it is not known whether these effects are maintained (European Commission, 2012). Due to similar reasons, the commercial beverage (7.07 ± 2.67cm) and regular breakfast (7.13 ± 2.83cm) showed a significantly pronounced VAS score rating for fullness than the other test meals, after consumption of breakfast.

After a 4-hr period following breakfast consumption, participants rated test meals on the four satiety responses before consuming their *ad libitum* lunch meal. As shown in Figure 9, reduction in huger was significantly greater with the high β-glucan beverage than the low β-glucan beverage ($p = 0.0012$), commercial beverage ($p < 0.0001$) and regular breakfast ($p = 0.0025$). A similar reduction in desire to eat was determined after consuming the high β-glucan beverage compared to the low β-glucan beverage ($p = 0.069$), commercial beverage ($p < 0.0001$) and regular breakfast ($p = 0.0007$). Four hours after consumption of breakfast meals, an increase in fullness was significantly greater with the high β-glucan beverage compared to the low β-glucan beverage ($p = 0.0007$), commercial beverage ($p < 0.0001$) and regular breakfast ($p = 0.0002$). A mixed model analysis of variance on the VAS ratings for prospective intake before lunch showed no significant difference between the high β-glucan beverage and low β-glucan beverage ($p = 0.0946$), however the high β-glucan beverage showed a significantly pronounced reduction in prospective intake compared to the commercial beverage ($p = 0.0002$) and regular breakfast ($p = 0.0031$).

The observations made in this study seemed to be consistent with previous studies as β-glucan was shown to corroborate the satiety response, similar to effects observed by

Rebello *et al*., (2016) in a study comparing the satiety effects of an instant oatmeal breakfast to a ready to eat cereal breakfast. In another study, breakfast meals containing varying levels of oat β-glucan , control (0 g), low (2.16 g), medium (3.82 g), and high (5.45 g), were compared for their influence on satiety (Beck *et al*., 2009). Their results revealed an increase in satiety at all doses compared with the control. Wood, (2007) and Beck *et al*., (2009) described the mechanism underlying the postprandial effect of β-glucan on satiety, where β-glucan is said to fundamentally increase gastrointestinal viscosity leading to solubilization of the food content and subsequent disruption of micelle formation and reduced contact with the intestinal walls. Absorbed nutrients, suppress the release of the hunger hormone ghrelin thereby leading to stimulation of the duodenal satiety hormone cholecystokinin (CCK) along with glucagon-like peptide 1 and peptide Y-Y3-36 (PYY3-36), resulting in a decrease in appetite. Furthermore, this study showed that the β-glucan meals took some time to initiate satiation effects compared with the other breakfast meals confirming the hypothesis made by Vitaglione *et al*., (2010) in a study on the satiating effect of a barley beta-glucan–enriched snack that, β-glucan entrapped in the cell walls (barley or oats) acts slowly compared with β-glucan used as extracts. In spite of its slow satiation effects, strong satiety effects of the high β-glucan beverage were observed during the 4-hr post-ingestive period similar to the study outcome reported by Juvonen *et al*., (2009).

In our study, regression analysis as presented in Figure 10, showed a non-significant correlation ($p = 0.4771$) between recorded hunger time over 4h postprandial (breakfast) period and mean ad libitum (lunch) intake ($R^2 = 0.2734$). However, increasing energy

intake in *ad libitum* lunch was seen to occur with decreasing minutes of recorded hunger time.

The high β-glucan beverage was seen to direct the lowest energy intake at lunch. A multiple comparison of one-way analysis of variance on energy intake showed no significant difference between energy intake at lunch following consumption of the high β-glucan beverage and low β-glucan beverage as breakfast meals (p = 0.0573). However, the commercial beverage (p = 0.0002) and regular breakfast (p = 0.0046) showed a significantly higher energy intake at lunch compared to the high β-glucan beverage.

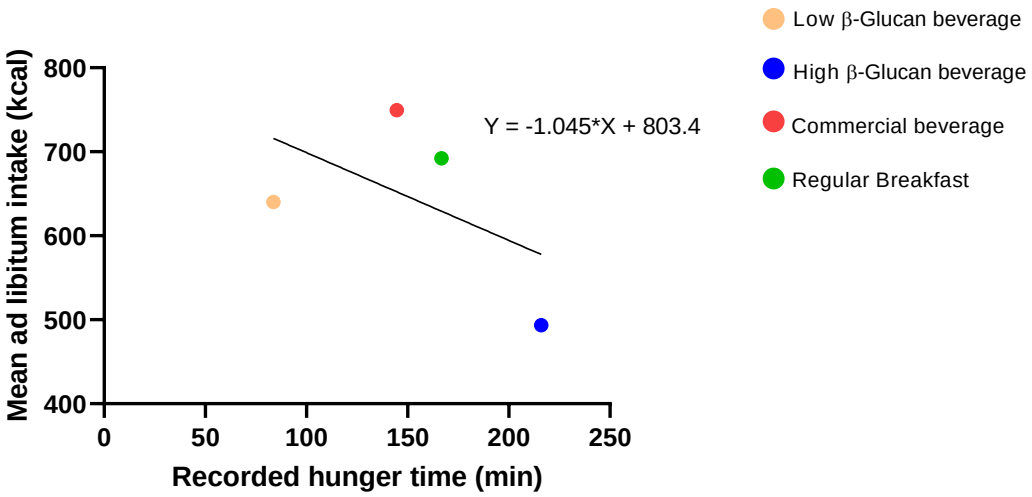

Figure 10. Correlation between recorded hunger time and Ad libitum (lunch) intake for each breakfast test meal. R^2 = 0.2734 for huger time vs. energy intake.

4.11 Further Analysis (Amino Acid Profile and Element Analysis)

To minimize time and cost constraints, amino acid profile and elemental profile analysis were only carried out on samples preferred by the consumer panel test and that were employed in the satiety studies. These samples included 80Oat and 20Barley beverages from both the GMI and Natty varieties, and the unhydrolyzed GMI and Natty beverage (controls).

4.11.1 Amino Acid Profile

Table 4.20 shows the amino acid profile of the partially hydrolyzed beverages, unhydrolyzed beverages and lactose free fat-free (LFFF) milk (control). The estimation of protein requirements considers not only their quantity but their quality as well. The requirements for dietary protein are aimed at providing the nine essential amino acids, which are only supplied in a balanced meal. Essential amino acids include histidine, isoleucine, leucine, valine, lysine, threonine, phenylalanine, methionine, and tryptophan (Young *et al*., 2000). Results are presented as grams of amino acid per 100 grams of sample on a % w/w basis. Grouping the amino acids based on their nutritional/ physiological roles, it was seen that the amount of non-essential amino acids (hydroxyproline, aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine, tyrosine and hydroxylysine) were significantly higher in the LFFF milk, ranging between 0.01g to 7.10g compared to the partially hydrolyzed high β-glucan beverage (0.01g to 2.26g), unhydrolyzed high β-glucan beverage (0.02g to 1.70g), partially hydrolyzed low β-glucan beverage (0.03g to 1.79g) and unhydrolyzed low β-glucan beverage (0.03g to 1.84g).

Table 4.20. Amino acid composition of partially hydrolyzed and unhydrolyzed beverages (experimental) and milk (control sample)

Amino acid (w/w%)	High β-G Beverage (80G20B)	Unhydrolyzed GMI Beverage	Low β-G Beverage (80N20B)	Unhydrolyzed Natty Beverage	Lactose Free Fat Free Milk (Control)
Taurine §	0.14 ± 0.00 ^{a}	0.05 ± 0.007 ^{b}	0.05 ± 0.00 ^{b}	0.06 ± 0.007 ^{b}	0.16 ± 0.007 ^{a}
Hydroxyproline	0.01 ± 0.00 ^{b}	0.05 ± 0.00 ^{a}	0.05 ± 0.00 ^{a}	0.05 ± 0.014 ^{a}	0.01 ± 0.014 ^{b}
Aspartic Acid	1.00 ± 0.00 ^{b}	0.93 ± 0.00 ^{cd}	0.91 ± 0.00 ^{d}	0.94 ± 0.00 ^{c}	2.565 ± 0.007 ^{a}
Threonine	0.49 ± 0.00 ^{b}	0.49 ± 0.00 ^{b}	0.50 ± 0.00 ^{b}	0.51 ± 0.00 ^{b}	1.45 ± 0.007 ^{a}
Serine	0.56 ± 0.007 ^{bc}	0.57 ± 0.00 ^{b}	0.56 ± 0.007 ^{bc}	0.58 ± 0.00 ^{b}	1.62 ± 0.014 ^{a}
Glutamic Acid	2.66 ± 0.00 ^{b}	1.70 ± 0.021 ^{e}	1.79 ± 0.021 ^{d}	1.84 ± 0.035 ^{c}	7.10 ± 0.021 ^{a}
Proline	1.03 ± 0.007 ^{b}	0.91 ± 0.007 ^{c}	1.01 ± 0.007 ^{b}	0.95 ± 0.00 ^{c}	3.28 ± 0.007 ^{a}
Lanthionine §	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
Glycine	0.41 ± 0.00 ^{b}	0.39 ± 0.00 ^{b}	0.38 ± 0.00 ^{bc}	0.38 ± 0.00 ^{bc}	0.64 ± 0.00 ^{a}
Alanine	0.49 ± 0.00 ^{b}	0.45 ± 0.00 ^{c}	0.45 ± 0.00 ^{c}	0.46 ± 0.00 ^{c}	1.09 ± 0.00 ^{a}
Cysteine	0.22 ± 0.007 ^{b}	0.24 ± 0.007 ^{b}	0.22 ± 0.007 ^{b}	0.23 ± 0.007 ^{b}	0.27 ± 0.00 ^{a}
Valine	0.78 ± 0.00 ^{b}	0.79 ± 0.00 ^{b}	0.80 ± 0.00 ^{b}	0.80 ± 0.007 ^{b}	2.16 ± 0.007 ^{a}
Methionine	0.28 ± 0.00 ^{bc}	0.31 ± 0.021 ^{b}	0.30 ± 0.007 ^{b}	0.31 ± 0.014 ^{b}	0.80 ± 0.007 ^{a}
Isoleucine	0.63 ± 0.00 ^{c}	0.67 ± 0.00 ^{b}	0.68 ± 0.00 ^{b}	0.67 ± 0.00 ^{b}	1.78 ± 0.021 ^{a}
Leucine	1.13 ± 0.007 ^{b}	1.11 ± 0.00 ^{b}	1.13 ± 0.00 ^{b}	1.14 ± 0.00 ^{b}	3.27 ± 0.007 ^{a}
Tyrosine	0.41 ± 0.007 ^{c}	0.44 ± 0.00 ^{b}	0.44 ± 0.007 ^{b}	0.46 ± 0.014 ^{b}	1.59 ± 0.014 ^{a}
Phenylalanine	0.66 ± 0.00 ^{b}	0.67 ± 0.00 ^{b}	0.67 ± 0.00 ^{b}	0.68 ± 0.00 ^{b}	1.65 ± 0.007 ^{a}
Hydroxylysine	0.01 ± 0.00 ^{ab}	0.02 ± 0.00 ^{a}	0.03 ± 0.007 ^{a}	0.03 ± 0.007 ^{a}	0.04 ± 0.007 ^{a}
Ornithine §	0.00 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.007 ^{a}	0.00 ± 0.00 ^{a}
Lysine	0.79 ± 0.00 ^{b}	0.72 ± 0.00 ^{d}	0.75 ± 0.00 ^{c}	0.77 ± 0.007 ^{c}	2.60 ± 0.007 ^{a}
Histidine	0.33 ± 0.00 ^{b}	0.33 ± 0.00 ^{b}	0.34 ± 0.00 ^{b}	0.34 ± 0.00 ^{b}	0.92 ± 0.00 ^{a}
Arginine	0.56 ± 0.007 ^{b}	0.48 ± 0.007^{d}	0.45 ± 0.007 ^{e}	0.51 ± 0.007 ^{c}	1.13 ± 0.00 ^{a}
Tryptophan	0.17 ± 0.007 ^{b}	0.16 ± 0.00 ^{b}	0.17 ± 0.007 ^{b}	0.16 ± 0.00 ^{b}	0.49 ± 0.007 ^{a}
Total	12.725 ± 0.02^{b}	11.46 ± 0.01^{e}	11.645 ± 0.02^{d}	11.84 ± 0.03^{c}	34.56 ± 0.08^{a}

Values are means ± standard deviation of two independent determinations. Means with different letters within rows are significantly different ($p < 0.05$). GMI = High β-glucan oat variety, Natty = Low β-glucan oat variety. Unhydrolyzed GMI and Natty beverage (controls) do not contain any proportion of barley. § = Non-proteinogenic amino acids.

Glutamic acid was found to be the most available non-essential amino acid in all the samples and hydroxylysine and hydroxyproline were found to be the most deficient. These observations evidenced findings by Rafiq et al., (2016), who also found glutamic acid to be the most abundant non-essential amino acid occurring in cow's milk (21.8g/100g). The high levels of glutamic acid could be due to experimental conditions that could cause the transformation of glutamine into glutamic acid (Mansouri *et al*., 2018). Non-essential amino acids are known to also play vital roles in physiological metabolism, including the following; cell signaling, DNA and protein synthesis pathways, gene expression and regulation, defining antioxidative responses to free radicals and roles in immunity, just to mention a Hou *et al*., (2015).

Non proteinogenic amino acids (taurine, ornithine and lanthionine) were the least available group of amino acids with lanthionine and ornithine being almost inaccessible in all samples analyzed. Taurine which was the most expressed non proteogenic was significantly higher in the LFFF milk (0.16g) and partially hydrolyzed high β-glucan beverage (0.14g) compared to the unhydrolyzed high β-glucan beverage (0.05g), partially hydrolyzed low β-glucan beverage (0.05g) and unhydrolyzed low β-glucan beverage (0.06g). Some nonproteinogenic amino acids (e.g. homoserine, ornithine) have been reported by Walsh *et al*., (2015) to be utilized as intermediates in primary metabolic pathways.

The lactose free fat free milk had the highest amounts of each of the analyzed essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and tryptophan). The most abundant essential amino acid occurring in all five analyzed samples was leucine, similar to results obtained by Rafiq et al., (2016).

In their study on the amino acid profile of casein and whey proteins, leucine was reported as the most abundant essential amino acid (10.8g/100g) in cow's milk. Leucine is also known to contribute essentially to the activation of the mammalian target of rapamycin (mTOR) signaling pathway, a pathway which also influences the recycling of amino acids, leading to protein synthesis and lean muscle building and fat reduction (Duan *et al*., 2015). LFFF milk ranged between 0.49g to 3.27g, partially hydrolyzed high β-glucan beverage (0.17g to 1.13g), unhydrolyzed high β-glucan beverage (0.16g to 1.11g), partially hydrolyzed low β-glucan beverage (0.17g to 1.13g) and unhydrolyzed low β-glucan beverage (0.16g to 1.14g), on the content of essential amino acids. Foods that contain all eight essential amino acids (EAA) are called "complete" proteins. Essential amino acids have been reported to have many biological effects including alleviating insomnia, aiding in the production of antibodies, hormones and enzymes, biosynthesis of carnitine in the liver and kidneys, among others (Petkova *et al*., 2013).

The total amino acid content was significantly higher in the control LFFF milk as expected, compared to the partially hydrolyzed high β-glucan beverage, unhydrolyzed high β-glucan beverage, partially hydrolyzed low β-glucan beverage, and unhydrolyzed low β-glucan beverage ($p < 0.0001$). Therefore it can be said that the LFFF milk being a 'complete' protein contributed essential amino acids to the beverage hence improving the overall protein quality of the developed beverages – the ability of the beverages to supply the amino acid needs of the body (Shaheen *et al*., 2016).

4.11.2 Elemental Analysis

Evaluating specific mineral element content in the developed beverage is of significant benefit since these elements in excess or in sparseness may affect human health (Khan *et*

al., 2014). In this study, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), zinc (Zn) and Iron (Fe) were analyzed in the developed partial-enzyme hydrolyzed beverages together with the unhydrolyzed controls. Table 4.21 provides the elemental content of these samples. According to the Food and Drug Administration (FDA), calcium is an essential macro element which is needed in the human body for bone and teeth formation, constriction and relaxation of blood vessels, blood clotting, hormone secretion, muscle contraction and nervous system function. This element has been termed as 'a nutrient of concern' by the FDA since most individuals do not consume enough to meet the recommended daily intake. The concentrations of calcium were the highest in all the developed beverages and there were no significant differences observed in the calcium content of the experimental and control samples. In previous studies focused on the determination of essential and trace mineral elements in plain milk, Avegliano *et al*., (2011) and Khan *et al*., (2013) reported the calcium content as 863.1 and 1085mg/100g, respectively. The results of our study are therefore seen to be comparable to these literature values. A similar trend was seen in the phosphorus content, as all beverage samples showed no significant differences in the concentrations of phosphorus. These values were however lower when compared with the above-mentioned literature, where Khan *et al*., (2013) reported a phosphorus content of 824.4 mg/100g for plain milk. Phosphorus, according to the FDA is needed in the body for acid-base balance, bone formation, energy production and hormone activation. Sodium is an essential element useful for fluid balance, muscle contraction and nervous system function in the body. However, the Food and Drug Administration reports that Americans consume nearly 50 percent more than the 2,300 mg daily limit recommended by federal guidelines and this

increase in sodium intake raises blood pressure, which is a major risk factor for heart disease and stroke. There is therefore a strong recommendation on the reduction of sodium intake. All beverage samples were seen to have a low sodium content ranging between (113 to 133mg) with no significant differences observed within the samples. Literature values reported by Avegliano *et al*., (2011) and Khan *et al*., (2013) for plain milk samples were 329.2 and 256.7 mg/100g, respectively. Concentration of iron and zinc in the beverage samples were found to be comparable to that reported in literature on plain milk, where Avegliano *et al*., (2011) reported the iron content as 2.6 mg/100g and the zinc content as 2.9 mg/100g.

Table 4.21. Elemental content of partially hydrolyzed and unhydrolyzed beverages (mg/100g).

Element type	High β-G Beverage (80G20B)	Unhydrolyzed GMI Beverage	Low β-G Beverage (80N20B)	Unhydrolyzed Natty Beverage
Calcium	991 ± 0.013[a]	965 ± 0.017[a]	980 ± 0.011[a]	982 ± 0.001[a]
Phosphorus	388 ± 0.001[a]	383 ± 0.005[a]	389 ± 0.001[a]	387 ± 0.004[a]
Sodium	133 ± 0.001[a]	116 ± 0.002[a]	125 ± 0.001[a]	113 ± 0.000[a]
Potassium	569 ± 0.004[a]	556 ± 0.002[a]	538 ± 0.007[a]	526 ± 0.005[a]
Zinc	2.04 ± 0.141[a]	2.03 ± 0.424[a]	1.98 ± 0.919[a]	1.95 ± 0.212[a]
Iron	2.72 ± 4.738[a]	2.90 ± 0.919[a]	3.12 ± 5.233[ab]	2.54 ± 1.414[a]

Values are mean ± standard deviation of two independent determinations. Means with different letters within rows are significantly different ($p < 0.05$). GMI = High β-glucan oat variety, Natty = Low β-glucan oat variety. β-G = β-Glucan. Unhydrolyzed GMI and Natty beverage (controls) do not contain any proportion of barley.

Comparison of nutritional information on oat/barley beverages and commercial beverage

Nutritional information of the developed beverages and the commercial control beverages have been compared in Table 4.22. The commercial beverage was shown to have a lower TDF (3.75g), carbohydrate (5g) and calcium (440 mg) content compared to the developed beverages. The commercial beverage had a higher protein content of 15g compared to the high and low β-glucan beverage which had a protein content of 11.96g and 11.24g, respectively. Also, the commercial beverage had a higher fat (7g), than the developed beverages which had a fat content of (1.85g; 1.15g). According to the FDA Code of Federal Regulations Title 21, Section 101.56, the developed beverages can be classified as 'low fat' food products since they both contain 3 g fat or less per reference amount customarily consumed. The high β-glucan beverage and low β-glucan beverage were shown to have a caloric content of 119.63 and 112.15 kcal, respectively. This was much lower than the caloric content of the commercial beverage (133kcal). The FDA Code of Federal Regulations Title 21, Section 101.60 states that "the terms 'low calorie,' 'few calories,' 'contains a small amount of calories,' 'low source of calories,' or 'low in calories' may be used in labeling of meal products which contains less than 120 calories or less per reference amounts customarily consumed (RACC)". Based on this recommendation by the FDA, both the high and low β-glucan developed beverages can be termed as low caloric food products.

Table 4.22. Nutritional information of formulated beverages and commercial control beverage on a ready to drink as is basis

Nutritional Parameter	80G20B (High β-glucan beverage)	80N20B (Low β-glucan beverage)	Commercial beverage (control)
Moisture (%)	62.23	62.77	N/A
Protein (g)	11.96	11.24	15
Fat (g)	1.85	1.15	7
TDF (g)	7.61	4.56	3.7
Carbohydrate (g)	9.98	11.93	5
Calcium (mg)	898	874	440
Phosphorus (mg)	353	347	550
Sodium (mg)	120	111	180
Potassium (mg)	515	480	780
Zinc (mg)	1.84	1.77	5.2
Iron (mg)	2.47	2.78	6
Total Caloric Content (kcal)	119.63	112.15	133
Calories from fat (kcal)	16.65	10.35	37

Moisture corrections were calculated with the formula $C_{wet} = C_{dry}/(100/(100\text{-moisture})$. Values presented are based on a 240ml serving size, following the National Health and Nutrition Examination Survey's RACC (Reference Amounts Customarily Consumed). Nutritional information on commercial beverage was obtained from product nutritional label. N/A = Not available.

4.12 Shelf-Life Analysis

Pasteurized partially hydrolyzed beverages and an unpasteurized sample which were stored at refrigerated conditions (4 - 6°C) were monitored over a four-week period on aerobic plate count, total coliform, Escherichia Coli (E. Coli), pH and sensory quality. Aerobic plate count (APC) as an indicator of food quality, provides useful information about the general quality and remaining shelf life of the beverage, and thus highlights possible problems of storage and handling (Center for Food Safety, 2014). APC gives an estimate of the total number of viable microorganisms in a sample, and has been reported to be an index that reflect conditions such as the microbial content of the raw materials and ingredients, the effectiveness of processing procedures, the sanitary condition of equipment and utensils, and the time-temperature profile of storage and distribution (National Research Council, 1985). As illustrated in Table 4.23, there was no significant difference in the APC levels of the pasteurized partially hydrolyzed high β-Glucan beverage and partially hydrolyzed low β-Glucan beverage ($p = 0.9981$). As the weeks progressed, the APC levels of these pasteurized samples were observed to increase (1.37×10^2 - 1.39×10^3 cfu/g). However, during the last week of storage the APC levels of both pasteurized beverages were still below the (2.0×10^4 cfu/g) detection limit proposed by the FDA. There was a significant difference in the APC levels of the pasteurized beverages and unpasteurized controls over the 4-week period, as the unpasteurized beverages were shown to have a significantly higher microbial count. Results from statistical analyses of our data showed the significant effect of pasteurization temperature ($p < 0.0001$) on the APC levels of the developed beverage.

Similar effects were observed in the coliform count of the beverages. Pasteurized beverages had coliform counts between 2-3 cfu/g over the 4-week period. This value was significantly lower ($p < 0.001$) than the 11 – 24cfu/g enumerated for the unpasteurized controls.

Table 4.23. Aerobic Plate Count, Coliform and E. Coli levels in selected beverage samples monitored over a 4-week refrigerated shelf life period

	Aerobic Plate Count (cfu/g)			Coliform Count (cfu/g)			*Escherichia* Coli Count (cfu/g)			pH		
	80G/ 20B	80N/ 20B	UnP Beverage	80G/ 20B	80N/ 20B	UnP Beverage	80G/ 20B	80N/ 20B	UnP Beverage	80G/ 20B	80N/ 20B	UnP Beverage
Week 0	1.37×10^2 ($\pm$ 11.33)^{b}	1.80×10^2 ($\pm$ 26.50)^{b}	1.86×10^3 ($\pm$ 193.04)^{a}	2 $\pm$ 0.00^{b}	2 $\pm$ 0.00^{b}	11 $\pm$ 1.41^{a}	0	0	0	6.74^{b} ($\pm$ 0.007)	6.84^{a} ($\pm$ 0.021)	6.53^{c} ($\pm$ 0.014)
Week 1	3.4×10^2 ($\pm$ 106.84)^{b}	3.33×10^2 ($\pm$ 37.47)^{b}	6.41×10^3 ($\pm$ 449.72)^{a}	2 $\pm$ 0.50^{b}	2 $\pm$ 0.50^{b}	13 $\pm$ 1.41^{a}	0	0	0	6.78^{a} ($\pm$ 0.021)	6.81^{a} ($\pm$ 0.014)	6.23^{b} ($\pm$ 0.007)
Week 2	5.75×10^2 ($\pm$ 27.86)^{b}	5.73×10^2 ($\pm$ 23.81)^{b}	7.36×10^3 ($\pm$ 128.69)^{a}	2 $\pm$ 0.50^{b}	3 $\pm$ 0.60^{b}	15 $\pm$ 1.41^{a}	0	0	0	6.72^{a} ($\pm$ 0.00)	6.78^{a} ($\pm$ 0.014)	5.73^{b} ($\pm$ 0.021)
Week 3	1.0×10^3 ($\pm$ 12.73)^{b}	1.03×10^3 ($\pm$ 32.35)^{b}	1.10×10^4 ($\pm$ 579.12)^{a}	3 $\pm$ 0.00^{b}	3 $\pm$ 0.00^{b}	19 $\pm$3.54^{a}	0	0	0	6.71^{a} ($\pm$ 0.014)	6.77^{a} ($\pm$ 0.021)	5.42^{b} ($\pm$ 0.014)
Week 4	1.31×10^3 ($\pm$ 22.05)^{b}	1.39×10^3 ($\pm$ 18.55)^{b}	1.46×10^4 ($\pm$ 514.07)^{a}	3 $\pm$ 0.00^{b}	3 $\pm$ 0.50^{b}	24 $\pm$ 2.12^{a}	0	0	0	6.69^{a} ($\pm$ 0.021)	6.75^{a} ($\pm$ 0.014)	4.98^{b} ($\pm$ 0.078)

UnP = Unpasteurized, 80G20B = Partially hydrolyzed high β-Glucan beverage, 80N20B = Partially hydrolyzed low β-Glucan beverage, cfu/g = colony forming units per gram; Values are mean microbial levels ± standard deviation of four independent determinations. Means with different letters in each parameter and row are significantly different ($p < 0.05$). Aerobic plate count limit = ($< 2.0 \times 10^4$ cfu/g); Coliform/Escherichia Coli limit = (10cfu/g).

Again, pasteurization was shown to be an effective method of reducing coliform count in food since the pasteurized beverage had counts significantly below the FDA detection limit of 10cfu/g. Coliform count is used as a microbiological criterion for many foods to indicate post-heat processing contamination. As they are immobilized by heat processes used in food production and are readily removed from equipment and environment by appropriate cleaning, their presence is often suggestive of post process contamination in heat-processed food (Nestlé, S.A., 2019). The presumptive E. Coli content has been described by the Center for Food Safety, 2014 as the best indicator of fecal contamination in foods. Several strains of E. Coli have been reported to cause gastrointestinal illnesses if consumed, and as such the detection limit in processed foods has been set to 10cfu/g by the FDA. As shown in Table 4.23 both pasteurized and unpasteurized beverages had no E. Coli count over the 4-week monitoring period.

Changes in pH over the four-week storage period are also represented on Table 4.23. The pH of milk or milk products provides information on the fresh state of the product, as the pH of fresh milk has been reported to be just below neutral (6.5 – 6.9) (Anderson *et al*., 2011). Directly after pasteurization on the basis of initial microbiological quality, the low β-glucan beverage had a significantly higher pH value ($p = 0.0034$) than the high β-glucan beverage. However, after the first week of storage no significant differences were observed between the two pasteurized beverages. The unpasteurized beverages over the monitoring period showed significantly lower pH values ($p < 0.0001$) compared to the pasteurized beverages. Generally, the pH values were seen to decrease in all the beverages as the weeks progressed, and this gave an indication of the action of fermentation of lactose present in milk into lactic acid by spoilage microorganisms.

Multiple ANOVA comparisons on the pasteurized beverages over the week showed no significant differences between pH values from week 0 through week 4. Significant decrease in pH values ($p < 0.0001$) were however observed in the unpasteurized control sample. Gaucher *et al*., (2008) also suggests that the A reduction in pH could arise from loss of positively charged amino acids, resulting from reaction of free ε-NH2 groups of lysine with lactose in a Maillard-type reaction.

Figure 11 provides the sensory test results relating to the 4-week shelf life study on 80Oat and 20Barley beverages from both the GMI and Natty varieties, and the unhydrolyzed GMI and Natty beverage (controls). The overall acceptability shelf life test was conducted using a five-point sensory hedonic scale. As seen in figure 16, over the weeks, consumers ($n = 10$) were seen to prefer the low β-glucan beverage over the other beverages developed. This could be attributed to its low viscosity stemming from its low β-glucan content. However, the sensory acceptability of the low β-glucan beverage was found to be comparable to high β-glucan beverage, as no significant statistical difference was observed during the shelf-life period. The unhydrolyzed beverage controls had significantly lower consumer acceptability ratings over the four-week shelf-life period.

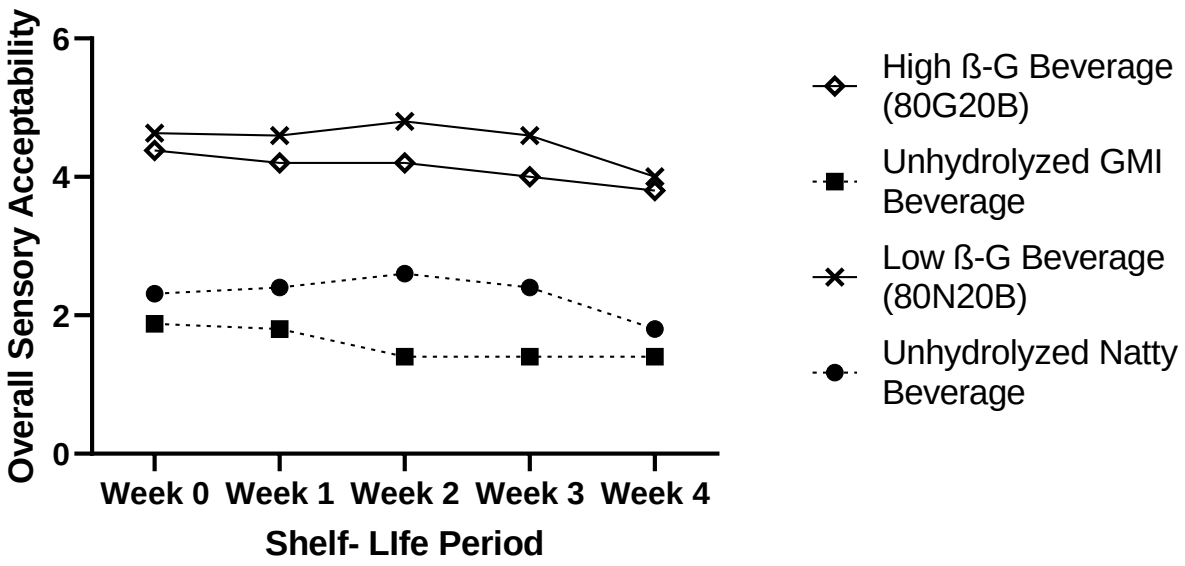

Figure 11. Overall sensory acceptability values of experimental and control beverages stored over a 4-week period at 4-6°C. Standard deviations are in the range of ± 0.40 to ± 0.92.

CHAPTER 5. CONCLUSION

Consumer demands for healthier, functional beverages continue to increase. This trend is driven mostly by individuals' alertness to healthier eating and well-being, due to rising levels of certain diseases like obesity and diabetes. Oat and barley, although containing the required nutrients needed for formulation of health promoting beverages, present problems with texture and viscosity in high moisture conditions. β-glucan, present in these cereals contribute to viscosity increases in food product containing the cereals. Partial enzyme and partial acid hydrolyses were utilized in this study to reduce viscosity of the beverage while maintaining its nutrition and functionality.

No statistical difference was observed between the nutritional composition of partial enzyme hydrolyzed β-glucan beverages and partial acid hydrolyzed β-glucan beverages. We therefore, accept the null hypothesis of Hypothesis 1. Partial enzyme hydrolysis reduced the viscosity of all the beverage formulations whilst partial acid hydrolysis did not decrease beverage viscosity, thus we accept the alternate hypothesis of Hypothesis 2. The partially hydrolyzed beverages were shown to have a significantly lower viscosity compared to the unhydrolyzed controls. The alternate hypothesis of Hypothesis 3 was thus accepted. Though partial enzyme hydrolysis lowered viscosity of the beverage, no significant difference in the β-glucan content was observed when compared to partial acid hydrolyzed and unhydrolyzed control beverages. This signaled a maintenance in β-glucan functionality of the beverages. Consumers preferred the partial-enzyme hydrolyzed 80%Oat20%Barley blends of both the high β-glucan oat variety (GMI423) and low β-glucan oat variety (Natty) during the sensory evaluation. Based on the results, it can be concluded that the main attributes influencing the overall sensory acceptability of the

beverages were texture, taste and hydrolysis method used. The alternate hypothesis of Hypothesis 4 was thus, accepted.

The satiety study indicated that the high β-glucan beverage suppresses appetite, increases satiety, and reduces subsequent energy intake, compared to the low β-glucan beverage, regular breakfast and commercially available hunger control beverage. We therefore, accept the alternate hypothesis of Hypothesis 5 and 6. Subsequent analysis showed that the developed beverages contained some amounts of essential and non-essential amino acids together with significant amounts of trace and macro elements. The beverages had low caloric content and proved to contain appreciable amounts of dietary fiber.

Shelf stability was achieved by thermal treatment in a pasteurizer. Compared to the unpasteurized beverages, pasteurized beverages maintained good microbiological quality over the 4-week refrigerated shelf life period. Analyzed indicator parameters of the pasteurized beverages were shown to be below the FDA detection limit. No significant variations in pH of the beverages were observed either. We therefore accept the alternate hypothesis of Hypothesis 7.

The present study demonstrates that through the use of partial enzyme hydrolysis, our developed shelf stable acceptable functional beverage meets the FDA claims of special dietary food (21 CFR 105.66), high dietary fiber and high protein content (21 CFR 101.54). The results presented in Table 4.22 support the above-mentioned claims and also shows that the developed beverages had a lower fat and caloric content than the commercially available beverage. The formulated high β-glucan beverage could therefore replace foods or meals in the diet and keep consumers full for an extended period while providing excellent nutrition needs of consumers.

Further clinical trials to assess the health impact of the formulated high β-glucan beverage on prevention and reduction of the prevalence of obesity are a good opportunity to conduct additional research.

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APPENDIX A: PRE-SCREENING FORM

MEDICAL HISTORY SCREENING QUESTIONNAIRE

Participant's Identification Number: Age:
Personal healthcare provider to contact in case of an emergency:
Name_____ Phone #:_____
City:_____

Have you been diagnosed, treated, medicated, and/or monitored for any of the conditions listed below within the time frame specified?
Only answer YES if a doctor or other licensed medical provider told you that you have or had this condition.
○ Yes If YES, fill in the circle next to the condition you have (or had) only if the applicable time frame applies to you.
○ No If NO continue on to the next section.

	In the last 12 months?	In the last 5 years
Stomach ulcer (example: peptic ulcer)	○ Yes	○ Yes
Other gastroenterological conditions (examples: abdominal pain, diarrheal infection)	○ Yes	○ Yes
Diabetes Type I	○ Yes	○ Yes
Diabetes Type II	○ Yes	○ Yes
Other metabolic disorders (examples: phenylketonuria (PKU), lactose intolerance)	○ Yes	○ Yes

ALLERGY, INTOLERANCE AND LIFESTYLE HISTORY

Many people have reactions after eating certain foods. A food allergy is a potentially life-threatening reaction that may involve hives, difficulty breathing, vomiting, or shock. A food intolerance is less severe, and may involve an upset stomach, behavioral changes, headache, chronic cold symptoms, or body ache. A common type of food allergy is peanut or tree nut allergy.

Do you have a diagnosis of an allergy from a healthcare provider? ___Yes ___No

Which of these are you allergic or intolerant to?

____ Oats

____ Barley

____ Peanut/Other nuts

___ Milk (if yes, are you allowed to take lactose free milk?) Yes/No_____

____ Certain Flavors (specify flavor type) _____

____Other (specify) _____

Do you currently smoke, or have you quit within the last six months? ___Yes ___No

Do you drink alcohol? ___Yes ___No If yes, how many drinks per week?

Do you have an Eating Disorder? e.g., anorexia nervosa, compulsive eating: ___Yes ___No

Has your body weight been stable over the past 6 months? _____Yes ____ No
If no, please explain_____

Have you had a weight gain or loss of ≥ 4 kg (app. 8.8lbs) in the last 3 months? ___Yes ___No

Are you currently taking of regular medications other than birth control or hormone replacement therapy?

Yes □ If yes, please list kind _____

No □

Fasting blood glucose results _____

Declaration:

I certify that my answers to the questions are complete, accurate and no information has been withheld. I understand that if this is later shown not to be the case it may result in the reconsideration of my suitability to continue participation in this research. The information supplied by you on this questionnaire will be used to assess your medical suitability to participate in this research.

When you sign this form, you are agreeing to take part in the screening protocol. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the Project Director to use your personal health information collected about you (without disclosure of personality) for research purposes within South Dakota State University.

Participant Identification Number Signature
Date

_____ _____
___/___/___

PRE-SCREENING FORM
(eligible participants only)

Participant's Identification Number:
Age:

Body Weight_____

Height_____

Calculated BMI (kg/m^2)_____

Waist circumference_____

Blood pressure_____

Pulse rate_____

Project Director's Signature _____
Date_____

APPENDIX B: SENSORY ANALYSIS FORM

SENSORY EVALUATION

Paired Preference Test

There are two small cups presented in each of the five groups (Groups A, B, C, D and E) Before starting, rinse mouth with water. For each of the groups, taste the two samples (rinse mouth with water in between samples) and circle sample you prefer.

	PAIRED TEST (circle/highlight samples you prefer)	
GROUP A	282	184
GROUP B	335	209
GROUP C	046	230
GROUP D	278	099
GROUP E	440	250

Quantitative Descriptive Analysis (Hedonic Scale)

There will be five samples presented in small cups

Taste each of the samples and rate the appearance, aroma, taste, texture and overall acceptability on the table beside it. (Rinse mouth with water and a cracker in between samples).

184

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

209

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

230

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

099

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

250

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

316

	Dislike extremely	Dislike slightly	Neither like nor dislike	Like slightly	Like extremely
Appearance					
Aroma					
Taste					
Texture					
Overall					

Which of these products will you normally prefer to consume, based on all the attributes above?_____

APPENDIX C: SATIETY TESTING FORM

SATIETY TESTING FOR DEVELOPED HIGH BETA GLUCAN BEVERAGE

Participant's Identification Number_____

Lunch Selection_____

Side Selection_____

Beverage Selection_____

Condiment Selection_____, _____, _____, _____

Day and Time Selection for First Satiety Testing_____

Fasting Blood Glucose for First Satiety Testing_____

Day and Time Selection for Second Satiety Testing_____

Fasting Blood Glucose for Second Satiety Testing_____

Day and Time Selection for Third Satiety Testing_____

Fasting Blood Glucose for Third Satiety Testing_____

Day and Time Selection for Fourth Satiety Testing_____

Fasting Blood Glucose for Fourth Satiety Testing_____

SATIETY TESTING

BEFORE BREAKFAST VISUAL ANALOGUE SCALE

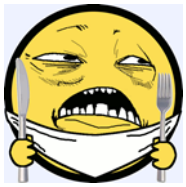
Instructions: Please provide feedback on the following sensations by placing a vertical line "I" at any point along the scale for each question.

1. How hungry do you feel right now?

Not hungry _____ Very hungry at all


2. How full do you feel right now?

Very Full _____ Not full at all

3. How strong is your desire to eat now?

Very weak _____ Very strong


4. How much food do you think you could eat right now?

Nothing at all_____ a large amount


AFTER BREAKFAST VISUAL ANALOGUE SCALE

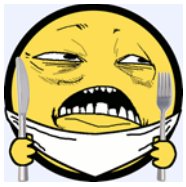
Instructions: Please provide feedback on the following sensations by placing a vertical line "I" at any point along the scale for each question.

1. How hungry do you feel right now?

Not hungry _____ Very hungry at all


2. How full do you feel right now?

Very Full _____ Not full at all

3. How strong is your desire to eat now?

Very weak _____ Very strong


4. How much food do you think you could eat right now?

Nothing at all_____ a large amount


BEFORE LUNCH VISUAL ANALOGUE SCALE

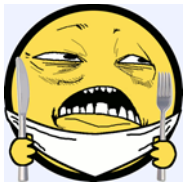
Instructions: Please provide feedback on the following sensations by placing a vertical line "I" at any point along the scale for each question.

1. How hungry do you feel right now?

Not hungry _____ Very hungry at all


2. How full do you feel right now?

Very Full _____ Not full at all

3. How strong is your desire to eat now?

Very weak _____ Very strong


4. How much food do you think you could eat right now?

Nothing at all_____ a large amount


Meal weight before lunch intake_____

Meal weight after lunch intake_____

APPENDIX D

Viscosity Charts & Conversion Tables

Liquid	Absolute Viscosity Centipoise	Temp. °F
DAIRY PRODUCTS		
Butter Fat	42	110
Butter Fat	20	150
Butter Deodorized	45	120
Cottage Cheese	30,000	65
Cocoa Butter	50	140
Cocoa Butter	0.5	210
Condensed Milk	40 – 80	100 – 120
Condensed Milk, 75% Solids	2,160	70
Cream, 30% Fat	14	60
Cream, 45% Fat	48	60
Cream, 50% Fat	112	60
Cream, 50% Fat	55	90
Milk	2.0	65
Milk	10	120
Milk Whey, 48% sugar	800 – 1,500	100
Process Cheese	6,500	175
Process Cheese	30,000	65
Whole Egg	150	40
Yogurt	152	105
FOOD PRODUCTS		
Batter	29,500	85
Baby Food	1,400	200
Beer	1.1	40
Beet Sauce	1,950	170
Brewers Yeast	368	65
Brewers Yeast, 80% Solids	16,000	40
Broth Mix	430	65
Cake Frosting	10,000	70
Caramel	400	140
Carob Bean Sauce	1,500	85
Chocolate	17,000	120
Chocolate Milk	280	120
Citrus Fruit Pulp	600	70
Coffee, 30-40% Liquor	10 – 100	70
Condensed Milk, s77% Sweetened	10,000	70
Cookie Cream Pre-mix	29,200	65
Corn Starch	300	85
Corn Syrup	12,000	130
Cream Style Corn	130	190
Custard	1,500	185 – 195
Edible Oil	65	70
Emulsifier	20	70
Gelatin, 37% Solids	1,190	110
Glucose	4,300 – 8,600	75 – 85
Gravy Slurry	110	175
Fruit Juice	55 – 75	65
Honey	1,500	100
Hot Fudge	36,000	120
Jam Garnish	8,440	60
Malt Extract, 80%	9,500	65
Malt Extract	3,000	140
Mashed Potato	20,000	100
Mayonnaise	20,000	70
Mincemeat	100,000	85
Molasses	1,400 – 13,000	100
Mousse Mix	1,200	40
Pabulum	4,500	100
Pear Pulp	4,000	160
Pectin	300	100
Pectin	345	80
Pet Food	11,000	40
Prune Juice	60	120
Orange Juice Concentrate (30 Brix)	630	70
Orange Juice Concentrate (30 Brix)	91	175
Orange Juice Concentrate (50 Brix)	2,410	70
Orange Juice Concentrate (50 Brix)	330	175
Rice Pudding	10,000	210
Salad Cream	1,300 – 2,600	65
Sauce-Apple	500	175
Sorbitol	200	70
Soybean Slurry	5,000 – 10,000	120 – 195
Tapioca Pudding	1,000	235
Toffee	87,000	100
Tomato Catsup	1,000	85
Tomato Paste, 30%	195	65
Vinegar	12 – 15	70
Yeast Slurry	20	65
VEGETABLE OILS		
Castor Oil	580	80
Caster Oil	36	175
Chinawood Oil	300	70
Coconut Oil	55	75
Coconut Oil	30	100
Corn Oil	28	135
Cotton Seed Oil	62	75
Cotton Seed Oil	24	125
Linseed Oil Raw	29	100
Olive Oil	40	100
Palm Oil	43	100
Peanut Oil	38	100
Soybean Oil	60	75
Soybean Oil	12	175
Turpentine	2.0	60
PHARMACEUTICALS		
Cough Syrup	190	85
Detergents	1,470	160
Face Cream	10,000	70
Hair Cream	5,000	70
Hand Cleaner	2,000	70
Hand Cream	780	65
Latex Emulsion	200	75
Latex Emulsion	48	150
Paraffin Emulsion	3,000	65
Pill Pastes	5,000	–
Shampoo	3,000	95
Soap Arylan	630	140
Soap Solution	82	140
Toothpaste	70,000 – 100,000	65
Wax	500	200
FISH & ANIMAL OILS		
Bone Oil	48	130
Cod Oil	32	100
Ground Beef Fat	11,000	60
Lard	62	100
Lard Oil	40 – 47	100
Meat Emulsion	22,000	40
Melted Animal Fat	43	110
Pork Fat Slurry	650	40
Sperm Oil	24	100
Whale Oil	25 – 39	100
INDUSTRIAL PRODUCTS		
Acetate Glue	1,200 – 1,400	70
Asphalt	500 – 2,500	–
Auto Lube Oil, SAE 40	200	100
Auto Trans Oil, SAE 90	320	100
Black Liquor	1,100	122
Black Liquor Soap	7,000	122
Black Liquor Tar	2,000	300
Box Glues	3,000	70
Clarified Sewage Sludge	2000 Range	–
Cresol Crystals	10	65
Diethylene Glycol	32	70
Dye	10	70
Ethylene Glycol	18	70
Fuel Oil #6	660	122
Glycerine, 100%	648	70
Glycerine, 100%	176	100
Gum	5,000	100
Isopropyl Alcohol	1.9	185
Kerosene	3	68
Lacquer, 25% Solids	3,000	65
Metallic Auto Paint	200	80
Paint Solvents	0.5 – 10	70
Paper Coating, 35%	400	–
Polyester	3,000	85
Polyester Resin	3,000	70
Polyisobutylene	12,500	185
Polypropylene	240,000	120
Polyvinyl Acetate Resin	65,000	70
Plastisol	28,000	65
Printers Ink	550 – 2,220	100
Printers Ink	238 – 660	130
Propylene Glycol	52	70
Resin Solution	880	75
Resin Solution	975	70
Resin Solution	7,140	65
Rubber & Solvent Cements	15,000	70
NaOH, 20%	1.0	65
NaOH, 30%	10	65
NaOH, 40%	20	65
Sulfide, 6%	1,600	–
Sulphonic Acid	125	85
Titanium Dioxide Slurry	10,000	–
Triacetate Dope	48,000 – 60,000	105
Triethylene Glycol	40	70
Varnish	140	100

CONVERSION FACTORS

Convert	To	Multiply By
centimeters	inches	.3937
cubic centimeters	cubic inches	.061
cubic inches	cubic centimeters	16.3872
cubic inches	gallons	.00433
cubic inches	liters	.01639
degrees °C	degrees °F	(°C x 1.8) + 32
degrees °F	degrees °C	(°F – 32°) x .5555
feet	meters	.3048
gallons	cubic inches	231
gallons	liters	3.78533
horsepower	kilowatts	.7457
inches H20	kiloPascals	4.0322
inches Hg	kiloPascals	.2953
kilometers	miles	.62137
kiloPascals	PSI	.145
kiloPascals	mm H20	.0098
kiloPascals	mm Hg	.1333
liters	cubic inches	61.025
liters	gallons	.26418
meters	feet	3.2808
meters	inches	39.37
miles	kilometers	1.6093
millimeters H20	kiloPascals	102.0408
millimeters Hg	kiloPascals	7.50
ounces (avoir)	grams	28.3495
ounces (fluid)	cubic centimeters	29.5737
ounces (fluid)	liters	.02957
pounds	kilograms	.45359
PSI	kiloPascals	6.895
square centimeters	square inches	.1550
square feet	square meters	.0929
square inches	square centimeters	6.4516
square meters	square inches	1550
square meters	square yards	1.9598
square yards	square meters	.83613
tons	kilograms	907.1846
yards	meters	.9144

This figure was adapted from (Basco Inc., 2004).