Development of a Functional Shelf Stable High Protein Dairy Beverage with Oat-β-glucan

THESIS

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Abstract

Soluble fiber components such as β-glucan in oats have been associated with the reduction of cholesterol and cardiovascular disease risk, and normalization of blood sugar levels. This ingredient is suitable for beverage applications. Oat-β-glucan can be used as a stabilizer that contributes to a clean label, while meeting a health claim. Moreover, milk proteins are a good source of essential amino acids and calcium. The objective of this study was to formulate an acceptable oat-β-glucan high protein dairy beverage containing at least 0.75 g of oat-β-glucan per serving size. Formulations adjusted to oat flour (1.50 – 2.30% w/w) and milk protein isolate (MPI) (2.50 – 4.00% w/w) were thermal processed (F₀= 10 minutes) in a rotary retort. The oat-β-glucan high protein beverages formulated presented a neutral pH and high suspension stability. The amount of oat flour seems to have a higher influence on the apparent viscosity of the beverages. Contents of β-glucan (0.5%) and protein (5.0 – 6.4%) allowed the coronary heart disease health risk (21 CFR 101.81) and “high” nutrient content (21 CFR 101.54) claims, respectively. Sensory evaluation indicated that beverages with <1.9% oat flour and <2.5% (thin liquids, <50mPas) were the most accepted. The increase of oat and MPI contents lead to nectar-like beverages (51-100 mPas). Perceived thickness, sweetness and aftertaste have the most influence on
acceptability. It was established that optimum product formulation contained oat flour (1.5 – 1.7%) and MPI (2.6 – 3.0%). This study demonstrates that it is possible to formulate an acceptable functional oat high protein dairy beverage that is also shelf stable.
I especially want to dedicate this work to my family. Eva, Nino, Momo and Papo, you are the entire inspiration of my life. Without you I would never be at this stage.

Los amo con todo mi corazón.
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Chapter 1: Introduction

Functional beverages are a growing trend in the food market. The additional health benefits and prevention of nutrition-related diseases are attractive for consumers that are seeking a healthy lifestyle and improvement of their eating habits. However, not many products in the market are able to completely meet the health benefits beyond normal nutritional standards as required by the Food and Drug Administration (FDA). There is an increasing interest in food industry innovation to offer products with demonstrable health benefits to the consumer. Because cardiovascular disease is one of the most widespread chronic health problems in the US, combating it with the development of functional foods that are appealing to the consumer is an emerging opportunity. Some studies have shown the beneficial effects of consuming β-glucan, a dietary fiber from oats. There is an FDA health claim (21 CFR 101.81) that allows products that contain 0.75 g β-glucan from oats per Reference Amount Customarily Consumed (RACC) to carry the claim that they may reduce the risk of cardiovascular disease. However, in the growing market of functional beverages, there is a lack of products that make use of this fiber.

One innovative approach would be the development of a high protein oat beverage as a total or partial replacement of more traditional breakfasts. According to market oriented research, products with increased protein content like meal replacement beverages
are preferred by younger consumers. These types of products are more likely to be consumed at breakfast time rather than lunch or dinner. The interest in the inclusion of protein as an ingredient is explained by the fact that increasing the amount of proteins with essential amino acid content can supplement the nutritional content of a beverage while enhancing its attributes. High protein beverages enhanced with soy, whey or milk proteins are gaining acceptance among the public. Commonly the smoothie-type and sport protein beverages are commercialized as ready-to-drink products or powder mixes.

Ingredients and processes directly impact the microstructure, texture, stability, flavor and quality attributes of the final product. The choice of the main protein sources is highly important in formulating a beverage with good sensorial quality and consumer acceptance. Common types of protein concentrates found in beverages are dairy (whey and caseins) and/or soy based. Heat treatments for shelf-life extension such as ultra-high temperature (UHT) or retorting processes are commonly performed. As previously stated, processing conditions influence the appearance of the product. In dairy drinks, milk proteins can agglomerate and precipitate due to the denaturalization and flocculation of whey proteins, in acidic conditions caseins can be aggregated. Other problems related to the denaturation of protein can also occur and affect the overall acceptance of this type of product. Stabilizing agents are incorporated in most dairy based beverages; however, the addition of a high number of additives has a negative impact on the consumer who looks for more natural options. The addition of phosphates can contribute to protein stability, while hydrocolloids could also act as stabilizers and as a source of dietary soluble fiber.
Gums, such as carrageenan and carboxymethyl cellulose, are commonly added to dairy products since thickening might be helpful for particle stabilization. Thus, the challenge is in balancing protein and additives in the formulation of high protein beverages to achieve an acceptable appearance, functionality and a cleaner label.

The use of natural ingredients with good functionality has been done purposefully to increase the “naturalness” of foods. Oats are an excellent alternative in the production of functional food due to their content of β-glucan. This non-starch polysaccharide increases viscosity at low concentrations and can stand a wide range of pH conditions. Supplementation with oat-β-glucan is an innovative approach to stabilize ready-to-drink (RTD) high protein beverages. Nonetheless, the thickening effect of β-glucan is also a challenge when it comes to beverage formulation. Health benefits are related to the increase in the higher molecular weight of β-glucan which affects not only the product’s viscosity but also viscosity in the gut lumen. Processing needs to be balanced in order to optimize both physicochemical and sensorial properties – the formulated product should be thick enough to be drinkable, while not negatively impacting its sensorial characteristics.
The purpose of this research is to make a functional oat-dairy based beverage. It is hypothesized that an acceptable dairy based beverage can have functional properties that meet the FDA approved cardiovascular disease risk reduction by adding oat flour and oat–β–glucan. The main objectives of this study are:

1. To formulate a consumer acceptable high protein dairy beverage enriched with oat and β–glucan
2. To identify the stabilization and processing conditions to make a shelf-stable product
3. To meet the “cardiovascular disease” health claim and “high protein” nutrient content labeling claim
Chapter 2: Literature Review

2.1 Functional Foods Trends

Functional foods promote health and provide additional health benefits beyond basic nutrition. In the U.S., functional food market sales reached $43.9 billion in 2012, and functional beverages were 59% of the market (Sloan 2012). Beverages are the most active product in the functional food market and breakfast is the preferred meal for incorporation of a functional food product. Currently, trends are focused on the use of specialty nutritional ingredients, claims to prevent or delay chronic diseases, food ingredient alternatives, sport nutrition products, high protein claim and weight management (Sloan 2014). Beverages represent an ideal matrix for micronutrients and bioactive compound supplementation. Possible perspectives for the development of functional beverages include supplementation with bioactive compounds and functional ingredients. Safety and quality issues related with the addition of compounds need to be addressed, as well as the understanding of ingredient stability during formulation and processing. In dairy beverages, there is an emerging opportunity for bioactive compound enrichment like food proteins, ω-3 fatty acids, plant sterols, vitamins, minerals, etc. (Corbo and others 2014). Further, incorporating functional ingredients in foods might imply numerous challenges for improvement of stability and sensorial properties such as flavor and mouthfeel.
2.2 Food Beverage Properties

Beverages or drinks mostly consist of water and can be divided into non-alcoholic and alcoholic categories. Regularly consumed products are fruit, energy and soft drinks, fruit juices, sport nutritional, milk and dairy-based and soy based beverages. The specific characteristics that describe a product are closely related to the ingredients present and will represent the overall quality of the product. For instance, properties like particle stabilization, sedimentation or phase separation and visual stability depend on the components’ interaction, and the processes and storage conditions, which might be used as an indication of a food beverage’s quality. Optimal formulations are based on the concept definition, ingredient selection, intensive formula testing, and processing and packaging and it is important to achieve a quality and stable shelf life product with an appearance that easily meets consumer requirements (Beristain and others 2006).

2.2.1 Physicochemical Properties and Stability

As previously mentioned, physicochemical characteristics are interrelated with the composition of each type of beverage. In products like fruit beverages, major physical properties are the acidity, loss of nutrition, vitamin C content and enzymes present. For soft drinks the main issues are carbonation, titratable acidity and pH. Others like dairy based beverages are frequently characterized for total solids, fat, pH, titratable acidity, protein and lactose content, viscosity, etc.
Particularly for protein beverages, ingredients’ solubility and pH conditions of the liquid matrix play an important role in the overall appearance. Dairy based beverages can be classified as acid or neutral. Stability of proteins is strongly influenced by pH conditions. High viscosity and precipitation of caseins at a pH near the isoelectric point (IP) of pH 4.6 are some of the difficulties that have been faced by manufactures when using increased milk protein content and longtime storage. Using stabilization agents like pectin, cellulose gum, addition of whey proteins and homogenization can help to overcome these problems (Agarwal and others 2015).

Texture properties have a major influence on beverage acceptability, mouthfeel, residual perception and overall appearance mostly when it comes to pouring a liquid product. In terms of viscosity (at the shear rate for swallowing: 50 s\(^{-1}\)), beverages can be classified as thin (1–50 mPas), nectar-like (51–350 mPas), honey-like (351–1750 mPas) and pudding-like (>1750 mPas) (NDD 2002). Homogeneous and smooth appearances are indicative of stabilization which is characterized as the absence of flocculation, sedimentation, phase separation or layer formation. Gravitational separation during storage is probably the main cause of instability in beverages. A three dimensional network helps suspend particles but viscosity in beverages should remain low compared with other food matrices. Therefore, stabilization of the beverage system is a critical part of beverage formulation. The interaction among all the components can lead to either under- or over-stabilized beverages (Beristain and others 2006; Mellema and Bot 2009).
Similarly, dairy beverages are likely to present aggregation and coagulation induced by a heat treatment. Whey proteins are heat sensitive and will denature at temperatures above about 70°C; without casein, whey proteins will form insoluble complexes. It is well known that there is a pH and temperature-dependence dissociation of casein protein from their micelles. UHT treatments in milk are also related with age gelation (Mellema and Bot 2009). Variations in the ratios of proteins for the whey fraction do not have an effect on the precipitation of caseinates during UHT processes (140°C for approximately 6.7 s). Above 70°C whey proteins are denatured and bond to other whey proteins and casein altering the stability during storage. Some authors also suggest that age gelation in high protein beverages can also be a consequence of additives, and not only the proteins’ interactions. (Grygorczyk 2009). Protein agglomeration and sedimentation of particles in a liquid matrix occurs over time due to a lack of stability in the food system. Also, suspension stability of the proteins might be influenced by heating and cooling process, protein concentration, pH, ionic strength, dielectric constant of the medium, and the types of stabilizer and emulsifiers used (Hinds 1997).

2.2.2 Appearance and Sensory Properties

Acceptability of any food product is strongly driven by taste and flavor. Thus, sensory properties directly influence the purchase and success of a product. Furthermore, most consumers have a high preference for sweet taste, which is context dependent and influenced by prior exposure to the specific foods (Drewnowski and others 2012). In
contrast, the presence of an aftertaste is negatively correlated with liking. In many cases, proteins may be associated with certain off-flavors. Well-known examples are the addition of whey protein isolates/concentrates. This can result in the detection of common off-flavors like cabbage, cardboard, metallic, sweet, soapy, salty, astringency, oxidized aftertaste and pasta water, among others (Wright and others 2006; Lammert, and others 2014; Whetstine, and others 2005). Acidic beverages (pH 3.4 to 2.6) differ from neutral beverages (~pH 6.8) in astringency and sourness. This is certainly a challenge in the development of high acidity drinks with whey protein, mostly due to astringency (Childs and others 2010). At low pH, whey proteins are positively charged, binding and aggregating with salivary proteins. The astringent characteristics do not decrease by increasing viscosity (1.6 to 7.7 mPa·s range) (Beecher and others 2008). It has also been reported that thermal treatments affect the flavor attributes. A common example is the formation of a “cooked milk flavor” in heat treated milk (above 90°C). Non-enzymatic browning processes in milk involves the formation of sulphur-containing compounds (Maillard reaction). The addition of antioxidants such as epicatechin and epigallocatechin gallate has been shown to inhibit the generation of these compounds (Colahan-Sederstrom and Peterson 2005).

In terms of appearance, color affects the perception of a food product’s quality characteristics, in particular, taste and flavor. For instance, it has been reported that there is a tendency to incorrectly identify flavors in beverages (fruit and non-fruit) that were inappropriately colored. Nevertheless, opacity can also play a role in the perception of
drinks such as milk (Zampini and other 2007; Dubose and others 1980). Texture properties in food also have great bearing on perception and acceptance. Flavor intensities might decrease as viscosity increases. Protein and carbohydrate contents might decrease the intensity of flavor additives due to their interaction. Lotong (2003) found that flavor perception was reduced in whole milk, apple and orange juice after the addition of thickening agents. Conversely, in viscous milk, flavor characteristics like metallic, astringent, and sour were increased (Lotong and other 2003). Similarly, Walker and Prescott evaluated the effect of carboxymethylcellulose (CMC), xanthan and pectin on odor and flavor attributes. Apple juices thickened with pectin showed a significant increase in cereal odor and sweetness but no significant differences were found for carboxymethylcellulose and xanthan (Walker and Prescott 2000).

2.3 Additives for Stability in Protein Beverages

Final attributes of the beverage are defined by the ingredient interactions. Formulations with gums and hydrocolloids are commonly used for protein and emulsion stabilization properties, as well as the mouthfeel and good suspension these additives produced in beverages. Hydrocolloids and gums are also a source of dietary soluble fiber (Beristain and others 2006; Mellema and Bot 2009).

Carrageenan and CMC are typically found in beverages. K-carrageenan is widely applied in milk products (evaporated milk and ice-cream mixes) because it requires the presence of calcium ions to generate a weak gel by interacting with the surface of the casein
micelles. When a slightly alkali treatment is applied to milk, fat and protein separation can be avoided. K-carrageenan interacts with casein protecting against calcium destabilization and giving heat stability. In milk-fruit beverages, locust bean is added to delay sedimentation of casein, but guar gum is not commonly used in acidic conditions because of phase separation close to its IP (Nussinovitch 1997). Whey protein complexes with maltodextrin present good emulsification properties at low pH (Akhtar and Dickinson 2007).

Modifications on the viscosity of beverages are common with CMC, which thickens and at the same time stabilizes suspended particles (Grygorczyk 2009). Dispersions of soy protein and xanthan gum have improved solubility and emulsifying properties. These properties were stable at a range of pH 3.0 to 9.0, ionic strength of 0.1 to 1.0M NaCl, and a heat treatment of 85°C during 1 h (Xie and Hettiarachchy 1997). Pectin is commonly used for thickening, gelling, or protein protection. The addition of pectin (at pH values close to IP) improves solubility by preventing soy protein aggregation. At neutral pH, there is reduction of soy protein isolates stability due to the lack of interaction with pectin (Lam and others 2007; Jaramillo and others 2011), which acts as a clouding agent for beverages. Pectin and protein ratios of 4:1 and 1:2 can give acceptable clouding stability and turbidity; for other applications, pectin can provide stabilization and prevents syneresis through the formation of a gel while it enhances creaminess (Klavons and others 1992). However, pectin has more affinity for water than for milk proteins; the addition of
pectin can lead to a membraneless osmosis process that will concentrate the protein phase and separate the solution into two phases (Nussinovitch 1997).

Other important additives that stabilize proteins are phosphates. Their use increases protein-protein repulsion and prevents gelation over time. Polyphosphates and sodium hexametaphosphates delay gelation of UHT-processed milk proteins, and sodium phosphate improves the heat stability (Datta and Deeth 2001; Kocak and Zadow 1986). Specifically, phosphates and citrates delay the dissociation of β-lactoglobulin and increase its stability (Kella and Kinsella 1988). Perez Hernandez (2005) studied additives that were used to improve the heat stability of sterilized whey protein (in emulsion) on high protein beverages. Phospholipids (hydrolyzed and acetylated lecithin) and polyphosphates were effective against this problem. Homogenization pressure did not affect the heat stability. Additionally, optimization parameters for emulsified beverages of 5% protein formulated with 0.3% lecithin without polyphosphates and homogenized at 90 MPa had the best stability after 28 days of storage (Perez Hernandez 2005).

2.4 Processing Conditions for Beverages

Beverages are frequently processed by means of homogenization and heat treatments. Homogenization is a common process where a liquid product is pushed at high pressure through a narrow aperture; high shear reduces fat droplets and protein particle sizes until a uniform fat distribution is achieved. The main objective of this process is particle size reduction, which also gives a smoother, creamier body and if required, a whiter
color. Effective pressure values are found below 100 MPa; at pressures above 150 MPa, a gel-like particulate network is formed as a result of the disrupted tertiary and quaternary structure of the protein. Furthermore, beverages require a thermal treatment to assure shelf stability and prevent pathogenic and food spoilage organisms. Commonly, processes used in the industry are UHT sterilization (typically 4–15 s at 135–150°C) that kills all spore forming microorganisms, pasteurization 15–20 s at 72–75°C, which inhibits vegetative microorganisms and retort processes (e.i. 45 min. at T ≥ 104°C; 10 min at 121°C), which destroy vegetative microbial cells, spores and enzymes. These treatments create a shelf stable product. Thermal process conditions and packaging conditions (aseptic or non-aseptic) directly affect the shelf life of a food product. For instance, 14 days of refrigerated shelf life is obtained when combining heat processing and non-aseptic packaging, 60 days of refrigerated shelf-life is obtained with UHT sterilization and non-aseptic packaging, and about 6 months of shelf stability is obtained with UHT sterilization and aseptic packaging (Mellema and Bot 2009).

Overall, commercial sterility is defined as the condition reached after a heat treatment that leads to a food free of microorganisms able to reproduce at normal non refrigerated conditions, and microbial cells or spores of public health significance (FDA 2016). The time required for sterilization is influenced by the heating conditions, physical state of the product (liquid or solid), headspace volume, fill weight, pH, size/shape/material of the container and the heat resistance of the microorganism targeted. Moreover, these variables have a substantial influence in heating rate and thus, lethality of the process.
Agitation is a good illustration of a variable in the system which has considerable impact on heat transfer. Internal flow or mixing pattern increases the rate of heating or convection in the case of liquid food products. This is useful for high viscosity products. Further, heat processes may promote chemical changes that can impact the flavor, color, nutritional values and stability of a beverage. Quality changes are dependent on how severe the heat treatment is since a processing time that can change the sensory characteristics of a product (also known as “cook-value”) is often longer than what is needed for inactivation of microorganisms. (Fellows 2009).

The rate of death of a microorganism depends on the specific substrate and temperature conditions to which it is exposed as well as its ability to form spores. The thermal destruction of microorganisms can be expressed logarithmically (number of survivors as log 10 CFU/mL vs time). Important parameters for designing effective heat treatments are the D- and z-values. In broad terms, D-value (decimal reduction time) can be defined as the time required to reduce by 1 log or destroy 90% of the microorganism and z-value corresponds to the temperature increase needed for a tenfold reduction of that D-value. These parameters can be determined from the surviving microorganisms vs time and temperature, respectively (FDA 2016; Forsythe 2011). The count reduction method for an inoculated product with vegetative and spore forms is commonly used to validate log-reduction protocols. Although Clostridium botulinum is the main concern for low acid food, the inoculation of this pathogen is not often used due to safety issues. Instead, surrogates of a higher heat resistance and nonpathogenic microorganism like Clostridium
sporogenes PA 3679 can be used for inoculation (10^3 and 10^5 spores per container). D- and z-values for both microorganisms are presented in Table 1.

Table 1. D- and z- values of Clostridium botulinum and Clostridium sporogenes

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Temperature (°C)</th>
<th>D-value (min)</th>
<th>z-value (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium botulinum</td>
<td>121.1</td>
<td>0.21</td>
<td>9.9</td>
<td>Lund (1975)</td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>121.1</td>
<td>0.1-0.15</td>
<td>9-13</td>
<td>Linton (2010)</td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>121.1</td>
<td>0.3−2.6</td>
<td>10.6</td>
<td>Linton (2010)</td>
</tr>
</tbody>
</table>

Furthermore, heat process evaluations are based on the equivalent minutes at a reference temperature (F-value or TDT-Thermal Death Time). The reference F-value (F_o) is the equivalent minutes at 121.1°C (250°F) and with a z-value of 10 °C. This is consistent with the standard temperature and z-value for destroying C. botulinum (Table 1).

The measurement and interpretation of heat penetration curves in the cold spot is an essential component in thermal process evaluation, and plays a key role in validation of a process. Generally accepted thermal process evaluations include the traditional graphical “General Method” developed by Bigelow and Etsy (1920), formula or mathematical method of Ball (1923) and numerical methods. The calculation of the process time by the general method is based on experimental work and it is considered the simplest method using graphical or numerical integration from heat penetration data. The lethal rate L, in minutes can be assigned to the temperature at the cold spot using Equation 1 (Pflug 1999):

$$L = 10^{\frac{(T-T_{ref})}{z}}$$  ……………..Equation 1
Where L is the lethal rate, z is the z-value, $T_{\text{ref}}$ is the reference temperature and T is the temperature at the cold spot. The critical z-value for the microorganism of concern and the reference temperature parameters must be previously established. The area under the curve (AUC) for lethality vs time represents the relative microbiological killing at different stages of the thermal process. In order to measure the AUC of the lethality curve, the trapezoidal rule or Simpson’s rule can be used. Mathematical methods use the heat penetration data to calculate mathematical models for the heating rate of the product. This has demonstrated the efficacy of using temperature as the variable of interest. The most well-known example is the Ball formula/mathematical method (1923). A graph of log ($T_1$-T) vs time is used to analyze experimental data of the straight-line portion of the heating and cooling profile (plotted on inverted semi-log graph). The Ball method is useful for extrapolating the process time at different retort and product initial temperatures (Pflug 1999; Lund 1975).

2.5 Cardiovascular Hearth Disease Risk Claim

Coronary heart disease (CHD) is the leading cause of death in the US and there is a marked interest in innovative ways to prevent this chronic disease. It is noteworthy that increased intake of dietary fiber has been associated with a reduction of coronary heart disease. Oats are considered a valuable source of soluble dietary fiber and mostly composed of β-glucans. The FDA (21 CFR 101.81) allows the claim that "a diet high in soluble fiber from whole oats and low in saturated fat and cholesterol may reduce the risk
of cardiovascular disease". At least 0.75 g of oat β-glucan per RACC can be claimed as having these health benefits; the minimum suggested daily intake of β-glucan is 3 g per day to obtain a health benefit (FDA 2013a).

2.6 Oat and β–glucan

Oats grains (*Avena sativa L*) possess a kernel (groat) with an edible outermost layer (oat bran) and lipid throughout the seed. Oat bran is obtained after grinding groats or rolled oats. The flour is made of ground dehulled oat groats (without a major loss of oat bran). Most oat varieties contain about 9-10 % water, 15-17% protein, 68% carbohydrates, 6-8% lipids, 15-22% dietary fiber, and also some vitamins, minerals, and phytochemicals (Butt and others 2008; Jing and Hu 2012; Marlett 1993); a detailed composition for whole oats, oat flour and oat bran is presented in Table 2. Oats are recognized among cereals for their high protein content. Protein fraction is composed of 70-80% globulins (Robert and others 1983), followed by prolamins (10-16%), glutenins (+ residues) (5%), and albumins (1%) (McMullen 2000), which results in an amino acid profile higher in amounts of lysine and threonine. (Klose and Arendt, 2012).
Table 2. Composition of whole oats, oat flour and oat bran.

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Fiber</th>
<th>Carbohydrate</th>
<th>Lipid</th>
<th>Ash</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole oat</td>
<td>7.7-14.8</td>
<td>6.5-12.8</td>
<td>53.0-65.8</td>
<td>4.3-</td>
<td>2.3-</td>
<td>Lásztity (1998)</td>
</tr>
<tr>
<td>Oat flour</td>
<td>14.7</td>
<td>~6.5</td>
<td>65.7</td>
<td>9.1</td>
<td>-</td>
<td>USDA (2016)</td>
</tr>
<tr>
<td>Oat bran</td>
<td>18.1</td>
<td>15.4</td>
<td>44.6</td>
<td>9.6</td>
<td>3.1</td>
<td>Marlett (1993)</td>
</tr>
<tr>
<td></td>
<td>12-26</td>
<td>6.6-7.4</td>
<td>47-53</td>
<td>2-11</td>
<td>2-9</td>
<td></td>
</tr>
</tbody>
</table>

Moreover, β-glucan is a linear nonstarch polysaccharide that composes about 75% of the oat endosperm cell walls. This soluble dietary fiber component is not only present in oats but also in barley, rye, some yeast, fungi and bacteria. Structural differences in β-glucans depend on the source; the form of (1→3)-β-D (30%) and (1→4)-β-D (70%) mixed linkage glucan is found in oats (Figure 1); (1→4) linked β-D-glucopyranosyl units occur in groups of two to four separated by single (1→3)–linkages, the chain is mostly composed of 3-O-β-D-cellobiosyl-D-glucose and 3-O-β-D-cellotriosyl-D-glucose units (Wood 1991; Ahmad 2012).

Figure 1. Structure of (1→3), (1→4)-β-D-glucan in oats.

Oat-β-glucan contents might range between 6-12 % and is dependent on cultivar and environmental conditions. Comparisons between several types of cultivars show a significant variation in the total β-glucan extracted. Miller and others (1993) evaluated the
β-glucan among 23 species, and two were found to have a significant amount of β-glucan: type A. hirtula and type A. hybrida. The lowest amount determined was 1.8 % in type A. heriantha. Concentrations of 0.76-3.68% β-glucan were found among 134 species; American oats (2.24%) showed larger amounts in comparison to Swedish species (1.43%). Additionally, molecular weight on β-glucan seems to be controlled mostly by environmental factors. Swedish and American cultivars presented extractable β-glucan that ranged between 1.3-1.8 x 10^6 g/mol to 1.4-1.8 x 10^6 g/mol, respectively (Ajithkumar 2005). Other reported values are between 2.7 x 10^6 to 3 x 10^6 g/mol (Wood and others 1991). Moreover, Yao and others (2007) monitored the MW of four species throughout two growing years with consistent values of 10 x 10^5 – 41 x 10^5 g/mol. Beer and others (1996) reported 2.1 to 1.7 x 10^6 g/mol. Oat gum extracted from oat bran (MW:882000 g /mol) contained 1.3% protein and 0.3% ash (Dawkins and Nnanna 1995).

2.6.1 Physicochemical Properties

Oat flour hydration is an important physicochemical property which may influence solubility, emulsification and texture. The water absorption capacity of oat flour is enhanced by higher β-glucan contents which can range from 2.8 to 3.4 g/g depending on the variety. Easier swelling of the oat slurry leads to a stable and viscous hot paste. Thus, on the basis of food applications, a larger water binding capacity helps to increase yield, consistency and body for a food system (Choi and others 2012). Indeed, oat β-glucan can be suitable as a thickening agent in beverages due to the viscosity forming properties, as
increases in the concentration of β-glucan cause an increase in viscosity. The higher molecular weight of β-glucan affects sensory sliminess and thickness in beverages. When compared with other thickening agents like gums, a 0.5% solution of oat gum is shown to be less viscous than a solution of xanthan or guar gum, but the oat gum is more viscous than locust bean gum and gum Arabic, as presented in Figure 2. Blends of oat gum and hydrocolloids at 50/50, 60/40 and 80/20 ratios suggested synergistic effects with locust bean and xanthan gums perceived as increase in viscosity at low shear. The interaction with guar gum is low and just slightly enhances viscosity at 60/40 and 0.5% total gum. Nnanna and others (1996) also indicated the stability of oat gum in high relative humidity (Nnanna and Dawkins 1996).

Deswal and others (2014) also found that above 0.5 % oat gum, the pH is stable. The viscosity also increased with sugar content until 65% sucrose, with an improvement of the pseudoplasticity for the oat slurry. The viscosity of oat gums (0.5%) is found to decrease by the addition of NaCl at 1%, but increase at low concentrations of NaCl (0.1%), or during heating or shear. Moreover, viscosity is stable over a wide range of pH (2–10) (Dawkins and Nnanna 1995; Autio and others 1987). Pseudoplastic properties of oats have been confirmed by Deswal and others (2014). Different concentrations (5-20 °Brix) and temperatures (10 - 40°C) have a significant effect on the rheological behavior of enzymatically processed, gelatinized and liquefied rolled oats at 70 – 75°C, also known as oat milk (Deswal and others 2014). Furthermore, protein dispersions of 2.5 - 40% protein made from oat protein concentrates (OPC) and oat protein isolates (OPI) exhibit
pseudoplastic flow behavior at different conditions, and compared with oat globulin dispersion, OPC and OPI have a higher consistency coefficient, apparent viscosities, and yield stress values due to the presence of a β-glucan content of around 7% in OPC (MA 1993).

Figure 2. A. Effect of concentration on oat gum viscosity (Dawkins and Nnanna 1995). B. Comparison of viscosity of oat gum, food-grade gums and binary mixtures with food-grade gums at 50/50 ratio at 0.5% gum concentration (Nnanna and Dawkins 1996).

*Measurements made at neutral pH and 25°C.
2.6.2 Health Promoting Properties

Oats possess a balanced nutritional content that promotes health and prevents diseases. The intake of β-glucan is beneficial in lowering coronary heart disease risk (Section Coronary Health Disease Claim: 21 CFR 101.81). Incorporation of β-glucan in the diet (3 g β-glucan/day) supports cardiovascular heart disease risk reduction related with reduction in cholesterol levels. β-glucan consumption has been linked with diabetes prevention (lowers postprandial glucose and insulin responses), a decrease in the risk of cancer and improving the immune functions The high viscosity promoted by β-glucan is important for serum cholesterol, insulin and glucose lowering effects (Daou and Zhang 2012; Jing and Hu 2012).

High cholesterol is a risk factor in the development of coronary heart disease, thus, decreases in total and low-density lipoprotein (LDL) cholesterol levels are associated with the reduction of the risk of cardiovascular diseases (0.06 mmol/L = 30% of reduction) (Law and others 1994). It is suggested that the high intestinal viscosity of β-glucan entraps bile acid micelles. The absorption of fats decreases when the interaction of bile acid with the intestinal lumen–membrane interface is avoided. As a result, properties that control viscosity such as molecular weight and solubility of the β-glucan might influence intestinal viscosity and its physical interaction in the small intestine (Daou and Zhang 2012).

The effect of incorporating oat fiber extracts in the diet has been reported to lower plasma lipids (0.8 to 1.2 g of oat β-glucan per day) (Behall and others 1997). Kerckhoffs and others (2003) evaluated a daily intake of 5.9 g β-glucan in bread, cookies and orange
juice. Consumption of the β-glucan drink lead to a 3.8 % total cholesterol reduction. However, when oat was included in solid matrices, no statistical significant was found, hence the processing and food matrix might influence the effectiveness in lowering cholesterol concentrations. Authors have found a decrease of 7.7% in LDL cholesterol after ingestion of a fruit drink enriched with 5 g β-glucan from oats during 5 weeks (Naumman and others 2006). Queenan and others (2007) found a relevant lowering effect for 6 g of β-glucan /day for six weeks. It has been suggested that the cholesterol lowering effect of β-glucan depends on the molecular weight of the β-glucan fraction ingested. Wolever and others (2010) showed that the intake of oat fiber (3 g β-glucan/day) with a high molecular weight (2,210,000 g/mol) was enough to cause a 5% decrease in cholesterol (0.2 mmol/L); however, 4 g β-glucan/day with a MW of 210,000 g/mol did not show significant effects. High viscosity in the intestine achieved with a high molecular weight oat additive will be more effective in providing health benefits. At least 3 g β-glucan/day reduce plasma total and low-density lipoprotein (LDL) cholesterol levels by 5-10% (Whitehead and others 2014). Furthermore, oat-β-glucan has been associated with attenuation of blood sugar levels and insulin response, thus reducing the risk of type 2 diabetes. Postprandial blood glucose response is related to viscosity and properties that influence it such as MW and concentration in solution. Braaten and others (1994) and Wood and others (1994) compared the attenuation of plasma glucose response with the consumption of high to low viscosity model drinks (20-8000 mPas at 30 s⁻¹). High viscosity beverages were shown to be more effective in decreasing plasma glucose and insulin rise, and thus, improving postprandial
glycemic control. Similar results have been found by Panahi (2007), when comparing oat solutions with different viscosities (~3000 mPas and ~45 mPas) but similar chemical compositions. Additionally, viscous soluble fiber provided by oats have been associated with a satiety-promoting effect; viscous fibers efficiently reduce appetite. Enriched juice with 4 g β-glucan was able to enhanced satiety by inducing a higher feeling of fullness compared to a solid enhanced food product. In general, fiber-containing beverages reduce hunger and promote satiety (Pentikäinen 2014; Rebello 2014). Other studies have also reported that oat fiber can improve intestinal regularity (Stephen and others 1997).

2.6.3 Effect of Processing on β–Glucan

While there has been recent interest in β-glucan as a functional ingredient in food, few authors have studied the behavior and transformation of this compound in a liquid matrix. However, some studies on the oxidation of β-glucan can be found. For instance, Kivelä and others (2012) have presented a comprehensive investigation on the carbonyl group formation along the β-glucan chain and its oxidation during heat treatments (95 and 120°C), high pressure homogenization (300 and 1000 bar), cold storage and addition of ascorbic acid (2, 10 and 50 mM) in pure and unpurified solutions of β-glucan. The analysis of carbonyl group distributions can be used as an indication of oxidation when it was found that the presence of transition metals, phosphorus compounds and proteins favored this process. Similarly, ferrous ions clearly degraded β-glucan by inducing oxidative cleavage (seen as reduction of molar mass) (Kivelä and others 2012) and ascorbic acid can also
induce molar mass decrease; the addition of sulfite and ascorbic acid might protect against the thermal degradation of β-glucan. The main reactions of heat degradation are free radical formation and oxidative cleavage (Kivelä and others 2011). The effect of heating (120°C for 5 min and 95°C for 30 min) on the viscosity and molar mass of 1.0–1.7 mg/ml –glucan extracts and highly purified commercial glucan (>99%) were assessed by Kivelä and others (2011). Unpurified solutions of β-glucan presented a decrease in viscosity of 10-20 % and pure solutions retained approximately 90% of their original viscosity. However, the molecular weight of pure solutions was decreased as the heat treatment time increased to 30 min. Interestingly, longer heat time exposure showed a lower concentration of carbonyl groups, probably because the longer treatment leaded to further degradation products. β-glucan has been shown to be relatively resistant to oxidation during a heat treatment of 95°C/30 min, since Kivelä and others (2012) found that co-extracted compounds (transition metals, phosphorous compounds and proteins) may contribute to oxidation. In general terms, heat treatments (120°C) can oxidize and consequently cause cleavage of the β-glucan chain. The addition of ascorbic acid decreased the molar mass of β-glucan; however, there is a formation of both antioxidative ascorbyl radicals and pro-oxidants that leads to a cross-over effect. The oxidation during homogenization of β-glucan was not a predominant degradation force (Kivelä and others 2012).
2.7 High Protein Content in Beverages

Based on the FDA (21 CFR 101.54), the term “High” can be used in food containing 20% or more of the Reference Daily Intake (RDI) per reference amount customarily consumed (Mellema and others 2009; FDA 2014). The recommended protein intake for a normal adult individual has been established to be 0.8 g kg\(^{-1}\) day\(^{-1}\) (RDI). Recommended Dietary Allowances (RDA) for adults (19 – 70 years) are approximately 46 and 56 g of protein per day for women and men, respectively (Trumbo and others 2002; Board 2002). Consequently, in order to be considered a high protein product, the amount of protein in a beverage should be more than 10 g per serving (20% for the 50 g of protein per day) (FDA 2013b).

2.8 Milk Proteins

Milk protein concentrates (MPC), milk protein isolates (MPI), whey protein concentrates (WPC), whey protein isolates (WPI), whey protein hydrolyzates (WPH) and caseinates are the commercially available concentrates of milk proteins. These are products obtained by the ultrafiltration and diafiltration (optional) of skim milk followed by spray-drying of the retentate. (Liang and others 2013). Powders with concentrations of up to 85% protein are known as MPI. An MPC with 65% protein normally will have around 3% fat and 20% lactose (Guzmán-González and others 1999). MPC’s and MPI’s possess the same ratio of caseins and whey proteins found in skim milk (Huppertz and others 2012). In general terms, caseins are all the phosphoproteins in the form of micelles that can be
precipitated from raw skim milk by acidification (pH 4.6 at 20°C). The casein fraction comprises at least 80% of total milk protein and it is mainly composed of \( \alpha_1 \)-, \( \alpha_2 \)-, \( \beta \)-, and \( \kappa \)-casein (CN). The remaining 20% corresponds to the whey fraction composed of \( \alpha \)-, \( \beta \)-lactoglobulin (LG), and serum albumin (SA). The CN fraction is constituted of 40% \( \alpha_1 \)-CN. Minor components are mostly immunoglobulin (Farrell and others 2004) (Table 3).

Table 3. Summary of composition of caseins and whey protein in skim milk obtained by Farrell and others (2004).

<table>
<thead>
<tr>
<th>Protein (abbreviation)</th>
<th>Composition in skim milk (g/L)</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_1 )-Casein (( \alpha_1 )-CN)</td>
<td>12–15</td>
<td>23,615</td>
</tr>
<tr>
<td>( \alpha_2 )-Casein (( \alpha_2 )-CN)</td>
<td>3–4</td>
<td>25,226</td>
</tr>
<tr>
<td>( \beta )-Casein (( \beta )-CN)</td>
<td>9–11</td>
<td>24,023</td>
</tr>
<tr>
<td>( \kappa )-Casein (( \kappa )-CN)</td>
<td>2–4</td>
<td>19,037</td>
</tr>
<tr>
<td>( \beta )-Lactoglobulin (( \beta )-LG)</td>
<td>2–4</td>
<td>18,363</td>
</tr>
<tr>
<td>( \alpha )-Lactalbumin (( \alpha )-LA)</td>
<td>0.6–1.7</td>
<td>14,178</td>
</tr>
<tr>
<td>Serum albumin (SA)</td>
<td>0.4</td>
<td>66,399</td>
</tr>
<tr>
<td>Immunoglobulin G1 (IgG1)</td>
<td>0.3–0.6</td>
<td>161,000</td>
</tr>
<tr>
<td>Immunoglobulin G2 (IgG2)</td>
<td>0.05</td>
<td>150,000</td>
</tr>
<tr>
<td>Immunoglobulin A7 (IgA)</td>
<td>0.01</td>
<td>385,000</td>
</tr>
<tr>
<td>Immunoglobulin M(IgM)</td>
<td>0.09</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Secretory component (SC)</td>
<td>0.02–0.1</td>
<td>63,750</td>
</tr>
<tr>
<td>Lactoferrin (LF)</td>
<td>0.02–0.1</td>
<td>76,110</td>
</tr>
</tbody>
</table>
2.8.1 Physicochemical Properties

MPC’s show a wide range in solubility depending on their protein content; larger percentages of protein tend to make them less soluble. Mineral composition such as higher sodium and lower calcium, magnesium and phosphorus contents lead to higher solubility (Sikand and others 2011; Anema and others 2006). Furthermore, factors involved in the rehydration process such as temperature of reconstitution, mixing conditions, heat treatment during spray-drying or mostly storage seem to have a significant effect on solubility over time (Haque and others 2010; Sikand and others 2011). Anema and others (2006) reported the reduction in solubility during MPC storage as a result of cross-linking of proteins at the interface and also showed that only casein became insoluble during storage (Anema and others 2006). Additionally, relative humidity has an effect on solubility over time; at constant temperature, a higher relative humidity and water activity accelerates the loss of solubility (Le and others 2011). It has also been reported that some modifications could enhance the solubility; in MPC80 the solubility increased from 63 to 100% by the addition of 50 to 150 mM NaCl (Anema and others 2006). Thus, high protein powders can be easily dispersed in high ionic strength mediums such as milk or NaCl solutions (Gaiani and others 2010). In agreement with this, increasing ionic strength and temperature were reflected in better rehydration properties and higher temperatures which also resulted in a decrease of sediments after reconstitution. A combination of both factors (addition of KCl and high temperature of 50°C) had a positive effect during rehydration leading to stable solutions (Crowley and others 2014.; Crowley and others 2016 ).
MPC emulsification depends on the aggregation state of the proteins such as αs1-, αs2-, β- and k-caseins, β-lactoglobulin and α-lactalbumin. Those proteins are normally present as micelles in MPC, and present larger emulsion droplets in comparison with only using whey proteins. As a result, emulsions with MPC require higher quantities of powder to obtain a stable emulsion leading to difficulties in their application as ingredients in food products. Furthermore, a close relation between calcium content and the aggregation of casein was found by Ye (2011). The author reported that casein micelles dissociate as the calcium content decreases, at the same time, less MPC is needed to obtained emulsion with small particle sizes and higher stability.

Individually both whey and caseins present in MPC can gel. Conditions such as heating, low pH, high pH and a not too low concentration of Ca⁺² make whey protein insoluble. Likewise, caseinates and denatured whey proteins give copious and stable foams when there is almost no lipid present (Walstra and others 2006). Surface properties of caseinates are mainly affected by β-casein. Caseinate and whey show opposite behaviors with pH; caseinates are effective foam formers far from the pI, and whey close to the pI value. It was found that higher concentrations of proteins have a positive relation with foamability and foam stability. Foaming attributes depend on the different molecular structures and ways of aggregation (Marinova and others 2009).
2.8.2 Health Promoting Properties of Milk Proteins

Protein fractions of milk provide high nutritional value as a source of essential amino acids. Casein provides all the essential amino acids but cysteine and whey has high contents of leucine, isoleucine and valine (Hall and others 2003). Overall, a favorable effect on metabolic control has been related to the ingestion of dairy products (McGregor and Poppitt 2013). Milk and dairy products are also a good source of calcium, which plays a role in processes and regulation of chemical reactions in the body such as muscle relaxation, nerve impulse transmission, blood clotting, etc. Similarly, other minerals such as phosphorus and iodine are present in significant proportions as well as some B vitamins (Kanekanian 2014). Consumption of milk can provide protein derived bioactive peptides that confer biological and physiological benefits. Several studies have related peptides in casein and whey with regulation of peripheral blood pressure (ACE inhibition), immunomodulation, angiotensin-converting-enzyme, opioid agonist peptides, lowering of cholesterol levels, and as an antithrombotic, among other functions (Yamamoto and others 1994; Maeno and others 1996; Schiffrin and others 1995; Teschemacher 1997; Fitzgerald and Meisel 2003). Caseins in bovine milk are considered the major source of bioactive peptides. It is therefore interesting that bioactive peptides from caseins are better regulators of blood pressure (ACE-inhibitor) than whey. Furthermore, biologically functional peptides can be found in dairy products due to the action of naturally occurring enzymes or can be produced during digestion by digestive enzymes. The released bioactive peptides should be absorbed in the gastrointestinal tract to provide the physiological effect in the
corresponding target organ. Milk protein hydrolysates are reported to be easily absorbed in comparison with intact proteins (Alhaj and others 2014; Nagpal and others 2011). Moreover, whey proteins have been proven to stimulate the uptake of amino acids and increase lean body mass when combined with resistance exercise and adequate training. (Phillips and others 2005; Tang and others 2009). It is suggested that dairy based drinks have a carbohydrate–protein balance that might promote recovery after exercise. A protein ingestion of 1.4-1.8 g kg$^{-1}$ day$^{-1}$ is considered optimal for athletes (Lemon 2000). Post-exercise rehydration is enhanced by whey protein isolate in beverages (20 g/L) when an equivalent volume of 150% of sweat loss is ingested in 1 h (James and others 2014). The abundance of electrolytes in milk may help increase normal hydration before exercise (Davison 2014). Other benefits of whey proteins include improving immune function, gastrointestinal health (Ha and Zemel 2003), and they might play a role in managing diabetes Type 2 (Frid and others 2005).
Chapter 3: Materials and Methods

3.1 Materials

The ingredients for oat β-glucan high protein dairy beverage formulations consisted of the following: Oat flour (Grain millers, Eugene, OR), oat-β-glucan isolate (>70%) (Garuda, Exeter, CA), milk protein isolate (MPI85) (Idaho Milk Products Inc., Jerome, ID), whey protein concentrate (Davisco Foods International, Le Sueur, MN), soy protein isolate (CHS, Inver Grove Heights, MN), mono-di-glycerides (Corbion, Leheda, KS), dipotassium phosphate (Innophos, Cranbury, NJ), K-carrageenan (Genulacta, CPKelco, Abugo, Philippines), dipotassium phosphate, sodium tripolyphosphate and Text Melt® (polyphosphoric acids sodium salt 50-80%, disodium phosphate 20-40% and trisodium phosphate 5-20%) were provided by Innophos, Inc., Cranbury, NJ. Raw milk was obtained from the Waterman Dairy Center, Columbus, OH. Standardized milk (2% milkfat) and milk cream (28% milkfat) were used. Salt (NaCl) and table sugar (sucrose) were purchased from a local supermarket in Columbus, OH.
3.2 Preliminary Work

3.2.1 Protein Source Determination

Preliminary beverage formulations were developed using laboratory-scale trials. In order to obtain a base formulation, experimental combinations with different levels of oat flour, water, skim milk, and cream were evaluated. Initial mixtures of 4% protein (Milk Protein Isolate, Soy Protein Isolate and Whey Protein Concentrate) were used. Dry ingredients were mixed and added to the liquid ingredients (blends of skim milk, cream, mono-di-glycerides, and salt) and then heated at 80 ± 5°C for 20 min. Physical appearance and bench-top sensory evaluations were used as accepting or rejecting criteria for these formulations. Modifications to the order of mixing and heating temperature were performed for the accepted formulations (data not reported). Concentrations for carrageenan, mono-di-glycerides, and the following four stabilizers; dipotassium phosphate, potassium citrate, sodium tripolyphosphate and Text Melt® (0.1%), were evaluated using the manufacturer’s recommended levels. Batches without stabilizer were used as control.

3.2.2 Bench-top Prototypes Processing

Formulations were adjusted to 2.5, 5.0 and 7.5% MPI and oat flour 2.0, 2.75 and 3.50% and processed as presented in Figure 3. Beverages were poured into size 300 x 407 (14-oz, #300) metal cans, sealed, and processed in a still retort (Dixie Canner Co, Bogart, GA) at a retort temperature of 121°C (250°F) for 17 minutes as defined by Walstra and
others (2006) and Cano-Ruiz and Richter (1998). Cans were cooled to 37.7°C (100°F) with water. Beverages were stored for at least 24 hours but no more than one week before analysis. Apparent viscosity was measured and five selected prototypes were examined using an untrained taste panel consisting of fifteen people. Initially, samples were presented individually and the panelists were asked to rank the prototypes for perceived viscosity on an unstructured 10-point scale. Then, each prototype was evaluated by overall liking measured on a 9-point hedonic scale and Just-About-Right (JAR) scale for perceived viscosity. Statistical significance (5% level) was calculated.

![Figure 3. Schematic diagram of bench-top beverage prototype production.](image)

3.3 Product Formulation, Scale-up and Processing

Pilot-plant scale processing protocol and minimum and maximum variable levels (oat flour and MPI) were selected based on the preliminary study (Section 3.2). The processing protocol for the beverage production is described in Figure 4. Dry ingredients (oat flour, β-glucan isolate, MPI, carrageenan and sucrose) and liquid ingredients (skim milk, cream, mono-di-glycerides, and water) were mixed separately (Table 4). Thereafter, all ingredients were mixed while heating to 80 ± 5°C for 10 minutes in a steam kettle, and
The resulting solution was homogenized at 30 MPa pressure and 60°C in a two-stage Lab 100 M-G homogenizer (Lubeck-Schlutut, Germany). Beverages (aprox. 355 mL) were hand-filled into 300 x 407 (14 fl-oz, #300) metal cans with a headspace of 15 mm and sealed in a steam injection closing machine (John J. Pledger Co., Chicago, IL).

Table 4. Formulation for oat-β-glucan high protein beverages.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage, % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk</td>
<td>75.00</td>
</tr>
<tr>
<td>Water</td>
<td>9.07-11.37</td>
</tr>
<tr>
<td>Cream</td>
<td>5.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.50</td>
</tr>
<tr>
<td>Milk Protein Isolate *</td>
<td>2.50-4.00</td>
</tr>
<tr>
<td>Oat Flour *</td>
<td>1.50-2.30</td>
</tr>
<tr>
<td>Oat-β-glucan Isolate</td>
<td>0.70</td>
</tr>
<tr>
<td>Mono-di-glycerides</td>
<td>0.20</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.10</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Percentages based on a Central Composite Design (Section 3.5)
Figure 4. Processing steps for oat-β-glucan high protein dairy beverages.
Beverages were sterilized in a rotary retort (Steritort™-FMC Corporation, San Jose, CA) at 121°C and operated at a reel rotational speed of 10 rpm. Sterilized samples were cooled to 32°C (110°F) with running water. An Fo value of 10 minutes was used as target (Richardson 2004). Heat penetration trials for the formulations were performed with thermocouples located in the geometric center (5 cm above the base) of the can. Temperature of the products and heating medium were recorded every 15 s using a CALPlex data logger and CALSoft II software (TechniCAL, New Orleans, LA). Calculation of the thermal process was carried out by the General Method for Process Calculation (Bigelow andetsy1920), assuming a z value of 10°C. Standard D value for Clostridium botulinum is D_{121.1°C} = 0.2 min (12D = 2.4 min) (Lund 1975).

3.4 Physicochemical Properties

The overall quality of the beverages was evaluated in terms of parameters such as contents of oat-β-glucan, protein, fat, suspension stability, soluble solids, pH and color.

3.4.1 Oat-β-glucan Content

The β-glucan content for oat flour, oat-β-glucan isolate, beverages, and a standard for oat flour was determined enzymatically by the AOAC method 995.16 using a Megazyme β-glucan (mixed linkage) assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Samples of oat flour (120 mg) and oat-β-glucan isolate (50 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). Each solution was then incubated in a boiling water bath for 3 min and equilibrated to 50°C. Beverage samples (3 mL) were heated in a water bath at 100°C for 5 min, cooled to room temperature and treated with 8 mL of ethanol (95%). After centrifugation at 3000 × g for 10 min, the supernatant was discarded and the pellets were suspended in 8 mL of aqueous ethanol (95%), again centrifuged, re-suspended in 20 mM sodium phosphate buffer (pH 6.5) to reach a volume of 4 mL and incubated at 50°C for 5 min. After incubation, all samples were treated with 10 U of lichenase and incubated at 50°C for 1 h with constant agitation. Then 5 mL of 200 mM sodium phosphate buffer (pH 4.0) was added and the mixture was centrifuged at 3000 × g for 10 min. Aliquots of 0.1 mL of sample were incubated with 0.2 U of β-glucosidase at 50°C for 10 min. Samples without β-glucosidase treatment were used as blanks. Then, 3 mL of GODOP reagent® (glucose oxidase and peroxidase) were added to each sample which were then incubated at 50°C for 1 h. Absorbance was measured at 510 nm in a UV–visible Spectrophotometer 2450 (Shimadzu Scientific Instruments, Inc., Columbia, MD).

3.4.2 Total Protein

Total protein content was determined by Kjeldahl Nitrogen analysis according to the AOAC official method 991.20. A catalyst tablet and 15 mL of concentrated sulfuric acid were added to 1.5 mL of beverage. Digestion of the samples was carried out at temperatures up to 380°C for up to 2 h and 30 minutes (samples turn clear green). After the digest was cooled to room temperature, 25 mL of water was added. In a distillation
system, boric acid solution was added, ammonia was formed and distilled, then titrated with standardized HCl. Total protein, expressed as protein equivalent, was calculated by the conversion factor of total nitrogen (mg/L \times 6.38/1000 = \text{g/L protein}).

3.4.3 Total Solids

Total solids were measured using a CEM SMART Trac II Analyzer (AOAC Official Method 2005.06) (CEM Corporation, Matthews, NC). 2-3 g of each sample was spread in the center of a glass fiber sample pad, covered with a second pad and dried (Cartwright and others 2005).

3.4.4 Fat Content

Following total solids analysis, the same samples were measured for fat content using a CEM SMART Trac II Analyzer (AOAC Official Method 2005.06). Sample pads were rolled and inserted into a “CEM trac tube” NMR system. The system uses Low-Resolution Time Domain Nuclear Magnetic Resonance (LR-NMR) to measure total lipid content (Cartwright and others 2005). The Babcock method (as cited in Carpenter 2010) with some modifications was used as a reference to develop a method for the SMART Trac II CEM (CEM Corp., Matthews, N.C., U.S.A.). Beverages with different levels of milk cream were added to an 8% Babcock bottle, incubated at 38°C for 15 min, mixed with 2 mL of ammonium hydroxide and 3 mL of N-butyl alcohol. Then, 17.6 mL of diluted sulfuric acid was added in three aliquots while gently mixing between additions. Bottles
were placed in a mechanical shaker for 5 min and centrifuged at 3000 g for 5 min. Samples were again centrifuged for 2 min after distilled water (51°C) was added to the base of the bottle neck. More distilled water was added to bring the fat column to the top of the scale, and samples were incubated in a water bath at 48°C for 5 min. Glymol was added and the distance from the lower meniscus to the upper meniscus was measured.

3.4.5 Apparent Viscosity

The rheological characteristics of the beverage samples were determined by using a controlled-strain modular compact rheometer (Anton Paar MCR 302 Rheometer, Ashland, VA) equipped with a Peltier temperature controller. The measurements were performed using a plate-plate geometry (plate diameter 40 mm, gap size 0.5 mm). 800 μL of each sample was placed on the plate of the rheometer. Apparent viscosities were evaluated as function of temperature range (4 – 25°C) at a shear rate of 50 s⁻¹. All measurements were carried out in quintuplicate.

3.4.6 Suspension Stability

Suspension stability (%SS) was measured as the ratio of total solids (TS) in the top (upper one-third) portion to the bottom (lower one-third) portion of 12 ml of beverage in a glass tube (Equation 2.) (Priepk and others 1980). All samples were centrifuged at 3000 x g for 20 min to accelerate settling (Sedmeyer and others 2004).
3.4.7 pH

The pH for the beverages was measured using a pH meter (Mettler-Toledo CO., Ltd) with a glass electrode standardized at 25°C over a pH range of 4.0 to 10.0.

3.4.8 Color Measurement

The parameters L*, a* and b* (CIELch) of the beverages were measured using a Hunter ColorQuest XE colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, USA). A higher L* value indicated a brighter (whiter) sample and values of a* and b* indicated red-green and yellow-blue colors, respectively.

3.5 Sensory Evaluation

Sensory evaluations of the oat high protein beverage were conducted to assess the overall liking, flavor, mouthfeel (thickness), sweetness, and aftertaste. About 1 oz of each refrigerated (at ~10°C) sample was served in plastic cups labeled with randomly generated codes of 3 digits and presented in a monadic sequential order to untrained panelists (n = 70). Cups of water and crackers were provided for palate cleansing. The sensory evaluation was performed as follows:

1. Panelists were asked to rank 3 formulations in order of their overall preference.
2. Acceptability tests were conducted for the 13 CCD formulations (Table 6) on overall liking, thickness (viscosity), flavor, sweetness and aftertaste on the basis of a 9-point hedonic scale (ranging from 1—dislike extremely to 9—like extremely).

3. Thickness (viscosity), flavor, sweetness and the presence of an aftertaste were evaluated in a five-point Just-About Right (JAR) scale (1 = much too low, 2 = slightly too low, 3 = just about right-JAR, 4 = slightly too much, and 5 = much too much).

4. Panelists provided their age, gender and ethnicity.

Moreover, penalty analysis or “mean drop” was calculated as described by Lawless and Heymann (2010). Data was separated in above-JAR (1,2), below-JAR (4,5), and JAR (3). Then, the mean hedonic score for overall liking was calculated for each group. The means from the above and below-JAR were subtracted from the mean of the JAR group. Finally, mean drops vs percentage of total panelists were plotted for each formulation (Table 6).

3.6 Experimental Design and Statistical Analysis

The experiments were based on a central composite design (CCD) for two factors at five levels. Therefore, 13 prototype formulations, including five replicates of the center point were carried out based on the CCD. The independent variables studied were: Oat flour content ($x_1=1.50–2.30\% \text{ w/w}$) and MPI content ($x_2 = 2.50–4.00\% \text{ w/w}$). Table 5 shows the values of the coded and uncoded forms of the design matrix. Additionally,
contents of oat flour and MPI corresponding to the matrix of the experimental CCD are presented in Table 6. Dependent variables measured were (y), five sensory responses: overall liking (y₁), perceived thickness (y₂), flavor (y₃), sweetness (y₄), and aftertaste (y₅), and analytical responses: protein content (y₆), total solids (y₇), and apparent viscosity at 5°C (y₈), 10°C (y₉), 15°C (y₁₀) and 20°C (y₁₁). MINITAB 17 statistical software package (Minitab Inc., Pennsylvania, USA) was used for the experimental design and SPSS Software was used for data analysis.

A prediction equation was generated for each response using the second order polynomials function as follows:

\[ y = b_o + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \]

Where, y is the dependent variable, b the regression coefficients and x the independent variables. The optimum region was identified by superimposing the contours for the response surfaces in an overlay plot. The shaded area represents the region of the optimum values for the responses.

Physicochemical measurements and sensory acceptance data was expressed as mean ± standard deviation and as mean ± standard error of the mean, respectively. All data was statistically analyzed with SPSS software (IBM, SPSS Incorporation, Chicago, IL, version 23.0) using a one-way ANOVA with a subsequent least significant difference (LSD) test, applied for multiple sample comparison, to test for any significant differences (p<0.05) in the mean values. All physicochemical measurements were done in triplicate for two separate cans.
Table 5. Experimental ranges and levels of independent variables for the CCD used in the RSM of an oat-β-glucan high protein beverage in terms of actual and coded factors.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1.41</td>
</tr>
<tr>
<td>Oat flour, %</td>
<td>1.50</td>
</tr>
<tr>
<td>Milk Protein Isolate, %</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Table 6. Matrix of the CCD for oat-β-glucan high protein beverages.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Oat flour, %</th>
<th>MPI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.62</td>
<td>2.72</td>
</tr>
<tr>
<td>2</td>
<td>2.18</td>
<td>2.72</td>
</tr>
<tr>
<td>3</td>
<td>1.62</td>
<td>3.78</td>
</tr>
<tr>
<td>4</td>
<td>2.18</td>
<td>3.78</td>
</tr>
<tr>
<td>5</td>
<td>1.50</td>
<td>3.25</td>
</tr>
<tr>
<td>6</td>
<td>2.30</td>
<td>3.25</td>
</tr>
<tr>
<td>7</td>
<td>1.90</td>
<td>2.50</td>
</tr>
<tr>
<td>8</td>
<td>1.90</td>
<td>4.00</td>
</tr>
<tr>
<td>9 (C)</td>
<td>1.90</td>
<td>3.25</td>
</tr>
<tr>
<td>10 (C)</td>
<td>1.90</td>
<td>3.25</td>
</tr>
<tr>
<td>11 (C)</td>
<td>1.90</td>
<td>3.25</td>
</tr>
<tr>
<td>12 (C)</td>
<td>1.90</td>
<td>3.25</td>
</tr>
<tr>
<td>13 (C)</td>
<td>1.90</td>
<td>3.25</td>
</tr>
</tbody>
</table>
4.1 Preliminary Work

Various sources of protein were evaluated. This included soy protein isolate (SPI), milk protein isolate (MPI) and whey protein concentrate (WPC). Initial trials were performed using 4% protein and 2% oat flour (Table 7). The heat treatment was aimed at solubilizing the oat flour. Liquid appearance was related with low denaturation of proteins. Oat beverages with MPI (<4.0%), SPI (<2.0%) and WPI (<1.3%) were found to have the most desirable characteristics. As shown in Table 7, the formulations with >1.3% of whey protein were not liquid. While addition of 4% SPI gives a liquid and stable beverage, soy protein presents an evident aftertaste. In a similar manner, authors have found that cardboard, cereal and flour-like flavors are common in beverages containing soy protein. Fortification with whey protein in beverages was related to off-flavor but also with sweet aromatic and vanillin flavors (Childs and others 2007, Lammert and others 2014).

Since according to Table 7, protein contents of MPI (0-4%), SPI (0 - 1.4%) and WPC (0 - 0.8%) might lead to acceptable formulations, those protein levels were evaluated. Values of viscosity <100 mPas at 20°C and a shear rate of 50 s⁻¹ and no aftertaste were desirable characteristics. As show in Table 8, the formulations with 4% MPI, and 2.0% MPI - 2.0% SPI reported lower viscosities (74 and 77 mPas) than the rest of the mixtures.
The addition of WPI even at low concentrations (0 - 1.4%) was observed to increase the viscosity due to protein denaturation. Comparisons in the addition of 0.1 % of different phosphates (Section 3.2.1) in 4% MPI formulations were performed. Interestingly, an unpleasant taste (“salty” and almost “metallic”) was also perceived in samples containing STPP or Text melt®. Beverages with dipotassium phosphate showed a lower viscosity in comparison with the same sample containing STPP, Text melt® or potassium citrate. Thus, disodium phosphate was chosen for the subsequent formulations. These observations are consistent with Vujicic and others (1968) that found the addition of phosphates increases viscosity in milk (Molins 1990). The addition of STPP has been related to age gelation and sedimentation (at 0.2%) of dairy retorted beverages (Cano-Ruiz and Richter 1998, Lin 2002).

Table 7. Protein mixture for selecting preliminary formulation.

<table>
<thead>
<tr>
<th>X1 (%MPI)</th>
<th>X2 (%SPI)</th>
<th>X3 (%WPC)</th>
<th>Liquid</th>
<th>Phase Separation</th>
<th>Aftertaste/Off-flavor</th>
<th>Accept/Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Accepted</td>
</tr>
<tr>
<td>0</td>
<td>4.0</td>
<td>0</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>4.0</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.0</td>
<td>2.0</td>
<td>0</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Accepted</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>2.0</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>Accepted</td>
</tr>
</tbody>
</table>

*MPI: Milk Protein Isolate; SPI: Soy Protein Isolate; WPC: Whey Protein Concentrate.
Table 8. Apparent viscosity and aftertaste perception for screening formulations with 4 % additional protein and 2% oat flour.

<table>
<thead>
<tr>
<th>%MPI</th>
<th>%SPI *</th>
<th>%WPC *</th>
<th>Viscosity (mPas)**</th>
<th>Aftertaste</th>
<th>Accept/Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>77.5 ± 0.4</td>
<td>X</td>
<td>Accepted</td>
</tr>
<tr>
<td>3.4</td>
<td>0.4</td>
<td>0.2</td>
<td>189.2 ± 5.8</td>
<td>X</td>
<td>Rejected</td>
</tr>
<tr>
<td>3.2</td>
<td>0.0</td>
<td>0.8</td>
<td>540.5 ± 5.6</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>3.0</td>
<td>0.4</td>
<td>0.6</td>
<td>586.3 ± 34.2</td>
<td>X</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.8</td>
<td>0.8</td>
<td>0.4</td>
<td>141.4 ± 1.2</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.4</td>
<td>1.4</td>
<td>0.2</td>
<td>365.5 ± 1.1</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.4</td>
<td>1.0</td>
<td>0.6</td>
<td>130.0 ± 1.5</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
<td>74.0 ± 1.5</td>
<td>✓</td>
<td>Rejected</td>
</tr>
<tr>
<td>2.0</td>
<td>1.2</td>
<td>0.8</td>
<td>447.9 ± 4.9</td>
<td>✓</td>
<td>Rejected</td>
</tr>
</tbody>
</table>

*MPI: Milk Protein Isolate; SPI: Soy Protein Isolate; WPC: Whey Protein Concentrate.
**Apparent viscosity measured at 20 °C with a shear rate of 50 s⁻¹

Then five different bench-top beverages with oat flour (2.00, 2.75 and 3.50 %) and MPI (2.50, 5.00 and 7.50%) were processed (Table 9). The addition of 0.7 % oat-β-glucan concentrate to the beverages allowed the β-glucan content to reach 0.48-0.54% (2.3-1.7 g in a 12 fl-oz serving size) in the final product. Protein concentration varied between 4.5-8.8%, thus, contents >10 g of protein/12 fl-oz were obtained. As for viscosity, concentrations >2.75% of oat flour were shown to promote gelation in the beverage matrix. The viscosity values indicate that only the formulation with the lowest content of oat flour (2.0%) and MPI (2.5%) with a viscosity of 70-113 mPas can be considered as a nectar-like drink (51-350 mPas) (NDD 2002). Formulations a and b presented ~60 % more viscosity when the temperature changed from 25°C to 10°C. The change for formulations c, d and e were less pronounced (~40% more viscous at 10°C). Conversely, a honey-like texture (351–1750 mPas) at 10°C was found when the protein was increased to 7.5% (b). The
prototypes c, d and e reached viscosities higher than 1750 mPas at 10°C; this range is considered as having a pudding-like consistency and it would not be desirable as a drinkable product (Table 9).

Preliminary evaluations of the overall liking and viscosity perception from the prototypes in a range of viscosity of 70 to 1850 mPas are illustrated in Figure 5 and 6. On one hand, formulations with 2% oat and 2.5 or 7.5 % MPI were the most preferred (overall liking score >6) and those were considered as “just about right” for perceived viscosity. On the other hand, the ranking for formulation a and b reveals a slight difference in perception of a beverage with viscosity of 70 mPa.s in comparison with 234 mPa.s (Figure 5). Additionally, formulations with the largest oat content (5.0 and 7.5%, d and e) were classified as too viscous (>739 mPa.s).

Table 9. Apparent viscosity, β-glucan and protein content for bench top formulations.

<table>
<thead>
<tr>
<th>Bench-top formulation*</th>
<th>Oat (%)</th>
<th>MPI (%)</th>
<th>β-glucan (%)</th>
<th>Protein (%)</th>
<th>Viscosity (10 °C, mPas)</th>
<th>Viscosity (25 °C, mPas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2.00</td>
<td>2.50</td>
<td>0.54</td>
<td>4.5</td>
<td>113</td>
<td>70</td>
</tr>
<tr>
<td>b</td>
<td>2.00</td>
<td>7.50</td>
<td>0.48</td>
<td>8.7</td>
<td>396</td>
<td>234</td>
</tr>
<tr>
<td>c</td>
<td>2.75</td>
<td>5.00</td>
<td>0.53</td>
<td>6.8</td>
<td>1960</td>
<td>739</td>
</tr>
<tr>
<td>d</td>
<td>3.50</td>
<td>2.50</td>
<td>0.51</td>
<td>5.1</td>
<td>2320</td>
<td>935</td>
</tr>
<tr>
<td>e</td>
<td>3.50</td>
<td>7.50</td>
<td>0.48</td>
<td>8.8</td>
<td>4570</td>
<td>1850</td>
</tr>
</tbody>
</table>

* Additional ingredients are described at Section 3.2.1.
Figure 5. Preliminary overall liking measured on a 9-point hedonic scale for the bench top formulations at 25 °C; bench-top formulations a, b, c, d and e. (n=15).

Figure 6. Preliminary ranking and Just-About-Right (JAR) scale for viscosity of bench top formulations at 25°C; bench-top formulations a, b, c, d and e (n=15).
4.2 Scale-up Processing

4.2.1 Product Formulation and Processing

Scale up prototypes were formulated within a narrow content of oat flour and MPI % based on Tables 4 and 6. Oat flour and oat-β-glucan were solubilized after a heat treatment to obtain an oat slurry. An efficient blending of the dry ingredients through the whole matrix was observed after cold mixing followed by the heat treatment (80 ± 5°C, 10 min). When the dry ingredients were mixed in hot temperature (>30°C), the oat flour β-glucan tended to form clumps. Similarly, Inglett (1989) used temperatures treatment between 70 to 100°C for solubilization of oats and gelatinization of the oat starch. Other authors have used gelatinization temperatures of 44.7 to 73.7°C (Tester and Karkalas 1996; Deswal and others 2014).

A sterilization process was performed. The heat penetration curve (Figure 7) shows that the come up time was approximately 5 minutes and the holding time was 8 minutes. Moreover, the lethal curve, which corresponds to the average calculated lethal rate against time for the 13 batches was plotted (Figure 8). The AUC for lethal curve calculated by the trapezoidal method indicated that heat treatment is equivalent to an $F_0= 10$ minutes. This oat-β-glucan high protein beverage can be defined as low acid food. Hence, the calculation was performed with the standard z value of 10°C and D value for *Clostridium botulinum* $D_{121.1^\circ C} = 0.2$ minutes (12D = 2.4 min), which suggests that an $F_0$-value of 10 minutes is considered as a safe target process for “adequate sterilization” of this dairy beverage. Furthermore, no significant variations were observed in the lethality calculated for the
minute-by-minute temperature in the heating and cooling rate for different formulation batches (Figure 8). Despite the different formulations (Table 6), the agitation caused by the rotational speed of 10 RPM might be enough to induce a similar heat rate for the samples regardless of the consistency. Similarly, Berry and Kohnhorst (1985) found no changes in $F_0$ value for dairy retorted beverages with different viscosities (7.3 to 15 mPas.) and 25% of total solids. The $F_0$ value coincided with the other dairy commercial products reported by Richardson (2004); $F_0$ values between 6-10 min are used for cream, evaporated milk and milk puddings. Previous studies for dairy beverages sterilized in a rotatory retort have used a process of 17 min at 128°C, 8 min at 123.9°C and $F_0$ of 9.8 and 12.3 minutes with a reel speed of 3.4 and 10.9 rpm, respectively. (Cano-Ruiz and Richter 1998; Richardson 2004; Berry and Kohnhorst 1985).

Figure 7. Heat penetration curve for oat-β-glucan high protein beverage in metal cans (#300). Average values for formulations #1-13.
4.3 Beverage Physicochemical Properties

4.3.1 β-glucan Content

The oat-β-glucan high protein beverages were formulated to contain 1.5 to 2.3% oat flour and 0.7% β-glucan isolate in the finished product. The content of β-glucan in those ingredients was 3.02 ± 0.05% in oat flour and 72 ± 0.2% in the oat-β-glucan isolate. The largest contribution was provided by oat β-glucan isolate. Figure 9. shows the β-glucan content in the beverages was in the range of 0.43 - 0.53%. There were no significant differences in the β-glucan content (p<0.05) of the beverages, except for formulations #1 and #5. Hence, no variation between batches of a same formula (#13) were found. The lowest content of β-glucan (0.43%) was correlated with the lowest amount of oat flour (1.5%, #5). Other researchers have enriched beverages with a similar amount of β-glucan.
For instance, a fruit-dairy juice with 0.5% β-glucan (Temelli and others 2004), a yogurt type drink made with 0.31 - 0.36% β-glucan and 4 - 5.5% oat flour (Angelov and others 2006), and milk fortified with 0.31% - 0.62 % β-glucan (Bangari and others 2011) have been formulated. In a serving size of 8 fl-oz (240 mL), the amount will range between 1.0 – 1.2 g of β-glucan (Table 10). Therefore, all the formulations of the oat-β-glucan high protein beverage meet the health claim 21 CFR 101.81 and provide at least 1 g of the 3 g of the required daily ingest of β-glucan. Moreover, increasing the serving size will allow a larger amount of β-glucan. Based on the contents found in this study, beverages with a 12 and 14 fl-oz serving size will contain 1.5 - 1.8 and 1.7 - 2.0 g of β-glucan.

![Figure 9](image_url)

Figure 9. Oat β-glucan content for the oat β-glucan high protein beverages.

- a, b, c: Different letters among the bars indicates significant difference (P < 0.05) (LSD test).
- *Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).
- ** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.3.2 Total Protein Content

Beverages were formulated to contain more than 10 g (>20% of DRI) protein per serving size. Hence, approximately 4% total protein is the minimum requirement for a high protein claim in 8 fl-oz beverages. The main ingredients of the study beverages including skim milk, MPI and oat flour contributed to the protein content. Skim milk as standard contains 3.4 % protein (USDA 2016). The protein in the MPI was 84.8 ± 0.8%, while for the oat flour was 11.5 ± 1.0 % (g protein/100 g of product). Figure 10 shows that the total protein content varied from 5.07 to 6.47%. These correspond to lower and higher amounts of MPI (2.5-4% MPI; #7 and #8).

![Figure 10. Total protein content for the oat β-glucan high protein beverages.](image)

Different letters among the bars indicates significant difference (P < 0.05) (LSD test).

*Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).

** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).

The most significant changes in protein content are influenced by MPI concentration. As expected, the higher MPI and oat content results in more protein content.

54
Samples containing similar amounts of MPI, concentrations of 3.78 - 4.00% (#3, 8 and 4) and 2.50 - 2.72% (#1, 7 and 2), did not present a significant difference in the protein content (p<0.05). Likewise, replicated batches (#9 - 13) showed no significant difference with formulation #6 (p>0.05). Samples #5 and #6 (3.25% MPI) presented a significant difference that might be due to their different oat flour content (1.50 and 2.30%). The equivalent amount of protein per serving size is summarized in Table 10. Overall, the beverages contain 12 to 14 g per 8 fl-oz. As a result, all formulations had a high protein level. The protein contents achieved for the beverages of this study are in the low range of commercial protein beverages, which commonly contain 10 to 60 g protein per serving size (Childs and others 2007).

Table 10. Equivalent amount in grams of β-glucan and protein for the oat β-glucan high protein beverages in a serving size of 8 fl-oz (240 mL).

<table>
<thead>
<tr>
<th>Formulations**</th>
<th>Oat-β-glucan</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.13 ± 0.05</td>
<td>12.38 ± 0.98</td>
</tr>
<tr>
<td>2</td>
<td>1.22 ± 0.05</td>
<td>13.42 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>1.20 ± 0.02</td>
<td>15.38 ± 0.17</td>
</tr>
<tr>
<td>4</td>
<td>1.25 ± 0.02</td>
<td>15.46 ± 0.26</td>
</tr>
<tr>
<td>5</td>
<td>1.03 ± 0.02</td>
<td>13.94 ± 0.22</td>
</tr>
<tr>
<td>6</td>
<td>1.25 ± 0.02</td>
<td>14.50 ± 0.10</td>
</tr>
<tr>
<td>7</td>
<td>1.25 ± 0.02</td>
<td>12.17 ± 0.79</td>
</tr>
<tr>
<td>8</td>
<td>1.20 ± 0.02</td>
<td>15.53 ± 0.22</td>
</tr>
<tr>
<td>9-13(C)</td>
<td>1.22 ± 0.02</td>
<td>14.35 ± 0.06</td>
</tr>
</tbody>
</table>

*Average determination of 2 determinations from 2 cans. Calculated from Section 4.3.1 and 4.3.2.

**Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6.).

#9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.3.3 Total Solids Content

The prototypes displayed total solids contents of 17.8 to 20.6 % (Figure 11). Beverage #8 (4% MPI) showed a higher total solids percentage value (20.7%) than the prototype made with higher content of oat flour (2.3%, #6). Although there was an increase in the total solids with the increase in oat flour, there was no difference between samples containing 1.9% and 2.3% oat flour (#6 and # 10, 12 and 13). Sample #5 shows both the lowest amount of solids (17.9%) and oat flour content. This indicates that not only the protein concentrate, but also the oat flour, can influence the total solids content. Similar levels of total solids (18-25%) are reported for sterilized dairy beverages (Berry and Kohnhorst (1985). Similar results were observed for some commercial protein beverages; the total solids range found was 7.4-23% (Table 12).

![Figure 11. Total solids for the oat β-glucan high protein beverages.](image)

* Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).
** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.3.4 pH

The mean pH value was 6.73 ± 0.03. Although there were barely detectable statistically significant differences in the pH value among the different formulations, the pH range found does not represent a significant effect on the physical properties of the beverage. Slight differences are observed in the replicate batch #9 versus replicates #11 and 12 (Figure 12). The pH can be affected by the amount of phosphate added. Vujicic and others (1968) described reductions in pH related with the concentration and type of phosphates added to milk. Overall, these differences were minimal. Therefore, it can be assumed that the range of pH found corresponds to a neutral dairy beverage.

![Figure 12. pH values of the oat β-glucan high protein beverages.](image)

Formulations

6.50 6.55 6.60 6.65 6.70 6.75 6.80 6.85
1 2 3 4 5 6 7 8 9 10 11 12 13

*Formulations #1-9 contain same base formula, but differ in oat and MPI content (Table 6).
** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.3.5 Fat Content

All beverages were formulated with 5% of cream (28% fat), thus, it is expected that the concentration of fat will almost be the same for all the prototypes. The oat flour and MPI used in this study have fat contents of approximately 9 and 1%, respectively (USDA 2016). Fat contents were found in the range of 2.14 to 1.84%. No significant differences (p>0.05) was found among formulations #2, 3, 4, 7, 9, 10, 11, 12, 13 (Figure 13), however, formulations #1 and #5 presented a slightly lower fat contents. Physical differences were not observed among the beverages.

Figure 13. Fat content for the oat β-glucan high protein beverages.

*a, b, c, d, e* Different letters among the bars indicates significant difference (P < 0.05) (LSD test).

*Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).*

**#9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI)."
4.3.6 Color

Data in Table 11 shows results of color measurements for the beverages in the parameters L*, a* and b* of the CIELch scale. Although there is a significant difference in the color parameters for formulations (p<0.05), the lightness (L*) of beverage # 5 was significantly higher than the rest of the formulations. This correlates with the lowest content of oat flour and MPI. The addition of MPI might contribute to achieving a whiter tonality, while oat content will reduce the L* value (darker color). A relatively small change in L* value can be influenced by both the amount of oat flour and MPI. In contrast, the a* value was statistically the same for the beverages #4, 2, 6, 8, 10, 11, 12 (contents ≥ 1.9% oat flour and ≥2.72% MPI). Slightly lower and non-significantly different values were found for beverages #3, 1, 5, and 7 (contents ≤ 1.9% oat flour and ≤3.78% MPI). The b* value showed a behavior similar to a* value; low content of oat flour seems to have a slightly low b*. However, visually there was no clear color difference observed among beverages. As a result, the impact of color on acceptability was not further evaluated within the sensorial panel.
Table 11. Color parameters (L*, a* and b*) for the oat β-glucan high protein beverages.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>L*</th>
<th>Color Parameters</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.51 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20± 0.28&lt;sup&gt;ace&lt;/sup&gt;</td>
<td>17.47 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>84.95 ± 0.12&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>3.73 ± 0.07&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>18.09 ± 0.15&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>86.19 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27 ± 0.31&lt;sup&gt;acg&lt;/sup&gt;</td>
<td>17.38 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>84.76 ± 0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.85 ± 0.24&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>18.41 ± 0.39&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>87.60± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.30 ± 0.12&lt;sup&gt;aceg&lt;/sup&gt;</td>
<td>17.06 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>85.29± 0.05&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>3.62 ± 0.20&lt;sup&gt;bdefg&lt;/sup&gt;</td>
<td>18.16 ± 0.26&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>86.49 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.56 ± 0.17&lt;sup&gt;bdefg&lt;/sup&gt;</td>
<td>17.45 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>86.74 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.80 ± 0.07&lt;sup&gt;bdefgh&lt;/sup&gt;</td>
<td>17.62 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>9 (C)</td>
<td>85.65 ± 0.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.40 ± 0.16&lt;sup&gt;acefg&lt;/sup&gt;</td>
<td>18.00 ± 0.29&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 (C)</td>
<td>85.30 ± 0.25&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>4.00 ± 0.26&lt;sup&gt;bdhi&lt;/sup&gt;</td>
<td>18.45 ± 0.35&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>11 (C)</td>
<td>84.68 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.11 ± 0.3&lt;sup&gt;dhi&lt;/sup&gt;</td>
<td>18.71 ± 0.68&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 (C)</td>
<td>84.78 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.08 ± 0.19&lt;sup&gt;dhi&lt;/sup&gt;</td>
<td>18.58 ± 0.41&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>13 (C)</td>
<td>84.60 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20 ± 0.18&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>18.69 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Different letters in the same column indicate significant difference (P < 0.05) (LSD test).
Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).
#9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).

4.3.7 Suspension Stability

Studies have related large amounts of milk protein with sedimentation over time (Harwalkar and others 1983, McKenna and Singh 1991). Accelerating the precipitation of particles in the liquid is indicative of the stability for the beverage. Although, there were some significant differences among the 13 beverages, all beverages showed stabilities higher than 80%. Therefore, all prototypes can be considered as stable. As expected, no significant differences were found in samples #9-13 (batch replicates). The lowest stability was 87.4 ± 5.9% (Figure 14) and corresponded to the formulation with the lowest amount of oat flour (1.50%, #5). Whereas the highest stability achieved (97.2 ± 0.7 %) was the sample among those with the highest amount of oat flour (2.30%, #6), there was no
statistical significant difference with samples with >1.9% oat flour (#8, 9, 10, 11, 12, 13). Priepke and others (1980) suggests that suspension stabilities lower than 70 % can be classified as non-stable beverages. The viscous/thicker texture developed by oat flour avoids the sedimentation of particles. This thickening effect of β-glucan, oat flour and carrageenan provides a stable matrix for milk proteins with no visual signs of separation.

In a similar matrix composed of milk and 0.3- 0.6% β-glucan, Bangaris and others (2011) observed that the addition of carrageenan favored the stability of the system. Samples without carrageenan added presented syneresis after 21 days. Other authors have indicated that a 0.2% β-glucan content lead to incompatibility with the milk protein and consequently caused phase separation.

Figure 14. Suspension stability for the oat-β-glucan high protein beverages.

ab Different letters among the bars indicates significant difference (P < 0.05) (LSD test).

*Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).

** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.3.8 Apparent Viscosity

Temperature has significant effects on the viscosity of each formulation. Figure 15, illustrates the viscosity values for the beverages at 4-25°C. As expected, there is relevant decrease in apparent viscosity values with the increase in temperature. Apparent viscosities >60 mPas at ~4°C were reduced to <40 mPas at room temperature (formulations #2, 3, 4, 6, 8 and center points #9-13). The decrease in apparent viscosity was lower for formulations #1, 3, 5, and 7 (Table 6). Values of 17 - 43 mPas at 4°C decreased to 5 - 15 mPas at 25°C; this corresponds to an approximate 29 - 34% less viscous product.

These findings suggest that all the beverage formulations had viscosities of thin liquids at room temperature (25°C), this means all viscosities will be <50 mPas (NDD 2002). Beverages with contents <1.90% oat flour and <2.5% MPI can be classified as thin beverages at any of the temperatures evaluated. On the contrary, formulations with contents >1.90% oat flour and >2.5% MPI are shown to be nectar-like liquids. Moreover, at a constant level of MPI (3.25%), there was a considerable change of the viscosity when oat flour was increased from 1.5% to >1.9%. Sample #5 (1.5% oat, 3.25% MPI) had a viscosity significantly different from samples #6, 9, 10, 11, 12, and 13 (p<0.05). As described before, within the 13 beverages samples tested, five of them were formulated with the same contents of oat flour 1.9% and MPI 3.25% (#9 - 13). The average viscosity value over the rage of temperature is slightly lower than formulations with higher oat and MPI (#4, 6 and 8). Figure 15 illustrates that the viscosity curves for formulations #4, 6 and 8 are
overlapped. This suggests no significant differences among these formulations (>1.90% oat flour and >3.25% MPI).

Oat flour had a significant influence in the beverages’ viscosity which is also increased by the presence of additional β-glucan. As discussed before, β-glucan has been reported to promote viscosity. All the beverages in this study have approximately 0.5% β-glucan (Figure 9). It has been reported that in beverages with oat bran rich in β-glucan, high thickness at concentrations above 0.5% β-glucan have generated problems in food processing. Concentrations higher than 0.622% β-glucan had caused gelation in milk (Bangari and others 2011). According to Lyly and others (2003) and Temelli and others
(2004) by replacing oat bran with purified β-glucan fractions it is possible to achieve concentrations of 2 % β-glucan without a remarkable effect in viscosity. Although, studies have reported the association of β-glucan content with the increase of viscosity, the presence of starch and protein also increased the viscosity in a liquid matrix. At constant β-glucan content, the change in viscosity might be attributed to other components present. The milk protein and oat flour, which provide protein and starch, respectively, have potential influence in the apparent viscosity. However, low increments of oat flour have more influence on viscosity because of the starch content. This interpretation might be supported by the work of Kim and White (2012). The authors evaluated the interaction of components in oat suspension (slurries) by enzymatic treatment. The addition of α-amylase for starch, lichenase for β-glucan, and proteinase for proteins showed that starch had the highest impact on viscosity. After the addition of amylase, the authors found an 89% decrease in viscosity. The protein from oats was reported to have minor influence on this property (Kim and White 2012).

The apparent viscosities (at 5 - 25°C) for commercial protein beverages are shown in Table 12. Results for commercial beverages with the highest protein content (30 and 40 g) showed viscosities of 13 - 4 mPas. These values were similar to formulation #5 (17 – 5 mPas). Similarly, some prototypes reached that range of viscosity when the temperature was increased. For instance, formulation #1 at >15°C presented 17-10 mPas, #3 at 25°C was 15 mPas, and #7 at >20°C was 13 - 10 mPas (Figure 15). Protein beverages with 10-20 g of protein/serving had the lowest viscosities (approximate range of 8 – 1.6 mPas),
except for the protein smoothie (12 g of protein), for which nectar-like thickness was achieved at temperatures of 5 - 15°C (70 - 50 mPas).

Table 12. Total solids, protein content and apparent viscosity (shear rate of 50 s\(^{-1}\) and temperature of 5, 10, 15, 20 and 25°C) of six commercial protein beverages.

<table>
<thead>
<tr>
<th>Commercial Product</th>
<th>Total Solids (%)</th>
<th>Protein Content (g per serving)*</th>
<th>Apparent Viscosity (mPas)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5°C</td>
</tr>
<tr>
<td>Milk Muscle®</td>
<td>13.4</td>
<td>40</td>
<td>13.0</td>
</tr>
<tr>
<td>Rocking Fuel®</td>
<td>12.7</td>
<td>30</td>
<td>12.2</td>
</tr>
<tr>
<td>Core Power®</td>
<td>11.2</td>
<td>20</td>
<td>4.1</td>
</tr>
<tr>
<td>Smoothie Carb®</td>
<td>7.4</td>
<td>12</td>
<td>70.3</td>
</tr>
<tr>
<td>Carnation® Breakfast</td>
<td>23.2</td>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>Kelloggs Breakfast</td>
<td>15.2</td>
<td>10</td>
<td>8.0</td>
</tr>
<tr>
<td>Shake®</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Declared in the label
4.4 Sensory Acceptability

4.4.1 Sensory Acceptability and Preference

Overall liking for the formulations seems be affected by oat flour and MPI concentration. The most acceptable prototypes (overall liking >5) were found to be #1, 3, 5 and 7 (Figure 16). Statistical analysis of data revealed that the overall liking of these formulations was significantly different (p<0.05) from the remaining formulations. However, the difference was not significant (p>0.05) among #1, 3 and #5. As expected, for mouthfeel (thickness), the same trend was observed: the scores slightly decreased with the increase of oat and MPI added (Figure 16). For samples with high oat and MPI concentrations (#4 and #6) no significant differences in mouthfeel acceptability between these two formulations were observed. For the higher MPI concentration (4%) samples a slightly higher acceptability was found. Beverages #1, 3, 5, and 7 were found to have the most acceptable mouthfeel (thickness) (p<0.05) (Figure 16). Overall, those formulations correspond to the lowest viscosity values. As previously mentioned, sensory tests were performed for beverages at ~10°C. At this temperature, the viscosities were: 21.7 ± 4.8, 36.4 ± 2.0, 14.5 ± 0.9 and 26.2 ± 0.9 mPas (# 1, 3, 5, and 7), all of which are categorized as thin liquids. The least acceptable beverage (#6) had a nectar-like viscosity (75.5 ± 3.7 mPas at 10°C). These findings suggest that the increase in viscosity is one of the main concerns when including a large amount of oat and protein in the beverage. High viscous beverages are difficult to swallow, which might result in a poor acceptability for the product.
Figure 16. Sensory acceptability of oat-β-glucan high protein beverages. A. Overall liking, and B. Viscosity acceptability (n=70).

Different letters among the bars indicates significant difference (P < 0.05) (LSD test).

*Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).

** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).

Therefore, there is a strong correlation between thickness acceptability and apparent viscosity measured at 10°C (Figure 17). Viscosities >40 mPas were not acceptable (hedonic score <5). Mouthfeel is influenced by different attributes that can act as drivers of texture acceptability. It had been reported that perceived thickness and sliminess were
strongly correlated with instrumental viscosity in beverages with contents of 0.5 - 1% β-glucan (Lyly and other 2003). However, not all the attributes related with mouthfeel can be correlated with an instrumental viscosity. According to de Wijk and Prinz 2005, other textural attributes like creaminess, smoothness or stickiness were not correlated with viscosity measurements at 50\(^{-1}\), which is the shear rate for swallowing. Moreover, total solids for the formulations correlated negatively with the mouthfeel acceptability; the more solids the less acceptable the beverage (Figure 18).

![Graph](image)

Figure 17. Mouthfeel (thickness) acceptability as function of apparent viscosity measured at 10°C and shear rate 50 s\(^{-1}\) for the oat-β-glucan high protein beverages (n=70).

*Formulations #1-13 (Table 6).*
Both preference and overall acceptability scores increase with a decrease in viscosity. The preference rankings performed for prototypes #2, 4 and 9 show that 50% of the panelists chose sample #2 (54 mPas) as their most preferred product, but for 19% this was their second preference and 31% chose this lower viscosity as the least preferred. A slightly higher percentage of panelists preferred sample #4 (80 mPas) over sample #9 (64 mPas) (Figure 19). Consequently, it is possible to consider two consumer segments, those who like the thick or viscous beverages and those who like thin beverages.

Moreover, the frequency of exposure to a certain taste or type of food is an important aspect of product acceptability/preference. Results from the demographic questions indicated that the panelists population was composed primarily females (56%) and 39% of them were between 18-23 years-old. The most prevalent ethnicity was white.
not Hispanic (61%); data for demographics of the panelists can be found in Appendix A. Panelists may not be familiar with this type of product, which can affect the acceptability scores. Preference/acceptability for a product might vary due to cultural differences. Thus far, a number of studies have examined the association of geographic origin and consumer acceptance of food products. The oat taste, as well as oats in a beverage type product can be considered as a novelty for the North American market. In this market oats are mostly consumed as a porridge (oat flakes and oat bran), snacks, Muesli, breakfast cereals, bread and biscuits (Sontag-Strohm and others 2008). However, sweetened oatmeal drinks are popular in other cultures like Hispanic, where oats are mostly consumed with milk or fruit juices, as a dessert or breakfast beverage known as “Avena” or “Atole” (Janer 2008). From the product development/marketing standpoint, deciding between more than one formulation may rely on the targeted consumer.

![Figure 19. Ranked preference for formulation # 2 (54 mPas), # 9 (64 mPas), and # 4 (80 mPas). Ranking from the most preferred (1st) to the less preferred formulation (3rd). Viscosity measured at 10°C.](image)

70
Flavor, sweetness and aftertaste attributes presented similar characteristics (Figure 20). This outcome is due to the narrow ranges used for oat flour % and MPI %. Overall, formulation #5 (1.5% oat flour, 3.25% MPI) had the most acceptable attributes. There were no significant differences in the attributes among the beverages with >1.9% oat flour and >3.25% MPI contents (p>0.05). Despite the fact that the level of sugar added for all the prototypes was the same (3.5% sucrose), acceptability of sweetness in samples #1, 5 and 7 was higher compared with the rest of the formulations. These formulations were shown to have the lowest levels of viscosity (15-27 mPas). The different viscosity of the beverages had a slight influence on the perception of sweetness. These observations are supported by evidence of increased hydrocolloid content contributing to decreased perceived taste in beverages (Stone and Oliver 1966, Pangborn and others 1978, Matta and others 2006).
Figure 20. Acceptability of flavor (A), sweetness (B), and aftertaste (C) of the oat-β-glucan high protein beverages (n=70).

a, b, c, d, e, f Different letters among the bars indicates significant difference (P < 0.05) (LSD test).

*Formulations #1-9 contain same base formula, but differ in oat, and MPI content (Table 6).

** #9-13 correspond to replicated batches of the central point (1.90% oat flour, 3.25% MPI).
4.4.2 Penalty analysis

A penalty analysis can be performed to recognize the main attributes that contribute to decreases in overall liking and JAR scores. Attributes with a large mean drop and a high percentage of panelists (upper right corner of the plot) imply a negative effect on the overall liking and are subject to reformulation. As shown in Appendix B, the position of the mean drop for the attributes evaluated suggested that samples with low overall acceptability presented similar characteristics. The penalty analysis for a non-acceptable (#6, overall liking <5) and the most acceptable (#5, overall liking >5) formulation are presented in Figure 21 and Figure 22.

Figure 21. Penalty analysis plot of a non-acceptable formulation of oat-β-glucan high protein beverages (#6: 2.30% oat flour, 3.25% MPI).
Figure 22. Penalty analysis plot of the most accepted formulation of oat-β-glucan high protein beverages (#5: 1.50% oat flour, 3.25% MPI).

Mouthfeel had the most effect on acceptability among other attributes. However, the decrease in acceptability was not only related to high thickness but also low sweetness and high aftertaste with a decrease in hedonic scores. Too much thickness (+) received the highest penalty with mean drops of ~2.0 for more than 60% of the panelists in formulations with contained contents higher than 1.9% oat flour and 3.25% of MPI. Too much thickness (+) did not account for any penalty in prototype #5. On the contrary, for a low percentage of panelists a decrease in overall liking was caused by the low thickness of this sample (30%, mean drop = 1).

Moreover, in terms of the taste, consumers penalized products because of a lack of sweetness. The mean drops for this attribute ranged from 0.6 to 2.3 for less than 47% of the panelists, when evaluating non-acceptable formulations. A slight increase in the sucrose
content will lead to higher overall linking scores. It is well known there is a strong influence of sweetness on food acceptability; there is a predisposition to prefer sweet tastes, which varies among individuals (Birch 1999; Kim and others 2014; Drewnowski and other 2012). However, the JAR scores for sweetness indicate that in most of the beverages, >50% of panelists considered the sweetness level used as adequate (Appendix B). Therefore, the sucrose added may be close to the ideal concentration. As stated before, a beverage with lower thickness may taste sweeter. Thus, when reformulating the product, it would be important to address potential increase of perceived sweetness with the reduction of thickness, to avoid a too much sweetness (+) penalty.

Another noteworthy penalty was due to perception of an aftertaste. Panelists (>40%) considered a strong aftertaste as a negative attribute with mean drops of 1.1 - 2.2. Surprisingly, there was a strong penalty for the absence of an aftertaste in the most accepted prototype (#5); however, the percentage of panelists was not significant for the overall liking of this prototype. The contradictory results for the presence of an aftertaste might be explained by the lack of understanding of what an aftertaste means. An untrained panel of consumers can relate any uncommon attribute to be a source of an aftertaste.

Additionally, a low percentage of panelists (11-30 %) scored both flavor attributes (not enough and too much) with strong penalties (mean drops = 1 to 3) for the beverages. For example, in formulation #8, 24% of panelists thought it had not enough flavor (mean drop 2.8) and 13% felt that it had too much flavor (mean drop 3.0) (Appendix B.). In most of the formulations, more than 50 % of the panelists chose the flavor attribute as JAR.
Addition of flavors commonly found in dairy beverages, such as vanilla or chocolate, will potentially increase the overall acceptance of the beverage.

4.5 Response Surface Models

The regression terms of the second order response surface models fitted for sensory acceptance and physicochemical properties are shown in Table 13 and Table 14. The response models were used to generate three dimensional response surfaces and contour plots (Figures 23, 24 and 25). All regression equations for responses showed no significant lack of fit (p>0.05), which implies that the models were accurate. The interaction effect (Oat x MPI) was not significant (p>0.05) for all response models except for protein content; thus, this term was dropped. It is suggested that a $R^2$ must be at least 0.80 to be considered as a good fit (Joglekar and May 1987). This indicates the fitted models for physicochemical properties as well as liking, thickness and flavor acceptance accounted for >80% of the variation in experimental data ($R^2$ >0.80) and were considered accurate (Table 13 and 14.).
Table 13. Regression coefficients, $R^2$, adjusted $R^2$, p-value and F-value (response) and lack of fit (p-value and F-value) for the final reduced sensory acceptance model equations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sensory Acceptance (9-Hedonic Scale) Model Equations*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall Liking ($y_1$)</td>
</tr>
<tr>
<td>$b_0$</td>
<td>26.63</td>
</tr>
<tr>
<td>$b_1$ (Oat)</td>
<td>-13.34</td>
</tr>
<tr>
<td>$b_2$ (MPI)</td>
<td>-4.63</td>
</tr>
<tr>
<td>$b_{11}$ (Oat*Oat)</td>
<td>3.181</td>
</tr>
<tr>
<td>$b_{22}$ (MPI*MPI)</td>
<td>0.676</td>
</tr>
<tr>
<td>$b_{12}$ (Oat*MPI)</td>
<td>-</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.8773</td>
</tr>
<tr>
<td>$R^2$ (adj)</td>
<td>0.8160</td>
</tr>
<tr>
<td>Response (p-value)</td>
<td>0.001</td>
</tr>
<tr>
<td>Response (F-value)</td>
<td>14.30</td>
</tr>
<tr>
<td>Lack of fit (p-value)</td>
<td>0.583</td>
</tr>
<tr>
<td>Lack of fit (F-value)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* $y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2$

$b_1$, the estimated regression coefficient for the main linear effects.

$b_{11}, b_{22}$ the estimated regression coefficient for the quadratic effects.

$b_{12}$, the estimated regression coefficient for the interaction effects.

Regression coefficients of all sensory acceptability responses (Table 13), show that main effects for the independent variable (Oat and MPI) had a significant negative effect on the overall acceptability, while the interaction effects of the independent variables (Oat*Oat; MPI*MPI) had a significant positive effect on the acceptability. From the response surface curves and contour plots (Figure 21 and 22), it is clear that a decrease in the levels of oat flour and MPI is related with an increase in overall liking and acceptability.
for thickness, flavor, aftertaste and sweetness. Specifically, levels of <1.7% of oat flour and < 3.3% MPI resulted in a sensory acceptance > 5 for all attributes. As in the case of mouthfeel (thickness) acceptance, this attribute showed slightly higher hedonic scores than the overall liking at the same concentrations of oat and MPI. The R² values were 0.877 and 0.900 for overall liking and thickness acceptance, respectively. The more significant coefficient was Oat, which presented a p=0.001 overall liking, and p=0.000 for the mouthfeel acceptance models.

A similar trend was also found in the surface response models for sweetness and flavor. The linear regression model for flavor was insignificant for MPI (p=0.057), however, this term was not dropped from the equation because the interaction MPI*MPI was significant at p=0.029. Although the sweetness model showed a low R² (0.7413), it is worth mentioning that the gradual increase in sweetness acceptability (>5) appears to correlate with a decrease in perceived thickness for the beverages. Moreover, aftertaste acceptability appears to decrease with increasing concentrations of oat flour. The adjusted R² of < 0.50 indicates that the model was not sufficiently accurate for predicting aftertaste acceptance. This may partly be explained by the lack of understanding in the definition of what an aftertaste was for the beverage. Without proper training, panelists will not be consistent in their evaluations. For instance, some of them might have focused on the typical chalky taste of protein while others might have focused on the oat taste. The contour plot for aftertaste reveals that the least acceptable “aftertaste” corresponds to formulations where the most oat flour was added (>2.2% oat flour). Despite the low correlation it is
interesting that this trend might indicate the panelists related “aftertaste” with oat taste. As described before, no flavors were added to the formulations. Thus, the oat taste might be more pronounced than expected for the panelists.

**Overall Liking**

![Liking Contour Plot](image)

**Mouthfeel (Thickness) Acceptance**

![Thickness Contour Plot](image)

Figure 23. Surface response and contour plots for overall liking and mouthfeel acceptance (thickness) as a function of varied levels of oat flour and MPI.
Figure 24. Surface response and contour plots for acceptability of flavor, sweetness, and aftertaste as a function of varied levels of oat flour and MPI.
The coefficients of determination (R²) for final reduced models equations for physicochemical properties (protein, total solids and apparent viscosity) were >0.80 (Table 14). As mentioned earlier, both oat flour and MPI have contents of protein, 11.5 ± 1.0 % and 84.8 ± 0.8%, respectively. Therefore, this suggests that these variables should be considered as significant primary factors affecting the protein content. Protein content was mostly influenced by MPI content (p=0.000). Oat flour had a less significant, but positive effect (p=0.004). As a contrast, oat content had a positive and slightly more significant (p<0.001) effect than MPI (p<0.003) on solids and viscosity at 5°C. The increase of oat flour and MPI leads to increase in total solids, viscosity and protein contents. Moreover, the coefficients for the quadratic model (Oat*Oat and MPI*MPI) had negative effect. For total solids, the MPI interaction (MPI*MPI) had a significant positive effect for the model but oat interaction (Oat*Oat) had a negative effect. Apparent viscosity of the formulations was more influenced by oat flour than MPI, however, both ingredients increase the viscosity of the beverages. Response surfaces and contour plots for viscosity at 5, 15 and 20°C are found in Appendix C.
Table 14. Regression coefficients, $R^2$, adjusted $R^2$, p-value (response) and lack of fit (p-value and F-value) for the final reduced models equations for physicochemical properties (protein, total solids and apparent viscosity).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Protein Content</th>
<th>Total Solids</th>
<th>5 °C ($y_8$)</th>
<th>10 °C ($y_9$)</th>
<th>15 °C ($y_{10}$)</th>
<th>20 °C ($y_{11}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>-5.33</td>
<td>3.21</td>
<td>-1018</td>
<td>-839</td>
<td>-643</td>
<td>-455.4</td>
</tr>
<tr>
<td>$b_1$ (Oat)</td>
<td>2.509</td>
<td>14.31</td>
<td>665</td>
<td>533</td>
<td>404.7</td>
<td>283.8</td>
</tr>
<tr>
<td>$b_2$ (MPI)</td>
<td>4.319</td>
<td>-0.85</td>
<td>199.0</td>
<td>176.1</td>
<td>137.0</td>
<td>97.8</td>
</tr>
<tr>
<td>$b_{11}$ (Oat*Oat)</td>
<td>-</td>
<td>-3.31</td>
<td>-151.1</td>
<td>-121.3</td>
<td>-92.2</td>
<td>-64.1</td>
</tr>
<tr>
<td>$b_{22}$ (MPI*MPI)</td>
<td>-0.3215</td>
<td>0.353</td>
<td>-25.4</td>
<td>-23.0</td>
<td>-17.97</td>
<td>-12.76</td>
</tr>
<tr>
<td>$b_{12}$ (Oat*MPI)</td>
<td>-0.666</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9838</td>
<td>0.8981</td>
<td>0.9298</td>
<td>0.9345</td>
<td>0.9369</td>
<td>0.9332</td>
</tr>
<tr>
<td>$R^2$ (adj)</td>
<td>0.9757</td>
<td>0.8472</td>
<td>0.8947</td>
<td>0.9017</td>
<td>0.9054</td>
<td>0.8998</td>
</tr>
<tr>
<td>Response (p-value)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Response (F-value)</td>
<td>121.54</td>
<td>17.63</td>
<td>26.50</td>
<td>28.53</td>
<td>29.70</td>
<td>27.95</td>
</tr>
<tr>
<td>Lack of fit (p-value)</td>
<td>0.804</td>
<td>0.302</td>
<td>0.451</td>
<td>0.641</td>
<td>0.625</td>
<td>0.464</td>
</tr>
<tr>
<td>Lack of fit (F-value)</td>
<td>0.40</td>
<td>1.74</td>
<td>1.14</td>
<td>0.68</td>
<td>0.71</td>
<td>1.10</td>
</tr>
</tbody>
</table>

* $y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2$

$b_1$, the estimated regression coefficient for the main linear effects.

$b_{11}, b_{22}$ the estimated regression coefficient for the quadratic effects.

$b_{12}$, the estimated regression coefficient for the interaction effects.
Figure 25. Surfaces responses and contour plots for protein content, total solids and apparent viscosity (at 10 °C) as a function of varied levels of oat flour and MPI.
4.6 Optimization of the Oat-β-glucan High Protein Beverage Formulation

The second order response surface models (Table 13 and 14.) were used for the generation of contour plots for optimization purposes. The optimum area for the oat high protein beverages was found by superimposing contour graphs for all of the responses evaluated as a function of oat flour and MPI content. Limits for the sensory hedonic score were established to achieve a formulation with a potential overall acceptability. Hence, it was assumed that scores >5 for all sensory responses were acceptable. The values of apparent viscosity at different temperatures were chosen based on the viscosities of commercial products measured (Table 12.). Formulations with apparent viscosities between 1-15 mPas at 5, 10, 15 and 20°C were selected. Likewise, a high protein content is desirable; levels of >5% protein (>13.5 g/8 fl-oz) were selected. It was desirable to maximize the amount of protein included in the beverage without negatively affecting the sensorial properties. According to Oltman and others (2015), consumers are aware of protein content; an increased amount of protein was an indication of satiety. Although it has been shown that consumers tend to prefer high protein claims, the sensorial attributes for beverages with 20 g per serving are less preferred in comparison to beverages with 15, 10 or 5 g of protein. Commercial beverages with 10 g of protein per serving show higher appearance liking than beverages with higher amounts of protein (Oltman and others 2015).

The optimum region that satisfies the constraints for the responses is shown in Figure 26, as the white shaded area of the overlay plot. Prototypes in this region might be considered the potential optimum acceptable beverages. The values of apparent viscosity
at 5°C (<15 mPas) were the limiting level for the increase in oat flour % and MPI %. Low concentrations of these ingredients were limited by viscosity at 20°C (>1.5 mPas). In order to achieve protein contents >5%, concentrations of MPI were restricted to <2.7%. Optimum formulations of the oat-β-glucan beverage were determined as approximate combinations of 1.5-1.7% oat flour and 2.6-3.0% MPI.

<table>
<thead>
<tr>
<th>Overall liking</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity Acceptance</td>
<td>5</td>
</tr>
<tr>
<td>Flavor Acceptance</td>
<td>5</td>
</tr>
<tr>
<td>Protein Content, %</td>
<td>5</td>
</tr>
<tr>
<td>Viscosity at 5 °C, mPas</td>
<td>1.5</td>
</tr>
<tr>
<td>Viscosity at 10 °C, mPas</td>
<td>1.5</td>
</tr>
<tr>
<td>Viscosity at 15 °C, mPas</td>
<td>1.5</td>
</tr>
<tr>
<td>Viscosity at 20°C, mPas</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Figure 26. Superimposed contour plots showing the white shaded overlapping area for optimum formulations.
Chapter 5: Conclusions

The present study indicates that it was possible to formulate an acceptable functional beverage that meets the cardiovascular disease risk reduction (21 CFR 101.81) and high protein content (21 CFR 101.54) claims by using oat flour, oat-β-glucan and milk protein.

Shelf stability was achieved by a thermal treatment in a rotary retort. Overall, the beverages show high suspension stability; the thickening effect of the oat flour prevented particle sedimentation. The pH was neutral and no significant variations in color were found.

It can be concluded that the main attributes influencing the acceptability of the beverages were mouthfeel (thickness), sweetness and aftertaste. The decrease in oat flour and MPI content was found to increase the overall sensory acceptability. This improvement was thought to be caused by the decrease in perceived thickness, which in agreement with viscosity and total solids values, yielded thinner beverages.

It was found that the optimum oat-β-glucan beverage contained 1.5-1.7 % oat flour and 2.6-3.0 % MPI. These formulations were shown to have good acceptance, high protein, and low viscosity.
The viscosity and stability of the finished products are good indicators of the potential that oat flour and oat-β-glucan may have to be used as stabilizers in dairy beverages. Additionally, clinical trials to assess the health benefits of oat-β-glucan enhanced products are a good opportunity to conduct further research.
References


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Perez Hernandez G. 2005 Use of ingredients and processing to control the stability of high whey protein concentration retort sterilized beverages. [DPhil dissertation]. College Station, TX:


Appendix A: Demographics
Appendix B: Penalty Analysis Plots
# 1: (1.62% Oat, 2.72% MPI)

# 2: (2.18% Oat, 2.72% MPI)

# 3: (1.62% Oat, 3.78% MPI)

# 4: (2.18% Oat, 3.78% MPI)

# 5: (1.50% Oat, 3.25% MPI)

# 6: (2.30% Oat, 3.25% MPI)

# 7: (1.90% Oat, 2.50% MPI)

# 8: (1.90% Oat, 4.00% MPI)
# 9: (1.90% Oat, 3.25% MPI)

# 10: (1.90% Oat, 3.25% MPI)

# 11: (1.90% Oat, 3.25% MPI)

# 12: (1.90% Oat, 3.25% MPI)

# 13: (1.90% Oat, 3.25% MPI)
Appendix C: Response Surfaces and Contour Plots for Viscosity (5, 15 and 20°C)
Viscosity at 5°C

Viscosity at 15°C

Viscosity at 20°C