Effects of Oat Beta Glucan on the Stability and Textural Properties of Beta Glucan Fortified Milk Beverage

by

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Abstract

Consumption of milk is declining while consumption of carbonated beverages is increasing. The rise in consumption of carbonated beverages has contributed to an increase in obesity in the United States. Milk based beverages can act as ideal vehicles for bioactive compounds. One such compound, beta glucan a viscous soluble dietary fiber, has received the FDA's approval as an agent that lowers serum cholesterol. Products that make beta glucan more available in the diet can help contribute to a reduction in coronary heart disease (CHD) and associated health issues. The objectives of this study are to evaluate the stability, texture and overall acceptance of flavored milk containing beta glucan and to evaluate processing parameters in maximizing the stability. The beta glucan fortified milk was made in one stage and two stages in a jacketed kettle. Stability and texture of the product can be described as a phase separation and viscosity of the beta glucan fortified milk. A significant difference ($p<0.05$) in viscosity between the beta glucan samples made using two protocols was observed. However, within each

protocol no significant difference between texture from day one and day six ($p>0.05$) was observed.

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I would like to dedicate this thesis to my beloved Parents.

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Chapter I: Introduction

Encouraging consumption of more nutritious food is important in the present United States market. Flavoring milk can be a potential strategy to introduce nutritious foods into diets of young adults (Johnson, Frary & Wang, 2002) and replace the consumption of high caloric beverages (Weaver & Boushey, 2003; Wyshak, 2000). However, in recent years the flavored milk market has shown a slow decline, due to lack of innovative flavors and value-added ingredients (Beverage Marketing Corporation, 2008). Despite the increasing interest in nutraceuticals and functional foods in the United States, the fortified milks at present are limited (Jerkins et al., 2002). The only bioactive ingredients used in functional dairy beverages in the present market include omega 3-fatty acids, plant sterols and bioactive peptides. Although the inherent health benefits of milk still remain largely unexploited, milk based beverages can act as ideal vehicles for newly discovered bioactive compounds (Sharma, 2005).

Bioactive compounds are believed to influence metabolic processes and eventually chronic diseases such as cancer, heart related diseases and diabetes (Jerkins et al., 1999; Roberfield, 1998; Saris et al., 1998; Ohigashi et al., 1996; Pick et al., 1996). However, there are few functional foods with low glycemic indices that can help in management of diabetes (American Diabetes Association, 1999). Therefore, it is essential to develop functional products which are consistent with diets that limit the rise of chronic diseases such as diabetes.

Dietary fiber from fruits, vegetables and especially from whole grains is believed to confer health benefits including lowered risk of diabetes. Oat bran, rich in beta glucan fiber has been shown to reduce cholesterol uptake (Anderson et al., 1990). Fortification of beta glucan in oat bran products resulted in lower glycemic values and reduced cholesterol uptake (Brown et al., 1999). In addition to oats, beta glucan from other sources such as barley, mushrooms and yeast have also shown similar effects (Mantovani et al., 2008).

Commercially, beta glucans are extracted from oats, barley, mushrooms, and yeasts. Initially, it was very expensive to extract and purify the beta glucan, but extraction processes have become more economical with the development of improved techniques. Inexpensive extraction of beta glucan from its source has allowed its incorporation into a variety of food products like milk and fruit beverages.

Statement of the Problem

Consumption of milk is declining while consumption of carbonated beverages is increasing. The rise in consumption of carbonated beverages contributes to an increase in obesity in the United States. This project is aimed at developing a milk based beverage combining the nutrient properties of milk and beta glucan. Beta glucan can impart a thick, gummy texture in many products such as milk and fruit beverages. In addition many hydrocolloids can reduce stability of milk proteins. The objectives of the study are to evaluate the stability, texture and overall acceptance of flavored milk containing beta glucan and to examine processing parameters in minimizing effects of beta glucan on casein stability.

Purpose of the Study

Soluble dietary fiber has become increasingly popular because its consumption is associated with improved health. *In vivo* and *in vitro* studies have demonstrated that certain dietary fibers can reduce the risk of developing cardiovascular diseases because they lead to decreased blood cholesterol, delayed carbohydrate digestion, regulation of postprandial blood glucose and insulin levels and the promotion of a healthy balance of gut microflora.

Teenagers and children in the United States are more inclined towards beverages such as soft drinks and fruit drinks. As a result they drink less milk (Johnson et al, 2002; Guthria & Morton, 2000). The lack of milk consumption can result in decreased bone density on a long term basis (Wyshak, 2000). In addition, obesity has been correlated with the rise in the consumption of high caloric soft drinks (Weaver & Boushey, 2003). Flavored milk has the potential to increase milk consumption by children and teenagers in school and at home (Johnson et al, 2002). Development of desirable and novel flavors can be an effective way to increase the consumption of this highly nutritious alternative to soft drinks. The proposed research attempts to develop a coffee flavored, oat beta glucan fortified milk.

Objectives

This project is aimed at developing a milk based beverage with enhanced nutrient properties of milk and beta glucan. The objectives of the study are to determine the stability, texture and overall acceptance of flavored milk containing beta glucan and to evaluate processing parameters to maximize the stability.

Definition of Terms

Beta glucan: β (1→3), β (1→4), β (1→6) glycoside of glucose found in oats, barley, mushrooms, and yeast.

Casein: Casein is a class of proteins found in milk. They are characterized by their lack of defined native structure, insolubility in the presence of calcium and their ability to form curds and gels.

Dietary fiber: Dietary fiber includes high molecular weight plant carbohydrates that resist digestion and are fermented in lower bowel. It plays an important role in maintaining health.

Functional foods: Functional foods are the foods with a potential positive impact on health. Though all foods can be considered functional, in a way that they have nutrients and other substances that provide energy, sustain growth and support vital processes, on the other hand functional foods generally offer further benefits that may be able to reduce the risk of a disease and in some cases may promote good health.

Hedonic Test: Rating scale used in an acceptance test from dislike extremely to like extremely.

Lifestyle diseases: They can be defined as the disease conditions which usually arise due to sedentary lifestyle and consumption of foods rich in calories. Such diseases include obesity, high blood pressure, high cholesterol and heart attack.

Viscosity: Viscosity is defined as the internal friction of a fluid resistance to flow. It is determined by plotting shear stress and shear rate and calculating the slope of the curve at any given point.

Chapter II: Literature Review

Healthy Dairy Beverage Market

Rapid increase in lifestyle diseases has fueled the current research and development of bioactive compounds and functional foods. The size of the functional foods category in the United States is more than $130 billion. Many companies desire to develop and market functional foods aimed at preventing or reducing nutrition based diseases (National Business Journal Healthy Foods Report, 2010; Sharma, 2005). Today supermarket shelves in the United States and Europe carry a range of functional dairy beverages containing omega 3-fatty acids, plant sterols, prebiotics, probiotics, and bioactive peptides. Many companies are developing novel functional dairy products in spite of the fact that formulation of functional dairy beverages with bioactive compounds can be challenging. Formulating flavored milk with functional dietary fiber is difficult because casein can be unstable in the presence of many hydrocolloids.

Dietary Fiber

Dietary fiber is primarily a class of storage and cell wall polysaccharides that are resistant to human digestive enzymes. A variety of definitions of dietary fiber exist. Some are based on methods of analysis, and others are based on physiological effects (Marlett et al., 2001; National Academy Press, 2001). For labeling purposes in the United States, the dietary fiber content of foods is defined as the material quantified by analytical methods approved by the Association of Official Analytical Chemists (AOAC) (992.28), Prosky (985.29) (National Academy Press, 2001). However, a variety of low-molecular weight carbohydrates (small polymers and oligosaccharides) that are not digested by human digestive enzymes, are not detected by the AOAC-approved methods for measuring dietary fiber. To resolve this discrepancy a new definition was developed by the American Association of Cereal Chemists. It defines dietary fiber as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine". Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber promotes beneficial physiological effects including laxation, blood cholesterol reduction, and blood glucose attenuation (Cereal foods world, 2000). The proposed definition of dietary fiber is intended to emphasize physiological action of fiber and its associated health benefits, and to identify dietary fiber as a constituent of the food (Marlett et al., 2001).

There is mounting evidence supporting the role of dietary fiber, especially viscous soluble fiber, in prevention or cure of many diseases such as cardiovascular disease, diabetes and related diseases (Pins & Kaur, 2006). Soluble fiber, in particular beta glucan found in oat and barley, can lower total and LDL cholesterol levels (Brown et al., 1999; FDA, 2003), improve blood glucose regulation (Kabir et al., 2002) and promote satiety (Marlett et al., 2002; Rigaud et al., 1998). In addition, soluble fiber intake may also prevent the increase in triglycerides and reduction of HDL in the blood that is usually associated with the consumption of easily digested carbohydrate rich diets.

Many health organizations promote the association of dietary fiber consumption with prevention of cardiovascular disease. The American Heart Association (AHA) dietary guidelines recommend a diet including a variety of high-fiber foods for achieving and maintaining cardiovascular health (Krauss et al., 2000). Similarly, National Cholesterol Education Program's (NCEP) ATP III guidelines also recognize the beneficial role of dietary fiber, specifically soluble fiber, in improving blood cholesterol levels by decreasing LDL in the blood, which is the primary target of cholesterol lowering therapy (NCEP, 2002). In addition to cholesterol reduction and cardiovascular health benefits of dietary fiber, many other health benefits such as prevention and management of diabetes mellitus, constipation, diverticulosis and cancer are associated with the consumption of dietary fiber. A diet including 20-30 g of dietary fiber and at least 5-10 g of soluble fiber per day is recommended for an average adult.

The increased connection between health benefits and the consumption of dietary fiber, especially viscous soluble dietary fiber such as beta glucans (Wrusch & Pi-Sunyer, 1997), suggests a need to develop more functional foods containing beta glucan.

Beta Glucan (β-glucan)

Beta Glucan is a polymer of D-glucose linked with glycosidic bonds at β (1→3), β (1→4), β (1→6) and is typically found in the endosperm cell wall in oats, barley. Beta glucan is commercially derived from oats, barley, mushrooms and some microorganisms. In microorganisms and mushrooms, these compounds are found to have a linear chain of D-glucose linked in the β (1 → 3) position with various sized D-glucose branches linked to the main chain by β (1 → 6) linkages (Figure 1). Beta glucans derived from cereals are polymers of D-glucose with β (1 → 3) and β (1→ 4) linkages (Figure 2). Beta glucan constitutes 1% of wheat grains, 3-7% of oats and 5-11% of barley (Skendi et al., 2003).

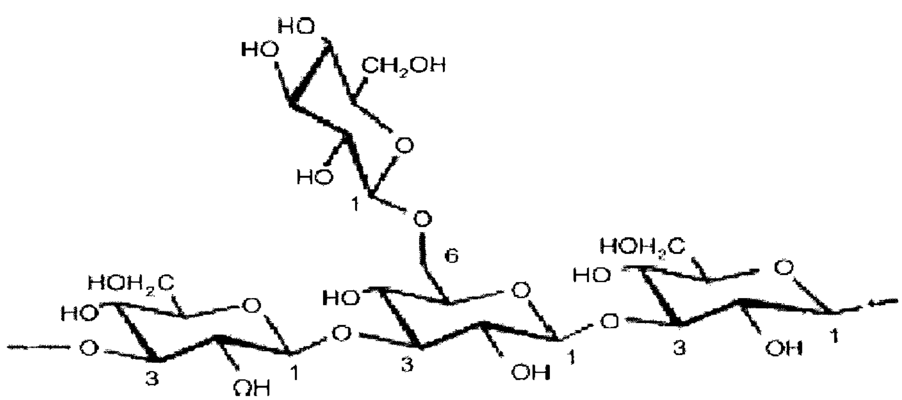

Figure1. Structure of (1 → 3) beta glucans with branched β (1 → 6) present in some microorganisms and mushrooms (Mantovani et al., 2007)

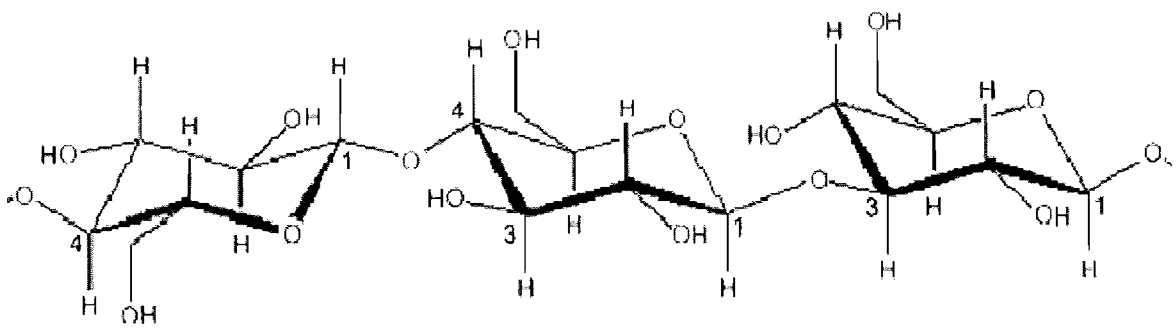

Figure 2. Structure of (1 → 3) beta glucans with branched β (1 → 4) present in oats and barley (Mantovani et al., 2007)

Major Beta glucan Sources

Barley (*Hordeum vulgare*)

Barley, one of the ancient crops in the world, belongs to the genus *hordeum* (Bothmer & Jacobson, 1985). It is used as animal feed, and for malting as brewing substrates for whiskey and beer fermentation and adjuvants for bread flour.

Oat (*Avena sativa*)

Oats belong to the genus *Avena* and are considered a minor cereal crop based on annual production. It is primarily used as an animal feed, but it is gaining popularity as a breakfast cereal in forms like oat meal, ready-to-eat cereal and cereal bars. Oats are a good source of beta glucan and as such a good source of dietary fiber (Weightman et al, 2002, 2004).

Availability of Beta Glucan in Cereal

The amount of the beta glucan in the barley grains affects malting potential and brewing yield by regulating the rate of endosperm modification (Bacic & Stone, 1980, 1981a, b; Bamforth & Martin, 1983; Bourne et al., 1982; Brennan et al., 1998; Edney & Mather, 2004). The amount of beta glucan varies with the types of the barley. Usually, beta glucan accounts for 5%-11% (w/w, on dry basis) in barley.

The beta glucan content in the barley and oats depends on the variety of the plant and the environmental conditions. While environmental conditions can impact the amount of the beta glucan, genetic type determines the ultimate beta glucan content. In general, six-row barley genotypes have less beta glucan than the two-row barley genotypes (Lehtonen & Ailasalo, 1987). Non-waxy cultivars have less beta glucan than waxy cultivars. Beta glucan content appears to correlate with amount of amylopectin in the endosperm (Ulrich et al., 1986; Yoon et al., 1995).

Soil moisture content prior to harvest affects the amount of beta glucan in the barley. Dry conditions during harvesting favor high beta glucan content (Bendelow, 1975), while, wet conditions result in lower beta glucan content (Aman et al., 1989; Stuart et al., 1988).

Characteristics of the 1, 3 and 1, 4 beta D glucan

The source fiber and method of isolation affect the distribution of branched units and length of the side chains of beta glucan. Consequently the tertiary structure and functionality of the molecule is impacted by isolation methods (Mantovani et al., 2008).

In barley, the non-starchy polysaccharide portion of the cell wall is composed of 1, 4 beta glucan(cellulose), 1, 3 and 1, 4 β-D glucan, glucomannan, arabinoxylan and the cytoplasmic fructans. Beta glucan has 30% of 1, 3 glycosidic bonds and 70% of 1, 4 glycosidic bonds (Bacic and Stone, 1981a; Fincher, 1975; Forrest and Wainwright, 1977). The starchy endosperm and aleurone appears to have similar beta glucan but different amounts (Bacic & Stone, 1981a,b; Wood et al., 1983;Woodward et al., 1983, 1988). Molecular weight of oat beta glucan, 0.065–3 X 10^6 g/mol, is higher than the barley beta glucan, 0.15–2.5 X 10^6 g/mol.

Rheological properties of beta glucan depend on degree of polymerization (DP), molecular weight (MW), charge of the polymer and structure in solution (Zekovic et al., 2005). Lazaridou and Biliaderis (2004) reported an increase in storage modulus (G') of beta glucan cryogels with decreasing molecular weight.

Extraction Procedures

Extraction of the beta glucan is difficult. In both barley and oats, beta glucan in combination with other non-starchy polysaccharides compartmentalizes the starch: protein matrix and the grain's lipid reserves. The effects of extracting of beta glucan on its physiochemical and physiological properties have been demonstrated in numerous studies (Bhatty, 1993, 1995; Burkus & Temelli, 1998; Fincher, 1975; Klopfenstein & Hoseney, 1987; Temelli, 1997; Woodward et al., 1983, 1988).

Extraction of beta glucan involves three steps; inactivation of endogenous enzymes, extraction of beta glucan and precipitation of beta glucan. Inactivation of the endogenous beta glucanase is necessary because it hydrolyzes beta glucan and results in reduced functional properties. Beta glucanase inactivation is usually accomplished by adding aqueous ethanol to the barley or treating the barley flour with ethanol and heating to 60°C. Extraction methodology was first reported by Wood et al., (1977). They assessed the effect of particle size, temperature, ionic strength and pH on beta glucan extraction yield in laboratory experiments. Hot, 75% ethanol was used to extract and inactivate endogenous enzymes. Sodium carbonate in solution at pH 10 precipitated 78% of beta glucan (Wood et al., 1989). Mc. Cleary (1988) demonstrated that sequential water extraction at 40, 65 and 95 °C increased the yield of extracted beta glucan to 90%. Addition of thermostable alpha-amylase during hot water extraction resulted in minimized contamination from starch and further optimized the purification of beta glucan (Saulnier et al., 1994). The main limitation of this extraction protocol by food industries is its cost. Therefore, preparation and use of beta glucan was often ignored as a potential functional food ingredient (Brennan & Cleary, 2005).

The nature of extraction protocol has a profound effect on the molecular weight, which in turn influences the functional properties of the extracted beta glucan (Brennan & Cleary, 2005).

The temperature used for the sequential water extraction of beta glucan has an influence on molecular weight, ratio between (1 →4) and (1 → 3) linkages and cellulose amount on the beta glucan chain (Storsley et al., 2003). Hence this subsequently affects viscosity, structural and nutritional effects on foods containing beta glucan as an ingredient. Care must be taken by monitoring the temperature during extraction of beta glucan to optimize the yield, rheological characteristics of the barley solution and to avoid depolymerisation during the extraction of beta glucan (Brennan & Cleary, 2005).

Health Benefits of Beta glucan

Beta glucan provides numerous beneficial health effects such as reduced bowel transit time (Feldheim & Wisker, 2000), prevention of constipation, reduced risk of colorectal cancer (Bingham, 1990; Faivre & Bonithin Kopp, 1999; Hill, 1997), lower blood cholesterol, better regulated blood glucose levels (Bornet et al., 1987; Frost et al., 1999; Gallagher et al., 1993; German et al., 1996), production of short chain fatty acids (Karppinen et al., 2000; Velasquez et al., 2000; Wisker et al., 2000), promotion of the growth of the beneficial bacteria in the colon and improved digestion(Crittenden et al., 2002; Tugland, 2003). Beta glucan in the diet contributes to the intestinal viscosity and thereby reduces cholesterol absorption (Bernardshaw et al., 2006).

Studies on beta glucan have shown that it is a promising substance in promoting many health benefits in humans, and further investigation of developing novel products containing beta glucan is suggested. United States Food and Drug Administration (FDA) permits products containing beta glucan to make health claims related to lower cholesterol and minimized risk of coronary heart diseases (CHD). To meet the daily dietary intake levels, one serving of the product containing 3g of beta glucan is necessary to make the health claims on the product.

Viscoelastic Properties of Beta glucan in Dairy Foods

In addition to the nutritional benefits, beta glucan also has significant viscoelastic properties. The nutritional qualities of beta glucan are related to the viscoelastic properties of the molecule when used in foods. The molecular weight of beta glucan varies with the source; it ranges from 80-2700 x 10^3, 65-3100 x 10^3, 209-416 x 10^3 and 21-1100 x 10^3 for barley, oat, wheat, and rye respectively (Lazardo & Biliaderis, 2007). Therefore, the source of beta glucan is an important factor in determining the rheological properties of beta glucan containing foods. Because the potential benefit of beta glucan in food is related to its rheological properties, mainly its ability to increase viscosity and increase gel forming capacity in the aqueous systems, the addition of beta glucans to dairy systems is an attractive option (Brennan, 2004). Many studies have demonstrated the use of beta glucan in yogurt and low fat ice creams. Brennan and Tudorica (2008), reported beta glucan in skim milk yogurt decreased syneresis when compared to yogurt made with full fat milk. The decrease in syneresis was attributed to beta glucan's ability to form a three-dimensional network entrapping water. In addition, it was found that more than 1% (w/w) beta glucan in low fat yogurt base significantly increased viscosity when compared to full fat yogurt (Brennan & Tudorica, 2008). The sensory studies further showed that addition of beta glucan to yogurt improved mouthfeel, scoopability, and sensory attributes consistent with full fat yogurt (Brennan, 2004).

The use of beta glucan in dairy beverages has not been extensively explored. At neutral pH, milk proteins and beta glucans are thermodynamically unstable leading to phase separation, a concentration dependent response (Vasiljevic et al., 2007). Vasiljevic et al., (2007) showed that yogurt base containing more than 0.3 % (w/w) beta glucan caused a phase separation resulting in protein rich and polysaccharide rich phases. The overall viscoelastic properties of the beta glucan and milk solids matrix are attributed to the thermodynamic incompatibility of proteins and polysaccharides (Tudorica et al., 2004). However, more research is needed to investigate the effects of process parameters and interactions from other ingredients on the rheological behavior of beta glucan in dairy products.

Interaction Between Kappa-Carrageenan and Milk Proteins

Kappa-carrageenan is a negatively charged sulphated polysaccharide made of galactose units that are joined by alternating α (1 $\rightarrow$ 3) and β (1 $\rightarrow$ 4) glycosidic linkages. Kappa-carrageenan from red algae is widely used in the food industry as a thickener, gelling agent and stabilizing agent (Baeza et al., 2002).

Kappa-carrageenan is widely used in dairy products, and much work has been done to understand the interaction between kappa-casein and milk proteins (Baeza et al., 2002). Casein instability resulting from addition of hydrocolloids can be prevented by the formation of weak carrageenan gel, which can suspend casein micelles (Bourriot et al., 1999), even when kappa-carrageenan is below its critical gelling concentration. The interaction of kappa-carrageenan and casein is affected by other factors such as pH, ionic strength and presence of other components.

At the natural pH of milk, kappa-carrageenan can effectively contribute to colloidal stabilization of casein micelles. It is also believed to protect calcium-sensitive casein from precipitation in the presence of calcium ions when other hydrocolloids and other natural gums are ineffective (Lin, 1977). The kappa-carrageenan interaction with milk proteins also inhibits phase separation between milk proteins and other polysaccharides. Spagnuolo et al. (2005) showed that helical aggregation of kappa-carrageenan has an ability to inhibit the phase separation between casein micelles and locust bean gum. Kappa-carrageenan might be a potential stabilizer for polysaccharides like beta glucan in dairy beverages where phase separation is a challenging issue.

Beta glucan Use in Dairy Industry

The use of the beta glucan in health based food supplements is growing, not only because of its health benefits, but also because of its use as a processing aid. In cheese making, beta glucan affects the curd formation by reducing the curd cutting time and increasing curd yield (Tudorica et al., 2004). Beta glucan has been used in the manufacture of low fat yogurt and ice cream to act as a fat mimetic in low fat dairy products by improving the mouth feel (Brennan et al., 2002).

However, beta glucan can have negative effects on flavor and mouth feel in cheese. "Nutrim", a commercial beta glucan reduced the firmness of cheddar cheese resulting in a paste rather than a curd (Volikakis et al., 2004). Addition of beta glucan, negatively affected the flavor and appearance of soft brined cheese (Volikakis et al., 2004).

Beta glucan can impart a thick, gummy texture in many products such as milk and fruit beverages. This project is aimed at developing a milk based beverage fortified with beta glucan. The objectives of the study are to evaluate the stability, texture and overall acceptance of flavored milk containing beta glucan and to examine processing parameters in maximizing stability.

Chapter III: Methodology

This chapter discusses different processing techniques such as one stage processing where k-carrageenan and beta glucan were added simultaneously and two stage processing where k-carrageenan and beta glucan were added separately during processing of oat beta glucan fortified milk beverage. It describes methods to measure viscosity and sensory evaluation.

Sample Selection and Description

Primary studies were conducted to standardize the process of manufacturing the flavored beta glucan enhanced milk. For this experiment, commercially available pasteurized skimmed milk and whole milk samples were obtained from local grocery stores. Oat beta glucan was obtained from the Corn Products International. Kappa-carrageenan was a gift from Danisco Inc. ingredients, and coffee, mocha flavors were donated by Sensient Flavors Inc.

Instrumentation

Stephan Mixer: In this study, a Stephan mixer (Stephan Machinery GmbH, Germany) with jacketed kettle was used in the process of making the flavored beta glucan milk for the purpose of pasteurization of the milk by continuous mixing which helps in the homogenization.

Viscometer: Brookfield a_n HB-DV-III Ultra rotoviscometer (Brookfield Engineering Labs. Inc., Stoughton, MA) was used measure the viscosity of the flavored beta glucan enhanced milk.

Processing Protocol for Beta glucan Added Flavored Milk

Flavored milk was processed using two processing protocols. The first protocol was a two stage heating regime (Figure 3) and the other protocol was a one stage heating regime (Figure 4). Formulas (Table 1) for control and beta glucan samples were identical in both protocols.

Table 1

Formulas for Dairy Based Flavored Beverages With and Without Beta Glucan

Ingredient	Control	0.311% beta glucan*	0.622% beta glucan
Skim milk	-	91.98%	91.98%
Whole milk	92.6%	-	-
k- Carrageenan	0.03%	0.03%	0.03%
Beta glucan	-	0.311%	0.622%
Sucrose	6.67%	6.67%	6.67%
Flavor (Coffee and/ or mocha)	0.5%	0.5%	0.5%
Creamy flavor	0.2%	0.2%	0.2%

* - this sample was made only during the sensory evaluation using protocol one (figure 3), no textural analysis was done on this sample

Two Stage Heating Regime (Separate Addition of Ingredients)

Skim milk in case of test sample or whole milk in case of control samples was mixed with k-carrageenan and pasteurized at 63°C for two minutes to completely hydrate the k-carrageenan and encourage interaction with the casein micelles then cooled to 25°C. Remaining ingredients; sucrose, flavor (coffee&mocha), beta glucan (only in test samples) were added with agitation after the milk reached 25°C. The total mixture was reheated to 73°C for an additional two minutes to ensure the proper hydration of beta glucan. The pasteurized flavored milk was hot filled into polyethylene tetraphthalate (PET) bottles and stored at 4°C for subsequent analysis. A brief flow diagram of two stage heating regime is given in Figure 3.

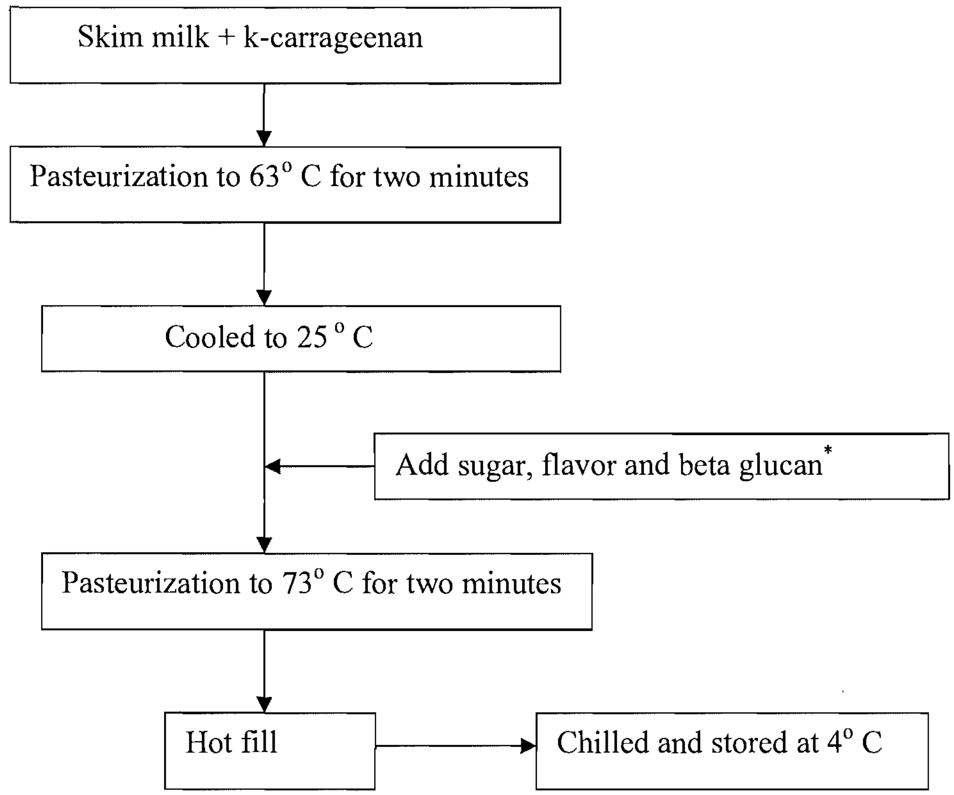

Figure 3. Two stage heating regime method to form a stable casein-carrageenan complex prior to addition of beta glucan.

*added only in test samples

One Stage Heating Regime (Simultaneous Addition of Ingredients)

Skim milk/whole milk samples were mixed with all the ingredients, k-carrageenan, sucrose, flavor (coffee, mocha & creamy), and beta glucan (only in test samples) and heated at 73°C for two minutes to hydrate both k-carrageenan casein and beta glucan. The pasteurized flavored milk was hot filled into PET bottles and stored at 4°C, until further analysis. A brief flow diagram of one stage heating regime is given in the Figure 4.

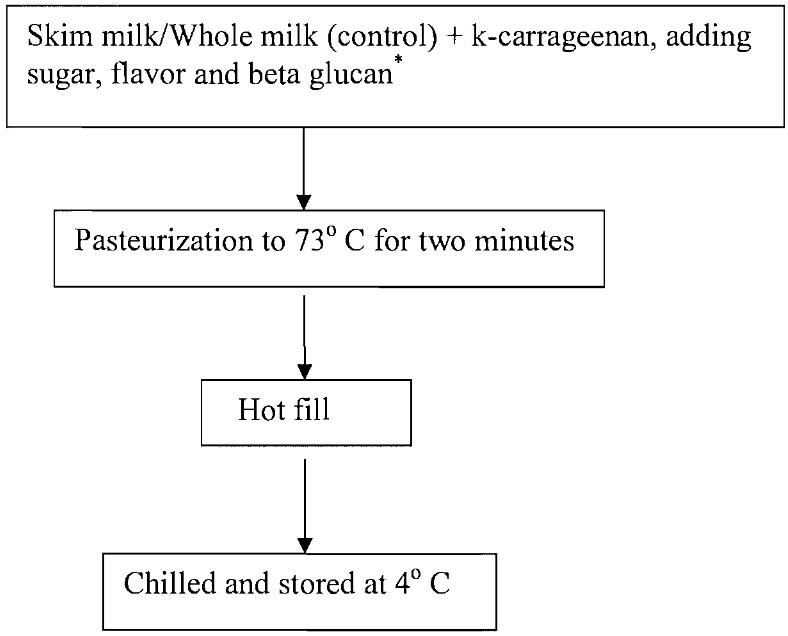

Figure 4. One stage heating regime method to form a stable casein-carrageenan complex.

*added only in test samples

Methods Used for Processing

Initially samples made according to the one stage and two stage heating regimes (figure 3 & figure 4) were statically heated in a hot water bath at 80°C. Later, samples were heated to 73°C for two minutes in a water jacketed kettle with low shear agitation at 300 rpm and kettle temperatures at 73°C.

Sensory Evaluation

Sensory analysis was carried out at University of Wisconsin-Stout after approval of University Institutional Review Board (IRB). The test consisted of three samples; control sample without beta glucan, experiment sample with 0.311% beta glucan and experiment sample with 0.622% beta glucan. Panelists were asked to taste each sample separately. They were asked a series of questions pertaining to color intensity, coffee flavor intensity, sweetness, smoothness of texture and overall acceptability of each sample. A 9-point hedonic scale was used in the sensory evaluation from dislike extremely to like extremely. An example of the score sheet is shown in Figure 5. Panelists were students and faculty of university who were recruited to participate in the sensory evaluation.

Sample Score Sheet

Sample: Flavored Milk Sample Number:312

Please rate the sample according to the following description: Taste the sample and check how much you like or dislike each characteristic. Use appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

Color

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Light Moderate Dark

Flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Light Moderate Strong

Sweetness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Low Moderate High

Mouthfeel

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Grainy Moderate Smooth

Overall Acceptance

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely Neither like or dislike Like extremely

Figure 5. Sensory ballot used to evaluate product attributes of flavored, fortified milk beverages

Data Analysis

Texture Analysis: Brookfield a_n HB-DV-III Ultra rotoviscometer (Brookfield Engineering Labs. Inc., Stoughton, MA) with a jacketed small sample adapter was used to measure viscosity, percent torque, shear rate, shear stress of samples on day one and day six. 9 mL of each sample were added to sample cup and allowed to relax for 60 seconds before measurements were taken. Readings were taken over a range of RPM's from 20 to 250. Duration at each RPM was 25 seconds. The instrument recorded viscosity, percent torque, shear rate and shear stress. Temperature of each sample was 10 ° C. The experiment was carried out in triplicates.

Stability

After making the oat beta glucan fortified milk, two samples were kept in the refrigerator at 3°C. Stability of the both samples was measured by means of physical appearance on days 3, 6, 9, 12, 15, 18 and 21 respectively.

Sensory Analysis

Sensory data was collected using Compusense Five Software (Compusense Inc, 2005) program for color, flavor, sweetness, mouthfeel and overall acceptability of the samples. Data was analyzed using ANOVA and multiple comparison tests, Tukeys HSD and Fisher's LSD to determine the consumer acceptance of all three samples.

Chapter IV: Results and Discussions

The present study aimed to develop a product by incorporating beta glucan into milk making it a healthy beverage. Since beta glucan can destabilize the casein, these experiments attempted to determine the effects of carrageenan and heating cycles on stability of beta glucan fortified milk. Preliminary experiments were conducted by using 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, and 2.0% of beta glucan in the milk to determine the maximum concentration of beta glucan that can be added to milk while not exceeding its gel threshold. It was determined that 0.622% was the maximum amount of beta glucan used in milk without gel formation.

Another sample with 0.311% of beta glucan was made using one stage heating regime for sensory evaluation, but no textural analysis was performed on this sample.

Water-Bath Method

The beta glucan enhanced milk beverage processed in this method resulted in the formation of the coagulated particles of beta glucan. To overcome this issue, water bath method was no longer used and a jacketed vessel with agitator was used in the second method.

Stephan Mixer Method

In this method a flavored, beta glucan milk beverage was made using both heating protocols (Figure 3 & Figure 4) and viscosity was measured using a Brookfield HB-DV-III Ultra rotoviscometer (Brookfield Engineering Labs. Inc., Stoughton, MA) on day 1 and day 6 for both samples. Stability was measured by means of physical appearance for every three days for 21 days for both the samples which were processed using both protocols. The viscosity readings are tabulated in Table 9. The viscosity of beta glucan sample made by two stage heating regime was more than one stage heating regime beta glucan sample. The viscosity readings at 150 rpm data were analyzed by using Analysis of Variance (ANOVA), Microsoft excel. The results showed that the viscosity of control sample was significantly low ($p<0.05$). There was no significant difference ($p>0.05$) in viscosity of oat beta glucan samples on day one or day six. However, the viscosity of oat beta glucan milk sample made using two stage heating regime was significantly ($p<0.05$) higher than one stage heating regime. Milk with 0.622% beta glucan in the milk was more slimy than required but it was observed that it's not a significant fat replacer.

Stability of the both samples was measured by measuring separation or formation of a layer of a syneresis on days 3, 6, 9, 12, 15, 18 and 21 respectively. It was observed that the sample made using the two stage regimen had consistent stability up to 21 days and started forming the layers of syneresis after 21 days. On the other hand, the sample made using single stage regimen separated on day 18 and collapsed completely by day 21. Thus the stability of the oat beta glucan milk can be increased by adding k-carrageenan and beta glucan to milk separately during the manufacturing process. The results showed that two stage heating regimen might increase the stability of oat beta glucan fortified milk, since k-carrageenan was completely hydrated and stabilizes the casein micelles in first step. Kappa-carrageenan was used in the formula to help stabilize casein against precipitation in the presence of beta glucan. Furthermore, two processes were used to determine if a stable interaction between carrageenan and casein could further enhance the stability. Two stage heating process showed that the carrageenan enhanced the stability of the oat beta glucan fortified milk.

Table 2

Viscosity, Percent Torque, Shear Rate, and Shear Stress for Control Sample Without Beta Glucan on Day 1

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	26.9	1.05	6.575	24.5
50	21	2.1	12.8	61.2
100	15.1	2.95	18.5	122
150	11.83	3.55	22.2	183
200	11.05	4.3	26.95	245
250	9.71	4.75	29.75	306

Table 3

Viscosity, Percent Torque, Shear Rate, and Shear Stress for 0.622% Beta Glucan Sample Made Using with Two Stage Heating Regime on Day 1

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	94.75	3.7	23.15	24.5
50	74.8	7.3	45.7	61.2
100	59.65	11.65	72.95	122
150	52.1	15.3	95.8	183
200	47.45	18.55	116.15	245
250	44.15	21.55	134.95	306

Table 4

Viscosity, Percent Torque, Shear Rate, and Shear Stress for 0.622% Beta Glucan Sample Made Using with Single Stage Heating Regime on Day 1

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	69.1	2.7	16.9	24.5
50	55.8	5.45	34.15	61.2
100	45.3	8.85	55.45	122
150	40.3	11.85	74.2	183
200	37.4	14.6	91.4	245
250	34.9	17.05	106.75	306

Table 5

Viscosity, Percent Torque, Shear Rate & Shear Stress of Control Sample Without Beta Glucan on Day 6

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	32	1.9	7.825	24.5
50	23.6	2.3	14.4	61.2
100	16.9	5	20.65	122
150	13.7	6.05	25.05	183
200	12.15	4.75	29.75	245
250	10.4	5.1	31.95	306

Table 6

Viscosity, Percent Torque, Shear Rate, and Shear Stress for 0.622% Beta Glucan Sample Made Using with Two Stage Heating Regime on Day 6

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	84.45	3.3	20.65	24.5
50	66.05	6.45	40.35	61.2
100	54.8	10.7	67	122
150	48.3	14.2	88.95	183
200	44.15	17.25	108	245
250	41.2	20.1	125.9	306

Table 7

Viscosity, Percent Torque, Shear Rate, and Shear Stress for 0.622% Beta Glucan Sample Made Using With Single Stage Heating Regime on Day 6

RPM	Viscosity (Pa)	Torque (%)	Shear stress	Shear rate
20	71.65	2.8	17.55	24.5
50	54.8	5.35	33.5	61.2
100	46.1	9.3	56.4	122
150	40.9	12.05	75.45	183
200	37.75	14.75	92.35	245
250	35.4	17.3	108.3	306

Table 8

Viscosity of the Control Sample, 0.622% Beta Glucan Sample Made Using Single and Two Stage Heating Regimes at Various RPM's on Day 1 and Day 6

RPM	1		2		3	
	Day 1	Day 6	Day 1	Day 6	Day 1	Day 6
20	26.9	32	94.75	84.45	69.1	71.65
50	21	23.6	74.8	66.05	55.8	54.8
100	15.1	16.9	59.65	54.8	45.3	46.1
150	11.83	13.7	52.1	48.3	40.3	40.9
200	11.05	12.15	47.45	44.15	37.4	37.75
250	9.71	10.4	44.15	41.2	34.9	35.4

1-control sample without beta glucan; 2- sample with 0.622% beta glucan made using two stage heating regime; 3- sample with 0.622% beta glucan made using one stage heating regime

Table 9

Viscosity Measured at 150 rpm for Samples With and Without Beta Glucan Using One and Two Stage Heating Regimes on Day One and Day Six

Sample	Mean	
	Day 1	Day6
Control (no beta glucan)	11.83	13.7
Two stage heating regime (0.622% bg*)	48.33	52.1
Single stage heating regime (0.622% bg*)	40.3	40.9

*bg - beta glucan

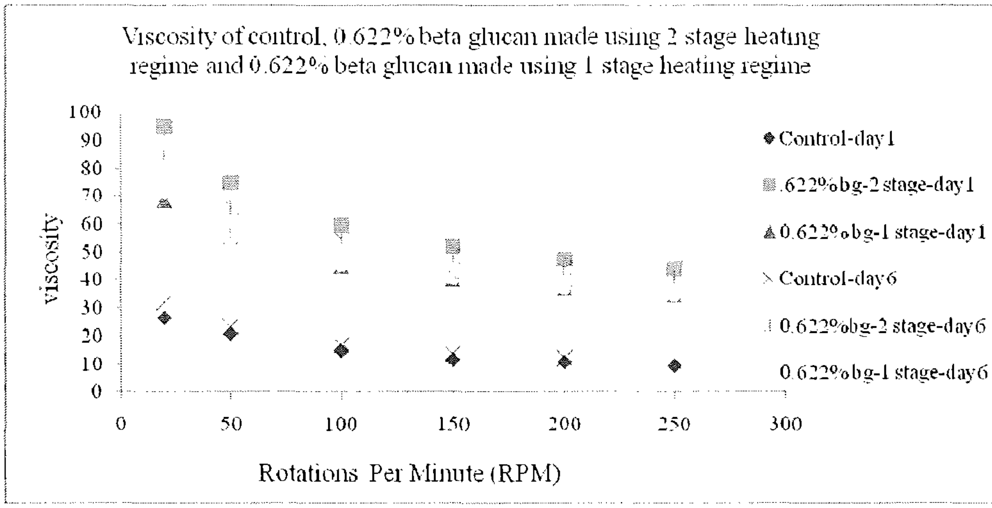

Figure 6. Viscosity of control sample, 0.622% beta glucan sample made using 2 stage heating regime and 0.622% beta glucan sample made using 1 stage heating regime

According to the Figure 6, the viscosity of samples containing beta glucan was higher when processed using the two stage heating regime. The results in Table 6 show that the viscosity of control sample was significantly lower ($p<0.05$). There was no significant difference ($p>0.05$) in viscosity of oat beta glucan samples on day one or day six. However, the viscosity of oat beta glucan milk sample made using two stage heating regime was significantly ($p<0.05$) higher than one stage heating regime. The reason might be that k-carrageenan was completely hydrated and interacting with the casein micelles during the first heating and cooling cycle. As a result the k-carrageenan/casein complex is more stable to beta glucan addition during the second heating and cooling cycle.

Sensory Evaluation

Sensory analysis was conducted to evaluate color, flavor, sweetness, smoothness and preference.

Table 10

Scores of the Tested Attributes for Control, 0.311% Beta Glucan Sample and 0.622% Beta Glucan Sample

Sample	Color	Flavor	Sweetness	Smoothness	O.A	Intent of purchase
Control	3.21[a]	5.14[a]	5.38[a]	7.11[a]	5.25[a]	2.16[a]
0.311% bg	4.78[b]	5.18[a]	5.48[a]	7.07[a]	4.99[a]	2.05[a]
0.622% bg	6.29[c]	4.29[b]	4.58[b]	6.65[a]	3.75[b]	1.61[b]

[a,b, c] represents the significant difference ($p<0.05$); bg-beta glucan; O.A-overall acceptance

There was a significant difference ($p<0.05$) in color between control without beta glucan, 0.311% beta glucan and 0.622% beta glucan samples with a difference of 2 hedonic points. According to the Table 10, as the amount of beta glucan in the sample increased the mean scores suggesting a darker color.

The sample with 0.622% beta glucan had lower flavor scores, lower overall acceptance and reduced intent to purchase compared to control sample and sample with 0.311% beta glucan. There was no significant difference between control sample and the sample with 0.311% of beta glucan for attributes like flavor intensity, sweetness, overall acceptance and intent to purchase.

Though the sucrose content was the same in all samples, the sweetness of the sample with 0.622% beta glucan was significantly lower compared to control sample and the sample with 0.311% beta glucan. Beta glucan at high levels can reduce the sweetness.

Beta glucan did not affect smoothness in milk beverages. Panelists were asked to rate the sample from grainy to smooth and all samples were rated very smooth. However, it was evident that samples with beta glucan had a more slimy mouthfeel. A question asking about mouthfeel preference or a slimy attribute might have been more informative.

The overall acceptance of the control sample with no beta glucan and the sample with 0.311% beta glucan was significantly higher than sample with 0.622% beta glucan with a score difference of 2 hedonic points. In Figure 6 it can be seen that the overall acceptance of the control sample with no beta glucan and sample with 0.311% of beta glucan was similar.

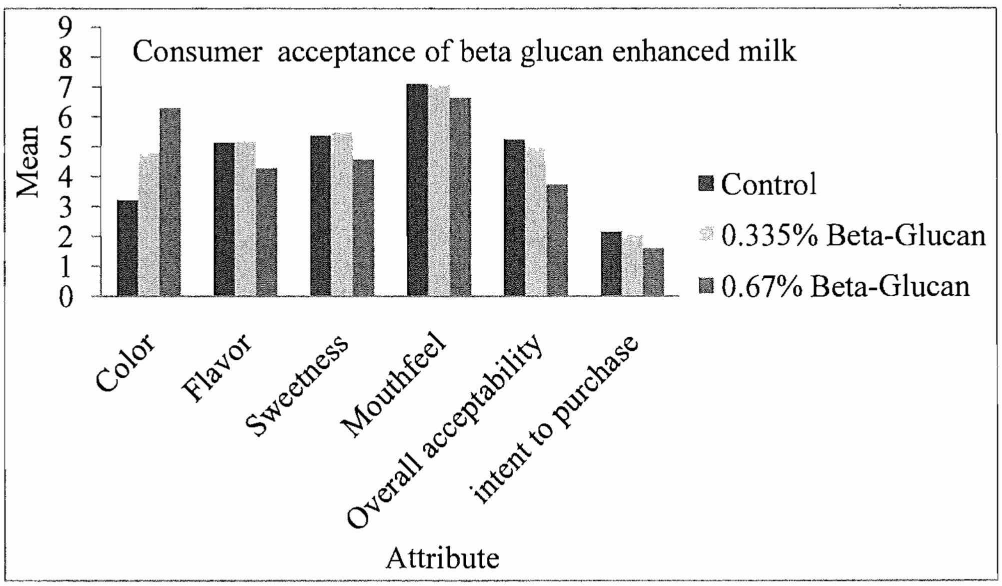

Figure 7: Graph between mean versus attribute explaining about consumer acceptance of beta glucan enhanced milk

Chapter V: Conclusion

Studies have shown that beta glucan has beneficial health effects related to the lower cholesterol and increased dietary fiber. A milk beverage containing beta glucan can be an innovative and healthy product for increasing beta glucan consumption in the United States.

Texture Analysis

Beta glucan fortified milk can be successfully developed using k-carrageenan to stabilize milk proteins. In this study stability of beta glucan fortified flavored milk was improved by using separate addition and hydration of k-carrageenan prior to the addition of beta glucan. This maintained even distribution of the particles and extended stability up to 21 days at 4°C. Eventually the milk began to coagulate as a result of destabilization caused by beta glucan. The viscosity of the control and beta glucan samples did not change overtime. Viscosity at day one was not different than viscosity at day six for each sample regardless of manufacturing protocol meaning that beta glucan did not effecting the viscosity overtime. However, the viscosity of oat beta glucan milk sample made using separate addition of k-carrageenan and beta glucan was significantly ($p<0.05$) higher than adding k-carrageenan and beta glucan simultaneously. The reason might be that k-carrageenan interacts with casein to achieve a higher viscosity prior to the addition of beta glucan particles. Thus it can be concluded that separate addition and hydration of k-carrageenan and beta glucan and interaction with casein has a significant effect on stability of the oat beta glucan fortified milk.

Sensory Evaluation

Sensory evaluation carried out to evaluate the consumer acceptance of the beta glucan enhanced flavored milk showed that beta glucan makes milk beverages darker. At low levels, beta glucan did not significantly affect flavor intensity, sweetness, overall acceptance and intent to purchase. There was a significant difference ($p<0.05$) between the sample with 0.622% beta glucan and other two samples in these attributes. Addition of beta glucan at levels of 0.622% significantly lowered sweetness perception even though all samples contain equal amounts of sugar. However, it was observed that as the amount of beta glucan increases the sliminess increases. The overall acceptance of the control sample and sample with 0.311% beta glucan was significantly higher than the sample with 0.622% beta glucan with a score difference of 2 hedonic points. Therefore, it can be concluded that an acceptable milk product containing beta glucan can be produced with lower amounts of beta glucan. However 0.311% beta glucan is half of the amount recommended by FDA to make the health claims.

Heating with low shear agitation and separate addition of k-carrageenan and beta glucan in two different steps resulted in increased beverage stability when compared to simultaneous addition of k-carrageenan and beta glucan. The water-bath method was insufficient because beta glucan formed lumps and aggregated particles because of improper mixing.

Beta glucan fortified milk can be developed successfully using carrageenan as an aid to stability of milk proteins. The beta glucan fortified, flavored milk made by using separate addition of k-carrageenan and beta glucan maintained even distribution of the particles and retained stability up to 21 days at $4^{o}C$.

Recommendations

The following recommendations were made for the future research in order to introduce the product into the market.

1. In this study oat beta glucan has been used in the product development, in future it is recommended to develop a product with barley beta glucan.
2. Oat beta glucan powder was used in the current study. Using hydrolyzed oat beta glucan may help in developing the stability and enhance the viscosity of the oat beta glucan fortified milk.

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Appendix: A - Sensory Evaluation Advertisement

Dept. of Food and Nutritional Sciences

UW-STOUT

Sensory Evaluation of Coffee Flavored Milk

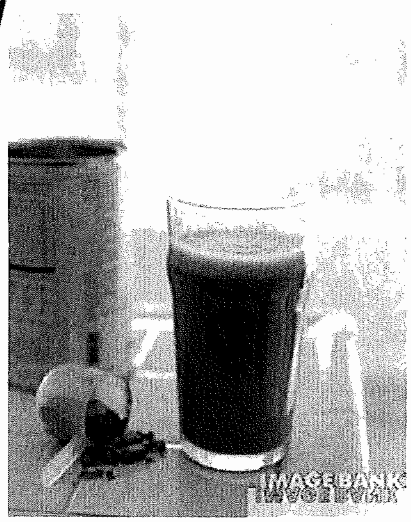
What : Flavored Milk

When : December 7, 2010,
Tuesday,
10:00 am -3:00 pm

Where : Heritage Hall, Rm# 252

Why : Graduate Research

Who : Srikanth Bangari



Get a Free Treat

Contact Information

Srikanth Bangari
E-mail: bangaris@my.uwstout.edu

Appendix: B – Sample Score sheet for sensory evaluation

Sample Score Sheet

Sample: Flavored Milk Sample Number:312

Please rate the sample according to the following description: Taste the sample and check how much you like or dislike each characteristic. Use appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

Color

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Light Moderate Dark

Flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Light Moderate Strong

Sweetness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Low Moderate High

Mouthfeel

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Grainy Moderate Smooth

Overall Acceptance

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely Neither like or dislike Like extremely