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Viability of bifidobacteria in yogurts containing oat beta-glucan and/or corn starch during cold storage

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Viability of bifidobacteria in yogurts containing oat beta-glucan and/or corn starch during cold storage

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INTRODUCTION

GENERAL INTRODUCTION

Beta-glucan, a soluble dietary fiber found in high concentration in oats and a known prebiotic, has many desirable physical and physiological characteristics. Beta-glucan has been shown to relieve constipation, reduce the risk of colorectal cancer, attenuate post prandial blood sugar, assist in the production of short chain fatty acids and promote the growth of beneficial gut microflora. However the most widely accepted benefit of beta-glucan is its ability to reduce serum cholesterol. Because of this significant physiological benefit, in 1997 the FDA approved a health claim for beta-glucan stating that when consumed in sufficient amounts, beta-glucan can reduce the risk of heart disease. Physically, beta-glucan is recognized to improve the texture and mouthfeel of low-fat dairy foods by acting as a fat mimetic. Therefore, beta-glucan is of great importance in both the medical field and food industry.

Bifidobacteria, beneficial bacteria with well known probiotic activity, have many known health benefits. These include prevention of diarrhea, positive immunomodulation, improved gut transit time, colon cancer prevention, and increased mineral absorption. In order to reap the benefits of bifidobacteria, they must be consumed at a level of 10^7 viable cells per ml or g of food. However, bifidobacteria often drop below this therapeutic level during refrigerated storage due to a host of factors that include pH, oxygen content, and osmotic pressure. In order to alleviate these stressors many techniques have been tested, including the addition of a prebiotic to the food.

Little is known about the effects of prebiotics, such as oat beta-glucan, on probiotic cultures survival outside of the gut, specifically during refrigerated storage before consumption. Cold storage is a critical time during the lifespan of a yogurt product as bacterial strains can be very cold liable. It is important to understand how cold effects probiotic strains in order to prevent significant probiotic decrease below a therapeutic level before a consumer purchases and consumes a yogurt product.

The objectives of this study were to evaluate the effect of two oat beta-glucan preparations, a modified corn starch and the combination of beta-glucan and corn starch on the survival of two bifidobacteria strains, as well as yogurt culture strains, during five weeks of storage at 4 °C. To achieve this objective, yogurts were created containing yogurt cultures, a beta-glucan or starch treatment, and one strain of bifidobacteria. The yogurts were fermented and stored at 4 °C for five weeks with aliquots taken weekly for bacterial enumeration.

THESIS ORGANIZATION

This thesis is organized in three chapters. The first chapter contains a general introduction of the subject material as well as a review of the peer-reviewed literature concerning the topic. The second chapter is a manuscript to be submitted to the Journal of Food Science, and contains a complete report of the research completed. The third chapter contains a general conclusion. Finally, there are two appendices located at the end of the thesis that give a brief overview of preliminary experiments designed to help understand the mechanisms for the observations noted in the manuscript. Although these findings were

inconclusive, they contain relevant information as it pertains to the primary study. After each chapter there is a list of references cited within that chapter.

LITERATURE REVIEW

BETA-GLUCAN

Beta-glucan is one of the major components of the starchy endosperm and aleurone cell walls of many commercially important cereal grains, such as oats, barley, rye and wheat (Lazaridou and Biliaderis 2007). Beta-glucan is composed of polymers of glucose connected by approximately 70% β-(1-4) and 30% of β-(1-3) linkages. The interruption of β-(1-4) linkages with β-(1-3) linkages makes beta-glucan much more flexible, soluble, and viscous than cellulose (Liu 2007). Beta-glucan content can range from 1% in wheat, 3-7% in oats and 5-11% in barley (Skendi and others 2003). Although the structure of beta-glucan is very similar between different genera of cereals, there is growing evidence that oat, barley and wheat beta-glucans are structurally distinct as determined by HPLC analysis of lichenase-released oligosaccharides (Lazaridou and Biliaderis 2007). This difference seems to lie within the relative amount of trisaccharide (cellotriosyl (DP3) and tetrasaccharide (cellotetraosyl (DP4)) produced by the lichenase action. Wheat has the greatest percentage of DP3 and lowest percentage of DP4, whereas oat has the lowest percentage of DP3 and the highest percentage of DP4. Barley distribution lies between those of wheat and oat (Lazaridou and Biliaderis 2007).

HEALTH BENEFITS

Cholesterol effects

Although there seem to be minor differences in the structure of beta-glucan originating from different species, the benefits associated with the consumption of beta-glucan are universal. Beta-glucan is classified as a soluble dietary fiber. According to the American Association of Cereal Chemists, dietary fiber 'is the edible parts of plants or analogous carbohydrates that are resistant to digestion and adsorption 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 fibers promote beneficial physiological effects, including laxation, and /or blood cholesterol attenuation, and/or blood glucose attenuation' (DeVries 2001). In addition, beta-glucan, as a component of dietary fiber, provides additional health benefits, including relief from constipation, reduction of risk of colorectal cancer, production of short-chain fatty acids, and promotes the growth of beneficial gut microflora (Bingham 1990; Karppinen and others 2000; Crittenden and others 2002). The most widely accepted benefit of the consumption of beta-glucan is the lowering of serum cholesterol. Results from studies using oat beta-glucan have shown an average reduction by 10% for total cholesterol and 8% for LDL cholesterol accompanied by a 16% elevation in HDL cholesterol after 4 weeks of consumption of a 1% (w/w) beta-glucan extract (Liu 2007; Behall and others 1997; Bell and others 1999).

There are many proposed mechanisms to account for how consumption of beta-glucan is able to lower cholesterol. The first, and most widely accepted, mechanism states that soluble fiber provides a very viscous environment in the intestinal lumen that is able to bind to bile acids in the intestine resulting in a total decrease in the available bile-acid pool that circulates back to the liver. The reduction in available bile stimulates the production of more bile acids which are derived from cholesterol either endogenously produced or adsorbed from circulation after a meal (Bell and others 1999). This binding action may also facilitate cholesterol elimination from the body (Liu 2007). In addition, the increased viscosity caused by beta-glucan may affect lipid emulsification by increasing the emulsion droplet size which may impair fat adsorption thus decreasing cholesterol adsorption (Pasquier and others 1996).   A second theory accounts for interaction of beta-glucan with gut microflora. As soluble fibers are fermented in the large intestine by colonic bacteria, short-chain fatty acids are produced. Short-chain fatty acids are taken up by the portal vein which inhibits hepatic cholesterol synthesis by way of limiting the action of HMG-CoA reductase or increasing the catabolism of LDL (Bell and others 1999). However this effect has only been confirmed for one of the short-chain fatty acids, propionate (Bell and others 1999). A third mechanism may involve the ability of beta-glucans to delay gastric emptying which reduces post-prandial insulin levels, in turn reducing hepatic cholesterol synthesis though mediation of HMG-CoA reductase (Bell and others, 1999).

In 1995, the Quaker Oats Company petitioned the FDA for permission to make a health claim that oat products may reduce the risk of heart disease. This petition was granted in January of 1997. The FDA claim states that "soluble fiber from oatmeal, as part of a low

saturated fat, low-cholesterol diet, may reduce the risk of heart disease" (FDA 1997). The FDA stated as part of the health claim that 3 g of beta-glucan per day lowers blood cholesterol. More specifically, the claim only relates to oat products that contain at least 0.75 g of beta-glucan per serving.

Blood sugar

Another well studied health benefit associated with the consumption of beta-glucan is its ability to attenuate post-prandial hyperglycemia and insulin response. In one study, frequent meals with or without beta-glucan resulted in similar carbohydrate metabolism, but ingestion of a single meal containing beta-glucan resulted in lowered post-prandial glucose concentrations (Battilana and others 2001) . These results suggest that beta-glucan may delay and reduce carbohydrate adsorption from the gut. It is also well known that the increased viscosity of the intestinal environment associated with beta-glucan consumption reduces digestion of carbohydrates by alpha-amylase. The addition of 5% of a beta-glucan-rich fraction in bread significantly reduced the release rate of reducing sugars after an *in vitro* digestion with pepsin and alpha-amylase (Symons and Brennan 2004). Beta-glucans may reduce the accessibility of starch degradation enzymes to their substrates by forming a gel matrix or by eliminating the water available for starch hydration and gelatinization (Lazaridou and Biliaderis 2007).

FUNCTIONAL FOODS

Because of the health claims associated with beta-glucan consumption, foods with incorporated beta-glucan are in demand as functional foods. Beta-glucans, as mentioned earlier, have unique rheological characteristics, such as the ability to gel and to increase the viscosity of aqueous solutions, which contributes to their health benefits. These same unique rheological characteristics can be used to benefit food products as well. When developing formulations that include beta-glucan it is important to consider not only the benefits but the problems that can arise with adding a sufficient amount to the food product to produce the desired physiological benefits without altering the food texture to a point where it would become unpalatable.

Commercial beta-glucan preparations

As a result of the health benefits of beta-glucan, there have been quite a few efforts to extract them on large scale for commercial use. There are a series of products with the suffix 'trim' which is an acronym for 'technical research involving metabolism' that contain varying amounts of beta-glucan invented by Dr. George Inglett at the USDA ARS Biopolymer Research Unit in Peoria, IL, USA (Harris and Smith 2006). One of these products, Oatrim, contains 5-10% beta-glucan and is made from oat bran or oat flour treated with thermostable alpha-amylase (Inglett 1993). Oatrim also contains small amounts of amylodextrins, lipid, protein and minerals (Inglett 1993). The most recent 'trim' product is C-trim which is prepared by steam-jet cooking and fractionating oat bran. Its beta-glucan concentration ranges from 15-30% (Lee and others 2005). Beta-glucan contained in the

'trim' preparations lower blood cholesterol and glucose concentrations, however Glucogel does not seem to provide these health benefits which may be due to the partial hydrolysis of the beta-glucans during extraction (Harris and Smith 2006). Recently, a very successful method has been developed for the extraction of beta-glucan from oat flour and the addition of this water soluble fiber to dairy products (Yao and White 2008).

Addition of Beta-glucan to Foods

Because of their ability to increase viscosity of aqueous solutions, beta-glucans can be used as thickening agents or as fat mimetics in the formulation of reduced calorie foods. They can control food texture and have been used to replace all or part of the fats in dairy, meat, and bakery products (Harris and Smith 2006). C-trim is used in the preparation of a low-fat cheddar cheese, and Glucogel can create edible films to control water transfer and improve shelf-life (Harris and Smith 2006; Morgan and Ofman 1998).

One of the largest areas for potential use of beta-glucan is in the dairy industry. Incorporation of beta-glucan with other soluble fiber into low-fat dairy products, such as ice-cream and yogurts, can improve their mouthfeel, scoopability and other sensory properties to more closely resemble full-fat products (Brennan and Cleary 2005). In addition, when beta-glucan is added to milk, the curd cutting time is reduced and curd yields are increased as a result of its ability to form a structured and elastic casein-protein-glucan matrix (Tudorica and others 2004). For example, an oat beta-glucan added to a low-fat white brined cheese at 0.7 and 1.4%, reduced the hardness of the cheese and lead to a product that more closely resembled the full-fat control (Volikakis and others 2004).

Because of the ability of beta-glucan to attenuate the glycemic response, there is much interest in incorporating beta-glucan into cereal-based products, such as pasta and breads. Recently, incorporation of high MW beta-glucans at 0.6% w/w improved the bread-making quality, specifically loaf volume, of a poor bread-making wheat cultivar that exceeded the quality of a good bread-making wheat cultivar (Lazaridou and Biliaderis 2007). The addition of beta-glucan of up to 5% into baked goods seems to show no affect on the overall acceptability of the products (Lazaridou and Biliaderis 2007). Furthermore, up to a 20% addition of barley beta-glucan to semolina or wheat flours produced pasta and noodles with acceptable sensory and cooking qualities; they did, however, have reduced brightness and yellowness and increased redness and 'speckiness' compared to the control (Hatcher and others 2005).

The addition of beta-glucan preparations also improved the quality of egg-yolk-stabilized emulsions, reduced-fat breakfast sausages, and lactose-free, non-dairy milk substitutes such as yogurt, ice-cream, oat-based cream, whipped cream and buttermilk (Lazaridou and Biliaderis 2007). In reduced-fat breakfast sausages, the addition of 0.3 % beta-glucan improved water binding without significant effects on product texture or flavor (Morin and others 2002). Additionally, the non-dairy milk substitutes listed previously benefit from the addition of beta-glucan as stabilizers and texturizers (Lazaridou and Biliaderis 2007)

INULIN

Inulin, a dietary fiber, is composed of a mixture of fructose chains that vary in length (from 2-60 fructose units) and have a terminal glucose residue (Niness 1999). Inulin is typically isolated in high amounts from chicory root, but also is found in artichoke, leek, onion, asparagus, wheat, barley, rye, garlic and bananas (Liu 2007). Inulin is the primary source of added dietary fiber in food products. It is often added to foods because of its sweet taste and desirable texture. Inulin is best noted for acting as a prebiotic. When ingested at a level of 15 g per day for 15 consecutive days, inulin was shown to increase the population of bifidobacteria, a probiotic, by 10% with a concomitant decrease in pathogenic bacteria (Liu 2007). Although similar to beta-glucan, in that it is a dietary fiber, inulin has not been recognized with an FDA health claim.

BIFIDOBACTERIA

Bifidobacteria are gram-positive, anaerobic bacteria that grow at temperatures between 37-41°C and at a pH between 6.5-7.0 (Sneath and others 1986). They are non-motile, nonspore-forming rods that show branching, bends, and protuberances, but can vary widely in morphology (Sneath and others 1986). Bifidobacteria naturally occur in the gut of both humans and animals and were first isolated from the feces of breastfed infants, in which it predominates, by Tissier in 1899 (Shah and Lankaputhra 2002). It is thought that breast milk provides the optimum combination of oligosaccharides that allow for bifidobacteria to flourish (Veereman 2007).

Although bifidobacteria predominate in the guts of infants, with age, they are soon joined by a variety of other bacteria such as *E. coli*, lactobacilli and clostridia that eventually outnumber bifidobacteria (Candy and others 2008). Gut colonization by bifidobacteria has been shown to fall in adults by 1000-fold after age 60 (van Tongeren and others 2005). A drop in bifidobacteria in the elderly is accompanied by an increase in less desirable Enterobacteriaceae, and this change in gut microbiota is associated with increased frailty (van Tongeren and others 2005). The shift in gut ecology with age draws attention to the role of bifidobacteria as a part of intestinal flora and its role in maintaining overall health and well-being.

Bifidobacteria are commonly called probiotics. Probiotics are defined as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host" (Schrezenmeir and de Vrese 2001). Many strains of bifidobacteria have been investigated for possible health benefits. Some of their better documented health benefits include decreasing frequency and duration of antibiotic associated diarrhea as well as positive immunomodulatory activity (Schrezenmeir and de Vrese 2001).

HEALTH BENEFITS

Prevention and Alleviation of Diarrhea

When the ecology of the gut is disrupted, as is the case with antibiotic treatment, the affect is often acute diarrhea. It is estimated that as many as 39% of patients who receive antibiotics will suffer from antibiotic-associated diarrhea (Surawicz 2003). This side affect occurs as a result of the death of beneficial bacteria, which are reduced in number by antibiotic treatment, leaving opportunity for the growth of strains such as *Clostridium*. Toxin production by *Clostridium difficile* is a serious and often common cause of antibiotic-associated diarrhea (Surawicz 2003). The probiotic actions of bifidobacteria continue to be investigated as a remedy for antibiotic associated diarrhea. Several studies have shown a reduction in both frequency and duration of antibiotic-associated diarrhea by *Bifidobacterium longum* (Colombel and others 1987; Orrhage and others 1994). Additionally, *Bifidobacterium bifidum* has been found to participate in toxin neutralization, which consequently leads to a reduction in the incidence of antibiotic-associated diarrhea (Plummer and others 2004).

Another common cause of diarrhea is exposure to a variety of pathogenic bacteria while traveling. Some of the more common strains involved in traveler's diarrhea include *Escherichia coli, Salmonella, Campylobacter* and *Shigella* strains (Ericsson 2003). In a study done with Egyptian tourists, *Bifidobacterium bifidum*, in combination with *S. thermophilus, L. bulgarius and L. acidophilus*, was shown to reduce the frequency of traveler's diarrhea from 71 to 43% (Black and others 1989). Rotavirus is a major cause of

diarrhea in infants and young children. When children were given *B. bifidum* in conjunction with *S. thermophilus* in formula, incidence of rotaviral infection was reduced (Saavedra and others 1994). In addition, *B. breve* was shown to inhibit the infectivity of rotavirus (Bae and others 2002). Finally, *B. lactis* strain Bb 12 when added to acidified formula, has been shown to have a protective effect against acute diarrhea in healthy children (Chouraqui and others 2004). All of these findings show potential for the use of bifidobacteria for the treatment and/or prevention of a variety of illnesses with associated diarrhea.

Immunomoduation

The gastrointestinal tract serves its main functions in absorption and digestion, however it is also the body's largest organ of host defense against antigens and the microflora of the gut are an essential part of this protection (Servin 2004). The addition of bifidobacteria can then either directly or indirectly affect the function of the gut barrier. Although the investigation of bifidobacteria's role in host protection is in its infancy, there are many proposed mechanisms as to how bifidobacteria are able to impart positive immunomodulatory effects. One commonly proposed mechanism states that colonization of bifidobacteria in the gut out-competes colonization by viruses or bacteria and is known as the barrier effect. Other mechanisms include production of inhibitory substances, blockade of adhesion sites, and stimulation of immunity. A variety of bifidobacteria stains have been found to produce and secrete inhibitory substances, including a protein factor produced by *Bifidobacterium longum* with a molecular weight of 100,000 that inhibited the adhesion of an entrotoxigenic E. coli strain to the intestinal mucosa (Fujiwara and others 1997). Similarly, bifidobacteria strains have been shown to produce antimicrobial compounds that inhibit the

growth of pathogens (Lievin and others 2000). Bifidobacteria may also provide improved gut barrier function by blocking epithelial adhesion sites from pathogenic viruses or bacteria (Picard and others 2005). Finally, bifidobacteria strains have been shown to stimulate immunity. Immune stimulation by bifidobacteria is accomplished by stabilizing the intestinal mucosa and normalizing intestinal permeability which decreases the overgrowth of pathogens (Picard and others 2005). Examples of immune stimulation include the ability of *B. longum* to increase the defensive functions of germ-free mice and *B. breve* to enhance the antigen specific IgA-antibody towards rotavirus in mice (Yamazaki and others 1985; Yasui and others 1995).

Improved Transit Time

There is evidence that bifidobacteria are able to prevent and/or relieve constipation by increasing gut transit time. The ability of bifidobacteria to speed gut transit time has been shown in a number of human studies. One such study found that the ingestion of 125 g/day of bifidobacterium-fermented milk reduced their oro-fecal transit time by 20-42% on average with the effect lasting from 2-4 weeks after cessation of the bifidobacterium-fermented milk ingestion (Bouvier and others 2001). The most common theory is that bifidobacteria, which are able to produce short-chain fatty acids in the gut, lower the pH of the intestinal lumen which, in turn, stimulates intestinal peristalsis, thus decreasing transit time (Candy and others 2008). A second theory is that bifidobacteria are able to improve transit time by increasing the fecal bacterial mass and bacterial metabolism of bile acids in the colon (Candy and others 2008). Finally, a third theory links improved transit time to the

stimulation of cholecystokinin which increases the stimulatory response of the smooth muscle of the gut (Candy and others 2008).

Cancer prevention

Colorectal cancer is the third most common cancer in men and women within the United States (U.S.C.S. 2007). Furthermore, a great percentage of human tumors are reported to be related to dietary habits (Zubillaga and others 2001). Certain strains of both lactic acid-producing bacteria as well as bifidobacteria that are used to ferment milk may be very promising antimutagenic and anticarcinogenic candidates (Zubillaga and others 2001). In a study done to investigate the relationship between ingestion of viable bifidobacteria and the incidence of aberrant crypts, which are precursor lesions of colon cancer, a diet including milk fermented by bifidobacteria reduced the incidence of aberrant crypts by 49% when compared to the control diet (Abdelali and others 1995). Thus, evidence is mounting that intestinal flora can impact carcinogenesis. Carcinogenesis as it relates to intestinal flora is hypothesized to be a result of the production of enzymes, such as β-glucuronidase, azoreductase and nitroreductase, by the bacteria that transform procarcinogens to active carcinogens (Picard and others 2005). There is some evidence that bifidobacteria may protect the host from carcinogens by reducing the production of carcinogens by way of reducing specific enzyme activity. In two similar studies, consumption of milk fermented with bifidobacteria lead to decreased β –glucuronidase and nitroreductase activity in humans (Marteau and others 1990; Bouhnik and others 1996).

Mineral Absorption and bone density

Recently, the consumption of bifidobacteria has been linked to improved intestinal mineral absorption and increased bone density. In a recent study, the oral application of *Bifidobacterium longum* was correlated with increased bone breaking strength in an osteoporosis rat model (Igarashi and others 1994). It is believed that increased bone breaking strength as it relates to bifidobacteria is the result of improved mineral adsorption and bone density. There are several factors possibly contributing to increased bone density, including increased mineral solubility as a result of increased bacterial production of short-chain fatty acids, enlargement of the adsorption surface by the proliferation of enterocytes which is mediated by the bacterial fermentation products lactate and butyrate, and increased expression of calcium binding proteins (Scholz-Ahrens and others 2007). Improved bone density may be a result of production, by probiotics, of vitamins such as C, D, K and folate involved in calcium metabolism (Scholz-Ahrens and others 2007).

VIABILITY OF BIFIDOBACTERIA IN FOODS

Yogurt, a fermented dairy food produced by the action of cultures of lactic acid bacteria, is produced and consumed worldwide and has been targeted as an ideal carrier for probiotic microorganisms, such as bifidobacteria, due to its ability to reach many consumers (Lourens-Hattingh and Viljoen 2001). It has been widely suggested, to achieve therapeutic benefits of bifidobacteria and other probiotic organisms, that consumption of the probiotic must be at least 10^7 viable cells per ml or g of product (Lourens-Hattingh and Viljoen 2001). Although bifidobacteria are easily added to dairy products such as yogurt their survival,

particularly during refrigerated storage of the food, is often poor. Many factors have been claimed to affect the survival of bifidobacteria in fermented milk products. These factors include pH, hydrogen peroxide level, storage temperature, oxygen content, lactic and acetic acid concentrations, sugar concentration (osmotic pressure), milk solids content, buffering capacity, β-galactosidase concentration, growth and inhibition factors, amount of inoculums, fermentation time, probiotic strain selection, and presence of other microorganisms (Dave and Shah 1997; Kailasapathy and others 2008; Donkor and others 2007). In order to overcome these factors various techniques have been employed including strategic culture selection, microencapsulation, and the addition of prebiotics (Lourens-Hattingh and Viljoen 2001; Bruno and others 2002; Capela and others 2006). The most commonly studied prebiotic, inulin, has been shown to improve the survival of a mixed culture of probiotics in yogurt during storage at 4 °C (Capela and others 2006). Beta-glucan, another well known prebiotic, has also been postulated to improve probiotic survival in yogurt. In a recent study, researchers found that addition of 0.5 % inulin, oat beta-glucan or barley beta-glucan to yogurt resulted in prolonged survival of *B. lactis* (Vasiljevic and others 2007). However, what was most interesting was that in this study oat beta-glucan out preformed both barley beta-glucan and inulin. Although well understood for the human digestive system, very little is known about the mechanism for a prebiotics effect on probiotics during refrigerated storage in a food system. It remains unclear as it if probiotics are able to metabolize the prebiotic during storage or if a prebiotic can modulate its surroundings such that it creates a more suitable environment for the probiotic.

Given the current understanding and great importance of prebiotics and probiotics, research was conducted to determine the affect of varying preparations of oat beta-glucan at the FDAs minimum therapeutic concentration on two probiotc bifidobacteria strains during storage at 4 °C. The effect of inclusion of a modified corn starch alone and in combination with beta-glucan on probiotic survival was also investigated.

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Viability of bifidobacteria strains in yogurt with added beta-glucan and corn starch during cold storage

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ABSTRACT: Probiotics must be consumed at a level of 10^7CFU/mL for successful

colonization of the gut. In yogurts containing beneficial cultures, the survival of probiotic

strains can quickly decline below this critical concentration during cold storage. The

inclusion in yogurt of beta-glucan, a possible prebiotic for bifidobacteria also known to have

heart-healthy effects, would increase the healthfulness of yogurt. We hypothesized that beta-

glucan would increase the viability of bifidobacteria strains in yogurt during cold storage.

Yogurts were produced containing 0.44% beta-glucan (concentrated or freeze-dried)

extracted from whole oat flour and/or 1.33% corn starch, and bifidobacteria (*B. breve* or *B.

longum*) at a concentration of at least 10^9 CFU/mL. All yogurts were stored at 4°C.

Bifidobacteria and yogurt cultures, *S. thermophilus and L. bulgaricus*, were enumerated from

undisturbed aliquots before fermentation, after fermentation, and once a week for five weeks.

S. thermophilus and L. bulgaricus maintained a concentration of at least 10^8 CFU/mL in

yogurts containing concentrated or freeze-dried beta-glucan regardless of starch addition, and in the control with no added beta-glucan or starch. Similarly, the probiotic, *B. breve,* survived above a therapeutic level in all treatments. The addition of beta-glucan prolonged the survival of *B. longum* at a concentration of at least 10^7 CFU/mL by up to two weeks on average beyond the control. Further, the inclusion of concentrated beta-glucan in yogurt improved survival of *B. longum* above 10^7 CFU/mL by one week longer than did freeze-dried beta-glucan.   Study results suggest that beta-glucan has a protective effect on bifidobacteria in yogurt when stressed by low-temperature storage. The combined benefits of the heart-healthy effects from beta-glucan and of the gut-health effects from bifidobacteria should provide the food industry with information needed to formulate more healthful yogurt products.

INTRODUCTION

Probiotics are defined as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host" (Schrezenmeir and de Vrese 2001). Bifidobacteria, known to act as probiotics, naturally occur in the gut of humans, and predominate in the guts of infants; however, as infants age their gut flora are soon joined by a variety of other bacteria such as *E. coli,* lactobacilli and clostridia that eventually outnumber bifidobacteria (Candy and others 2008). Gut colonization by bifidobacteria falls in adults by 1000-fold after age 60 (van Tongeren and others 2005). This drop in bifidobacteria is associated with an increase in less desirable enterobacteriaceae. Many strains of bifidobacteria have been investigated for possible health

benefits, with some of the better documented health benefits including decreased frequency and duration of antibiotic-associated diarrhea, as well as positive immunomodulatory activity. Also, colonization in the human gut of the bifidobacteria is postulated to increase mineral absorption, prevent hypercholesterolemia, enhance immunity and provide anticarcinogenic activity (Fuller 1989; Schrezenmeir and de Vrese 2001). Indeed, the role of bifidobacteria as a part of intestinal flora in maintaining overall health and well-being is not fully understood; however, it is recognized that probiotic cultures must be consumed at a level of at least 10^7 CFU/mL to provide the therapeutic benefits listed previously (Lourens-Hattingh and Viljoen 2001). When probiotics are incorporated into food products, culture viability often decreases as a result of low pH, cold temperature stress during storage, lack of nutrients, and oxidative stress; thus, it can be a challenge to maintain proper levels of the bacteria in food products so the food can deliver the desired amount. The addition of a prebiotic to foods may reduce these stressors and increase probiotic viability during storage (Bruno and others 2002; Capela and others 2006; Corcoran and others 2004) .

Prebiotics are defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid 1995). Currently, the predominate prebiotic used in high fiber foods is the oligofructan, inulin. Oat beta-glucan has potential advantages over inulin as a prebiotic. Oat beta-glucan is a soluble dietary fiber that reduces serum cholesterol, decreases insulin response and can provide prolonged satiety (DeVries 2001; Bell and others 1999)   The Food and Drug Administration (FDA) recognizes oat-beta glucan for its cholesterol-lowering effects with a health claim (CFR 21, Section 101-81). According

to the FDA, the amount of beta-glucan needed to achieve health benefits in yogurt is 0.75g/6 oz serving which equates to 0.44% w/w beta-glucan. In addition, oat beta-glucan has a unique high viscosity that can provide desirable textural properties to yogurt (Skendi and others 2003). As a prebiotic, beta-glucan is postulated to improve probiotic survival in foods, such as yogurt, however little is known about the relationship between bifidobacteria and beta-glucan in a food system during cold storage (Vasiljevic and others 2007). Additionally, modified corn starch is often added to yogurts to improve texture. In particular, corn starch greatly improved yogurt viscosity and syneresis when oat beta-glucan was included in the formulation (White and Yao 2007).   The impact of corn starch on bifidobacteria survival in yogurt was not evaluated. This study was undertaken to explore the relationship between oat beta-glucan, modified corn starch, and bifidobacteria survival in yogurt during refrigerated storage.

The objectives of this study were to investigate the effects of adding oat beta-glucan and/or corn starch on the viability of two strains of bifidobacteria in a set-style yogurt during 5 wk of refrigerated storage.

MATERIALS AND METHODS

Propagation of Cultures

Pure strains of *Bifidobacterium breve* R0070, *Bifidobacterium longum* R0175, *Bifidobacterium infantis* R0033, and *Bifidobacterium bifidum* R0071 (Institut Rosell Inc., Montreal, QC) were grown anaerobically in de Man Rogosa and Sharpe (MRS broth) broth (Fisher Scientific, Pittsburgh, PA) supplemented with 0.22 µm-filter sterilized 0.05% L-

cysteine (Sigma-Aldrich, St. Louis, MO) for 24 h at 37 °C for two consecutive passes. The cultures were then concentrated, 20% glycerol (v/v) was added, and samples were stored in cyrovials at -75 °C. Preliminary results eliminated the use of *B. infantis* and *B. bifidum* in this study because of their profound fragility during storage at 4 °C. Yogurt starter culture strains, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YC-180, Chr. Hansen, Milwaukee, WI), were stored at -20 °C. Prior to yogurt production both bifidobacteria strains were inoculated and propagated twice in MRS broth at 37 °C for 24 h.

Beta-glucan extraction

An experimental oat line (N979-5-2-4), developed at Iowa State University to have a high beta-glucan concentration (7.88%), was grown and harvested at the Agronomy and Agricultural Engineering Field Research Center near Ames, Iowa in 2006 (Kim and White 2009).   The oats were dried and shipped to the Quaker Oats Inc. pilot plant in Cedar Rapids, Iowa where they were steamed for one min at 80 °C and rolled to a flake thickness of 0.61 mm followed by grinding in a Hammer Mill though a 0.56 mm screen. Beta-glucan was extracted from the whole oat flour (Yao and White 2008) and the supernatant collected.. Extracted beta-glucan was concentrated in a rotary evaporator at 75 °C and 200 mbar until the solution reached one-third its starting mass or freeze-dried (-20 °C pre-chill, -26 °C for 24 h followed by 22 °C for remainder of drying, 80 mbar). The beta-glucan concentration of both treatments was determined by using the Megazyme mixed-linkage beta-glucan assay procedure (AACC method 32-23, AOAC method 995.16).

Yogurt Preparation

Yogurt containing 1.5% fat and 11.5% milk-solids not-fat (MSNF) provided the base for the treatments (Table 1). Yogurt treatments containing beta-glucan received a final concentration of 0.44% beta-glucan (w/w). Yogurt treatments containing modified waxy corn starch (Thermflo®, National Starch, Bridgewater, NJ) received a final concentration of 1.33% starch (w/w). The ingredients were combined, homogenized, and heated at 85 °C for 30 min. The yogurt was then cooled to 43 °C and inoculated with yogurt starter culture (200 mg/L). The bifidobacteria strains, *B. breve*, and *B. longum,* were added, individually, to each yogurt treatment at a concentration of at least 10^9 CFU/mL. Each treatment was divided into 10-mL aliquots and fermented at 42 °C until reaching a pH <4.6, cooled to 4 °C, and stored for 5 wks.

Culture viability determination

To determine cell viability, cell counts were made from aliquots taken prior to fermentation (day 0), the day following fermentation (day 1), and once every week for five total weeks (days 7-35), each from previously undisturbed yogurt. Each sample was serially diluted in 0.1% peptone water, plated in duplicate on modified MRS agar containing X-α-Gal (5-bromo-4-chloro-3-indolyl-α-galactopyranoside) and incubated anaerobically at 37 °C for 48 h. The plates were then incubated aerobically an additional 24 h at 22 °C to allow for the bifidobacteria color change reaction to occur. Bifidobacteria (blue) and yogurt culture (white) colonies were counted after 72 h (Chevalier and others 1991).

Experimental Design

For each bacterial strain (*B. longum*, *B. breve*, no bifidobacteria added), the experiment was conducted as a three-way factorial, with factors (and levels) beta-glucan (present, absent), starch (present, absent) and time (0, 1, 7, 14, 21, 28, 35 days). Combinations of beta-glucan and starch levels acted as blocks for time. Each time measurement was taken from an independent vial. Because of space and time constraints, half the treatments were completed during one time period and the other half were completed during a separate time period. It is assumed there was no systematic difference in lab conditions between the two experimental time periods. There were three replications for each treatment. Plate counts were done in duplicate for each enumeration time point and treatment, with the average $\log_{10}$ colony count used as the response variable for each treatment in each replication.

Statistical Analysis

Data for each bacterial strain were analyzed separately. It was not possible to monitor bifidobacteria culture survival below 10^7 CFU/mL, which affected how data were analyzed for each bacterial treatment. For *B. longum* and yogurt cultures without bifidobacteria, $\log_{10}$ colony counts were analyzed using a linear mixed model with normal errors (PROC MIXED with restricted maximum likelihood option, SAS 9.1). Random effects for replicates were nested in each experimental time period to reflect the design structure. Analysis for yogurts containing *B. longum* was based on a logistic random effects model using an indicator

variable with a value of 1 if the vial contained a log count above the minimum detection level and a value of 0 if the log count was below the minimum detection level. The parameter estimates were obtained using proc GLIMMIX (with restricted maximum likelihood option, SAS 9.1). Thus, for *B. longum,* observational results as well as estimated detection probabilities are reported. For all cultures, the initial time point before fermentation was excluded from statistical analysis. Significance was set at $P \leq 0.05$ using ANOVA F-tests and tests of linear contrasts (SAS 9.1).

RESULTS AND DISCUSSION

Viability of *B. breve*

B. breve counts remained above the therapeutic level of 10^7 CFU/mL over the 5 weeks of storage for all beta-glucan and starch treatments. The addition of beta-glucan to yogurt containing *B. breve* had no effect ($p = 0.31$) on culture viability during cold storage (Figure 1). Furthermore, the addition of starch to yogurt containing *B. breve* also had no effect on culture survival during cold storage ($p = 0.85$). However, culture counts during cold storage were time dependent ($p < 0.01$). This was expected as bifidobacteria are, in general, susceptible to cell damage or death when exposed to decreased temperature and pH over time. However, even though *B. breve* survival was time dependent, average counts remained well above the therapeutic threshold, and so, this time dependency is not of practical significance.

The average cell count of *B. breve* in yogurt containing concentrated or freeze-dried beta-glucan was not different from the average count for yogurt containing no beta-glucan ($p = 0.14$ and 0.52, respectively). Similarly, no differences occurred between the average

survival of *B. breve* in yogurt containing concentrated beta-glucan and the average survival of *B. breve* in yogurt containing freeze-dried beta-glucan (p = 0.36). In addition, there were no significant differences in average *B. breve* survival between yogurts that did or did not contain starch regardless of beta-glucan treatment (p = 0.85).   For all beta-glucan and starch treatments the estimated count decreased by 0.00385 log CFU/mL per day. The lack of differences between treatments is not surprising considering the hardiness of this *B. breve* strain. These results are consistent with a previous study showing that another *B. breve* strain (ATCC 15700) did not differ in growth when grown either aerobically or anaerobically in MRS (Bolduc and others 2006). The lack of differences between treatments containing *B. breve* could indicate that the *B. breve* strain used may be very aero-tolerant resulting in improved survival during the storage period. The use of an aero-tolerent *B. breve* strain could indicate that any differences in probiotic survival between yogurt treatments may be a result of an effect on the dissolved oxygen content of the yogurt or the cultures' ability to provide defense against dissolved oxygen.

Viability of *B. longum*

　　B. longum was much less cold resistant than either *B. breve* or the mixed yogurt cultures. *B. longum* did not survive in any treatment for the entire duration of cold storage (Table 2). The longest average survival above 10^7 CFU/mL occurred in yogurt that contained concentrated beta-glucan, either with or without starch, at 21 days. In yogurt containing freeze-dried beta-glucan, with or without starch, the average survival above 10^7 CFU/ml was 14 days. In yogurt containing no beta-glucan or starch, the average survival above 10^7 CFU/mL was 7 days, whereas in yogurt containing starch and no beta-glucan, the

average survival above 10^7 CFU/mL was 1 day. Because of the sensitivity of *B. longum* to cold storage, many treatment effects were detected. The data provide strong evidence of an effect of both time and beta-glucan treatment on the probability that a count will reach the observable level ($p < 0.01$ and 0.02, respectively).

These data indicated that the addition of beta-glucan to yogurt increased the survival probability of *B. longum* regardless of starch status, demonstrating that starch, when added in combination with beta-glucan or without beta-glucan, did not affect the survival probability of *B. longum*. The estimated survival probability for *B. longum* was greater in yogurt containing either concentrated or freeze-dried beta-glucan than in yogurt containing no beta-glucan ($p < 0.01$ and < 0.01, respectively). This result is consistent with a study done by Vasiljevic and others (2007) in which improved survival occurred of *B. animalis* (Bb-12™) in yogurt containing oat beta-glucan during prolonged cold storage.   *B. longum* survival above 10^7 CFU/mL was increased by one week on average in yogurt with concentrated beta-glucan when compared to yogurt with freeze-dried beta-glucan. Furthermore, the estimated survival probability of *B. longum* in yogurt containing concentrated beta-glucan was greater than in yogurt containing freeze-dried beta-glucan ($p = 0.03$). The mechanism surrounding improved survival of *B. longum* in the presence of oat beta-glucan remains unknown (Vasiljevic and others 2007).

It is clear that bifidobacteria do not actively divide during cold storage, therefore the protective effect of beta-glucan may result from a physical alteration of the environment surrounding the probiotic, or from a protective component released during fermentation. Thus, the impact might be a protective effect, rather than a prebiotic effect. As seen previously, *B. longum* was more susceptible to oxygen stress than was *B. breve* (Bolduc and

others 2006). Another *B. longum* strain (ATCC 15708) had improved growth in media supplemented with ascorbic acid, a known oxygen scavenger, indicating that oxygen status is critical for *B. longum* survival (Bolduc et al., 2006). Beta-glucan is well known for its viscosity-increasing properties. It could be hypothesized that this physical change may affect the matrix such that oxygen penetration is decreased within the yogurt, thus creating a more hospitable environment for *B. longum*. A benefit to bifidobacteria based on the physical alteration of the yogurt environment could explain the benefit of concentrated beta-glucan, which remained hydrated and was more easily dispersed, over freeze-dried beta-glucan.

Viability of mixed yogurt cultures *S. thermophilus* and *L. bulgaricus*

In yogurt containing no bifidobacteria, *S. thermophilus* and *L. bulgaricus* increased in number by an average of 1.7 $\log_{10}$ cycles during fermentation (Figure 2). During 5 wks of storage, the mixed yogurt cultures, *S. thermophilus* and *L. bulgaricus,* survived at a level well above the therapeutic level of 10^7 CFU/mL. High survival of yogurt cultures is consistent with studies indicating that *S. thermophilus* and *L. bulgaricus* strains survive well during cold storage at lowered pH (Saccaro and others 2009; Dave and Shah, 1997; Martensson and others 2002). The cultures, however, positively benefitted from the addition of starch ($p < 0.01$). There was no benefit from the addition of beta-glucan to the yogurt cultures ($p = 0.13$), and no difference between average cell counts between yogurt containing concentrated and freeze-dried beta-glucan ($p = 0.09$). The data also suggest an effect of time on average cell counts ($p = 0.06$) with average cell counts decreasing by 0.00185 $\log_{10}$ CFU/mL each day.

Yogurt containing *B. breve*, *S. thermophilus* and *L. bulgaricus* increased in number by an average of 0.88 $\log_{10}$ cycles during fermentation (Figure 3). Similarly, yogurt containing *B. longum*, *S. thermophilus* and *L. bulgaricus* increased in number by an average of 0.61 $\log_{10}$ cycles during fermentation (Figure 4). Thus, the increase during fermentation was 0.82 $\log_{10}$ cycles less for yogurt cultures in the presence of *B. breve* and 1.09 $\log_{10}$ cycles less for yogurt cultures in the presence of *B. longum*, indicating a competitive environment in these treatment. However, yogurt cultures mixed with *B. breve* or *B. longum* remained well above 10^7 CFU/mL for the entire the study.

For yogurt cultures in the presence of *B. breve*, there was no evidence of an effect of beta-glucan, starch, or time on the mean $\log_{10}$ count. For yogurt cultures in the presence of *B. longum* the data provide no evidence for an effect of starch, but do provide evidence of an effect of beta-glucan (p = 0.27 and 0.05 respectively). The mean $\log_{10}$ count was significantly lower for the freeze-dried beta-glucan treatment than for the control (p = 0.02). There was also suggestive evidence that the mean $\log_{10}$ count of the yogurt cultures was lower when concentrated beta-glucan was added than when no beta-glucan was added (p = 0.08). There was no evidence of a difference in the mean $\log_{10}$ counts of the yogurt cultures in the presence of *B. longum* for the concentrated and freeze-dried beta-glucan treatments (p = 0.39). From these results we can conclude that in a competitive environment, beta-glucan and starch do not provide a benefit to the yogurt cultures when the competitive strain is B. breve or B. longum. Additionally, in a competitive environment where *B. longum* is the competitive strain, beta-glucan may result in lowered average yogurt culture counts.

CONCLUSIONS

Beta-glucan imparted a protective effect on bifidobacteria strains in yogurt when stressed by prolonged cold storage. Inclusion of beta-glucan in yogurt improved *B. longum* survival during storage at 4 °C. Choice of the bifidobacteria strain as well as the beta-glucan preparation method was vital to the increased survival of the probiotic. For example, in the case of a very cold sensitive strain, such as *B. longum*, the addition of beta-glucan increased the likelihood that this probiotic would be delivered at a physiologically beneficial level even after three weeks of storage at 4 °C. Although starch had a positive effect on the survival of *S. thermophilus* and *L. bulgaricus*, there was no effect of starch on the survival of the selected bifidobacteria strains. Questions remain regarding the mechanisms associated with the observations in this study; however, these results indicate that the gut-health benefits associated with probiotics can be complemented by the heart-health benefits of beta-glucan in a yogurt system to provide a doubly healthful product.

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Table 1. Yogurt base formulations listed as percent of total by mass

Ingredient (%)	No β-glucan/ No starch	No β-glucan/ Starch	Concentrated β-glucan/ No Starch	Concentrated β-glucan/ Starch	Freeze Dried β-glucan/ No Starch	Freeze Dried β-glucan/ Starch
Cream	3.80	3.80	3.80	3.80	3.80	3.80
Water	82.00	82.00	80.22	80.22	80.22	80.22
NFDM	11.38	11.38	11.38	11.38	11.38	11.38
Sugar	2.83	2.83	2.83	2.83	2.83	2.83
Beta-glucan	-	-	0.44	0.44	0.44	0.44
Corn Starch	-	1.33	-	1.33	-	1.33

Table 2. Average viability of *B. longum* in yogurt treatments with and without beta-glucan (BG) and starch during

4 °C storage. Dash indicates that average of three replications fell below the quantifiable level ($<10^7$ CFU/mL).

Standard error not shown due to binary nature of data.

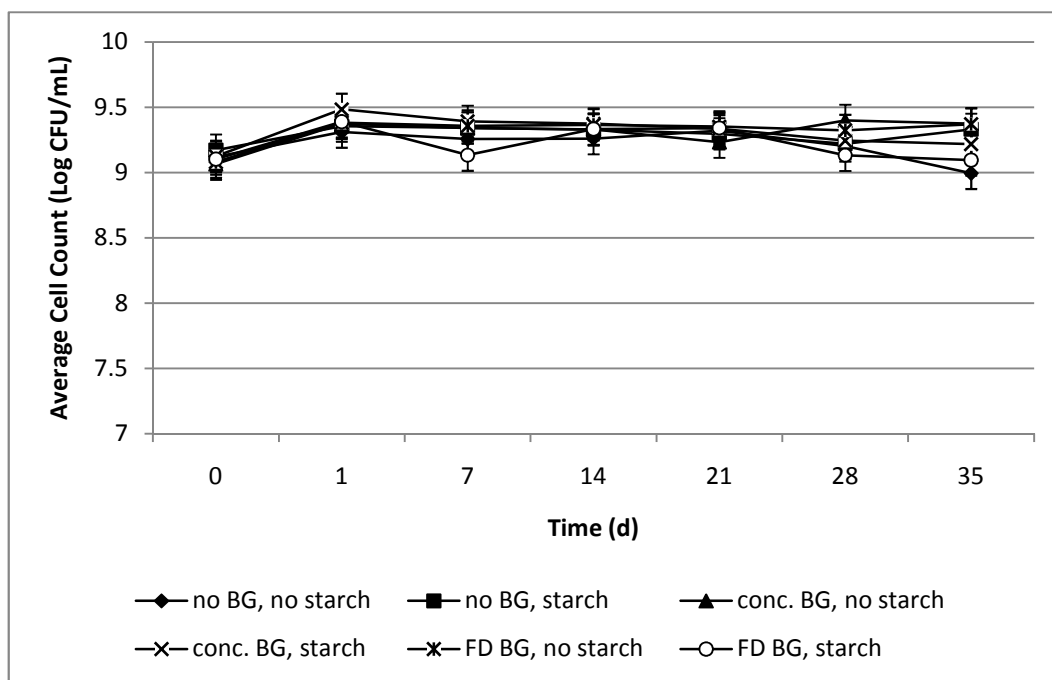|	Average Cell Count ($\log_{10}$ CFU/mL)					
Day	No β-glucan no starch	No β-glucan starch	concentrated β-glucan, no starch	concentrated β-glucan, starch	Freeze-dried β-glucan, no starch	Freeze-dried β-glucan, starch
0	9.3	9.2	9.4	9.4	9.3	9.5
1	9.3	9.4	9.5	9.2	9.5	9.4
7	8.5	-	9.3	8.8	9.5	9.0
14	-	-	8.8	7.7	8.7	7.8
21	-	-	7.7	8.1	-	-
28	-	-	-	-	-	-
35	-	-	-	-	-	-

Figure 1. Viability of *B. breve* in yogurt treatments during 4 °C storage. BG = beta-glucan, conc. = concentrated, FD = freeze-dried. Error bars represent pooled standard error of the mean, SEM = 0.12.
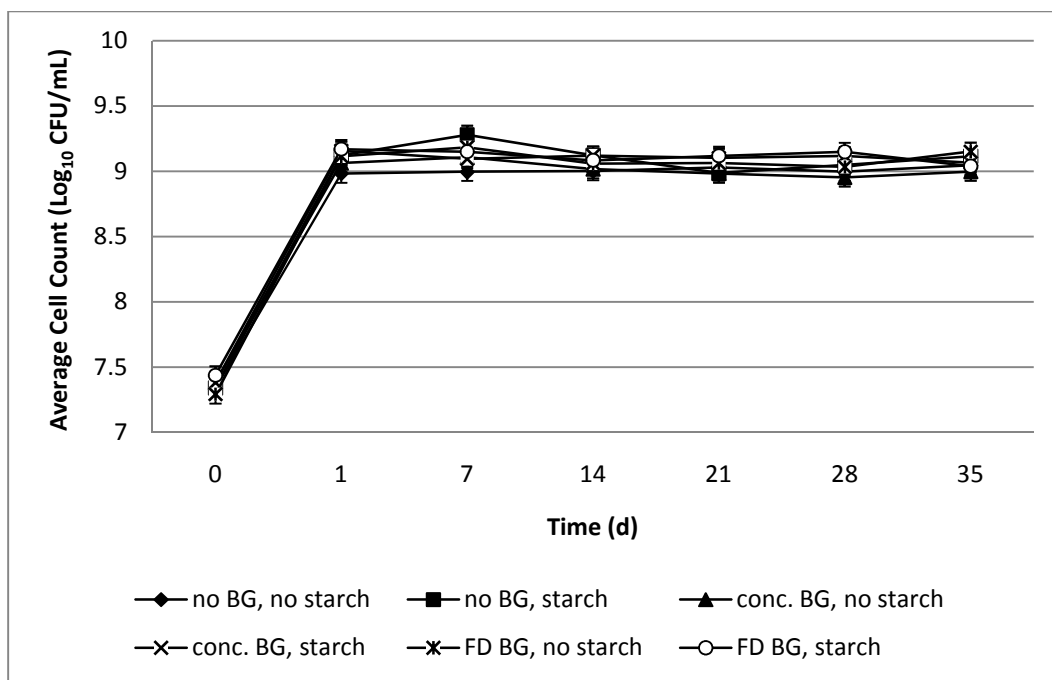
Figure 2. Viability of *S. thermophilus* and *L. bulgaricus* in yogurt treatments not containing

bifidobacteria during 4 °C storage. BG = beta-glucan, conc. = concentrated, FD = freeze-

dried. Error bars represent pooled standard error of the mean, SEM = 0.07.
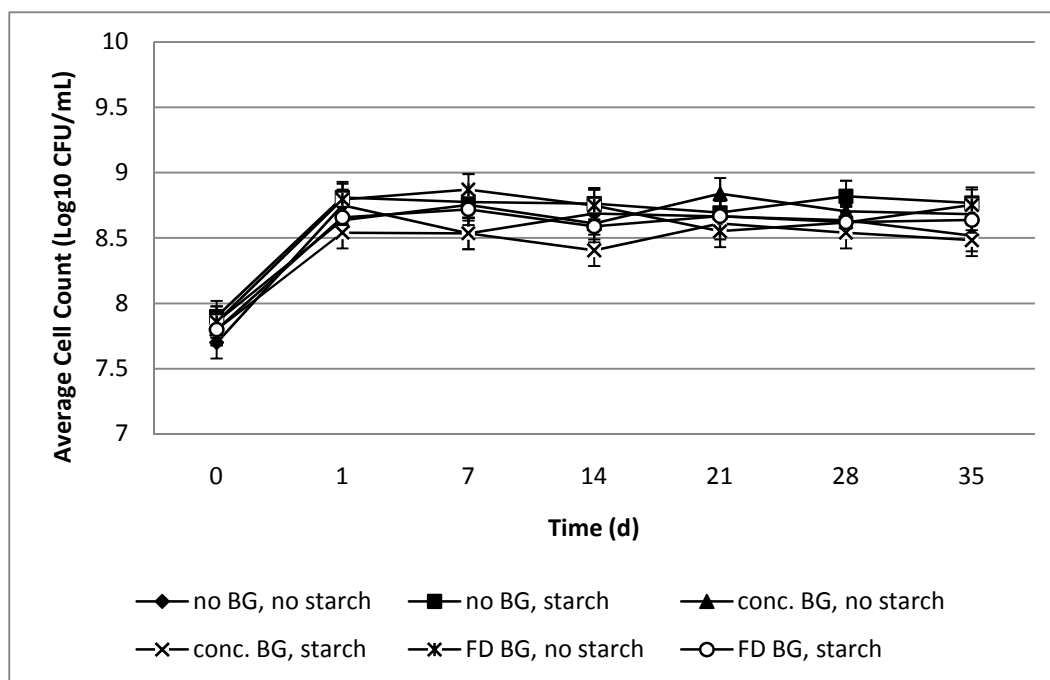
Figure 3. Viability of *S. thermophilus* and *L. bulgaricus* in yogurt treatments containing *B. breve* during 4 °C storage. BG = beta-glucan, conc. = concentrated, FD = freeze-dried. Error bars represent pooled standard error of the mean, SEM = 0.12.
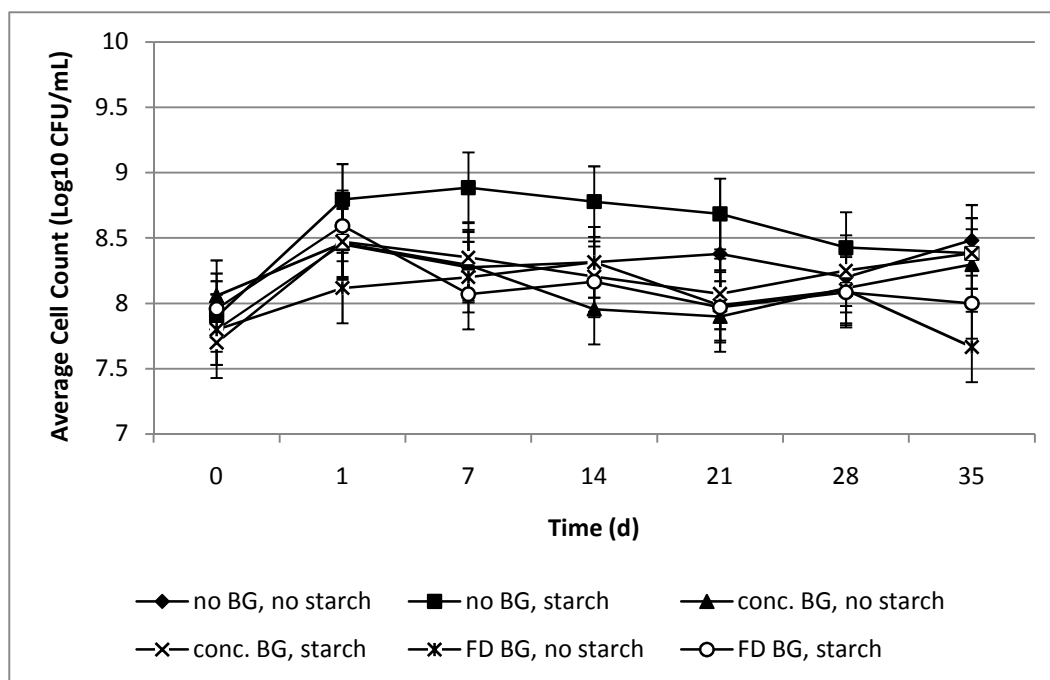
Figure 4. Viability of *S. thermophilus* and *L. bulgaricus* in yogurt treatments containing *B. longum* during 4 °C storage. BG = beta-glucan, conc. = concentrated, FD = freeze-dried. Error bars represent pooled standard error of the mean, SEM = 0.27.

GENERAL CONCLUSIONS

This study demonstrated that the use of oat beta-glucan in yogurt can improve the viability of bifidobacteria during refrigerated storage. *B. longum* survival above 10^7 CFU/mL was improved by one week for treatments containing freeze-dried beta-glucan and two weeks for treatments that contained concentrated beta-glucan when compared to the control. Improved survival of *B. longum* in yogurt with concentrated versus freeze-dried beta-glucan may be a result of better beta-glucan dispersion within the yogurt base for the concentrated preparation. Additionally, this study confirmed that for hardy bacteria strains, such as *B. breve* and yogurt cultures, addition of beta-glucan had no detrimental effect on cell viability. Furthermore this study demonstrated that the addition of corn starch, in combination with beta-glucan, did not effect bifidobacteria survival during cold storage.

Mechanisms surrounding the ability of oat beta-glucan to prolong survival of sensitive bifidobacteria strains during cold storage still remain unclear. Mechanisms may include modulation of the environment, such that there are more favorable growth conditions, possibly decreased dissolved oxygen content, or the mechanism may involve the digestion of beta-glucan or the production of a bioactive compound during fermentation.

Recommendations for future research include measurement of dissolved oxygen content to determine if the mechanism is linked to modulation of the environment and creation of a more suitable growth environment by way of decreased dissolved oxygen. Additionally,measurements of physical parameters, such as viscosity and/or texture could provide useful data. If the mechanism is related to viscosity, it would be important to know how this measurement compared between the treatments. Finally, it would be prudent to

monitor the beta-glucan content after fermentation and during refrigerated storage. There would be a decrease in beta-glucan concentration if beta-glucan is consumed by the cultures as an energy source. Continued research is needed to elucidate the appropriate mechanisms.

This study showed that heart healthy oat beta-glucan can complement the gut-health benefits of bifidobacteria to produce a healthful yogurt product.

APPENDIX 1

SURVIVAL OF BIFIDOBACTERIA IN LIQUID MEDIA WITH ADDED BETA-GLUCAN DURING COLD STORAGE

REASONING

In yogurt prepared with beta-glucan, survival of bifidobacteria was lengthened during storage at 4 °C when compared with yogurt with no added beta-glucan. This experiment was designed to test the ability of beta-glucan to protect bifidobacteria from cold stress. Healthy bifidobacteria are able to grow on MRS agar and MRS + 1% NaCl. Cells weakened or damaged by the effects of cold storage are unable to grow on MRS + 1% NaCl. By comparing colony growth on these two types of agar we will be able to differentiate between the number of healthy and damaged cells for each treatment. The difference in plate counts between the two types of plating media will tell us if the presence of beta-glucan can decrease the number of damaged cells and, in turn, decrease cell death during cold storage.

METHODS

A series of plating tests were done using fresh and aged (storage for 28 days at 4 °C) bifidobacteria cultures to determine the correct salt concentration needed to allow for the growth of healthy bifidobacteria yet inhibit the growth of damaged bifidobacteria on MRS agar (data not shown). This concentration was found to be 1% NaCl. Bifidobacteria cultures were obtained and cultured as in chapter 2 methods. Beta-glucan also was obtained and measured as in chapter 2, however, following extraction and concentration or freeze-

drying, aliquots were vacuumed sealed in plastic sheeting and irradiated at 30.75 kGy/min. This was done to ensure sterility during storage. de Man Rogosa and Sharpe (MRS) broth (Fisher Scientific, Pittsburgh, PA) was prepared and treatments were created by the aseptic addition and dispersion of concentrated beta-glucan or freeze-dried beta-glucan (0.44% w/v). Plain MRS broth served as a control. Each media treatment was inoculated with either *B. breve* or *B. longum* (~10^9 CFU/mL) and 10 mL aliquots were aseptically transferred to sterile screw-cap tubes. Tubes were stored anaerobically at 4 °C. At each enumeration time point each treatment was serially diluted in 0.1% peptone water and plated in duplicate on MRS agar and MRS + 1% NaCl agar. Plates were incubated anaerobically at 37 °C for 48 h and colony counts were recorded. One replication was completed.

RESULTS AND DISCUSSION

B. breve viability stayed above 10^7 CFU/mL for all beta-glucan treatments though week 4 (Table 1). At the week 5 time point, *B. breve* counts dropped below 10^7 CFU/mL for cultures plated on the non-selective MRS agar for all beta-glucan treatments. However, counts above 10^7 CFU/mL were observed for cultures plated on the selective MRS + 1% NaCl agar for all beta-glucan treatments for all time points. Similarly, *B. longum* counts stayed above 10^7 CFU/mL for all beta-glucan treatments though week 3 (Table 2). At the week 4 time point, *B. longum* counts dropped below 10^7 CFU/mL for cultures plated on the non-selective MRS agar for all beta-glucan treatments. Again, counts above 10^7 CFU/mL were observed for cultures plated on the selective MRS + 1% NaCl agar for all beta-glucan treatments for all time points. These are highly unexpected results, as the MRS + 1% NaCl agar was designed to be a much more stressful media for bifidobacteria to grow on.

Therefore, we would not expect to see *B. breve* or *B. longum* grow on MRS + 1% NaCl and not grow on MRS. From these results we can conclude that the selectivity of the agar was not successful. We cannot, however, make inferences concerning the effect of beta-glucan treatments on cold stress or cold damage to *B. breve* or *B. longum*.

CONCLUSION

It was postulated that beta-glucan may have a protective effect on cellular stress or damage caused by prolonged cold storage. Due to the inability to distinguish between damaged and healthy bifidobacteria, we were unable to determine if this protective effect exists. It would be prudent to determine another method for examining the effect beta-glucan may have on cellular cold stress and damage.

Table 1. Survival of *B. breve* ($\log_{10}$ CFU/mL) in MRS treated either with or without beta-glucan plated on non-selective (MRS) and selective (MRS+1%NaCl) media after storage at 4°C. Dash indicates a count that fell below quantifiable level ($<10^7$ CFU/mL).

	Beta-glucan treatment					
	No beta-glucan		Freeze-dried beta-glucan		Concentrated beta-glucan	
	Plating media					
Week	MRS	MRS+1%NaCl	MRS	MRS+1%NaCl	MRS	MRS+1%NaCl
0	9.0	9.0	8.9	9.0	8.8	8.8
1	9.0	8.9	9.0	8.9	8.9	8.8
2	9.1	9.0	9.0	9.0	9.0	9.0
3	9.1	9.0	9.0	8.8	9.0	9.0
4	7.2	7.9	7.3	7.8	7.2	7.9
5	-	7.3	-	7.3	-	7.4

Table 2. Survival of *B. longum* (log$_{10}$ CFU/mL) in MRS treated either with or without beta-glucan plated on non-selective (MRS) and selective (MRS+1%NaCl) media after storage at 4°C. Dash indicates a count that fell below quantifiable level ($<10^7$ CFU/mL).

	Beta-glucan treatment					
	No beta-glucan		Freeze-dried beta-glucan		Concentrated beta-glucan	
	Plating media					
Week	MRS	MRS+1%NaCl	MRS	MRS+1%NaCl	MRS	MRS+1%NaCl
0	9.2	9.2	9.3	9.3	9.4	9.3
1	9.0	8.7	9.2	9.1	9.0	9.0
2	9.1	9.0	9.1	9.0	9.1	9.1
3	8.6	8.9	8.9	8.3	8.9	8.6
4	-	7.5	-	8.1	-	8.0
5	-	7.8	-	7.6	-	7.8

APPENDIX 2

SURVIVAL OF BIFIDOBACTERIA WITH ADDED BETA-GLUCAN THROUGH A SIMULATED GASTRIC SYSTEM

REASONING

Beta-glucan has been shown to improve probiotic survival in the large intestine. As a dietary fiber, beta-glucan evades digestion in the stomach and small intestine and is available for fermentation by probiotics, such as bifidobacteria, in the large intestine which is known as the prebiotic effect. Studies also suggest that beta-glucan can increase probiotic survival during refrigerated storage in yogurt. The objectives of this study were to determine if beta-glucan can improve bifidobacteria survival through a simulated human gastric system.

METHODS

Cultures of *B. breve* and *B. longum* were obtained and cultured as in methods from chapter 2. Beta-glucan was also obtained and measured as in chapter 2, however, following extraction and concentration or freeze-drying aliquots were vacuumed sealed in plastic sheeting and irradiated at 30.75 kGy/min. This was done to ensure beta-glucan sterility. Media was prepared to represent the human gastrointestinal tract in the following way: stomach simulated with de Man Rogosa and Sharpe (MRS) broth (Fisher Scientific, Pittsburgh, PA) adjusted to pH 3.0 with 20% HCl, upper intestine simulated with MRS broth with 0.6% (w/v) oxgall (dehydrated fresh bovine bile) (Difco, Franklin Lakes, NJ) and the

colon was simulated with MRS broth with 0.3% (w/v) oxgall. For treatments containing

beta-glucan, either concentrated or freeze-dried beta-glucan (0.44% w/w) was added to the

appropriate media immediately before incubation. Control media contained no beta-glucan.

Incubation time in stomach media was 3 hours aerobically, upper intestinal media for 4 hours

aerobically and colon media for 24 hours anaerobically. Between each stage of the simulated

gastrointestinal tract cultures were sampled for plate counts, centrifuged (4300 rpm, 15 min),

the supernatant was discarded, and the next media was added accordingly. Aliquots taken

after each incubation period were diluted in 0.1% peptone water, plated in duplicate on MRS

agar and incubated anaerobically at 37 °C for 48 hrs after which colonies were counted and

recorded. One replication was completed.

RESULTS AND DISCUSSION

Survival of *B. breve* though the simulated gastric system remained above 10^8

CFU/mL for all treatments (Table 1). There was no appreciable difference in survival though

the gastric system between the beta-glucan treatments for *B. breve*. These results are not

surprising considering that previous work has shown that *B. breve* is a very resilient strain.

There was no difference in survival of *B. longum* between the beta-glucan treatments after

incubation in MRS broth at pH 3 (stomach media). After incubation in MRS broth +0.6 %

bile (upper intestine media) *B. longum* survival remained at 6.0 $\log_{10}$ CFU/mL for the control

treatment. However, after this incubation step there was no indication that *B. longum* had

survived in treatments that contained either concentrated or freeze-dried beta-glucan. After

incubation in MRS + 0.3% bile (colon media) *B. longum* was found to be 6.8 $\log_{10}$ CFU/mL

for the control and 2.0 and 1.2 $\log_{10}$ CFU/mL for media containing concentrated and freeze-

dried beta-glucan respectively. It was surprising to observe no survival of *B. longum* through a simulated upper intestine and decreased survival through the simulated colon for treatments containing beta-glucan. Although data indicate that beta-glucan may be detrimental to survival of probiotic though the gastric system, this may not be so. The method used in this study involved adding beta-glucan to each media before incubation. Because beta-glucan is water soluble, it should have been discarded after each centrifugation. Visual observations during this experiment, however, indicated that the beta-glucan may not actually be discarded with the supernatant after centrifugation. Instead the beta-glucan may remain after centrifugation, thus leading to an increasing concentration through the simulated gastric system. It is possible that an increasing beta-glucan concentration was responsible for hindering the survival of *B. longum*, which has been shown to be sensitive to environmental conditions, and not necessarily the presence of beta-glucan which was intended to be at a constant 0.44 % (w/w) concentration.

Observations of possible beta-glucan accumulation prompted measurement of beta-glucan in the supernatant from each stage of the simulated gastric system. As suspected, results indicated that very little beta-glucan was found in the supernatant after incubation for 24 h in MRS and no beta-glucan was found in the supernatant after incubation in stomach acid media and 0.6% bile media (Table 2).

CONCLUSION

Beta-glucan is known to act as a prebiotic for probiotics once it reaches the large intestine. It is possible that though modulation of its fluid environment, beta-glucan may have protective effects for probiotics passing through the gastric system. Unfortunately,

these study results do not indicate a benefit to bifidobacteria survival though a simulated gastric system given the current methods. Through the appropriate modifications to the methods, to ensure consistent beta-glucan concentration though the gastric system, there may be better opportunity to observe a possible protective effect of beta-glucan.

Table 1. Survival ($\log_{10}$ CFU/mL) of *B. breve* and *B. longum* through simulated gastric system with and without beta-glucan.

Incubation Media	*B. breve*			*B. longum*		
	No beta-glucan	Concentrated beta-glucan	Freeze-dried beta-glucan	No beta-glucan	Concentrated beta-glucan	Freeze-dried beta-glucan
MRS Broth (Initial Count)	8.5	8.8	8.8	9.0	9.0	8.9
MRS Broth pH 3	8.4	8.8	8.5	9.0	9.0	9.0
MRS Broth + 0.6% Bile	8.4	8.8	8.7	6.0	0.0	0.0
MRS Broth + 0.3% Bile	8.2	8.3	8.0	6.8	2.0	1.2

Table 2. Percent beta-glucan in supernatant after and centrifugation in designated incubation

media. Percent is average of three measurements taken from cultures of *B. breve* plus/minus

standard deviation.

	Incubation Media		
Beta-glucan Treatment	MRS Broth	MRS Broth pH 3	MRS Broth 0.6% bile
No Beta-glucan	-0.040 ± 0.024	-0.064 ± 0.007	-0.042 ± 0.010
Concentrated Beta-glucan	0.126 ± 0.035	-0.004 ± 0.073	0.000 ± 0.037
Freeze-dried Beta-glucan	0.177 ± 0.080	-0.044 ± 0.052	-0.013 ± 0.025

ACKNOWLEDGMENTS