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Barley β-glucan in bread: The journey from production to consumption

by

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ABSTRACT

β-Glucan is a soluble fibre shown to help regulate blood sugar and lower cholesterol. Incorporation into food, particularly bread, may affect β-glucan's physicochemical properties and health benefits. The journey of β-glucan through the mixing, fermenting, baking, and storage of bread was evaluated, in terms of its solubility and viscosity under *in vitro* physiological conditions, at levels most likely to be presented to consumers (0.75, 1.0, 1.5 g β-glucan/serving). Satiety and glycemic response measures, in addition to the quality and consumer acceptability of the bread, were also investigated.

In dough, viscosity of the physiological extract was impacted by β-glucan level, fermentation time, and endogenous flour enzymes. Fermentation decreased β-glucan solubility indicating that the reduction in viscosity depends on both molecular degradation and solubility reduction. Dough rheological properties and microstructure, characterized using an oscillatory rheometer and fluorescence microscopy, respectively, showed that β-glucan may interfere with the gluten network, though gluten addition may help improve this. The bread's physical properties supported these observations, as β-glucan decreased loaf volume and height, while gluten addition corrected this.

Baking increased β-glucan solubilization to 58-60%, compared to 9% in dough. Gluten addition increased solubility further (67-68%). Similar trends were seen for extract viscosity and were supported by fluorescence microscopy images. Storage at ambient, refrigeration and frozen conditions showed that bread

with β-glucan should be consumed fresh to maintain highest bread quality and β-glucan solubility and viscosity.

Bread with β-glucan kept panelists full, longer. Reducing sugar release values implied that satiety may depend on digesta viscosity and/or rate of sugar release from the bread. Bread with β-glucan produced the most leveled glucose curve; though areas under the 2 hr plasma glucose curves were similar. Consumers liked the 0.75 g β-glucan/serving bread and the control more than the 1.5 g β-glucan/serving bread, though provision of health information improved bread liking to similar values.

The findings demonstrate that low solubility β-glucan concentrate that gets solubilized upon baking is well suited for bakery applications and that a successful β-glucan-fortified bread product is possible. Commercialization of bread fortified with β-glucan would provide consumers an additional source of dietary fibre to assist them in coming closer to recommended daily intakes.

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INTRODUCTION AND OBJECTIVES

Barley is a versatile cereal crop that can be grown in a wide range of soil and environmental conditions. Despite barley's abundance as a primary crop in North America, this cereal is underutilized in human food production. This is unfortunate as the β-glucan present in barley grain has been shown to regulate blood glucose levels (Wood et al 1994, Yokoyama et al 1997, Bourdon et al 1999) and also to lower blood cholesterol levels (Gallaher et al 1992, Martinez et al 1992, Newman et al 1992, Jackson et al 1994, Wang et al 1997, Bourdon et al 1999). In fact, the U.S. Food and Drug Administration (FDA 2005) has approved a health claim for the association between the consumption of products containing β-glucan from barley and a reduction in the risk of heart disease, which is an extension of the health claim originally approved for β-glucan from oats (FDA 1997). This claim is based on the consumption of at least 3 g of β-glucan per day or 4 servings of a food product that supplies at least 0.75 g of β-glucan per serving (FDA 2005).

Cholesterol reduction and glucose regulation are important factors in the prevention and management of heart disease and Type 2 diabetes, which are two of the largest drains on our health care system and the health of North Americans (Canadian Diabetes Association 2007, Heart and Stroke Foundation 2007). Interest is growing in the production of β-glucan-fortified foods, ranging from muffins (Beer et al 1997) to beverages (Temelli et al 2004) in efforts to bring the

health promoting properties of β-glucan closer to home. β-Glucan's cholesterol lowering and blood glucose regulating effects are primarily related to its viscosity, which can be negatively influenced by certain processing and storage conditions (Wood et al 2000). Depending on the conditions applied, such treatments can lower β-glucan's solubility or bring about depolymerization of the molecule, causing it to become less physiologically effective, though no clear conclusions have been drawn (Gallaher et al 1993, Wood et al 1994, Yokoyama et al 1998, Wood et al 2000). For instance, Keogh et al (2003) and Kerckhoffs et al (2003) did not show positive results upon incorporation of β-glucan into various food products, which was mainly attributed to changes in the physicohemical properties of β-glucan. However, our understanding of such changes in various food systems is limited.

In the interest of developing a food product that effectively imparts the benefits of β-glucan, it is important to investigate such products with the level of β-glucan addition most likely to be utilized by the food industry. Thus, due to the marketability of an associated health claim on the product package and the high cost of fortification, it is likely that commercial production of β-glucan-fortified products will incorporate the minimum amount, 0.75 g β-glucan/serving, deemed necessary by the FDA (2005). It is also important to consider formulations that have the ability to impart the beneficial effects of β-glucan on the greatest number of people. Thus, bread, a staple food in North America, which also has a wide and well established share of the food market, shows great potential for β-glucan incorporation. However, previous studies have indicated that the beneficial

effects of β-glucan are decreased when incorporated into the bread system (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008) and that β-glucan addition may be detrimental to bread loaf volume (Cavallero et al 2002, Gujral et al 2003, Trogh et al 2004), though quality defects may be improved with vital gluten addition (Gujral et al 2003, Mohamed et al 2008). However, the majority of these studies have utilized highly soluble β-glucan extracts and/or incorporated β-glucan at levels that are substantially higher than those likely to be adopted by the food industry, in addition to utilizing experimental methods that are not reflective of the physiological system in which the desired health benefits are hoped to be seen.

The journey of β-glucan through the mixing, fermenting, baking, and storage of bread using low solubility β-glucan concentrate obtained using a new technology (Vasanthan and Temelli 2009), evaluated under approximated physiological conditions, has not been fully investigated, particularly at levels of addition most likely to be presented to consumers. It is essential to identify the step(s) within the bread production regime that are detrimental to β-glucan so recommendations can be made to help maintain its highest quality. In addition, consumer desire for high quality products cannot be overlooked, as an inferior product, despite its nutritional value, will not be consumed. For successful product development, the quality and consumer acceptability of fortified bread should be well characterized. Providing information regarding the FDA approved health claim, which would accompany such products in a retail setting, may also

play an important role in acceptability. Therefore, due to the importance of developing a widely accepted food product with health benefits, the main objectives of this PhD thesis were:

1. to investigate the effect of formulation and processing treatments on the solubility and viscosity of β-glucan, extracted under approximated physiological conditions, upon incorporation into bread dough at levels likely to be utilized by the food industry (Chapter 3),
2. to identify the impact of β-glucan and gluten addition on the mixing characteristics, rheological properties, and microstructure of bread dough, (Chapter 4).
3. to determine the effect of baking on the solubility and viscosity of β-glucan upon incorporation into bread and also its impact on the bread's physicochemical properties (Chapter 5),
4. to assess the effect of storage conditions, similar to those seen in the market place and also in clinical studies, on the solubility and viscosity of β-glucan in bread, as well as β-glucan's impact on bread quality characteristics (Chapter 6),
5. to evaluate the impact of β-glucan concentrate incorporation into the bread system on its physiological effectiveness, in terms of perceived satiety, glycemic response and *in vitro* reducing sugar release (Chapter 7), and
6. to investigate the consumer acceptability and purchase intent of bread fortified with β-glucan concentrate at levels likely to be utilized at

commercial scale in the presence and absence of health information (Chapter 8).

1.1. REFERENCES

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Beer, M.U., Wood, P.J., Weisz, J., Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-glucan extracted from oat products by an *in vitro* digestion system. Cereal Chem. 74(6):705-709.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr. 69:55-63.

Canadian Diabetes Association. 2007. The Prevalence and costs of diabetes. Accessed September 20, 2007. http://www.diabetes.ca/Section_About/prevalence.asp

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chem. 102:889-897.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Food and Drug Administration. 2005. Food labeling: health claims; soluble dietary fibre from certain foods and coronary heart disease; Final Rule Federal Register Doc. 2004P-0512, filed 12-23-05.

Food and Drug Administration. 1997. Food labeling: health claims; oat and coronary heart disease; Final Rule Federal Register Doc. 97-1598, filed 1-22-97.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

Gallaher, D.D., Locket, P.L., Gallaher, C.M. 1992. Bile acid metabolism in rats fed two levels of corn oil and brans of oat, rye, and barley and sugar beet fibre. J Nutr. 122:473-481.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. J Nutr. 123:244-252.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Heart and Stroke Foundation. 2007. Incidence of cardiovascular disease. Accessed September 20, 2007. http://ww2.heartandstroke.ca/Page.asp ?PageID=33&ArticleID=1077&Src=news &From= SubCategory

Jackson, K.A., Suter, D.A.I., Topping, D.L. 1994. Oat bran, barley, and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J Nutr. 124:1678-1684.

Keogh, G.F., Cooper, G., Mulvey, T.B., McArdle, B.H., Coles, G.D., Monro, J.A., Poppitt, S.D. 2003. Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease reisk factors in mildly hypercholesterolemic men. Am J Clin Nutr. 78:711-718.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Martinez, V.M., Newman, R.K., Newman, C.W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. J Nutr. 122:1070-1076.

Mohamed, A., Rayas-Duarte, P., Xu, J. Hard Red Spring wheat/C-TRIM 20 bread: Formulation, processing and texture analysis. Food Chem. 107:516-524.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., Hofer, P.J. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Temelli, F., Bansema, C., Stobbe, K. 2004. Development of an orange-flavored barley β-glucan beverage. Cereal Chem. 81(4):499-503.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Wang, L., Behr, S.R., Newman, R.K., Newman, C.V. 1997. Comparative cholesterol-lowering effects of barley β-glucan and barley oil in golden Syrian hamsters. Nutr Res. 17(1):77-88.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Wood, P.J., Beer, M.U., Butler, G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnlund, J.R., Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74(3):293-296.

Yokoyama, W.H., Knuckles, B.E., Stafford, A., Inglett, G. 1998. Raw and processed oat ingredients lower plasma cholesterol in the hamster. J Food Sci. 63(4):713-715.

Chapter 2

LITERATURE REVIEW

2.1. BARLEY

Barley (*Hordeum vulgare*) is a versatile cereal crop that can be grown in a wide range of soil and environmental conditions. It accounts for 12% of the world's cereal production, ranking fourth after wheat, rice, and corn (Jadhav et al 1998). Canada is the second largest barley producer in the world (Alberta Barley Commission 2008), with Alberta contributing almost 50% of the total production (Statistics Canada 2001). Of this total, 75% of barley is used for feed, 25% is used for malting, and less than 5% is used in food products (Bhatty 1986) such as baby food or pearled barley in soups. Though past research on barley has mainly focused on its malting and brewing properties (Bathgate et al 1974, Bamforth et al 1982, Henry 1988, Brennan et al 1996, Brennan et al 1997, Edney et al 1998, Yadav et al 2001, Haraldsson et al 2004), current research is renewing interest in this grain as a highly nutritious commodity due to realization of its attributed health benefits. Despite barley's abundance as a primary crop in North America and its nutritional qualities, this cereal is vastly underutilized in human food production. However, the mounting research supporting its health benefits and the development of processing techniques to further utilize the kernel and its components may hold the key to unlocking barley's enormous potential within the food industry.

2.1.1. STRUCTURE

The barley grain is comprised of four physiological parts: the hull, the bran, the endosperm, and the germ. The hull segment consists of the husk, which is the outermost layer of the barley seed. This portion comprises 23% (w/w) of the kernel and completely covers the grain with its leaf-like structures termed the lemma and the palea (Magness et al 1971). The husk is firmly attached to the kernel by the cementing layer called the pericarp. Prior to processing, the husk, which is primarily composed of insoluble dietary fibre, is generally removed. Alternatively, hull-less varieties are available and are characterised by a weakly attached husk due to the significantly smaller amount of pericarp. As a result, the husk of hull-less barley tends to fall off during harvesting.

The bran is composed primarily of the aleurone and subaleurone layers that represent 8-13% (w/w) of the grain. In addition to fibre components, the bran portion also contains lipids and protein, though much of the protein is concentrated in the aleurone layer, contained in vacuoles called aleurone grains (MacGregor and Fincher 1993). The endosperm portion is the largest, comprising 75-80% (w/w) of the kernel. This tissue is the primary storage area for starch, housing approximately 75-85% of the kernel's supply (Lekhi 2004). This segment also contains protein, which surround the starch granules in a protein matrix primarily in the peripheral cells of the endosperm (Hoseney 1994). These proteins are mainly storage proteins, the hordeins and the glutelins, each representing 35-45% of the total proteins in the grain (Lekhi 2004). The

endosperm also houses the majority of the grain's β-glucan, which makes up 70% of the cell walls in the endosperm (Jadhav et al 1998).

The final segment, the germ, comprises only 2-4% of the kernel (MacGregor and Fincher 1993). This portion is high in lipids (13-17%) and proteins (34%) and houses the majority of the nonsaponifiable constituents, namely the tocopherol components of vitamin E (Newman and Newman 1991, Lekhi 2004).

2.1.2. COMPOSITION

The primary constituents of the barley kernel are starch (52-71%), protein (8-13%), lipids (2-3%), and non-starch polysaccharides, of which most notable is the β-glucan portion that accounts for 3-11% (Bhatty 1992, MacGregor and Fincher 1993). As mentioned previously, the endosperm houses the majority of the starch and β-glucan, while the protein is more concentrated within the aleurone layer. The germ is characteristically high in lipids. Depending on the growing conditions and genetic factors, however, variations in the proportions of each constituent may exist. For instance, Oscarsson et al (1997) found that by increasing the nitrogen fertilization rate of barley kernels, the crude protein content of the grain increased. In terms of genetic factors, waxy barley varieties (low amylose, high amylopectin) and the high amylose varieties exhibit higher β-glucan contents than normal starch varieties (Andersson et al 1999, Elfverson et al 1999).

2.2. β-GLUCAN

2.2.1. PHYSICOCHEMICAL PROPERTIES

β-Glucan is a soluble fibre found in appreciable amounts in both barley (3-11%) and oats (3-7%) (Bhatty 1992, Lee et al 1992, Skendi et al 2003). Barley β-glucan is found primarily in the cell walls of the endosperm (Miller and Fulcher 1994). Structurally, it is a (1→3)(1→4)-β-D-glucan with runs of consecutive (1→4)-linked residues forming cellulose-like sections separated by single (1→3)-links (Lazaridou and Biliaderis 2007). Though most of the cellulose-like sections are composed of trimers and tetramers, longer sections ranging from 4 to 14 residues have also been reported (Cui et al 2001, Lazaridou et al 2003). Interruption of the cellulose-like sections by the (1→3)-linkages creates a kink in the structure, permitting β-glucan to form viscous solutions upon solubilization through hydrogen bonding between water molecules and β-glucan's numerous hydroxyl groups. Thus, β-glucan is classified as a soluble dietary fibre.

The structural characteristics of β-glucan can be inferred using the ratio of cellotriosyl to cellotetraosyl residues, which are commonly referred to as DP3 and DP4 oligomers (Fig. 2-1). These oligomers are the primary hydrolysis products released upon β-glucan hydrolysis by lichenase. The ratio of DP3 to DP4 for barley is between 1.8-3.5 (Lazaridou and Biliaderis 2007), which is higher than that of oat (Lazaridou et al 2003). The molecular weight of barley β-glucan has been reported to be in the range of 31-2700 x 10^3, where the large discrepancy in size is due primarily to different environmental conditions, extraction procedures and analytical techniques (Lazaridou and Biliaderis 2007). The higher end of this

molecular weight range may be indicative of the pre-processing molecular weight of β-glucan in the original grain.

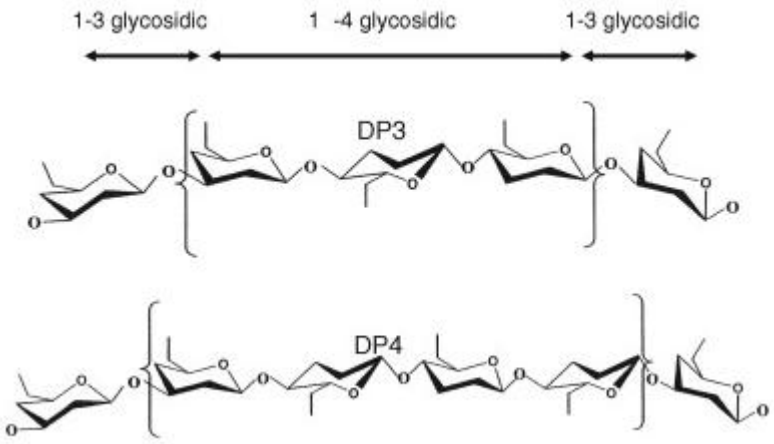


Fig. 2-1 Structure of cereal β-glucan (Vasanthan and Temelli 2008)

The viscosity of β-glucan in solution is influenced by its concentration, temperature and the length of the β-glucan chain, with the longest chains resulting in the highest viscosity. Viscosity is the resistance to flow, described as the ratio of shear stress to shear rate for Newtonian fluids, and is a common way to characterize liquids. At concentrations above 0.27%, β-glucan solutions typically exhibit non-Newtonian behavior (Ren et al 2003). This behaviour is described as pseudoplastic, meaning viscosity decreases when the solution is subjected to increasing rates of shear. This is a reversible process provided the shear rate is not high enough to degrade the β-glucan molecule. Advantages exist in industry if the pseudoplastic nature of β-glucan gum is manipulated properly, as utilization of high shear rates, without degrading the molecule, will allow the gum solutions to be transported through pipes with the application of less force. As such, when

the gum solution reaches its target location, decreasing the shear rate will allow this solution to return to its original viscosity. Concentration of β-glucan in solution is dictated by its solubilization from a starting material whether it is the grain, β-glucan conccnetrate or a food product fortified with β-glucan. Throughout this thesis β-glucan solubility refers to the extractability of β-glucan from a given material under a defined set of conditions.

Upon storage, β-glucan has also been shown to self associate and gel under certain conditions. Intra and intermolecular bonding between β-glucan molecules may occur due to reorientation of β-glucan molecules within solution to promote hydrogen bonding with each other on the linear, cellulose-like portions of each molecule (Fincher and Stone 1986), or, as more recently proposed, via association of consecutive cellotriose units linked by (1→3) bonds (Böhm and Kulicke 1999, Lazaridou et al 2004, Tosh et al 2004). The gelling ability of β-glucan relates to its molecular weight and structural characteristics as those with low molecular weight and increased cellotriosyl (DP3) units show greater gelling ability (Lazaridou et al 2003 and 2004, Vaikousi et al 2004).

2.2.2. PHYSICAL FUNCTIONALITY

The functional properties of barley β-glucan include its water binding, thickening, foam and emulsion stabilizing, and gelling capacities. As such, β-glucan shows great potential for inclusion into food products. β-Glucan is a hydrocolloid, which means it is able to bind exceptionally large amounts of water. Hydrocolloids with numerous hydroxyl groups in the structure exhibited the greatest water absorption values due to interactions between the hydrocolloid and

water through hydrogen bonding (Rosell et al 2001, Lazaridou et al 2007). β-Glucan has high water binding capacity due to an abundance of hydroxyl groups within its structure. This functional property allows for improved moisture content and retention upon incorporation into food products, which is useful within the food industry to increase product yield as an extender. Hydrocolloids are also widely used to thicken food systems. The ability of β-glucan to increase viscosity of aqueous solutions dictates its thickening capabilities. Recent research (Lyly et al 2003 and 2004) indicates that increased molecular weight of β-glucan in solution increases viscosity and also the perceived thickness of the solution upon sensory evaluation.

Little has been published on the foam and emulsion stabilizing capabilities of β-glucan. Hydrocolloids are added to foams and emulsions in order to increase the viscosity of the continuous phase (Temelli 1997). The increased viscosity of the aqueous phase slows down the movement of air bubbles in foams and oil droplets in emulsions, reducing coalescence and increasing the stability of the system. Previous research (Temelli 1997) indicated that β-glucan shows potential as a foam and emulsion stabilizer, though other components present in β-glucan concentrates, possibly starch, pentosans, or proteins, likely also contribute to the stability of these systems. Burkus and Temelli (2000) showed that volume and stability of foams significantly increased when barley β-glucan gum was used as a stabilizer. The mechanism of stabilization was also shown to result from increases in the viscosity of the aqueous phase (Burkus and Temelli 2000). The length of the β-glucan chain influences viscosity and emulsion capacity, with

longer β-glucan chains requiring longer to form stabilizing networks (Burkus and Temelli 2000). In addition, Kontogiorgos et al (2004) showed that low molecular weight β-glucan may also influence emulsion stability through network formation in the continuous phase.

The formation of a 3-dimensional continuous network resulting from associations and cross-linking between polymer chains, which is capable of trapping and immobilizing liquid and is resistant to flow under applied force, is referred to as gelation (Glicksman 1982). The gelling characteristics of barley β-glucan concentrate were described by Burkus and Temelli (1999). Low viscosity β-glucan was shown to gel at concentrations ≥5% and gel strength increased with concentration. In addition, gel setting at ambient conditions was optimal compared to setting at 4^{o}C, while β-glucan hydration temperature did not affect gelling capacity. Overall, β-glucan's functional properties may allow for its use a thickener, extender, emulsifier, and stabilizer in a variety of food products including sauces, soups, salad dressings, desserts, and meat products, among others. In addition to β-glucan's ability to impart a variety of important structural and textural aspects to food products, its incorporation would also improve nutritional value due to its classification as a soluble dietary fibre.

2.2.3. PHYSIOLOGICAL FUNCTIONALITY

2.2.3.1. CHOLESTEROL REDUCTION AND ROLE IN HEART DISEASE

Cereal β-glucan has a well established cholesterol lowering effect in both animals and humans (Newman and Newman 1991, Ranhotra et al 1991, Gallaher

et al 1992, Malkki et al 1992, Martinez et al 1992, Newman et al 1992, Kahlon et al 1993, Jackson et al 1994, Wang et al 1997, Bourdon et al 1999, Delaney et al 2003, Behall et al 2004, Karmally et al 2005, Naumann et al 2006, Queenan et al 2007). The mechanism of β-glucan's cholesterol lowering action is thought to be multifactorial. Lupton and Turner (2000) suggest that the most important characteristics of soluble fibre are its viscosity and fermentability in the colon. Due to its viscosity, β-glucan may delay gastric emptying, resulting in a more uniform presentation of the meal to the small intestine for digestion and absorption, and also delay the absorption of nutrients from the small intestine by entrapping nutrients and reducing access of digestive enzymes to nutrients (Lupton and Turner 2000).

The benefits of delayed nutrient absorption include not only an improvement in blood sugar control, but also a subsequent decrease in serum cholesterol levels. When glucose is absorbed in small amounts over an extended period, such as the case with viscous fibres, the insulin response is reduced (Pick et al 1996). This is because high amounts of glucose appear to trigger sustained insulin secretion, which stimulates 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity (the rate limiting enzyme in cholesterol synthesis) (Lupton and Turner 2000). Therefore, it appears that this soluble fibre may have the ability to reduce blood cholesterol levels via its normalizing effect on blood sugar (Lupton and Turner 2000).

β-Glucan may also decrease blood cholesterol levels by interfering with cholesterol absorption and by increasing the excretion of bile acids from the body

by entrapping them within the viscous digesta (Story and Kritchevsky 1976, Story and Lord 1987, Lia et al 1995). Because bile acids are normally reabsorbed within the intestine and recirculated, new bile acids are synthesized from cholesterol to restore those that were lost, subsequently lowering the body's cholesterol pool. In addition, sequestration of bile acids also causes less to be available for micelle formation, thereby reducing the absorption of cholesterol and triglycerides (Lia et al 1995). An additional hypothesis is the production of the short-chain fatty acids such as propionate from the fermentation of β-glucan in the colon (Lupton and Turner 2000). Propionate, which is absorbed from the colon through the portal vein, has been shown by some investigators to inhibit HMG CoA reductase, the rate limiting enzyme in the production of cholesterol mentioned previously (Lupton and Turner 2000).

The cholesterol lowering effect of β-glucan is particularly important today as there is a causal relationship between high blood cholesterol and heart disease (Heart and Stroke Foundation 1999). Due to the substantial body of evidence supporting β-glucan's cholesterol lowering effects, the U.S. Food and Drug Administration (FDA 2005) has approved a health claim for the association between the consumption of products containing β-glucan from barley and a reduction in the risk of heart disease, which is an extension of the health claim originally approved for β-glucan from oats (FDA 1997). This claim is based on the consumption of at least 3 g of β-glucan per day or 4 servings of a food product that supplies at least 0.75 g of β-glucan per serving (FDA 2005). However, criticism of the health claim exists because many of the studies assessed by the

FDA did not even adequately measure the amount of β-glucan present in the products evaluated or characterize its physicochemical properties (Ripsin et al 1992). Approval of a similar claim in Canada is currently under review by Health Canada.

Heart disease is the leading cause of death in Canada, accounting for 37% of total deaths, $7.3 billion in total direct, and $12.3 billion in total indirect health care costs (Heart and Stroke Foundation 1999). High blood cholesterol is shown to cause and worsen heart disease (Heart and Stroke Foundation 1999). The National Cholesterol Education Program (Watson 1987) recommends that dietary treatment for high cholesterol levels should be undertaken for a minimum of six months before deciding whether to begin pharmaceutical treatment. Therefore, β-glucan may provide a non-pharmacological means of lowering blood cholesterol for the eight in ten Canadians at risk of heart disease (Heart and Stroke Foundation 2003).

2.2.3.2. BLOOD SUGAR REGULATION AND ROLE IN DIABETES

β-Glucan has been shown to reduce glycemic response (Braaten et al 1991, Wood 1994, Yokoyama et al 1997, Bourdon et al 1999, Panahi et al 2007, Kim et al 2009). Though the mechanism is not completely understood, it is believed that β-glucan's ability to increase the viscosity of the digesta may slow gastric emptying, resulting in a more uniform presentation of the meal to the small intestine for absorption (Lupton and Turner 2000). In addition, β-glucan may interfere with the digestion and absorption of nutrients from the small intestine due to increased viscosity of the digesta by impeding digestive enzyme access to

carbohydrates within the meal. This would subsequently reduce products of digestion and/or their diffusion to the surface of the small intestine for absorption, thereby decreasing glycemic response (Flourie et al 1984, Lupton and Turner 2000).

The incidence of diabetes amongst our population is alarming and growing. Currently, diabetes is estimated to affect more than two million Canadians and more than twenty million Americans. Only 10% of these numbers is currently attributable to Type 1 diabetes, which is usually diagnosed in children and adolescents and is not preventable (CDA 2006, ADA 2006). However, Type 2 diabetes usually develops in adulthood around the age of 40, though the incidence of this disease in younger populations, particularly in children, is increasing as well (CDA 2003). In the Type 2 diabetic, the pancreas is usually not producing enough insulin or the body is not using its insulin properly (CDA 2006). As a result, the unused glucose builds up in the blood instead of being used by the body's cells for energy (CDA 2006).

β-Glucan's ability to normalize blood sugar response is very important as randomized, controlled trials have provided undeniable evidence that long-term complications of diabetes mellitus can be reduced by tight blood sugar control (CDA 2003). When compared to a more relaxed approach to blood sugar management in those with Type 2 diabetes, intensive treatment regimes aimed at lowering blood sugar values to a constant, normal level have been associated with a reduction in microvascular complications (Ohkubo et al 1995, UKPDS 1998). Such complications include problems related to vision loss and eventual legal

blindness due to retinopathy (Klein and Klein 1995), a decline in kidney function, as diabetic nephropathy is the most common cause of kidney failure in North America (CORR 2001), and the frequent pain of nerve dysfunction from neuropathy (Partanen et al 1995). The cellular damage responsible for such complications is thought to be induced by prolonged high blood sugar levels causing irreversible alterations of structural proteins, oxidative damage, and decreased blood flow (Taguchi and Brownlee 2003). In addition, more than 80% of people with diabetes will die as a result of heart disease or stroke, with diabetes considered a contributing factor in the deaths of approximately 41,500 Canadians each year (CDA 2006).

The financial burden of diabetes on the individual and the Canadian healthcare system is also enormous. Diabetes and its complications are estimated to cost the Canadian health care system approximately $13.2 billion every year, with estimates that this figure will reach $15.6 billion by 2010 and $19.2 billion by 2020 (CDA 2006). Daily consumption of soluble dietary fibre, like β-glucan, can help to reduce the risk of Type 2 diabetes (Wursch and Pi-Sunyer 1997). However, despite the preventability of Type 2 diabetes, its prevalence is expected to increase by as much as 50% by the end of the decade (CDA 2006).

2.2.3.3. IMPROVED SATIETY AND ROLE IN OBESITY

Due to its soluble nature, β-glucan may also possess the ability to increase feelings of satiety (Lupton and Turner 2000, Lyly et al 2009, Stevenson and Inglett 2009, Willis et al 2009) due to prolonged gastric distention resulting from a reduction in gastric emptying rate based on the increased viscosity of the digesta

(French and Read 1994, Lupton and Turner 2000). Satiety may also be related to blood sugar levels since reductions in blood sugar increase feelings of hunger (Mayer 1955, Chaput and Tremblay 2009). Therefore, by slowing gastric emptying and causing a more uniform presentation of the meal to the small intestine for absorption β-glucan may be important in appetite control (Mayer 1955, Holt et al 1992, Chaput and Tremblay 2009).

Because β-glucan may improve satiety and produce a more leveled blood glucose curve upon consumption, it may be helpful in weight loss and preventing weight gain due to improvements in appetite control (Holt et al 1992). The prevention of overweight and obesity is becoming increasingly important today as nearly 60% of Canadian adults (Statistics Canada 2004) and greater than 65% of Americans (Tjepkema 2005, Ogden et al 2006) are considered overweight or obese. Obesity is a direct risk factor for heart disease (Heart and Stroke Foundation 1999) and Type 2 diabetes (CDA 2003). To be considered overweight or obese an individual must have a calculated body mass index (a simple calculation involving weight in kilograms divided by height^2 in meters) exceeding 24.9 or 29.9, respectively. Obesity and overweight are becoming more and more common amongst our population.

2.2.3.4. EFFECTIVENESS OF β-GLUCAN IN ESTABLISHING HEALTH BENEFITS

As discussed previously, β-glucan's key health benefits are thought to be primarily dependant on its viscosity. The viscosity of β-glucan in solution is mainly controlled by concentration and its molecular weight (Wood et al 2000).

As such, conditions that lower β-glucan's solubility or bring about depolymerization of the molecule may cause it to become less physiologically effective, though no clear conclusions have been drawn (Gallaher et al 1993, Wood et al 1994, Yokoyama et al 1998, Wood et al 2000, Smith et al 2008). Recent studies (Östman et al 2006, Lan-Pidhainy et al 2007, Tosh et al 2008) owe support to the role of β-glucan solubility, depolymerization and viscosity in glycemic response, while the impact of these variables on serum cholesterol is less clear (Jenkins et al 1978, Braaten et al 1994, Frank et al 2004, Keenan et al 2007, Smith et al 2008). For instance, Wood et al (1994), Östman et al (2006), and Panahi et al (2007) demonstrated that the ability of β-glucan to lower postprandial glycemia is strongly correlated with its viscosity. Wood et al (1994) also highlighted the dependence of blood glucose reduction on β-glucan dose. Wood et al (2000) showed that blood glucose response is also dependent on β-glucan molecular weight and its concentration within solution, while others highlighted β-glucan solubility as an important factor (Lan-Pidhainy et al 2007). Because all of these measures are interdependent, it is essential to characterize these variables in order to gauge the physiological effectiveness of β-glucan. Various conditions, such as β-glucan extraction procedures (Vasanthan and Temelli 2008) or incorporation into different food matrices (Brennan and Cleary 2005), may lower β-glucan solubility or bring about depolymerization of the molecule. As such, attention must be paid to ensure that β-glucan quality in the raw material is maintained throughout processing. Failure in the past by clinicians to properly characterize the physicochemical properties of the β-glucan

and food products being studied may explain why some of the clinical trials evaluated by the FDA prior to the approval of the health claim for the association between the consumption of products containing β-glucan and a reduction in the risk of heart disease showed no significant reduction in serum cholesterol upon consumption. Recently, Keogh et al (2003) and Kerckhoffs et al (2003) did not show reductions in serum cholesterol upon incorporation of β-glucan into various food products including, waffles, cookies and bread. However, despite reductions in β-glucan molecular size, it has still been shown to improve plasma cholesterol, glucose and insulin responses in some studies (Sundberg et al 1995, Yokoyama et al 1998, Wilson et al 2004, Keenan et al 2007, Smith et al 2008). It is clear that more research within this area is needed to fully understand the effect of the physicochemical properties of β-glucan on its physiological functionality.

Various methods exist to identify the effect processing and storage conditions have on the viscosity and physiological effectiveness of β-glucan. Laboratory measures of β-glucan molecular weight and viscosity have been used as a means of estimating its physiological effectiveness and also the effect various processing conditions have on the molecule (Beer et al 1997, Burkus and Temelli 1998, Wood et al 2000). However, it is important to extract β-glucan utilizing experimental methods that are reflective of the physiological system in which the desired health benefits are hoped to be seen, such as *in vitro* extraction under approximated physiological conditions, which is designed to simulate the conditions of the digestive tract. The glycemic index and glycemic response both evaluate the effect different carbohydrate containing foods have on blood sugar

levels in comparison to a standard food (commonly glucose or white bread). These measures are affected by the viscosity of the guts contents. Thus, measurements of the glycemic index and glycemic response of β-glucan containing foods that have been subjected to various treatments may be an effective method to establish the physiological effectiveness of these products.

2.2.3.5. OTHER BENEFITS

Recent research has shown that β-glucan may also be important as a prebiotic (Snart et al 2006). Prebiotics are substances that pass undigested through the small intestine and into the large bowel where they may become sources of carbon and energy for the intestinal microflora and promote the growth of beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli* (Gibson and Roberfroid 1995). Cereal β-glucan may also have immune stimulating effects (Yun et al 1997, Yun et al 1998, Davis et al 2004), which were previously thought to only be elicited by yeast and fungal β-glucan due to differences in structure. Additional studies are needed, however, to further support these health benefiting roles in humans.

2.2.4. EXTRACTION OF β-GLUCAN

Barley and oats are both good sources of β-glucan and comprise the industry's main source of this valuable component (Bhatty 1992, Lee et al 1992, Skendi et al 2003). Interest in the production of β-glucan-fortified foods is growing, however, incorporation of large amounts of barley or oat flour needed to fortify products with a sufficient amount of β-glucan to meet the guidelines set out by the FDA for use of the health claim can lead to quality defects. Therefore,

different commercial technologies (Vasanthan and Temelli 2008) have been developed to obtain more concentrated sources of β-glucan and thus help reduce or even eliminate any negative effects on quality. Dry separation processes, including pearling and air classification of meal or flour, are commonly employed to help concentrate β-glucan. These methods are relatively simple and inexpensive, though the β-glucan content of the fibre concentrates is usually ≤30% (Vasanthan and Bhatty 1995, Zheng et al 2000). In order to obtain much higher β-glucan concentrations wet separation techniques have traditionally been used. Such processes utilize water, acidified water and/or aqueous alkali as solvents to hydrate and solubilize the β-glucan from the cell walls of barley or oat. Solubilization is followed by centrifugation and ethanol precipitation in order to separate the β-glucan from the slurry (Vasanthan et al 2004). Though very high β-glucan concentrations are achieved with this method, many technical problems exist with extraction procedures that focus on the solubilization of β-glucan. Such disadvantages include high susceptibility of the solubilized β-glucan to enzymatic degradation by the grain's endogenous β-glucanases in the aqueous system and β-glucan's susceptibility to shear induced molecular fragmentation during the mixing and centrifugation steps (Vasanthan et al 2004). In addition, similar degradation may be seen upon its incorporation into a variety of food matrices due to enzymes present in the food preparation and/or during mixing and processing steps (Gallaher et al 1993, Wood et al 1994, Wood et al 2000, Frank et al 2004, Pins and Kaur 2006). Both enzymatic degradation and shear fragmentation would lead to a reduction in β-glucan molecular weight and, as a result, its viscosity. β-

Glucan extracts obtained using such methods show lower viscosity upon resolubilization in water (Panahi et al 2007). Aqueous extraction techniques also come with a high cost at commercial scale due to large water and ethanol requirements, which has also impeded the wide-spread use of β-glucan in common food products (Vasanthan and Temelli 2008). Despite these disadvantages, research has relied heavily on the use of solubilized β-glucan extracts to achieve appropriate levels of fortification.

Recently, Vasanthan and Temelli (2009) introduced a new technique that did not require the solubilization of β-glucan for its isolation. Instead this aqueous ethanol based enzymatic process was designed to concentrate β-glucan in its native form (Vasanthan and Temelli 2009). This new technology yields a final product of β-glucan-rich cell wall fibre particulates with up to 65% β-glucan (Vasanthan and Temelli 2009). The structure of the barley β-glucan concentrate is displayed in Figure 2-2. Because the β-glucan is not solubilized and remains intact within the cell walls, it is protected from shear fragmentation and enzymatic degradation. Thus, the final product exhibits high molecular weight and solution viscosity (Vasanthan and Temelli 2009). To concentrate the β-glucan, flour is slurried with aqueous ethanol, allowing the β-glucan to remain intact within the cell walls. The slurry is also treated with enzymes to remove the flour's protein and starch. The β-glucan-enriched cell walls are then recovered by filtration and/or centrifugation. Barley β-glucan concentrates obtained using this technology can have low solubility under physiological conditions and require further treatment to enhance its solubility, whereas those obtained using

traditional techniques have high solubility at room and body temperature. However, both would completely solubilize upon heating at 85^{o}C for 1 hr. Recent studies (Panahi et al 2007) emphasize the importance of extraction methods that preserve viscosity in order to increase β-glucan's efficacy as a physiologically functional ingredient.

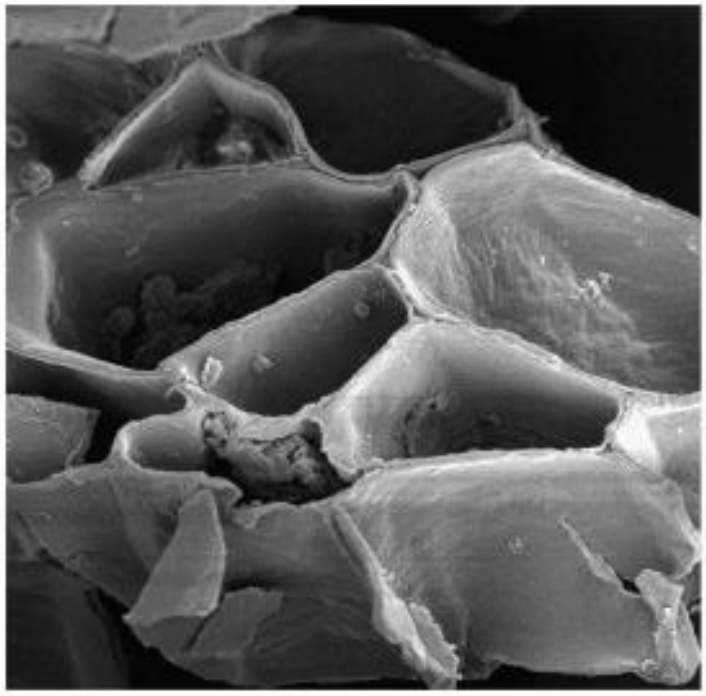


Fig. 2-2 Scanning electron micrograph of barley β-glucan concentrate obtained via aqueous-alcohol based enzymatic extraction (Vasanthan and Temelli 2008)

2.3. FORTIFICATION OF FOOD PRODUCTS WITH β-GLUCAN

Due to its health benefits, functional properties, and the availability of concentrated extracts, there is growing interest in the fortification of foods with β-glucan. As such, β-glucan shows promise in various food systems including beverages, meat products, and bakery products, among others. Newman et al (1998) incorporated a barley shorts fraction to double the soluble dietary fibre

content of biscuits, sugar cookies, and muffins. The addition of barley fibre to muffins was also investigated by Hudson et al (1992), who showed that the maximum level of barley fibre incorporation was 40% to avoid a sticky, hard to mix dough.

β-Glucan has a smooth mouthfeel that makes it well suited for use in beverages (Temelli et al 2004). Temelli et al (2004) formulated an orange-flavored beverage utilizing barley β-glucan at levels of 0.3, 0.5, and 0.7% (w/w). Analysis of the physical characteristics of the beverage and sensory evaluation by trained panelists indicated that the viscosity increased with gum concentration (Temelli et al 2004), though panelists did not detect an increase in viscosity for beverages that contained up to 0.5% β-glucan (Temelli et al 2004). The addition of β-glucan did not suppress the flavor intensity likely because the neutral nature of the β-glucan presented less opportunity for it to bind flavor components (Temelli et al 2004). The addition of β-glucan to a beverage containing whey protein isolate (WPI) from milk has also been investigated (Temelli et al 2004). An antifoaming agent had to be utilized in the formulation as β-glucan increased the stability of the whey protein foams (Temelli et al 2004). β-Glucan has also been added to low-fat ice cream, yogurt (Gee 2005, Brennan et al 2002) and cheese (Tudorica et al 2004) to help improve textural aspects.

β-Glucan has been tested in meat products. Morin et al (2002) incorporated β-glucan into reduced-fat breakfast sausage. Due to β-glucan's highly viscous nature, gelling capacity, foam and emulsion stabilizing capabilities (Temelli 1997) and lubricating mouthfeel, resulting from its pseudoplastic nature (Glicksman

1991), it is possible to use it as a fat replacer in processed foods. At a level of 0.3% β-glucan, the reduced fat sausages (12% fat) were similar in firmness to the high-fat control sausages (22% fat), while at 0.8% inclusion, the sausages were evaluated by the consumer panel as being significantly ($p \leq 0.05$) less firm than the control (Morin et al 2004). β-Glucan increased the water-binding ability of the meat system while not interfering with the protein structure (Morin et al 2002), which resulted in a higher cook yield (Morin et al 2004).

Extrusion cooking may present an additional option for utilizing β-glucan in food products. Extrusion cooking is a popular food processing technique especially in the production of fibre-rich products such as breakfast cereals, flat breads, and dextrinized or cooked flour, among others (Yeung 2001). Extrusion of barley flour increased the solubility of β-glucan, where solubility increased with increasing moisture levels (Yeung 2001). Yao et al (2006) formulated an extruded cereal using high β-glucan oats, though the experimental products were browner, harder, coarser, and crunchier than a control oat breakfast cereal. β-Glucan has also been incorporated into pasta by Marconi et al (2000) using barley pearling by-products. Although the pasta showed poor rheological properties, it was improved upon the addition of vital gluten.

Maintenance of β-glucan health benefits was also demonstrated for a variety of food matrices formulated with β-glucan. Inclusion of β-glucan into breakfast cereals, even at low levels (below 5%), was shown to reduce glycemic response by up to 50% (Tappy et al 1996). Cleary and Brennan (2006) found a decrease in *in vitro* sugar release of β-glucan-fortified durum wheat pasta. Additionally,

Hallfrisch et al (2003) reported that subjects fed pudding containing highly soluble β-glucan from oats and barley exhibited blood glucose responses that were significantly lower than those generated by pure glucose. Lan-Pidhainy et al (2007) reported a decrease in the area under the 2 hr blood glucose curve upon consumption of muffins with high levels of β-glucan. Bourdon et al (1999) showed a reduction in cholesterol concentration upon feeding men barley pasta enriched with β-glucan, while Braaten et al (1994) showed a similar response when β-glucan was consumed in a drink by subjects with hypercholesterolemia.

Despite the abundance of research supporting the health benefits of β-glucan, the effect of different food matrices and subsequent processing and storage treatments on its physiological effects is still not fully understood. Some food processing treatments have been shown to lower β-glucan solubility or bring about depolymerization of the molecule, reducing its physiological effectiveness (Wood et al 2000). For example, Kerckhoffs et al (2003) revealed that serum LDL cholesterol concentrations were substantially lower after consumption of orange juice but not bread and cookies containing nearly 6 g of β-glucan/day. Casiraghi et al (2006) showed that β-glucan incorporation into cookies and crackers affected the blood glucose and insulin responses positively, though cookies elicited a more favorable response. In addition, Beer et al (1997) evaluated the effect cooking and storage had on the solubility and the molecular weight of β-glucan incorporated into muffins. The results suggested that the *in vitro* extractability of β-glucan varies depending on the formulation, as the extractability rates of two muffin recipes differed by >25%. In addition, the

formulation and processing of both muffin types caused a reduction in β-glucan's molecular weight, decreasing its viscosity.

Storage of β-glucan-fortified food products may also impact its physiological effectiveness. After frozen storage of β-glucan-enriched muffins for eight weeks, there was no change in the molecular weight of β-glucan, though its extractability decreased by >50% in one muffin recipe and dropped to less than 25% in the other (Beer et al 1997). Lan-Pidhainy et al (2007) showed that subjecting the β-glucan-fortified muffins to 4 freeze-thaw cycles reduced β-glucan solubility from 30–40% in fresh muffins to just 10%. Following consumption, the blood glucose response was also significantly lower for fresh muffins than that after consumption of muffins treated with the freeze-thaw cycles. The amount of soluble β-glucan has been highly correlated with reductions in glycemic response (Makelainen et al 2006). The results indicate that the long-term, frozen storage of β-glucan-enriched products may cause a substantial decrease in the physiological effectiveness of the β-glucan (Beer et al 1997). Despite this, long term frozen storage is often employed for food products used in clinical trials.

The type of β-glucan extract or concentrate incorporated and consumed may also affect β-glucan's physiological response. Panahi et al (2007) showed that depending on the extraction conditions and the resultant quality of the β-glucan, differences in the glycemic response can be seen. Consumption of the β-glucan concentrate extracted under conditions that maintained its viscosity (alcohol-based enzymatic process) showed a greater reduction in glycemic response than the concentrate obtained using aqueous extraction (Panahi et al 2007). Other

studies have also correlated decreases in blood sugar response to the average viscosity of the β-glucan (Wood et al 2000, Juvonen et al 2009). However, conflicting results appear in the literature regarding the ability of low molecular weight β-glucan extracts to lower blood cholesterol upon incorporation into food products (Keogh et al 2003, Keenan et al 2007).

β-Glucan's ability to decrease blood sugar responses may be further optimized following its combination with other food ingredients. For instance, Behall et al (2006) showed that the consumption of resistant starch and β-glucan together improved the postprandial blood glucose levels in normal weight and overweight women at levels greater than that of each substance alone. However, it must be noted that the test meals, which combined both β-glucan and resistant starch, contained substantially more fibre than the tests conducted with each component individually. The amount of β-glucan incorporated into the food system will also affect the results. Kim et al (2006) showed that consumption of cooked cereal products containing 2 g of β-glucan significantly reduced postprandial glucose responses, in comparison to a control, while consumption of 1 g β-glucan did not. An obvious negative trend in blood glucose was also reported in response to increasing levels of β-glucan incorporation by Wursch and Pi-Sunyer (1997) and Tappy et al (1996) using breakfast cereal as the delivery vehicle.

2.3.1. β-GLUCAN IN BREAD

When deciding on foods to fortify with β-glucan, it is important to consider formulations that have the ability to impart the beneficial effects of β-

glucan on the greatest number of people. Thus, bread, a staple food in North America and which also has a wide and well established share of the food market, is an optimal choice for β-glucan incorporation as the benefits of β-glucan-fortified bread would have the potential to reach a huge segment of the population. However, as discussed previously, β-glucan's health benefits are primarily related to its viscosity, which is governed by the molecular weight of the β-glucan and the amount solubilized (Beer et al 1997). Unfortunately, reductions in β-glucan molecular weight and viscosity were reported upon incorporation into bread (Gallaher et al 1993, Wood et al 1994, Wood et al 2000, Frank et al 2004, Pins and Kaur 2006) and the beneficial effects of β-glucan may be decreased (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008). The amount of β-glucan incorporated into the products may help to attenuate the detrimental impact of β-glucan degradation in bread products. For instance, Cavallero et al (2002) showed a reduction in area under the 2 hr blood glucose curve upon fortification of bread products with 6.7% β-glucan, while fortification levels from 2.4-4.3% showed results similar to the control. In addition, Tosh et al (2008) only showed a reduction in area under the 2 hr blood glucose curve upon the consumption of 8 g, but not 4 g, of β-glucan incorporated into muffins.

A high correlation has been reported between the viscosity of *in vitro* digests from breads fortified with β-glucan and reductions in glycemic response (Östman et al 2006). In addition, breads with high and low molecular weight β-

glucan (4.5% β-glucan with ~11 g of total dietary fibre) have been shown to attenuate reducing sugar release 90 min into a 300 min *in vitro* digestion process in comparison to a control (Cleary et al 2007). Symons and Brennan (2004) also investigated the *in vitro* sugar release of white wheat breads formulated with 2.5 or 5% β-glucan-rich extracts from barley (70% β-glucan) and showed that 5% inclusion resulted in a significant decrease in the release of reducing sugars over a 300 min digestion process compared with the control, though substitution at 2.5% did not have an effect. The mechanism of this action is not clear, though Symons and Brennan (2004) hypothesize that this may be the result of the altered rheological properties of the pastes and doughs or possibly from the formation of a β-glucan gel matrix, which may have inhibited enzyme accessibility to partially gelatinized starch granules.

It is thought that the majority of β-glucan degradation in bread occurs during the mixing step as Andersson et al (2004) reported that there was no significant difference in the molecular weight of the β-glucan between the final dough and the baked bread. Cleary et al (2007) suggested that the enzymatic hydrolysis of β-glucan during bread processing was most likely due to enzymes present in the added yeast. However, the effect of contaminant enzymes within flour or enzyme preparations has also been established as a problem (Åman et al 2004, Andersson et al 2008). In fact, some researchers have recommended that fortification of bread products with β-glucan be abandoned due to the detrimental effect of native flour enzymes (Andersson et al 2004). Enzyme activity occurs very quickly within dough (Andersson et al 2004, Trogh 2004, Flander et al 2007)

and increasing bread dough fermentation time results in a greater reduction in β-glucan viscosity (Åman et al 2004, Andersson et al 2004, Trogh et al 2004, Andersson et al 2008).

Incorporation of highly soluble β-glucan extracts into bread results in degradation of the molecule. Therefore, fortification of bread products using solubilized β-glucan extract would warrant the initial recommendation by previous researchers to abandon the use of β-glucan in yeast-leavened bread in order to avoid substantial reductions in β-glucan's viscosity and health benefits (Andersson et al 2004). However, despite the reduction in β-glucan solubility and viscosity seen upon incorporation of β-glucan into bread, it is still unclear as to what extent the quality of β-glucan can be degraded and still maintain its physiological effects (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008). Furthermore, fortification of bread products using β-glucan concentrates of lower solubility has not been investigated and may allow for greater retention of β-glucan quality.

There are very few reports on the effect of storage conditions on β-glucan solubility and viscosity in food products. As mentioned previously, decreases in β-glucan solubility were seen upon long term frozen storage of muffins (Beer et al 1997) and in oat bran muffins upon increases in the number of freeze-thaw cycles (Lan-Pidhainy et al 2007). It is likely that similar effects will be seen within the bread system, though no research within this area has been performed.

2.3.1.1. BREAD DEVELOPMENT AND STORAGE

The primary ingredients in a typical wheat bread are flour, water, sugar, salt and yeast and development of bread structure is described in detail by Stear (1990). The initial stages of dough formulation involves first moistening flour particles and as mixing proceeds solubilization and swelling of the water soluble flour components occurs. Swelling of the prolamines and glutelins increases dough volume by 2-3 times. The starch granules also adsorb water (undamaged fraction taking up 30% of its own weight, resulting in a volume increase of about 75%). The swelling ability of the starch granules is more rapid than that of the proteins. The final stage of dough mixing involves restructuring of the gluten proteins, which are converted from swollen flour proteins by the energy of mixing into polypeptide chains that are aligned in a linear, film forming fashion. Inter- and intramolecular chemical bonds, hydrogen bonds, hydrophobic bonding, and the most important in dough formation being the breaking of disulphide bonds and their reformation, are created via the energy of continued mixing eventually building the viscoelastic gluten network. Wheat is unique in that it contains gluten, made up of glutenin and gliadin proteins, which is capable of retaining gases and leavening doughs. The gluten network makes up the solid phase of the dough, with starch granules and insoluble flour components embedded in it, while the liquid phase consists of water and the water-soluble dough components.

The formed dough is fermented using Baker's yeast (*Saccharomyces cerevisiae*) to convert sugar primarily to moisture and CO_2, though some ethanol

is produced, thereby contributing to leavening. A series of punching and molding steps are performed to ensure uniform air cell formation in the crumb. When fermentation is complete the dough is baked. Upon initial exposure to oven heat, the dough undergoes the final phases of swelling and solubilization. The outer surface of the bread reaches 100^{o}C in about 3 min when placed in an oven at 203^{o}C and rapidly increases to about 130^{o}C after 20 min (Stear 1990). The heat-transfer to the center of the crumb, which never exceeds 100^{o}C, is relatively slow due to the temperature gradient between the dough surface and the crumb center. From 30 to 40^{o}C, swelling, enzymatic activity, and yeast growth all accelerate. At 55^{o}C, the wheat starch begins to gelatinize, continuing until about 65^{o}C. To facilitate starch swelling and gelatinization, water migrates from other dough components to the starch granule, causing partial dehydration of the gluten, increasing its rigidity and causing the gluten strands to become increasingly more viscous and elastic. Protein coagulation occurs between 50 and 70^{o}C, which is also the range where the dough and yeast enzymes become thermally deactivated. The dough assumes a more rigid state at 70^{o}C resulting from protein coagulation and partial gelatinization of the starch. The maximum rate of moisture evaporation, starch gelatinization and coagulation of dough proteins occurs at 98-99^{o}C, progressively resulting in a baked crumb structure. At 100^{o}C, the majority of free moisture in the crumb evaporates through pores in the crust.

The quality shelf life of bread is quite short due to the staling process. Bread staling refers to changes that take place after baking other than spoilage by microorganisms (Bechtel et al 1953). Staling of bread is complex and not fully

understood. During storage the most pronounced changes are related to losses in moisture content and hardening of the crumb in bread (Guarda et al 2004), though flavour and aroma deteriorate as well (Eliasson and Larsson 1993). Firmness measurements of the crumb may be used as a method to determine the degree of staling in bread, though moisture content can be another measure since moisture loss is also thought to reduce quality (Stear 1990, Mohamed et al 2008, Kalinga and Mishra 2009). It is believed that bread staling is closely associated with starch retrogradation (recrystallization), involving the primary components of starch, namely amylose and amylopectin. Much of the amylose retrogradation occurs within the first day after baking (Kim and D'Appolonia 1977), though amylopectin retrogradation is thought to be the major cause of bread firming (Ribotta and Bail 2007). The progressive loss of moisture at room temperature occurs due to diffusion from the crumb to the crust (Stear 1990). Some of the changes that occur during staling can be reversed by reheating to temperatures of 50–70°C (Eliasson and Larsson 1993).

2.3.1.2. INFLUENCE OF β-GLUCAN ADDITION ON BREAD QUALITY

Limited work has been done to investigate the impact of β-glucan on dough quality. Previous research has shown that the quality of bread dough is decreased upon the addition of brewers' spent barley grains (Prentice and D'Appolonia 1977), which are high in fibre, and barley flour (Bhatty 1993) to bread dough by negatively affecting the development time of the dough and its stability. A similar effect was seen upon the addition of barley bran into biscuits (Sudha et al

2007). However, gluten addition to such doughs may help to increase dough quality parameters (Preston and Tipples 1980). The addition of high solubility β-glucan extract to bread dough increased dough elasticity and extensibility (Symons and Brennan 2004), though it was not associated with an increase in loaf height or overall loaf volume. This was thought to be due to a lack of water available for the development of the gluten network resulting from preferential hydration of β-glucan (Symons and Brennan 2004). In addition, preferential hydration of β-glucan over starch was limiting the swelling of starch granules and reducing gelatinization (Symons and Brennan 2004). However, this effect may be overcome with adjustments to water addition to correct for the increase in dough water absorption upon β-glucan addition.

Breads made with high fibre ingredients are often characterized as having reduced loaf volume, reduced crumb softness and a dark crumb and crust (Heiniö 2006). Fortification of bread with β-glucan has a similar effect. Numerous researchers have reported that the addition of barley flour and high β-glucan fractions from barley decreased bread volume in comparison to a wheat control (Cavallero et al 2002, Symons and Brennan 2004, Trogh et al 2005). Interestingly, Cleary et al (2007) reported that the greatest loss in bread quality resulted from the addition of high molecular weight β-glucan to bread due to its ability to bind large amounts of water versus the addition of low molecular weight β-glucan. By increasing the amount of water-extracted β-glucan added to bread, Jacobs et al (2008) reported a progressive decrease in loaf volume, while Gill et al (2002) showed a similar trend with increasing barley flour substitution.

Subsequently, increases in bread firmness were also reported (Gill et al 2002, Symons and Brennan 2004, Jacobs et al 2008). This demonstrates the challenge of producing a high-quality β-glucan enriched western style bread. Gujral et al (2003) and Mohamed et al (2008) also experienced a reduction in loaf volume and increased bread firmness upon the addition of barley flour and β-glucan extract, respectively, to bread, which was corrected upon the addition of gluten. Reductions in loaf volume and height upon the addition of barley flour and β-glucan extracts was thought to result from the binding of the water needed for gluten development by β-glucan (Gill et al 2002). In addition, steam is also an important leavening agent and, due to β-glucan's high affinity for water, the amount of steam generated was likely reduced (Gill et al 2002).

Despite its negative effects on quality, β-glucan may have a positive effect by increasing the shelf life of bread. Hydrocolloids help to reduce the rate of firmness increase and crumb dehydration during bread storage (Davidou et al 1996, Guarda et al 2004). Gujral et al (2003) and Mohamed et al (2008) reported a delay in firming upon ambient storage of bread with added β-glucan and gluten in comparison to a control. He and Hoseney (1990) showed that breads with higher moisture contents firm at a slower rate. Mohamed et al (2008) also reported stability in firmness values under refrigerated storage conditions upon incorporation of β-glucan and gluten into bread. This was in part a result of an alteration in water migration attributable to the increased protein and β-glucan contents of the breads (Mohamed et al 2008), though the change in crumb

moisture content upon storage was not measured. This was unexpected as refrigeration temperatures are the most conducive to staling.

Freezing is commonly used as a bread preservation method due to improved moisture retention and reduced movement of molecules within the bread system, thereby retarding staling (Stear 1990). Hydrocolloids help to stabilize water movement within bread during freezing. Mohamed et al (2008) showed that frozen storage of bread with added β-glucan and gluten over one week produced the least change in firmness when compared to storage at ambient and refrigerated temperatures. In addition, Kalinga and Mishra (2009) reported that frozen storage of low-fat cakes with β-glucan produced the least change in hardness over a 21 day storage period, in comparison to ambient and refrigeration temperatures. Despite the increases in bread shelf life that can decrease waste cost, the impact storage conditions may have on the physiological effectiveness of β-glucan cannot be overlooked. Further research within this area is vital to make recommendations for storage of β-glucan-enriched products in order to maintain its health promoting properties.

2.4. MARKETABILITY OF FOOD PRODUCTS WITH ADDED FIBRE

The field of functional and fortified foods aimed at improving various aspects of health has been growing steadily worldwide. Over the next 10 years, functional foods and whole food nutrition is expected to be one of the strongest health trends (Sloan 2009). Functional foods are characterised as those that provide health benefits beyond basic nutrition. Last year, the majority of

consumers made a strong effort or some effort to eat fortified foods (Sloan 2009). Consumers are becoming more and more aware of functional foods (Heller 2009) and as a result research within this area has grown enormously. In a recent survey, fibre was named a "top functional food" by consumers and ranked second, preceded only by calcium, as a top food component consumers look for when choosing foods (Heller 2009). As such, growing interest of food processors in dietary fibre is not surprising as it is estimated that whole grain and high fibre foods will reach sales of $7.5 billion this year (Packaged Facts 2005).

Fibre has multifaceted health benefits including digestive health, weight loss, heart health, blood sugar regulation and cancer prevention (Lupton and Turner 2000). The recommended daily intake of dietary fibre is 21-38 g per day, though on average consumers are receiving less than half of this recommendation (Heart and Stroke Foundation 2009). A lack of sufficient fibre in the diet is suspected to be a probable cause of many chronic diseases (Selvendran 1984, Eastwood 1987, Schneeman 1989, Asp 1994, McDougall et al 1996). The looming risk and prevalence of heart disease and Type 2 diabetes for the majority of North Americans and the demand for condition specific foods have both been growing steadily (Sloan 2006). As such, the functional food market is being driven by the growing prevalence of chronic disease, which is not expected to decrease any time soon.

Giuglian et al (2006) indicates that the typical Western diet is high in foods associated with increased risks of developing various chronic diseases. However, an effective and convenient solution to the growing burden of chronic diseases

may be found within the field of functional foods, which has the potential to allow many consumers, who are unable or unwilling to adapt their diets to meet the suggested guidelines for disease prevention, a convenient and effective way to improve their health by opting for the functional and healthy versions of their favorite products. In addition, the majority of consumers state that they are trying to live preventive lifestyles, which would allow fortified foods and beverages to become a common addition to everyday life (Sloan 2008). Due to its health benefits, β-glucan shows great potential within the current functional food market, with a large focus on improving health. However, it is important to ensure that the highest level of consumer acceptance of new food products is achieved during the product development cycle prior to market launch due to the exceptionally high failure rates within the food industry that can exceed 90% of new product introductions (Martinez 2007).

2.4.1. ACCEPTABILITY OF FOOD PRODUCTS

The main quality attributes used to characterize foods are appearance, flavour and texture. The intensity of such sensory attributes plays a substantial role in the overall perception and acceptance of food products (Cardello 1994). Previous research has shown that colour and appearance may elicit a halo effect that can modify food acceptability and flavour perception (Kostyla and Clydesdale 1978, Hutchings 1994). However, when consumers are making food choices, taste and perceived sensory quality of food products are considered the most important aspects in food choice (Wandel and Bugge 1997, Glanz et al 1998, Chryssohoidis and Krystallis 2005, Kihlberg et al 2005, Radder and la Roux

2005). In addition, Bower et al (2003) reported higher purchase intent by consumers for the product that was liked more. Sensory evaluation without revealing the identities of the products allows consumer acceptability to be determined based on the sensory properties of the product alone, without the bias of conceptual claims that are often present on labels (Lawless and Heymann 1998).

The key attributes that influence consumer acceptability of bread are flavour and texture (Heiniö 2006). However, bread freshness, colour, and texture may also influence overall perception. Incorporation of large amounts of barley or oat flour needed to fortify bakery products with a sufficient amount of β-glucan to meet the guidelines set out by the FDA approved health claim and the common use of solubilized β-glucan extracts, particularly in bread, can lead to quality defects and a reduction in product sensory parameters (Bhatty 1986, Knuckles et al 1997, Dhingra and Jood 2004, Skribic et al 2009). The use of β-glucan concentrate of low solubility under physiological conditions that gets solubilized upon baking to fortify bread products has not been evaluated. To maximize product acceptance, it is essential to evaluate the effect of β-glucan concentrate on the sensorial aspects of bread. However, the perception of a food product may also be affected by external stimuli, including labeling information and nutritional content (Krondl and Lau 1978 and 1982, Raats et al 1995), which must also be taken into account.

2.4.2. INFLUENCE OF INFORMATION ON ACCEPTABILITY

In addition to sensory qualities, food choice is also affected by the presentation context of the food (Shepherd 1989, Kahkonen et al 1997). The provision of nutrition information is very common in retail market settings and, depending on the product, may have a large impact on consumer acceptability. Previous studies have reported that the provision of nutritional information during sensory evaluation may increase, decrease, or simply not affect consumer acceptability depending on the nature of the food product (Goerlitz and Delwiche 2004, Wansink et al 2004, van Kleef et al 2005). van Kleef et al (2005) reported that consumers tend to prefer functional food concepts that communicate disease-related health benefits in carriers with a healthy image, though previous research has shown that health information has little impact on the perceived taste of foods that are typically expected to already be healthy (Wansink et al 2004). Wansink et al (2004) indicated that in order for health or diet labels to have a positive impact the label needs to create a diminished expectation of the food and there needs to be a very positive disconfirmation. Oliver (1980) suggested that when such foods are presented to consumers, the resulting disconfirmation of expectation would result in a more favorable rating compared with the rating of an unlabeled version of the same food. However, this type of disconfirmation is rare when the foods are not highly favorable (Wansink et al 2004).

For bakery products, Biaxauli et al (2008) found that the liking of multigrain muffins increased when information about the dietary fibre content was provided, though Mialon et al (2002) reported that liking of multigrain bread

did not differ. In addition, Ginon et al (2009) reported that consumers' willingness to pay for French baguettes increased if the baguette was considered a 'source of fibre'. The contextual effect of providing nutritional information on the acceptability of β-glucan-fortified bread has not been investigated. Due to the health information provided by the FDA health claim, which would accompany products that meet the FDA's guidelines, it is essential to see if it has an effect on consumer acceptability of bread fortified with β-glucan.

2.5. REFERENCES

Alberta Barley Commission. 2008. Accessed August 17, 2009 at http://www.albertabarley.com/barley/barley/barley.html.

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

American Diabetes Association. 2006. Diabetes Statistics. Accessed November 15, 2006. http://www.diabetes.org/diabetes-statistics/prevalence.jsp.

Andersson, A.A., Elfverson, C., Andersson, R., Regner, S., Aman, P. 1999. Chemical and physical characteristics of different barley samples. J Sci Food Agr. 79:979-986.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Andersson, A.A., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Asp, N.G. 1994. Nutritional classification and analysis of food carbohydrates. Am J Clin Nutr. 59(suppl):679S-681S.

Bamforth, C.W. 1982. Barley β-glucans, their role in malting and brewing. Brew Digest. 57:22-27.

Bathgate, G.N., Palmer, G.N., Wilson, G. 1974. The action of endo-β-glucanases on barley and malt β-glucans. J Inst Brew. 80:278-285.

Bechtel, W.G., Meisner, D. F., Bradley, W. B. 1953. The effect of the crust on the staling of bread. Cereal Chem. 30:160-168.

Beer, M.U., Wood, P.J., Weisz, J., Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by an in vitro digestion system. Cereal Chem. 76:705-709.

Behall, K.M., Scholfield, D.J., Hallfrisch, J. 2004. Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. Am J Clin Nutr. 80:1185-93.

Behall, K.M., Scholfield, D.J., Hallfrisch, J.G., Liljeberg-Elmstahl, H.G. 2006. Consumption of both resistant starch and β-glucan improves postprandial plasma glucose and insulin in women. Diabetes Care. 29(5):976-81.

Bhatty, R.S. 1993. Extraction and enrichment of (1→3)(1→4)-β-D-glucan from barley and oat brans. Cereal Chem. 70:73-77.

Bhatty, R.S. 1992. β-Glucan content and viscosities of barleys and their roller-milled flour and bran products. Cereal Chem. 69:469-471.

Bhatty, R.S. 1986. The potential of hull-less barley-A review. Cereal Chem. 63(2):97-103.

Biaxauli, R., Salvador, A., Hough, G., Fiszman, S.M. 2008. How information about fibre (traditional and resistant starch) influences consumer acceptance of muffins. Food Qual Pref. 19(7):628-635.

Bohme, N., Kulicke, W.M. 1999. Rheological studies of barley (1→3)(1→4)-β-glucan concentrated solution: mechanistic and kinetic investigation of the gel formation. Carb Res. 315:302-311.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr. 69:55-63.

Bower, J.A., Saadat, M.A. 1998. Consumer preference for retail fat spreads: an olive oil based product compared with market dominant brands. Food Qual Pref. 9(5):367-376.

Braaten JT, Wood PJ, Scott FW, Riedel D, Poste LM, Collins MW. 1991. Oat gum lowers glucose and insulin after an oral glucose load. Am J Clin Nutr. 53:1425-1430.

Braaten, J.T., Wood, P.J., Scott, F.W., Wolynetz, M.S., Lowe, M.K., Bradley-White, P., Collins, M.W. 1994. Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J Clin Nutr. 48:465-474.

Brennan, C.S., Amor, M.A., Harris, N., Smith, D., Cantrell, I., Griggs, D., Shewry, P.R. 1997. Cultivar differences in modification patterns of protein and carbohydrate reserves during malting of barley. J Cereal Sci. 26:83-93.

Brennan, C.S., Cleary, L.J. 2005. The potential use of cereal (1→3, 1→4)-β-D-glucans as functional food ingredients. J Cereal Sci. 42:1-13.

Brennan, C.S., Harris, N., Smith, D., Shewry, P.R. 1996. Structural differences in the mature endosperms of good and poor malting barley cultivars. J Cereal Sci. 24:171-178.

Brennan, C.S., Tudorica, C.M., Kuri, V. 2002. Soluble and insoluble dietary fibres (non-starch polysaccharides) and their effects on food structure and nutrition. Food Ind J. 5:261-272.

Burkus, Z., Temelli, F. 2000. Stabilization of emulsions and foams using barley β-glucan. Food Res Int. 33:27-33

Burkus, Z., Temelli, F. 1999. Gelation of barley β-glucan concentrate. J Food Sci. 33:27-33.

Burkus, Z., Temelli, F. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum. Cereal Chem. 75(6):805-809.

Canadian Diabetes Association Clinical Practice Guideline Expert Committee. 2003. Canadian Diabetes Association 2003 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada. Can J Diabetes. 27(Suppl 2):S1-152.

Canadian Diabetes Association. 2006. Accessed November 15, 2006. http://www.diabetes.ca.

Canadian Diabetes Association. 2009. Managing your blood glucose. Accessed July 31, 2009 at http://www.diabetes.ca/about-diabetes/living/management/manage-glucose/.

Canadian Organ Replacement Registry (CORR). 2001. Annual Report. Ottawa, ON, Canada: Canadian Institute for Health Information.

Cardello, A.V. 1994. Consumer expectations and their role in food acceptance. In MacFie, H.J.H, Thomson, D.M.H (eds). Measurement of Food Preferences. Blackie Academic and Professional, Glasgow. pp.253-297.

Casiraghi, M.C., Garsetti, M., Testolin, G., Brighenti, F. 2006. Post-prandial responses to cereal products enriched with barley β-glucan. J Am College Nutr. 25(4):313-320.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Chaput, J.P., Tremblay, A. 2009. The glucostatic theory of appetite control and the risk of obesity and diabetes. Int J Obesity. 33:46-53.

Chryssohoidis, G.M., Krystallis, A. 2005. Organic consumers' personal values research: Testing and validating the list of values (LOV) scale and implementing a value-based segmentation task. Food Qual Pref. 16:585-599.

Cleary, L., Brennan, C. 2006. The influence of a (1→3)(1→4)-β-D-glucan rich fraction from barley on the physico-chemical properties and in vitro reducing sugars release of durum wheat pasta. Int J Food Sci Technol. 41(8):910-918.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and in vitro digestion. Food Chem. 102:889-897.

Cui, S.W. 2001. Polysaccharide Gums from Agricultural Products: Processing, Structure and Functionality. Technomic Publishing Company, Lancaster, PA., USA.

Davidou, S., Le Meste, M., Debever, E., Bekaert, D. 1996. A contribution of the study of staling of white bread: Effect of water and hydrocolloid. Food Hydrocoll. 10:375-383.

Davis, J.M., Murphy, E.A., Brown, A.S., Carmichael, M.D., Ghaffar, A., Mayer, E.P. 2004. Effects of moderate exercise and oat ß-glucan on innate immune function and susceptibility to respiratory infection. Am J Physiol Regul Integr Comp Physiol. 286:R366-R372.

Delaney, B., Nicolosi, R.J., Wilson, T.A., Calson, T., Frazer, S., Zheng, G., Hess, R., Ostergren, K., Haworth, J., Knutson, N. 2003. β-Glucan fractions from

barley and oats are similarly antiatheriogenic in hypercholesterolemic Syrian golden hamsters. J Nutr. 133:468-495.

Dhingra, S., Jood, S. 2004. Effect of flour blending on functional, baking and organoleptic characteristics of bread. Int J Food Sci Technol. 39(2):213-222.

Eastwood, M. 1987. Physiological properties of dietary fibre. Molec Aspects Med. 9:31-40.

Edney, M.J., LaBerge, D.E., Langrell, D.E. 1998. Relationships among the β-glucan contents of barley, malt, malt congress extract, and beer. J Am Soc Brew Chem. 56(4):164-168.

Elfverson, C., Andersson, A.A., Aman, P., Regner, S. 1999. Chemical composition of barley cultivars fractionated by weighing, pneumatic classification, sieving, and sorting on a specific gravity table. Cereal Chem. 73(6):434-438.

Eliasson, A.C., Larsson, K. 1993. Bread. In: Eliasson, A.C., Larsson, K. (eds). Cereals in Breadmaking, Marcel Dekker Inc., New York. pp: 325–370.

Fincher, G. B., Stone, B.A., 1986. Cell walls and their components in cereal grain technology, Vol 8. Pomeranz, Y. (ed.) Am Assoc Cereal Chem, St. Paul, MN., USA. pp:206-207.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Flourie, B., Vidon, N., Florent, C.H., Bernier, J.J. 1984. Effect of Pectin on jejuna glucose absorption and unstirred layer thickness in normal man. Gut. 25:936-941.

Food and Drug Administration. 2005. Food labeling: health claims; soluble dietary fibre from certain foods and coronary heart disease; Final Rule Federal Register Doc. 2004P-0512, filed 12-23-05.

Food and Drug Administration. 1997. Food labeling: health claims; oat and coronary heart disease; Final Rule Federal Register Doc. 97-1598, filed 1-22-97.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

French, S.J., Read, N.W. 1994. Effect of guar gum on hunger and satiety after meals of differing fat content: relationship with gastric emptying. Am J Clin Nutr. 59:87-91.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. J Nutr. 123:244-252.

Gallaher, D.D., Locket, P.L., Gallaher, C.M. 1992. Bile acid metabolism in rats fed two levels of corn oil and brans of oat, rye, and barley and sugar beet fibre. J Nutr. 122:473-481.

Gee, V.L. 2005. Utilization of barley β-glucan in dairy products. MSc Thesis, University of Alberta, Edmonton, AB.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125:1401-1412

Gill, S., Vasanthan, T., Ooraikul, B., Rossnagel, B. 2002. Wheat bread quality as influenced by substitution of waxy and regular barley flours in their native and extruded forms. J Cereal Sci. 36:219-237.

Ginon, E., Loheac, Y., Martic, C., Combris, P., Issanchou, S. 2009. Effect of fibre information on consumer willingness to pay for French baguettes. Food Qual Pref. 20(5):343-352.

Giugliano D, Ceriello A, Esposito K. 2006. The effects of diet on inflammation: emphasis on Metabolic Syndrome. JACC. 48(4):677-685.

Glanz, K., Basil, M., Maibach, E., Goldberg, J., Snyder, D. 1998. Why Americans eat what they do: Taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. J Am Diet Assoc. 98(10):1118-1126.

Glicksman, M. 1982. Functional properties of hydrocolloids. In: Food Hydrocoll. Glicksman, M. (ed.), CRC Press Inc, Boca Raton, FL. pp: 49-93.

Glicksman, M. 1991. Hydrocolloids and the search for the "oily" grail. Food Technol. 45(10): 94-103.

Goerlitz, C.D., Delwiche, J.F. 2004. Impact of label information on consumer assessment of soy-enhanced tomato juice. J Food Sci. 69(9):S376-S379.

Guarda, A., Rosell, C.M., Benedito, C., Galotto, J. 2004. Different hydrocolloids as bread improvers and antistaling agents. Food Hydrocoll. 18(2):241-247.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Hallfrisch J, Scholfield DJ, Behall KM. 2003. Physiological responses of men and women to barley and oat extracts (Nu-trimX). II. Comparison of glucose and insulin response. Cereal Chem. 80(1):80-83.

Haraldsson, A., Rimsten, L., Alminger, M.L., Andersson, R., Andlid, T., Åman, P., Sandberg, A. 2004. Phytate content is reduced and β-glucanase activity suppressed in malted barley steeped with lactic acid at high temperature. J Sci Food Agric. 84:653-662.

He, H., Hoseney, R.C. 1990. Changes in bread firmness and moisture during long-term storage. Cereal Chem. 67(6):603-605.

Heart and Stroke Foundation. 2009. Fibre, whole grains and carbohydrates. Accessed August 21, 2009. http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3484239/k.E1F9/Healthy_living__Fibre_whole_grains_and_carbohydrates.htm

Heart and Stroke Foundation of Canada. 2003. The Growing Burden of Heart Disease and Stroke in Canada 2003. Ottawa, ON, Canada.

Heart and Stroke Foundation of Canada. 1999. The changing face of heart disease and stroke in Canada 2000. Ottawa, ON, Canada.

Heiniö, R.L. 2006. Sensory attributes of bakery products. In: Bakery Products: Science and Technology. Hui, Y.H. (ed.), Blackwell Publishing, Ames. pp: 285–298.

Heller, L. 2009. How consumers approach functional foods: survey. Nutra Ingredients-USA. Accessed August 11, 2009 at http://www.nutraingredients-usa.com/Consumer-Trends/How-consumers-approach-functional-foods-Survey.

Henry, R.J. 1988. Changes in β-glucan and other carbohydrate components of barley during malting. J Sci Food Agric. 42:333-341.

Holt, S., Brand, J., Soveny, C., Hansky, J. 1992. Relationship of satiety to postprandial glycemic, insulin and cholecystokinin responses. Appetite. 18:129-141

Hoseney, R.C. 1994. Principles of Cereal Science and Technology, 2nd edition. ST Paul, MN., USA. AACC.

Hudson, C.A., Chiu, M.M., Knuckles, B.E. 1992. Development of high-fibre muffins with oat bran, rice bran, and barley fibre fractions. Cereal Foods World. 37(5):373-376.

Hutchings, J.B. 1994. Sensory assessment of appearance – methodology. In: Food Colour and Appearance. J.B. Hutchings (ed.), Aspen Publishers, Inc., Gaithersburg, MD, pp. 105-41.

Jackson, K.A., Suter, D.A.I., Topping, D.L. 1994. Oat bran, barley, and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J Nutr. 124:1678-1684.

Jacobs, M.S., Izydorczyk, M.S., Preston, K.R., Dexter, J.E. 2008. Evaluation of baking procedures for incorporation of barley roller milling fractions containing high levels of dietary fibre into bread. J Sci Food Agric. 88:558-556.

Jadhav, S.J., Lutz, S.E., Ghorpade, V.M., Salunkhe, D.K. 1998. Barley: Chemistry and value-added processing. Crit Rev Food Sci. 38:123-171.

Jenkins, D.J., Goff, D.V., Leeds, G.M., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., Alberti, K.G. 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Brit Med J. 1:1392-1394.

Juvonen, K., Purhonen, A., Salmenkallio-Marttila, M., Lähteenmäki, L., Laaksonen, D., Herzig, K., Uusitupa, M., Poutanen, K., Karhunen, L. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J Nutr. 139(3):461-466.

Kahkonen, P., Tuorila, H., Lawless, H. 1997. Lack of effect of taste and nutrition claims on sensory and hedonic responses to a fat-free yogurt. Food Qual Pref. 8(2):125-130.

Kahlon, T.S., Chow, F.I., Knuckles, B.E., Chiu, M.M. 1993. Cholesterol lowering effects in hamsters of β-glucan-enriched barley fraction, dehulled whole barley, rice bran, and oat bran and their combinations. Cereal Chem. 70:435-440.

Kalinga, D., Mishra, V.K. 2009. Rheological and physical properties of low fat cakes produced by addition of cereal β-glucan concentrates. J Food Proc Preser. 33:384-400.

Karmally W, Montez MG, Palmas W. 2005. Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. J Am Diet Assoc. 105:967–70.

Keenan, J.M., Goulson, M., Shamliyan, T., Knutson, N., Kolberg, L., Curry, L. 2007. The effects of concentrated barley β-glucan on blood lipids in a population of hypercholesterolemic men and women. Brit J Nutr. 97(6):1162-1168.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Keogh, G.F., Cooper, G., Mulvey, T.B., McArdle, B.H., Coles, G.D., Monro, J.A., Poppitt, S.D. 2003. Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease reisk factors in mildly hypercholesterolemic men. Am J Clin Nutr. 78:711-718.

Kihlberg, I., Johansson, L., Langsrud, O., Risvik, E. 2005. Effects of information on liking of bread. Food Qual Pref. 16(1):25-35.

Kim, H., Behall, K.M., Vinyard, B., Conway, J. 2006. Short-term satiety and glycemic response after consumption of whole grains with various amounts of β-glucan. Cereal Foods World 51:29–33.

Kim, H., Stokes, K.S., Behall, K.M., Spears, K., Vinyard, B., Conway, J.M. 2009. Glucose and insulin responses to wholegrain breakfasts varying in soluble fibre, β-glucan. Eur J Nutr. 48(3):170-175.

Kim, S.K., D'Appolonia, B.L. 1977. Bread staling studies. II. Effect of protein content and storage temperature on the role of starch. Cereal Chem. 54:216-224.

Klein, R., Klein, B.E. 1995. Vision disorders in diabetes. In Diabetes in America. 2nd edition. Bethesda, M.D., (ed). National Diabetes Data Group, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. 293-338.

Knuckles, B.E., Hudson, C.A., Chiu, M.M. Sayre, R.N. 1997. Effect of β-glucan barley fractions in high-fibre bread and pasta. Cereal Foods World. 42(2):94-99.

Kontogiorgos, V., Biliaderisk, C.G., Kiosseoglou, V., Doxastakis, G. 2004. Stability and rheology of egg-yolk-stabilized concentrated emulsions containing cereal β-glucans of varying molecular size. Food Hydrocoll. 18:987-998.

Kostyla, A.S., Clydesdale, F.M. 1978. The psychophysical relationships between colour and flavour. Food Sci Nutr. 10:303-319.

Krondl, M., Lau, D. 1978. Food habit modification as a public health measure. Can J Public Health. 69:39-48.

Krondl, M., Lau, D. 1982. Social determinants in human food selection. In Barker, L. (ed). The Psychobiology of Human Food Selection, Ellis Horwood Ltd, Chichester. pp:139-151.

Lan-Pidhainy, X., Brummer, Y., Tosh, S., Wolever, T.M., Wood, P. 2007. Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. Cereal Chem. 84(5):512-517.

Lawless, H.T., Heymann, H. 1998. Sensory Evaluation of Food: Principles and Practices. Heldman, D.R. (ed.). Chapman and Hall. New York, NY, USA.

Lazaridou, A., Biliaderis, C.G., Izydorczyk, M.S. 2003. Molecular size effects on the rheological properties of oat β-glucans in solution and gels. Food Hydrocoll. 17:693-712.

Lazaridou, A., Biliaderis, C.G. 2007. Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. J Cereal Sci. 101-118.

Lazaridou, A., Biliaderis, C.G. 2004. Cryogelation of cereal β-glucans: structure and molecular size effects. Food Hydrocoll. 18:933-947.

Lee, S.C., Prosky, L., deVries, J.W. 1992. Determination of total soluble, and insoluble dietary fibre in foods-enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. J AOAC Int. 75:395-402.

Lekhi, A. 2004. Extraction and Functional Properties of Barley Proteins. M.Sc. Thesis. University of Alberta, AB.

Lia, A., Hallmans, G., Sandberg, A.S., Sundberg, B., Åman, P., Andersson, H. 1995. Oat β-glucan increases bile acid excretion and a fibre-rich barley fraction increases cholesterol excretion in ileostomy subjects. Am J Clin Nutr. 62:1245-1251.

Lupton, J.R., Turner, N.D. 2000. Dietary Fibre. In: Biochemical and Physiological Aspects of Human Nutrition. M.H. Stipanuk (ed.), W.B. Saunders Co. Philadelphia, PA. pp:143-154.

Lyly, M., Liukkonen, K.H., Salmenkallio-Marttila, M., Karhunen, L., Poutanen, K., Lahteenmaki, L. 2009. Fibre in beverages can enhance perceived satiety. Eur J Nutr. 48(4):251-258.

Lyly, M., Salmenkallio-Marttila, M., Suortti, T., Autio, K., Poutanen, K., Lahteenmaki, L. 2003. Influence of oat β-glucan preparation on the

perception of mouthfeel and on rheological properties in beverage prototypes. Cereal Chem. 80(5):536-541.

Lyly, M., Salmenkallio-Marttila, M., Suortti, T., Autio, K., Poutanen, K., Lahteenmaki, L. 2004. The sensory characteristics and rheological properties of soups containing oat and barley β-glucan before and after freezing. Lebens Wiss-u Technol. 37(7):749-761.

MacGregor, A.W., Fincher, G.B. 1993. Carbohydrates of the barley grain. In: MacGregor, A.W., Bhatty, R.S. (eds.) Barley: Chemistry and Technology. St. Paul: AACC. Pp. 73-130.

Magness, J.R., Markle, G.M., Compton, C.C. 1971. Food and free crops of the United States. Interregional Research Project. IR Bulletin.

Makelainen, H., Anttila, H., Sihvonen, J., Hietanen, R.M., Tahvonen, R., Salminen, E., Mikola, M. 2006. The effect of β-glucan on the glycemic and insulin index. Eur J Clin Nutr. 1-7.

Malkki, Y., Autio, K., Hanninen, O., Myllymaki, O., Pelkonen, K., Suortii, T., Torren, R. 1992. Oat bran concentrates: Physical properties of β-glucan and hypocholesterolemic effects in rats. Cereal Chem. 69(6):647-653.

Marconi, E., Graziano, M., Cubadda, R. 2000. Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fibre and β-glucans. Cereal Chem. 77(2):133-139.

Martinez, S.W. 2007. The U.S. Food Marketing System: Recent Developments, 1997-2006, ERR-42, USDA, Economic Research Service, May 2007. Accessed August 10, 2009 from: http://www.ers.usda.gov/publications/err42/.

Martinez, V.M., Newman, R.K., Newman, C.W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. J Nutr. 122:1070-1076.

Mayer, J. 1955. Regulation of energy intake and the body weight, the glucostatic theory and the lipostatic hypothesis. Ann NY Acad Sci. 63:15–43.

McDougall, G.J., Morrison, I.M., Stewart, D., Hillman, J.R. 1996. Plant cell walls as dietary fibre: Range, structure, processing and function. J Sci Food Agric. 70:133-150.

Mialon, V.S., Clark, M.R., Leppard, P.I. Cox, D.N. 2002. The effect of dietary fibre information on consumer responses to breads and "English" muffins: a cross-cultural study. Food Qual Pref. 13(1):1-12

Miller, S.S., Fulcher, R.G. 1994. Distribution of (1→3), (1→4)-β-D-glucan in kernals of oats and barley using microspectrofluormetry. Cereal Chem. 71(1):64-68.

Mohamed, A., Rayas-Duarte, P., Xu, J. 2008. Hard Red Spring wheat/C-TRIM 20 bread: Formulation, processing and texture analysis. Food Chem. 107:516-524.

Morin, L.A., Temelli, F., McMullen, L. 2004. Interactions between meat proteins and barley (Hordeum spp.) β-glucan within a reduced-fat breakfast sausage system. Meat Sci. 68:419-430.

Morin, L.A., Temelli, F., McMullen, L. 2002. Physical and sensory characteristics of reduced-fat breakfast sausages formulated with barley β-glucan. J Food Sci. 67(6):2391-2396.

Naumann, E., van Rees, A.B., Onning, G., Oste, R., Wydra, M., Mensink, P. 2006. β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations. Am J Clin Nutr. 83:601-605.

Newman RK, Newman CW. 1991. Barley as a food grain. Cereal Foods World. 36(9):800-805.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., Hofer, P.J. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Newman, R.K., Ore, K.C., Abott, J., Newman, C.W. 1998. Fibre enrichment of baked products with a barley milling fraction. Cereal Foods World. 43(1):23-25.

Ogden, C.L., Carroll, M.D., Curtin, L.R., McDowell, M.A., Tabak, C.J., Flegal, K.M. 2006. Prevalence of overweight and obesity in the United States, 1999-2004. J Am Med Assoc. 295:1549-1555.

Ohkubo, Y., Kishikawa, H., Araki, E., Miyata, T., Isami, S., Motoyoshi, S., Kojima, Y., Furuyoshi, N., Shichiri, M. 1995. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res Clin Pract. 28:103-117.

Oliver, R.L. 1980. A cognitive model of the antecedents and consequences of satisfaction decisions. J Market Res. 17:460-469.

Oscarsson, M., Parkkonen, T., Autio, K., Aman, P. 1997. Research Note: Composition and microstructure of waxy, normal and high amylose barley samples. J Cereal Sci. 26:259-264.

Östman, E., Rossi, E., Larsson, H., Brighenti, F., Björck, I. 2006. Glucose and insulin responses in healthy men to barley bread with different levels of (1→3, 1→4)-β-glucans; predictions using fluidity measurements of in vitro enzyme digests. J Cereal Sci. 43:230-235.

Packaged Facts. 2005. Baby Boomers and the U.S. Food and Beverage Industry: Trends and Opportunities in Health, Natural and Organic, Gourmet, Ethnic and Convenience Foods, www.packagedfacts.com.

Panahi, S., Ezatagha, A., Temelli, F., Vasanthan, T., Vuksan, V. 2007. β-Glucan from two sources of oat concentrates affect postprandial glycemia in relation to level of viscosity. J Am College Nutr. 26(6):639-644.

Partanen, J, Niskanen, L., Lehtinen, J. 1995. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 333:89-94.

Pick, M.E., Hawrysh, Z.J., Gee, M.I., Toth, E., Garg, M.L., Hardin, R.T. 1996. Oat bran concentrated bread products improve long-term control of diabetes: a pilot study. J Am Diet Assoc. 96(12):1254-1261.

Pins, J.J., Kaur, H. 2006. A review of the effects of barley β-glucan on cardiovascular and diabetes risk. Cereal Foods World. 51(1):8-11.

Prentice, N., D'Appolonia, B.L. 1977. High-fiber bread containing brewer's spent grain. Cereal Chem. 54:1084-1095.

Preston, K.R., Tipples, K.H. 1980. Effects of acid-soluble and acid-insoluble gluten proteins and baking properties of wheat flours. Cereal Chem. 57:314-320.

Queenan, K.M., Stewart, M.L., Smith, K.N., Thomas, W., Fulcher, R.G., Slavin, J.L. 2007. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. Nutr J. 6:6.

Raats, M., Daillant-Spinnler, B., Deliza, R., MacFie, H. 1995. Are sensory properties relevant to consumer food choice? Food Choice Consum. 239-263.

Radder, L., le Roux R. 2005. Factors affecting food choice in relation to venison: A South Africa example. Meat Sci. 71:583-589.

Ranhotra, G.S., Gelroth, J.A., Astroth, K., Bhatty, R.S. 1991. Relative lipidemic responses in rats fed barley and oat meals and their fractions. Cereal Chem. 68:548-551.

Ren, Y., Ellis, P.R., Ross-Murphy, S.B., Wang, Q., Wood, P.J. 2003. Dilute and semi-dilute solution properties of (1→3), (1→4)-β-D-glucan, the endosperm cell wall polysaccharide of oats (Avena sativa L.). Carb Polym. 53:401-408.

Ribotta, P., Bail, A. 2007. Thermo-physical assessment of bread during staling. Lebesmitt Wiss-u Technol. 40(5):879–884.

Ripsin, C.M., Keenan, J.M., Jacobs, D.R., Elmer, P.J., Welch, R.R., Van Horn, L., Liu, K., Turnbull, W.H., Thye, F.W., Kestin, M., Hegsted, M., Davidson, D.M., Davidson, M.H., Dugan, L.D., Demark-Wahnefried, W.,, Beling, S. 1992. Oat products and lipid lowering: A meta-analysis. J Am Med Assoc. 267(24):3317-3325.

Rosell, C.M., Rojas, J.A., Benedito de Barber, C. 2001. Influence of hydrocolloids on dough rheology and bread quality. Food Hydrocoll. 15:75-81.

Schneeman, B.O. 1989. Dietary fibre: A scientific summary by the Institute of Food Technologists' expert panel of food safety and nutrition. Food Technol. 43:133-139.

Selvendran, R.R. 1984. The plant cell wall as a source of dietary fibre: Chemistry and structure. Am J Clin Nutr. 39:320-337.

Shepherd, R. 1989. Factors influencing food preferences and choices. In Shepherd, R. (ed). Handbook of the Psychology of Human Eating. John Wiley and Sons Inc, Chichester. pp:3-24.

Skendi, A., Biliaderis, C.G., Lazaridou, A., Izydorczyk, M.S. 2003. Structure and rheological properties of water soluble β-glucans from oat cultivars of Avena sativa and Avena bysantina, J Cereal Sci. 38:15-31.

Skribic, B., Milovac, S., Dodig, D., Filipcev, B. 2009. Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. Food Chem. 115:982-988.

Sloan AE. 2009. Top 10 functional food trends. Food Technol. 63(4):22-42.

Sloan AE. 2008. Top 10 functional food trends. Food Technology. 62(4):24-44.

Sloan AE. 2006. Top 10 functional food trends. Food Technology. 60(4):22-40.

Smith, K.N., Queenan, K.M., Thomas, W., Fulcher, R.G., Slavin, J.L. 2008. Physiological effects of concentrated barley β-glucan in mildly hypercholesterolemic adults. J Am College Nutr. 27(3):434-440.

Snart, J., Bibiloni, R., Grayson, T., Lay, C., Zhang, H., Allison, G., Laverdiere, J.K., Temelli, F., Vasanthan, T., Bell, R., Tannock, G.W. 2006. Supplementation of the diet with high-viscosity β-glucan results in enrichment for Lactobacilli in the rat cecum. Appl Env Micro. 72(3):1925-1931.

Statistics Canada. 2001. Field crop series. 80(8). Statistics Canada. Ottawa, Ontario.

Stear, C. 1990. Handbook of Breadmaking Technology. Elsevier Applied Science. London and New York. pp:649-708.

Stevenson, D.G., Inglett, G.E. 2009. Cereal β-glucans. In: Handbook of Hydrocolloids. G.O. Phillips and P.A. Williams (eds.), Woodhead Publishing Ltd. Boca Raton, FL. pp:615-639.

Story, J.A., Kritchevsky, D. 1976. Comparison of the binding of various bile acids and bile salts in vitro by several types of fibre. J Nutr. 106:1292-1294.

Story, J.A., Lord, S.L. 1987. Bile salts: In vitro studies with fibre components. Scand J Gastroenter. 22:S129-S174.

Sudha, M.L., Vetrimani, R., Leelavathi, K. 2007. Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chem. 100:1365-1370.

Sundberg, B., Pettersson, D., Aman, P. 1995. Nutritional properties of fibre-rich barley products fed to broiler chickens. J Sci Food Agric. 67(4):469-476.

Symons, L.J., Brennan, C.S. 2004. The influence of (1→3)(1→4)-β-D-glucan-rich fractions from barley on the physicochemical properties and in vitro reducing sugar release of white wheat breads. J Food Sci. 69(4):C463-C467.

Taguchi, T., Brownlee, M. 2003. The biochemical mechanisms of diabetic tissue damage. In: Pickups, J.C., Williams, G. (eds) Textbook of Diabetes 2. Blackwell Publishing Ltd. Malden, MA, 47.1-47.17.

Tappy, L., Gugolz, E., Wursch, P. 1996. Effects of breakfast cereals containing various amounts of β-glucan fibres on plasma glucose and insulin responses in NIDDM subjects. Diabetes Care. 19:831-834.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J Food Sci. 62:1194-1201.

Temelli, F., Bansema, C., Stobbe, K. 2004. Development of an orange-flavoured barley β-glucan beverage with added whey protein isolate. J Food Sci. 69(7):S237-S242.

Tjepkema, M. 2005. Adult obesity in Canada: measured height and weight. Statistics Canada. Accessed July 31, 2009: http://www.statcan.gc.ca/pub/82-620-m/2005001/article/adults-adultes/../../pdf/4224906-eng.pdf (PDF Version, 300 kb)

Tosh, S., Brummer, Y., Wolever T.M., Wood, P.J. 2008. Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. Cereal Chem. 85(2):211-217.

Tosh, S., Brummer, Y., Wood, P.J., Wang, Q., Weisz, J. 2004. Evaluation of structure in the formation of gels by structurally diverse (1→3)(1→4)-β-D-glucans from four cereal and one lichen species. Carb Polym. 57(3):249-259.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Trogh, I., Courtin, C.M., Goesaert, H., Delcour, J.A., Andersson, A.A.M., Aman, P., Fredriksson, H., Pyle, D.L., Sorensen, J.F. 2005. From hull-less barley and wheat to soluble dietary fibre-enriched bread. Cereal Foods World. 50:253-260.

Tudorica, C.M., Jones, E., Kuri, V., Brennan, C.S. 2004. The effects of refined barley β-glucan on the physic-structural properties of low-fat dairy products: curd yield, microstructure, texture and rheology. J Sci Food Agric. 84:1159-1169.

UK Prospective Diabetes Study (UKPDS) Group. 1998. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). Lancet. 352:837-853.

Vaikousi, H., Biliaderis, C.G., Izydorczyk, M.S. 2004. Solution flow behavior and gelling properties of water-soluble barley (1→3, 1→4)-β-glucans varying in molecular size. J Cereal Sci. 39:119-137.

van Kleef, E., Van Trijp, H.C., Luning, P. 2005. Functional foods: health claim-food product compatibility and the impact of health claim framing on consumer evaluation. Appetite. 44:299-308.

Vasanthan, T., Bhatty, R.S. 1995. Starch purification after pin milling and air classification of waxy, normal, and high amylose. Cereal Chem.72:379-384.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Vasanthan, T., Temelli, F. 2008. Grain fractionation technologies for cereal β-glucan concentration. Food Res Int. 876-881.

Vasanthan, T., Temelli, F., Burkus, Z. 2004. Preparation of high viscosity β-glucan concentrates. Patent application, International Publication Number WO/2004/085484. International publication date-2004 July.

Wandel, M., Bugge, A. 1997. Environmental concern in consumer evaluation of food quality. Food Qual Pref. 8(1):19-26.

Wang, L., Behr, S.R., Newman, R.K., Newman, C.V. 1997. Comparative cholesterol-lowering effects of barley β-glucan and barley oil in golden Syrian hamsters. Nutr Research. 17(1):77-88.

Wansink, B., van Ittersum, K., Painter, J.E. 2004. How diet and health labels influence taste and satiation. J Food Sci. 69(9):S340-S346.

Watson, J.E. 1987. National Cholesterol Education Program: Guidelines for treating high blood cholesterol in adults. J Am Acad Nurse Pract. 1(1):14-19.

Willis, H.J., Eldridge, A.L., Beiselgel, J., Thomas, W., Slavin, J.L. 2009. Greater satiety response with resistant starch and corn bran in human subjects. Nutr Res. 29(2):100-105.

Wilson, T.A., Nicolosi, R.J., Delaney, B., Chadwell, K, Moolchandani, V., Kotyla, T., Ponduru, S., Zheng, G., Hess, R., Knutson, N., Curry, L., Kolberg, L., Goulson, M., Ostergren, K. 2004. Reduced and high molecular weight barley ß-glucans decrease plasma total and non-HDL-cholesterol in hypercholesterolemic Syrian Golden Hamsters. J Nutr. 134:2617-2622.

Wood, P.J., Beer, M.U., Butler, G. 2000. Evaluation of role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-743.

Wursch P., Pi-Sunyer, F.X. 1997. The role of viscous soluble fibre in the metabolic control of diabetes. Diabetes Care. 20:1774-1780.

Yadav, S.K., Luthra, Y.P., Sood, D.R., Aggarwal, N.K. 2001. Malting potential of husked barley in relation to proanthocyanidins. J Food Sci Technol. 38(1):71-74.

Yao, N., Jannink, J.L., Alavi, S., White, P.J. 2006. Physical and sensory characteristics of extruded products made from two oat lines with different β-glucan concentrations. Cereal Chem. 83(6):692-699.

Yeung, J.S. 2001. Extrusion Cooking of Barley Flour with and without Thermostable α-Amylase. MSc Thesis, University of Alberta, Edmonton, AB.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnlund, J.R., Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74(3):293-296.

Yokoyama, W.H., Knuckles, B.E., Stafford, A., Inglett, G. 1998. Raw and processed oat ingredients lower plasma cholesterol in the hamster. J Food Sci. 63(4):713-715.

Yun, C.H., Estrada, A., van Kessel, A., Gajadhar, A., Redmond, M., Laarveld, B. 1997. β-(1-3, 1-4) oat ß-glucan enhances resistance to Eimeria vermiformis infection in immunosuppressed mice. Int J Parasit. 27: 329-337

Yun, C.H., Estrada, A., van Kessel, A., Gajadhar, A., Redmond, M., Laarveld, B. 1998. Immunomodulatory effects of oat ß-glucan administered intragastrically or parenterally on mice infected with *Eimeria vermiformis*. Microbial Immunol. 42:457-465.

Zheng, G.H., Rossnagel, B.G., Tyler, R.T., Bhatty, R.S. 2000. Distribution of β-glucan in the grain of hull-less barley. Cereal Chem. 77:140-144.

Chapter 3

EFFECT OF FORMULATION AND PROCESSING TREATMENTS ON THE VISCOSITY AND SOLUBILITY OF EXTRACTABLE β-GLUCAN IN BREAD DOUGH EVALUATED UNDER *IN VITRO* CONDITIONS[1]

3.1. INTRODUCTION

β-Glucan's cholesterol lowering and blood glucose regulating effects are primarily related to its viscosity, which can be negatively influenced by certain processing and storage conditions. Depending on the conditions applied, such treatments can lower β-glucan's solubility or bring about depolymerization of the molecule, causing it to become less physiologically effective, though no clear conclusions have been drawn (Gallaher et al 1993, Wood et al 1994, Yokoyama et al 1998, Wood et al 2000). Thus, it is essential to investigate the effects of processing and storage conditions on the quality of β-glucan following incorporation into a variety of food matrices. Bread, a staple food in North America that has a wide and well established share of the food market, has great potential for β-glucan incorporation. However, previous studies have indicated that the beneficial effects of β-glucan are decreased when incorporated into the bread system (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007,

[1] A version of this chapter has been submitted to Cereal Chemistry for consideration for publication.

Andersson et al 2008). In addition, the majority of these studies have incorporated β-glucan at levels that are substantially higher than those likely to be adopted by the food industry and have utilized experimental methods that are not reflective of the physiological system in which the desired health benefits are hoped to be seen.

It is essential to identify the step(s) within the bread production regime that are detrimental to β-glucan viscosity so that the full potential of β-glucan-fortified bread can be recognized as an effective and reliable means of lowering blood cholesterol and regulating blood glucose levels. The journey of β-glucan through the dough mixing and fermentation stages of bread making has not been fully investigated, particularly at levels of addition most likely to be presented to consumers. Therefore, the main objective of this study was to investigate the effects of β-glucan concentration, gluten addition, fermentation time, premixing, enzyme inactivation, and yeast addition on the viscosity of β-glucan extracted under approximated physiological conditions following incorporation into the bread dough system at 0.75, 1.0, and 1.5 g/serving. In addition, the effect of incorporation into the dough matrix and fermentation, in the presence and absence of yeast, on the solubility of β-glucan under approximated physiological conditions was also evaluated.

3.2. MATERIALS AND METHODS

3.2.1. MATERIALS

White wheat bread flour (Robin Hood Best for Bread Flour, Smucker Foods of Canada Co., Markham, ON), granulated white sugar, and traditional active dry yeast (Fleischmann's, Ach Food Companies, Inc., Oakville, ON) were purchased from a local grocery store. Additionally, vital wheat gluten (Permolex, Red Deer, AB), NaCl (Fisher Scientific, Fair Lawn, NJ) and barley β-glucan concentrate (BBG conc) were incorporated into bread dough. The BBG conc was supplied by Cevena Bioproducts Inc., who uses a patented process (Vasanthan and Temelli 2009) for β-glucan concentration. The BBG conc contained 50% (w/w) β-glucan, approximately 3% starch, 6% protein, 4% ash, and 0% lipid with the remainder comprised of other fibre components.

3.2.2. BREAD DOUGH PREPARATION

The moisture content of the BBG conc, vital gluten, and the bread flour was determined according to Method 44-15A (AACC International 2000). Bread dough was prepared using Method 10-09 (AACC International 2000) with modifications. As shown in Table 3-1, a control batch and 6 dough treatments were formulated with the BBG conc at levels that corresponded to 0, 0.75, 1.0, or 1.5 g β-glucan/serving of bread, via direct (w/w) substitution of the bread flour. The actual levels of BBG conc inclusion were calculated to reflect the amount of β-glucan required to produce a standard commercial loaf (containing approximately 15 slices with 2 slices per serving) providing the desired amount of β-glucan per serving. Because the experimental dough would only produce a loaf

approximately 1/3 the size of a commercial loaf and taking into account the β-glucan concentration in the BBG conc, the actual level of BBG conc inclusion was 3.75, 5.0, and 7.5 g/loaf, which corresponded to a final bread product containing 0.75, 1.0 and 1.5 g β-glucan/serving, respectively. As well, vital gluten was added at 0 g or 2.18 g per g of β-glucan for each level of β-glucan addition.

TABLE 3-1
Formulations for Bread Dough

	Amount of β-glucan / serving						
Ingredient (g)	**0 g**	**0.75 g**		**1.0 g**		**1.5 g**	
Yeast[a]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Wheat Flour	100.0	96.25	96.25	95.0	95.0	92.5	92.5
Vital Gluten	0	0	4.1	0	5.5	0	8.2
Sugar	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Distilled Water	68.0	79.0	86.4	81.8	92.0	86.0	99.0
β-Glucan Concentrate	0	3.75	3.75	5.0	5.0	7.5	7.5

[a]Yeast was activated by mixing with a solution of water (18.75 g) and sugar (1.69 g) at 38°C and allowing it to stand for 10 min according to manufacture's recommendations. If the solution doubled in volume within this time it was considered active. The added sugar and water are accounted for in the formulation table.

All dry ingredients were sifted together immediately prior to dough formulation. Optimal dough hydration was determined using a farinograph (Brabender Farino/Resistograph, Model FA/R-2, C.W. Brabender Instruments Inc., South Hackensack, NJ) equipped with a 50 g mixing bowl. The temperature was maintained at 30°C with a circulating water bath (MGW Lauda, Model RM6, Konigshofen, Germany) and the optimal amount of distilled water addition was determined as the point at which the consistency of the sample reached 500 BU. After sifting of the dry ingredients, all of the ingredients were combined and mixed using an electric mixer (Model K45SSWH, KitchenAid, St. Joseph, MI)

with a bread hook for 7 min. The prepared dough was then subjected to fermentation times of either 0 min or 235 min. Fermentation took place within a fermentation cabinet (Model 121-1816, Crescent Metal Products Inc, Cleveland, OH) maintained at 30^{o}C and 85% relative humidity.

To determine the effects of premixing the dry ingredients, inactivating the enzymes in the flour, and yeast addition, bread dough was prepared utilizing the recipe for 1.5 g of barley β-glucan/serving without the addition of gluten (Table 3-1). Deviations from the original dough recipe included premixing the dry ingredients either 36 or 168 hr prior to dough formulation, replacing the bread flour (w/w) in the recipe with bread flour that had been refluxed in ethanol to inactivate enzymes, or omitting the yeast. To obtain the refluxed bread flour, the method of Nilsson et al (2000) was used with modifications. Bread flour (85 g) was suspended in 95% aqueous ethanol (170 mL) and refluxed for 2 hr. The slurry was then dispersed into loaf pans and dried for 48 hr in a fume hood. The refluxed flour was ground to a powder prior to use.

3.2.3. *IN VITRO* PHYSIOLOGICAL EXTRACTION OF β-GLUCAN

To obtain the approximated physiological extract, the experimental dough was prepared and immediately subjected to the *in vitro* digestion procedure outlined by Beer et al (1997) with modifications to accommodate the limitations of a dough system. A dough sample, that supplied 1/10 of the barley β-glucan originally incorporated into the whole dough (0.1875, 0.25, and 0.375 g for the 0.75, 1.0, and 1.5 g β-glucan/serving of bread, respectively), was broken into 1 cm^3 pieces by hand and mixed in an Erlenmeyer flask with 50 mL of 20 m*M*

sodium phosphate buffer (pH 6.9) containing 10 m*M* NaCl. The solution was stirred slowly for 15 min at 37°C, after which 500 µL of human salivary α-amylase solution (5 mg/mL in 3.6 m*M* $CaCl_2$; A-1031, Sigma-Aldrich, St. Louis, MO) was added. The mixture was stirred for another 15 min. Following this, the pH was adjusted to 2.0 (±0.1) with 4 *M* HCl and 1250 µL of porcine pepsin solution (0.5 mg/mL in 0.9% NaCl, P7012, Sigma-Aldrich) was added. The mixture was stirred further for 30 min at 37°C. The pH was then neutralized to pH 6.9 (±0.1) using 4 *M* NaOH and 2500 µL of the pancreatin solution was added (0.5 mg/mL in 20 m*M* sodium phosphate buffer containing 10 m*M* NaCl, P-7545, Sigma-Aldrich). The solution was then incubated for 90 min and centrifuged (7,000 x *g*, 10 min). The supernatant was taken as the physiological extract of the dough that would contain the solubilized portion of the β-glucan, which was quantified as described later in Section 3.2.6. The physiological extract of the original BBG conc was also obtained using this method.

3.2.4. HOT WATER EXTRACTION OF β-GLUCAN

To serve as a control, the hot water extract of the BBG conc was also obtained. The targeted amount of β-glucan in solution was 0.06% and 0.13%. These levels were used to mimic the concentration of β-glucan solubilized within the physiological extracts of the dough fortified with 1.5 g β-glucan/serving of bread that was either fermented or not fermented, respectively. The BBG conc was mixed with 20 g of water and heat stable α-amylase (50 µL) and incubated for 1 hr at 85°C. The solution was centrifuged (7,000 x *g*, 10 min) and the supernatant was taken as the hot water extract of the BBG conc.

3.2.5. VISCOSITY DETERMINATION

To determine the effect of the experimental treatments on the extract viscosity as an indirect measure of changes in β-glucan molecular weight (MW), viscosity tests were performed on the physiological extracts. Viscosity was determined at consecutive fixed shear rates of 1.29-129 sec^{-1} (1-100 rpm) using a Paar Physica UDS 200 rheometer (Glenn, VA), which was equipped with a Peltier heating system that controlled the sample temperature. All viscosity tests were performed at 37°C using a DG 27 cup and bob geometry with 7.05±0.01 g sample. Shear rate was reported in sec^{-1} after multiplying rpm by the conversion factor of 1.29 sec^{-1} supplied by the manufacturer.

3.2.6. SOLUBILITY DETERMINATION

To determine the effect of incorporation into the dough system and fermentation on the solubility (extractability) of barley β-glucan under approximated physiological conditions, the method of McCleary and Glennie-Holmes (1985) using the β-glucan enzymatic assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) was used, with modifications to accommodate the liquid extract. An aliquot (3.0 mL) of the physiological extract was added to 5.0 mL of 20 m*M* sodium phosphate buffer (pH 6.5) and mixed. Lichenase (200 μL, 50 U/mL, Megazyme International Ireland Ltd.) was added and the solution was incubated at 40°C for 60 min in a water bath. The solution was diluted to 15 mL with distilled water, mixed, and an aliquot of each solution was centrifuged at 1,000 x *g* for 10 min. Following this, each sample (100 μL) was incubated (15 min, 40°C) with 100 μL, each, of acetate buffer (pH 4.0) and β-glucosidase (2

U/mL, Megazyme International Ltd.). Glucose Oxidase Peroxidase (GOPOD, Megazyme International Ltd) was then added (100 μL), the solution was incubated (20 min, 40^{o}C) and the absorbance was read at 510 nm against a reagent blank.

The following equation was used, accounting for the aqueous nature of the extract, dilution factors, and the initial β-glucan amount, to determine the amount of β-glucan solubilized in the physiological extract as a percentage of the total amount of β-glucan in the original sample:

Physiological solubilization (%) = ΔA*F*150*1/1000*100/W*162/180*54.25/3

where ΔA is the difference between the absorbance reading of the sample and the sample blank, F is 100/average absorbance of 100 μg of glucose, W is the amount of β-glucan in the original sample, and 150 x (54.25/3) is the dilution factor.

3.2.7. STATISTICAL ANALYSIS

Bread samples were prepared with two complete replications. Extraction, viscosity, and solubility determination of each sample were performed in duplicate. Results were analyzed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Several models were employed to determine the effects of different variables investigated. The first model consisted of the effects of β-glucan concentration, gluten addition, and fermentation on the viscosity of the physiological extracts. An additional model consisted of the effects of premixing, yeast addition, fermentation, and enzyme inactivation on the solubility of β-glucan. A final model analyzed the effect of incorporation into fermented and unfermented dough on the solubility of β-

glucan. Means were compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$.

3.3. RESULTS AND DISCUSSION

The levels of incorporation used in this study (0.75, 1.0, and 1.5 g β-glucan/serving of bread) reflect a probable range likely to be utilized by food manufacturers. An *in vitro* digestion system reported by Beer et al (1997) was adopted to evaluate the effects of the bread dough production process on β-glucan viscosity. Though the digestion system at 37^{o}C only approximates what is actually occurring in the body (Beer et al 1997), this method provides a more realistic view of the impact of the food system and processing variables on the β-glucan's effectiveness under physiological conditions upon consumption compared to extractions using boiling water or aqueous alkali, which employ conditions not reflective of the human body.

3.3.1. EFFECTS OF β-GLUCAN LEVEL, GLUTEN ADDITION AND FERMENTATION TREATMENT WITH YEAST ON VISCOSITY

The impact of β-glucan concentration, fermentation, and gluten addition on the viscosity of the physiological extracts of dough is presented in Table 3-2. Even though viscosity was measured over a shear rate range of 1.29-129 sec^{-1}, only the results obtained at 12.9 sec^{-1} (10 rpm) are presented as the physiological extracts of the breads exhibited Newtonian-like behavior and showed only a slight decrease in viscosity upon increasing shear rate. Gujral et al (2003) indicated that

TABLE 3-2
Viscosity of the Physiological Extracts of Bread Dough with Varied Levels of β-Glucan, With or Without Vital Gluten Addition[1], and Fermentation Treatments with Yeast

Dough Treatment	β-Glucan (g) / serving of bread	Viscosity (mPa.sec) at 12.9 sec^{-1} / 37°C
Not Fermented	Control	0.81 (± 0.02)^{e,d}
Without Gluten	0.75	1.03 (± 0.01)^{c}
	1.0	1.38 (± 0.06)^{b}
	1.5	1.56 (± 0.01)^{a}
With Gluten	0.75	1.03 (± 0.01)^{c}
	1.0	1.38 (± 0.04)^{b}
	1.5	1.67 (± 0.09)^{a}
Fermented	Control	0.72 (± 0.01)^{e}
Without Gluten	0.75	0.86 (± 0.03)^{d,e}
	1.0	0.87 (± 0.00)^{d}
	1.5	0.92 (± 0.05)^{c,d}
With Gluten	0.75	0.86 (± 0.02)^{d,e}
	1.0	0.87 (± 0.05)^{d}
	1.5	0.99 (± 0.01)^{c,d}

[1]Gluten added at levels of 0 or 2.18 g per g of added β-glucan
[a-e] Means (± standard deviation) within the same column with the same letter are not significantly different ($p > 0.05$)

gluten may offset the detrimental effect β-glucan addition may have on bread loaf volume, as reported in the past (Cavallero et al 2002, Gujral et al 2003). Gluten was added at 2.18 g per g of barley β-glucan based on extrapolating Gujral et al's (2003) data, as well as a positive trial run. There was no ($p>0.05$) effect of gluten addition on the viscosity of the extracts at each level of β-glucan. Thus, vital gluten may be incorporated into future bread formulations to offset any detrimental effect β-glucan addition may have on loaf volume as it does not appear to compromise the viscosity.

There was, however, a significant effect ($p \leq 0.05$) of β-glucan concentration and fermentation on the viscosity of the physiological extracts, as well as a significant interaction between the two. The extract viscosity of the unfermented dough, regardless of gluten addition, increased ($p \leq 0.05$) with β-glucan level with the 0.75, 1.0, and 1.5 g β-glucan/serving levels exhibiting viscosities of 1.03, 1.38, and 1.56-1.67 mPa·sec, respectively. Such increases in viscosity with increased β-glucan concentration have been illustrated in the past (Ren et al 2003, Lazaridou et al 2004, Burkus and Temelli 2006). Despite the fact that the BBG conc contained small amounts of starch and protein, it is unlikely that these would have contributed significantly to increases in extract viscosity as these substances would have been digested to the same extent as those present in the control formulation by the enzymes in the *in vitro* digestion system. There may have been a small contribution to viscosity by other soluble fibre components present in the BBG conc, particularly arabinoxylans, though this contribution would be minimal as arabinoxylans can only be solubilized to any appreciable extent under alkali conditions (Izydorczyk and Dexter 2008). Thus, very little would have been solubilized under the approximated physiological conditions.

The characteristic increase in solution viscosity with increasing β-glucan concentration was not seen when the dough was allowed to ferment for 235 min (Table 3-2). Following fermentation, the extracts of dough fortified with β-glucan at 0.75, 1.0, and 1.5 g β-glucan/serving all showed similar ($p > 0.05$) viscosities, which were all lower ($p \leq 0.05$) than the viscosities exhibited by the corresponding unfermented dough. This observation is supported by previous reports (Åman et

al 2004, Cleary et al 2007, Andersson et al 2008). Cleary et al (2007) suggested that the enzymatic hydrolysis of β-glucan during bread processing was most likely due to enzymes present in the added yeast, though, it is also probable that the flour's endogenous β-glucanase enzymes or those that may have contaminated the amylase added to the bread flour by the manufacturer are responsible for the decrease in viscosity due to depolymerization of the β-glucan molecule, leading to a reduction in MW (Irakli et al 2004). The effect of contaminant enzymes within flour or enzyme preparations has already been established as a problem (Åman et al 2004, Andersson et al 2008) as previous studies have recommended that β-glucan fortification of bread products be abandoned due to the detrimental effect of native β-glucanases (Andersson et al 2004). Burkus and Temelli (1998) determined that a high level of enzymatic degradation from β-glucanase occurs at pH 4.5. Furthermore, increasing bread dough fermentation time results in a greater reduction in the β-glucan viscosity (Åman et al 2004, Andersson et al 2004, Trogh et al 2004, Andersson et al 2008). As such, the conditions within the dough system and the lengthy fermentation time (235 min) would facilitate the degradation of the β-glucan by the action of β-glucanase enzymes within the flour. The reduction in the extract viscosity may have also been the result of a reduction in β-glucan solubility as its viscosity is also governed by the amount of β-glucan present in solution (Beer et al 1997).

3.3.2. EFFECTS OF REFLUXING FLOUR, YEAST ADDITION AND FERMENTATION TIME ON VISCOSITY

To better understand the effect of the enzymes in the flour on the viscosity of β-glucan extracts, physiological extracts were obtained from the dough corresponding to 1.5 g β-glucan/serving that was formulated with either the regular bread flour or bread flour that had undergone ethanol refluxing for 2 hr, with or without yeast addition, and subjected to a fermentation time of 0 or 235 min. The results (Table 3-3) indicate that the addition of yeast to the dough, fermented for 0 or 235 min, does not have an effect ($p>0.05$) on the extract viscosity, which is also supported by the findings of Andersson et al (2004). Furthermore, the results of the current study indicate that the enzymes in the flour are a key contributor to the reduction of β-glucan viscosity as refluxing the flour to inactivate such enzymes increased ($p\leq0.05$) viscosity in comparison to those obtained from dough formulated with regular bread flour.

Overall, refluxing the flour with ethanol prior to preparation of the dough resulted in higher ($p\leq0.05$) viscosity, while fermentation resulted in lower ($p\leq0.05$) viscosity. There was also interaction ($p\leq0.05$) between refluxing the flour and fermentation. All of the fermented dough extracts showed lower ($p\leq0.05$) viscosities than their unfermented equivalents. The unfermented doughs prepared with the refluxed flour showed the highest extract viscosities (3.23-3.34 mPa·sec). Interestingly, the dough formulated with the regular bread flour that did

TABLE 3-3
Viscosity of the Physiological Extracts of Bread Dough Formulated with 1.5 g β-Glucan/ Serving, With or Without Refluxing, Yeast Addition, and Varied Incubation Time

Flour Treatment	Yeast Added (Y/N)	Fermentation Time (min)	Viscosity (mPa · sec) at 12.9 sec^{-1}/ 37°C
Without Refluxing	N	0	1.47 (± 0.02) [b]
	Y	0	1.56 (± 0.06) [b]
	N	235	0.85 (± 0.01) [d]
	Y	235	0.92 (± 0.05) [d]
With Refluxing	N	0	3.34 (± 0.09) [a]
	Y	0	3.23 (± 0.04) [a]
	N	235	1.25 (± 0.09) [c]
	Y	235	1.25 (± 0.02) [c]

[a-d] Means (± standard deviation) within the same column with the same letter are not significantly different ($p > 0.05$)

not undergo fermentation produced extracts with viscosities less than half of this, ranging from 1.47-1.56 mPa·sec. The viscosity of the extracts from the dough formulated with the refluxed flour that had undergone fermentation was lower ($p \leq 0.05$) at 1.25 mPa·sec, though this value was higher ($p \leq 0.05$) than the viscosities of the extracts from the fermented dough formulated with the regular bread flour (0.85-0.92 mPa·sec).

The above results show that inactivating the enzymes in the flour prior to dough mixing maintains β-glucan viscosity when the dough is unfermented and helps in viscosity retention throughout fermentation. When the BBG conc was subjected to the same *in vitro* extraction procedure at the 1.5 g β-glucan/serving level, the physiological extract had a viscosity of 3.33 mPa·sec. Statistical comparison of the extract viscosities for all the dough treatments at the 1.5 g β-glucan level revealed that all of the extracts had lower ($p \leq 0.05$) viscosities than

that of the BBG conc, with the exception of the extract from the unfermented dough formulated with the refluxed flour, which was similar ($p>0.05$). The importance of inactivating the β-glucanase enzymes in grains prior to the extraction of β-glucan in order to maintain its MW and viscosity in solution has been highlighted previously (Wood et al 1978, Symons and Brennan 2004, Burkus and Temelli 2006). Refluxing with aqueous ethanol is a common approach to inactivate enzymes (Wood et al 1978). However, as Burkus and Temelli (1998) observed, ethanol refluxing alone may not be sufficient to provide a stable, high-viscosity product. In the current study, refluxing the flour prior to dough formulation, in the absence of fermentation, did result in extract viscosities that were similar ($p>0.05$) to those from the original β-glucan concentrate, while the dough formulated with the untreated bread flour showed extract viscosities that were 64% less. This finding supports the observation of Andersson et al (2004) that simply mixing the dough components together results in a decrease in the MW and thus the viscosity of the β-glucan. In addition, the first 30 min of the approximated physiological extraction process, up until decreasing the pH to 2.0, likely provided an environment that facilitated the action of the β-glucanase enzymes present in the flour and, therefore, may also contribute to the reduced extract viscosity for the dough produced with the flour that had not been refluxed.

Despite the high viscosity obtained by refluxing the flour prior to dough formulation, this treatment may not be realistic for commercial bread production, not only due to the additional cost, but also due to the fact that the gliadin portion of gluten is soluble in ethanol since it falls under the prolamine classification of

proteins. Thus, this treatment would result in low quality bread by preventing the formation of an adequate gluten network, which is primarily responsible for the gas holding capacity and resultant loaf volume in bread. However, the results allow for a greater understanding of β-glucan's journey through the dough making process and indicate that the enzymes in the flour are a key contributor to the degradation of β-glucan.

3.3.3. EFFECTS OF PREMIXING AND FERMENTATION WITH YEAST ON VISCOSITY

To complete the investigation of β-glucan throughout the bread dough making process, the effect of premixing the dry ingredients on the viscosity of the β-glucan extract was evaluated. This investigation was warranted as previous reports determined that the history of the β-glucan played a significant role in its network formation in solution (Burkus and Temelli 2006) and also that long-term storage of milled products at ambient temperature may lead to a decrease in the MW of extractable β-glucan (Beer et al 1997). The viscosities of the physiological extracts obtained from the dough fortified at the 1.5 g β-glucan/serving level that was formulated using the premixed dry ingredients are presented in Table 3-4. Sifting the dry ingredients together 0, 36, or 168 hr prior to dough preparation did not cause any decrease ($p>0.05$) in the extract viscosity, in either the unfermented or the fermented dough, with values ranging from 1.56-1.67 and 0.88-0.96 mPa·sec, respectively. As expected, fermentation resulted in a decrease ($p\leq0.05$) in viscosity. There was no interaction ($p>0.05$) between premixing and fermentation. The bread flour and the vital gluten had moisture

contents of 11.9% and 11.2%, respectively, which were considerably higher than that of the BBG conc, at 6.4%. This indicates that the 11.9% moisture present in bread flour was not sufficient to stimulate enzymatic cleavage of the β-glucan under dry storage conditions. This has positive implications for the food industry as the premixing of ingredients is a common time saving practice.

TABLE 3-4
Viscosity of the Physiological Extracts of Bread Dough Formulated with 1.5 g β-Glucan/Bread Serving Following Varied Premixing Time and With or Without Fermentation with Yeast

Dough Treatment	Dry Ingredient Premixing Time (hr)	Viscosity (mPa · sec) at 12.9 sec^{-1}/ 37°C
Not Fermented	0	1.56 (± 0.01)[a]
	36	1.67 (± 0.05)[a]
	168	1.60 (± 0.04)[a]
Fermented	0	0.92 (± 0.05)[b]
	36	0.96 (± 0.04)[b]
	168	0.88 (± 0.03)[b]

[a-b] Means (± standard deviation) within the same column with the same letter are not significantly different ($p > 0.05$)

3.3.4. SOLUBILITY OF β-GLUCAN FROM BBG CONC, UNFERMENTED DOUGH AND FERMENTED DOUGH WITH AND WITHOUT YEAST

In addition to viscosity, it is also important to investigate the solubility of β-glucan as increased solubility results in a greater β-glucan concentration in solution and thus a greater solution viscosity (Beer et al 1997, Lan-Pidhainy et al 2007). The results of the solubility test are presented in Figure 3-1 as percentage of β-glucan solubilized under approximated physiological conditions. Fermented

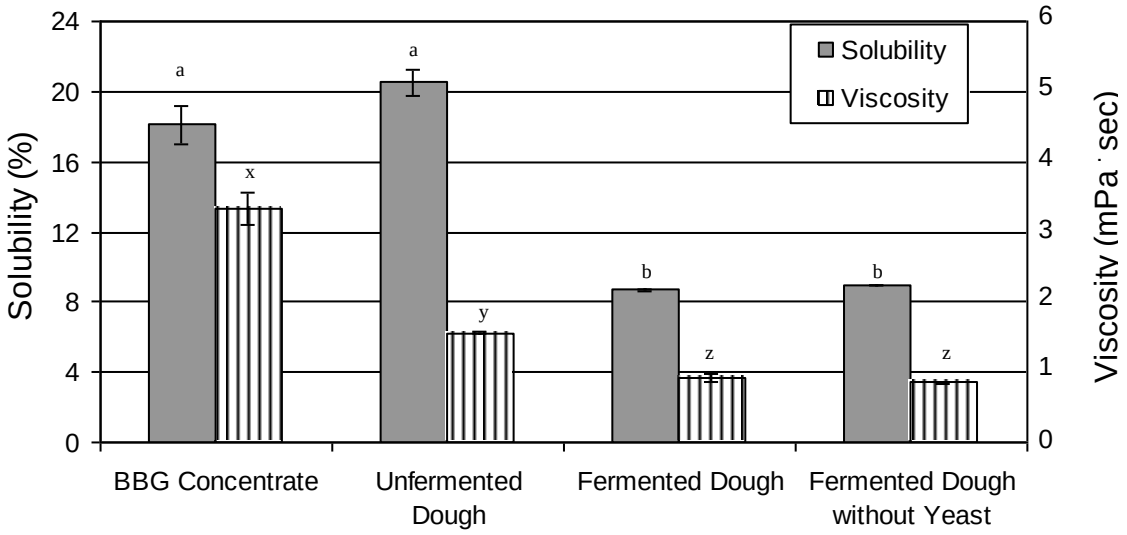


Fig. 3-1. Viscosity (mPa·sec) at 12.9 sec^{-1} and β-glucan solubility (%) for the physiological extracts of the barley β-glucan (BBG) concentrate, the fermented dough, with and without yeast, and the unfermented dough fortified with 1.5 g β-glucan/bread serving.
[a-b] Columns with the same letter are not significantly different ($p > 0.05$) in solubility.
[x-z] Columns with the same letter are not significantly different ($p > 0.05$) in viscosity.

dough formulated with 1.5 g β-glucan/serving, with and without yeast, had similar ($p>0.05$) and the lowest ($p\leq0.05$) β-glucan solubility at 8.7% and 9.0%, respectively. The similarity of these values also supports the observation that the presence of yeast does not impact the viscosity of the β-glucan extract from bread dough due to molecular degradation and/or a reduction in β-glucan solubility. Dough formulated with the same level of β-glucan that had not been fermented showed solubility that was more than double this value at 20.5%, which was similar ($p>0.05$) to the 18.1% solubility of the original BBG conc. Beer et al (1997) reported similar values as physiological extraction of β-glucan from oat bran and oat meal samples resulted in solubility values ranging between 12.9-28.7%.

Andersson et al (2004) observed that mixing for 10 min followed by fermentation increased the hot water extractability of β-glucan in bread dough in comparison to that in unfermented dough. However, under the approximated physiological conditions used in the current study, the opposite was seen as the fermentation process significantly decreased ($p \leq 0.05$) β-glucan solubility from 20.5% to only 8.7%. This inconsistency may have resulted from differing extraction methods. Performing extraction and solubility measurements under physiological conditions that approximates those of the body (versus extraction conditions exceeding 37^{o}C) on β-glucan-fortified food products may produce results that are a more realistic representation of how well a certain food system is able to elicit the desired health benefits within the body. The discrepancies in the results between the studies emphasize the importance of choosing the experimental conditions that are most applicable to the system in question in an effort to produce results that are the most reflective of the intended end goal.

The decrease in solubility upon fermentation as reflected in the extracts obtained under approximated physiological conditions may have occurred for a variety of reasons. The lengthy fermentation time may have allowed for insoluble complexes to form through interactions between the β-glucan and the dough components, thereby reducing solubility. Trogh et al (2004) and Andersson et al (2008), who saw a reduction in the extractability of β-glucan in bread dough upon fermentation, also acknowledged the possibility of such complexes. Izydorczyk and MacGregor (2000) provided evidence that spontaneous and strong intermolecular associations were formed between unsubstituted regions of the

xylan chains in arabinoxylans (AX) and the cellulose-like sections of β-glucan to produce insoluble complexes, likely through hydrogen bonding. Therefore, higher temperature extraction may have been sufficient to break such bonds, thereby resulting in the greater solubility and extractability values observed by Andersson et al (2004).

The lengthy fermentation time and reduction in β-glucan molecular size due to enzyme action, either from the flour's native enzymes or those that may have contaminated the amylase added to the flour by the manufacturer, and subsequent increase in the availability of small linear chains may have enhanced orientation between β-glucan and the gluten matrix through hydrogen bonding, in a manner similar to the interactions reported between starch and gluten (Wesley and Blakeney 2001). In addition, L-cysteine hydrochloride, a reducing agent added to the flour by the manufacturer, may have facilitated such a reaction. Although several protein hydrolyzing enzymes were added during the *in vitro* digestion protocol, it is likely that some of the protein may have remained undigested thereby allowing associations, if any, between β-glucan and the dough proteins to remain, though more research is needed to better understand such interactions. Kontogiorgos et al (2006) indicated that β-glucan and proteins phase separate, though this research was done in an aqueous system using sodium caseinate as the protein source. Interactions between β-glucan and gluten have not been investigated as research on the interactions between β-glucan and proteins in general is still in its infancy.

Smaller β-glucan fragments may have also reoriented and hydrogen bonded with each other on the linear, cellulose-like portions of each molecule (Fincher and Stone 1986), or, as more recently proposed, via association of consecutive cellotriose units linked by (1→3) bonds (Böhm and Kulicke 1999, Lazaridou et al 2004, Tosh et al 2004), thus reducing its solubility. This type of β-glucan reorientation was also seen by Beer et al (1997) and Lan-Pidhainy et al (2007). The presence of damaged starch granules within the flour and/or the primary end products of α-amylase digestion produced by the digestive enzymes in the *in vitro* digestion process, which are maltose, maltotriose and α-dextrins (Gray 2000) may have allowed for some interaction with the β-glucan. However, interactions within the actual human digestion tract are unclear as the products of α-amylase digestion are hydrolyzed into their component monosaccharides by enzymes expressed within the brush border of the small intestine for absorption (Gray 2000). Due to the uncertainty, it is apparent that further research is warranted to characterize the interactions between β-glucan, gluten, AX, and possibly other components found within the bread dough system. Furthermore, the results of this study indicate that the decrease in viscosity during fermentation appears highly dependent on both a reduction in MW from enzymatic depolymerization and a reduction in β-glucan solubility. Associations between lower MW and increased solubility have been noted previously (Wood et al 1991, Tosh et al 2008) and it is thought that increased β-glucan solubility would compensate for decreased viscosity (Beer et al 1997). However, this was not observed as the reduction in β-glucan viscosity, thought to be the result of a

decrease in MW, did not increase β-glucan solubility, as illustrated in Figure 3-1. Tosh et al (2008) reported that a reduction in β-glucan molecular weight from 2,200,000 to 400,000 initially increased β-glucan solubility; however, further reduction to 120,000 caused a reduction in β-glucan solubility, thought to be due to an increase in β-glucan self association because of increased mobility.

3.3.5. RELATIONSHIP BETWEEN β-GLUCAN EXTRACTION CONDITIONS, VISCOSITY AND SOLUBILITY

The viscosities of the extracts obtained by either the *in vitro* physiological extraction procedure (37^{o}C, 2.5 hr) or by hot water extraction (85^{o}C, 1 hr) are presented in Table 3-5. The average solubility of the β-glucan from the unfermented dough and the BBG conc was 19.3%. At this solubility level, the concentration of β-glucan within solution would be approximately 0.13%. Following hot water extraction, a solution of this concentration exhibited viscosity of 4.33 mPa·sec, which was 23% higher than the viscosity of the extract following *in vitro* extraction. Because the β-glucan concentrate and the β-glucan extracted from the unfermented dough showed the same solubility and thus the same concentration of β-glucan within solution, the 53% reduction in viscosity, from 3.33 mPa·sec to 1.56 mPa·sec, may only be attributed to the enzymatic degradation facilitated during dough mixing and the first stages of the *in vitro* digestion process. Despite the fact that the dough was unfermented, Andersson et al (2004), Flander et al (2007), and Trogh (2004) indicate that enzyme activity occurs very quickly within the dough and hydrolysis would have continued within the *in vitro* digestion process up to the α-amylase step.

TABLE 3-5
Viscosity (measured at 12.9 sec^{-1} and 37°C) of the Physiological Extracts (37°C, 2.5 hr) and Hot Water extracts (85°C, 1 hr) of the BBG Conc and Fortified Dough at Levels Corresponding to 1.5 g β-Glucan/Bread Serving, Taking into Account Solubility Differences

	Physiological Solubility (%)	β-Glucan Concentration[a] (%)	Physiological Extract Viscosity[b] (mPa · sec)	Hot Water Extract Viscosity[c] (mPa · sec)
BBG Concentrate	19.3	0.13	3.33	4.33
Unfermented Dough	19.3	0.13	1.56	4.33
Fermented Dough	8.7	0.06	0.92	1.62

[a] β-Glucan concentration in the physiological extract
[b] Extracts prepared from adequate amount of BBG conc and dough samples to provide 375 mg β-glucan
[c] Extracts prepared from BBG conc only with β-glucan amounts corresponding to the concentrations in the physiological extracts

Additionally, the solubility of the β-glucan from the fermented dough was 8.7%. At this solubility level, the concentration of β-glucan within solution would be approximately 0.06%. Following hot water extraction, a solution of this concentration exhibited viscosity of 1.62 mPa·sec, which was 57% higher than the viscosity of the physiological extract, at 0.92 mPa·sec. Because the decrease in viscosity due to the reduction in β-glucan solubility is already accounted for, the substantially lower viscosity of the physiological extract in comparison to the hot water extract suggests that enzymatic degradation of the β-glucan molecule is a key contributor to the reduction in extract viscosity during fermentation. Overall, the results indicated that the reduction in extract viscosity following dough mixing is primarily the result of enzymatic degradation of the β-glucan molecule by the enzymes present in the flour. The further decrease in extract viscosity following fermentation appears to be equitably attributable to enzymatic degradation of β-glucan and a reduction in β-glucan solubility. For comparison purposes, an additional hot water extraction of a 0.5% β-glucan solution from BBG conc was

performed, which exhibited a viscosity of 314 mPa·sec. By finding solutions to maintaining the MW and the solubility of β-glucan throughout the dough making process, substantially higher viscosities and possibly greater retention of physiological benefits may be obtained.

3.4. CONCLUSIONS

Under approximated physiological conditions, the viscosity development of barley β-glucan bread fortified at levels likely to be utilized by the food industry at commercial scale is dependent on the level of β-glucan addition, fermentation time, and inactivation of the enzymes present within the flour. The viscosity of the β-glucan extract was not affected by gluten addition, yeast addition, or premixing the dry dough ingredients. In terms of β-glucan solubility, fermenting the dough substantially lowered values, though simply incorporating β-glucan into the dough system did not affect solubility. This indicates that interactions between β-glucan molecules through self association and perhaps with various dough components may be to blame for the decrease in solubility levels. It is important to use realistic and applicable experimental procedures such as *in vitro* digestion when determining the characteristics of products targeted for human consumption, as some traditional experimental methods (hot water extraction) may yield different and less realistic results. In addition, the results of the current study indicate that the decrease in viscosity that has been primarily attributed to fermentation and a subsequent reduction in the β-glucan MW in the past may also be highly dependent on a decrease in β-glucan solubility. Therefore, in addition

to viscosity determination, it is essential to perform solubility tests in order to characterize the changes in β-glucan.

3.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 44-15A. The Association: St. Paul, MN.

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Beer, M.U., Wood, P.J., Weisz, J., Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-glucan extracted from oat products by an *in vitro* digestion system. Cereal Chem. 74(6):705-709.

Böhm, N. and Kulicke, W.M. 1999. Rheological studies of barley (1→3)(1→4)-β-D-glucan in concentrated solution: mechanistic and kinetic investigation of the gel formation. Carb Res. 315:302–311.

Burkus, Z., Temelli, F. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum. Cereal Chem. 75(6):805-809.

Burkus, Z., Temelli, F. 2006. Network formation by pilot plant and laboratory-extracted barley β-glucan and its rheological properties in aqueous solutions. Cereal Chem. 83(6):584-589.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chem. 102:889-897.

Fincher, G. B., Stone, B.A., 1986. Cell walls and their components. In: Cereal Grain Technology, Vol 8. Pomeranz, Y. (ed.) Am Assoc Cereal Chem, St. Paul, MN., USA. pp:206-207.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. J Nutr. 123:244-252.

Gray, G. 2000. Digestion and absorption of carbohydrates. In: Biochemical and Physiological Aspects of Human Nutrition. M.H. Stipanuk (ed.), W.B. Saunders Co. Philadelphia, PA, pp. 91-101.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Irakli, M., Biliaderis, C.G., Izydorczyk, M.S., Papadoyannis, L.N. 2004. Isolation, structural features and rheological properties of water-extractable β-glucans from different Greek barley cultivars. J Sci Food Agric. 84:1170-1178.

Izydorczyk, M.S., MacGregor, A.W. 2000. Evidence of intermolecular interactions of β-glucan and arabinoxylans. Carb Polym. 41:417-420.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Kontogiorgos, V., Ritzoulis, C., Biliaderis, C.G., Kasapis, S. 2006. Effect of barley β-glucan concentration on the microstructural and mechanical behaviour of acid-set sodium caseinate gels. Food Hydrocoll. 20:749-756.

Lan-Pidhainy, X., Brummer, Y., Tosh, S., Wolever, T.M., Wood, P. 2007. Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. Cereal Chem. 84(5):512-517.

Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M., Steele, B.R. 2004. A comparative study on structure-function relations of mixed-linkage (1→3), (1→4) linear β-D-glucans. Food Hydrocoll. 18:837-855.

McLeary, B.V., Glennie-Holmes, M. 1985. Enzymatic quantification of (1→3)(1→4) β-D-glucan in barley and malt. J Inst Brew. 91:285-295.

Nilsson, M., Andersson, R., Andersson, R.E., Autio, K., Åman, P. 2000. Heterogeneity in a water-extractable rye arabinoxylan with a low degree of disubstitution. Carb Polym. 41:397-405.

Ren, Y., Ellis, P.R., Ross-Murphy, S.B., Wang, Q., Wood, P.J. 2003. Dilute and semi-dilute solution properties of (1→3), (1→4)-β-D-glucan, the endosperm cell wall polysaccharide of oats (*Avena sativa* L.). Carb Polym. 53:401-408.

Symons, L.J., Brennan, C.S. 2004. The effect of barley β-glucan fibre fractions on starch gelatinization and pasting characteristics. J Food Sci. 69(4):C257-C261.

Tosh, S., Brummer, Y., Wolever T.M., Wood, P.J. 2008. Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. Cereal Chem. 85(2):211-217.

Tosh, S., Brummer, Y., Wood, P.J., Wang, Q., Weisz, J. 2004. Evaluation of structure in the formation of gels by structurally diverse (1→3)(1→4)-β-D-glucans from four cereal and one lichen species. Carb Polym. 57(3):249-259.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Wesley, I.J., Blakeney, A.B. 2001. Investigation of starch-protein-water mixtures using dynamic near infrared spectroscopy. J Near Infr Spec. 9(3):211-220.

Wood, P.J., Siddiqui, I.R., Palon, D. 1978. Extraction of high-viscosity gums from oats. Cereal Chem. 55(6):1038-1049.

Wood, P.J., Weisz, J., Mahn, W. 1991. Molecular characterization of cereal β-glucans. II. Size-exclusion chromatography for comparison of molecular weight. Cereal Chem. 86:530-536.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Wood, P.J., Beer, M.U., Butler, G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Yokoyama, W.H., Knuckles, B.E., Stafford, A., Inglett, G. 1998. Raw and processed oat ingredients lower plasma cholesterol in the hamster. J Food Sci. 63(4):713-715.

Chapter 4

MIXING CHARACTERISTICS AND RHEOLOGICAL PROPERTIES OF BREAD DOUGH WITH BARLEY β-GLUCAN[2]

4.1. INTRODUCTION

β-Glucan is a (1→3)(1→4)-β-D-glucan with cellulose-like sections linked through (1→3) glycosidic bonds, which allow the β-glucan to form viscous solutions upon solubilization (Burkus and Temelli 2005), as described in Chapter 2. Growing interest in incorporating β-glucan into food products stems primarily from its reported health benefits, including better regulation of blood glucose levels (Wood et al 1994, Yokoyama et al 1997, Bourdon et al 1999) and the lowering of blood cholesterol levels (Gallaher et al 1992, Martinez et al 1992, Newman et al 1992, Jackson et al 1994, Wang et al 1997, Bourdon et al 1999). However, the demand for products of high quality by consumers must not be overlooked (Sloan 2005).

Various studies incorporating β-glucan into bread have shown that β-glucan addition may be detrimental to bread loaf volume (Cavallero et al 2002, Gujral et al 2003, Trogh et al 2004), though none have fully evaluated the impact of β-glucan addition on dough mixing and rheological properties. It is essential to explore the effect of β-glucan incorporation on the mixing and rheological quality of bread dough to better understand the fundamental molecular interactions and

[2] A version of this chapter has been submitted to Journal of Cereal Science for consideration for publication.

the effects on quality to achieve the ultimate goal of a readily acceptable, high quality product. Therefore, the main objective of this study was to investigate the effects of β-glucan concentration and gluten addition on the mixing characteristics and rheological properties of bread dough following β-glucan incorporation at 0.75, 1.0, and 1.5 g β-glucan/serving of bread. In addition, the effect of β-glucan and gluten addition on the microstructure of the dough was also evaluated.

4.2. MATERIALS AND METHODS

4.2.1. MATERIALS

The materials incorporated into the bread dough are consistent with those described in Section 3.2.1.

4.2.2. DETERMINATION OF WATER HOLDING CAPACITY (WHC)

The WHC of 5.0 g of the β-glucan concentrate (BBG conc), the bread flour and the vital gluten was determined according to Method 88-04 (AACC International, 2000). A 5.0 g mixture of each sample was hydrated to a paste-like consistency with distilled water and centrifuged. The process was repeated and the approximate WHC, which was established as the maximum volume of water addition at which no supernatant was separated, was determined for each sample. Volumes of water 1.5 and 0.5 mL more and 1.5 and 0.5 mL less than the approximate WHC was then vigorously mixed for 2 min with each of the samples and then centrifuged. The two consecutive tubes, one with and one without supernatant, represented the range in which the WHC limit was reached. The

WHC is presented as the midpoint between those two volumes divided by g of sample.

4.2.3. BREAD DOUGH PREPARATION

Bread dough was prepared using Method 10-09 (AACC International 2000) with modifications. As shown in Table 4-1, a control batch and 6 dough treatments were formulated with the BBG conc at levels that corresponded to 0, 0.75, 1.0, or 1.5 g β-glucan/serving of bread, via direct (w/w) substitution of the bread flour. The actual levels of BBG conc inclusion were described in Section 3.2.2. Vital gluten was also added at 0 or 2.18 g added vital gluten per g of β-glucan for each level of β-glucan addition. All dry ingredients were sifted together immediately prior to dough formulation.

TABLE 4-1
Formulations for Bread Dough[1]

	Amount of β-glucan/serving						
Ingredient (g)	**0 g**	**0.75 g**		**1.0 g**		**1.5 g**	
Wheat Flour	100.0	96.25	96.25	95.0	95.0	92.5	92.5
Vital Gluten	0	0	4.1	0	5.5	0	8.2
Sugar	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Distilled Water	68.0	79.0	86.4	81.8	92.0	86.0	99.0
β-Glucan Concentrate	0	3.75	3.75	5.0	5.0	7.5	7.5

[1]Dough ingredients for small amplitude oscillatory measurements were mixed with a bread hook for 7 min on low speed using a KitchenAid mixer (Model KSM150SWH, KitchenAid Canada, Mississauga, ON)

4.2.4. DETERMINATION OF DOUGH MIXING CHARACTERISTICS

The mixing characteristics of the bread dough with the various amounts of β-glucan concentrate and vital gluten discussed previously were determined using a farinograph (Brabender Farino/Resistograph, Model FA/R-2, C.W. Brabender Instruments Inc., South Hackensack, NJ) equipped with a 50 g mixing bowl. The temperature was maintained at 30^{o}C with a circulating water bath (MGW Lauda, Model RM6, Konigshofen, Germany). Measurements of the dough development time (DDT) in min (measured as the time between the origin and the maximum development), the mixing tolerance index (MTI) in BU (measured as the difference in consistency between the peak consistency and 5 min after reaching the peak), dough stability (DS) in min (measured as the difference between the time the dough first reads 500 BU and the time it falls below), and time to breakdown (TTB) in min (measured as the time between the origin and 30 BU below the peak consistency) were measured according to Method 54-21 (AACC International, 2000). Samples were hydrated by adding the optimal amount of distilled water determined previously as the point at which the sample reached a consistency of 500 BU.

4.2.5. SMALL AMPLITUDE OSCILLATORY MEASUREMENTS

To determine the effect of the experimental treatments on the rheological properties of the experimental dough, small oscillatory shear measurements were performed using a Paar Physica UDS 200 rheometer (Glenn, VA), which was equipped with a Peltier heating system that controlled the sample temperature.

Rheological tests were performed at 25^{o}C using parallel plate geometry (25 mm diameter and 2 mm gap) with sanded surface probe to prevent slippage. After loading, the sample was left to rest for 15 min. To avoid moisture loss, the excess dough was trimmed just before the measurement and food grade petroleum jelly was used to thinly coat the sample's edges. Dough properties were analyzed using G' (the storage modulus), which represents the elastic behaviour of a test material, and G'' (the loss modulus), which represents the viscous behaviour. A strain sweep test was performed over the range of 0.01-100% at 1 Hz frequency to identify the linear viscoelastic (LVE) region. The % strain that exceeded the LVE region's tolerated 10% deviation was also obtained from this test. In addition, a frequency sweep test, ranging from 0.1 to 10 Hz, was performed on fresh dough using 0.01% strain, which was determined to be well within the LVE region from the results of the strain sweep test. The tests were performed on fresh dough samples prepared as described above.

4.2.6. FLUORESCENCE MICROSCOPY

Experimental bread dough structure was examined by fluorescence microscopy. Using the method described by Moore et al (2004) with modifications, Safranin O dye (S2255, Sigma-Aldrich, St. Louis, MO) was added to the control dough (0 g β-glucan/serving) and the 1.5 g β-glucan/serving dough, with and without added gluten, at a level of 0.002% (w/w flour basis). This was done to stain the starch and polysaccharides within the dough system so the position of the primary components could be determined. The dye was solubilized in the water required for dough formulation before mixing to ensure

homogenous distribution. A sample of fresh dough was placed on a microscope slide and was covered uniformly by a glass coverslip. A fluorescence microscope (Axio Imager M1m, Zeiss, Göttingen, Germany) with x10 objective was used to obtain the dough images using excitation and emission wavelengths of 470 ± 40 nm and 525 ± 50 nm, respectively.

4.2.7. STATISTICAL ANALYSIS

Samples were prepared with two complete replications. Determination of dough mixing characteristics, WHC, and small amplitude oscillatory measurements were all performed in duplicate. The WHC results, dough mixing characteristics, and the LVE limits were analyzed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Means were compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$.

4.3. RESULTS AND DISCUSSION

4.3.1. HYDRATION CHARACTERISTICS

The WHC of the BBG conc, bread flour, and vital gluten was determined. The BBG conc bound significantly ($p \leq 0.05$) more water than the vital gluten and the bread flour, with a WHC of 4.6 g H_2O/g of concentrate. Bhatty (1993) indicated that the high WHC of barley flour (4.5% β-glucan) and bran (7.7% β-glucan), 2.5 and 3.7 g H_2O/g of material, respectively, was primarily due to the high β-glucan content of barley. Similarly, Dhingra and Jood (2004) found that the water absorption of wheat flour was increased upon the addition of barley

flour. However, despite this comparatively high water binding capacity, the BBG conc utilized for this study is of relatively low solubility under approximated physiological conditions.

The bread flour bound the least ($p \leq 0.05$) amount of water in comparison to the BBG conc and the gluten, with a WHC of 0.8 g H_2O/g of flour, which was comparable to the 1.0 g H_20/g of flour reported by Bhatty (1993). WHC is influenced by protein content (Bhatty, 1993), thus vital gluten showed a slightly higher WHC of 1.1 g H_2O/g of vital gluten. This value is comparable to the WHC found by Chen et al (1988) of 1.42 g H_2O/g of vital gluten; however, their procedure was different from that used in this study, which may explain the slightly higher value.

The water absorption values increased ($p \leq 0.05$) upon the addition of β-glucan at all levels to obtain dough with 500 BU consistency. The control dough required the addition of 34 mL of water, while the doughs with 0.75, 1.0, and 1.5 g β-glucan/serving all required higher ($p \leq 0.05$) water levels, at 39.5, 40.9, and 43 mL, respectively. Increases in water absorption upon incorporation of β-glucan into bread formulations were also seen upon the replacement of white wheat flour with barley meal and oatmeal (Prentice et al 1979) and β-glucan extract from oat (Mohamed et al 2008). The addition of barley bran to biscuits was also shown to substantially increase water absorption (Sudha et al 2007). Because β-glucan has an abundance of exposed hydroxyl groups within its structure, an increase in water absorption upon its addition to dough may be expected. The addition of gluten further increased ($p \leq 0.05$) water absorption, as the dough with 0.75, 1.0,

and 1.5 g β-glucan/serving required the addition of 43.2, 46, and 49.5 mL of water, respectively. This observation is in agreement with Preston and Tipples (1980) who also demonstrated an increase in water absorption upon gluten addition.

4.3.2. MIXING CHARACTERISTICS

The mixing characteristics of the experimental dough are presented in Table 4-2. The dough development time (DDT) increased ($p \leq 0.05$) upon β-glucan addition at all levels with the control dough exhibiting DDT of 6.8 min, while the dough with 1.5 g β-glucan/serving had a DDT nearly 3 times this value at 20.8 min. The addition of brewers' spent grains high in fibre to bread dough also increased DDT (Prentice and D'Appolonia, 1977). The increase in DDT is in agreement with the observations of Markley and Bailey (1938) who showed that DDT increases with increasing water absorption, though this is often associated with an increase in wheat protein.

Very little work has been done on the mixing characteristics of β-glucan-fortified dough; however, when pentosans were added to dough a similar increase in DDT was seen (Jelaca and Hlynka 1971). Very strong flours that are higher in protein and mainly used for blending characteristically have a long DDT (>10 min) (Preston and Kilborn 1984). According to Bhatty (1993), barley flour (containing 4.5% β-glucan) showed a DDT of only 2 min. As such, it may be beneficial to incorporate BBG conc into bread flour due to the improved DDT versus trying to obtain a high β-glucan product through substitution of wheat flour with high amounts of barley flour. However, very long DDT is not desirable

TABLE 4-2
Mixing characteristics of Bread Dough with Varied Levels of β-Glucan and With or Without Vital Gluten Addition[1]

	β-glucan (g)/serving	DDT[2] (min)	Dough Stability (min)	MTI[3] (BU)	Time to Breakdown (min)
Control	0	6.8[g]	2.8[g]	40[a]	8.8[f]
Without Gluten	0.75	11.5[f]	7.3[f]	15[b]	14.8[e]
	1.0	17.5[e]	10.8[e]	5[c]	24[d]
	1.5	20.8[d]	13.3[d]	0[d]	30.5[c]
With Gluten[4]	0.75	28.5[c]	20.5[c]	5[c]	37.5[b]
	1.0	32[b]	22[b]	5[c]	37.5[b]
	1.5	45[a]	35[a]	0[d]	50[a]

[a-g]Means within the same column with the same letter are not significantly different ($p > 0.05$)
[1]Standard deviation was less than 10% for all values presented
[2]DDT:dough development time
[3]MTI: mixing tolerance index
[4]Vital gluten added at levels of 0 or 2.18 g per g of added β-glucan

as this may lead to increased energy cost. Gluten addition further increased ($p \leq 0.05$) DDT of the dough with 0.75, 1.0, and 1.5 g β-glucan/serving to 28.5, 32, and 45 min, respectively (Table 4-2). Preston and Tipples (1980) and Haraszi et al (2004) also reported increased DDT upon the addition of gluten prolamines to dough. These results are also comparable to observations made by Mohamed et al (2008) upon the addition of a β-glucan extract from oat and gluten to bread dough.

The control dough had the lowest ($p \leq 0.05$) dough stability, at 2.8 min. β-Glucan addition at all levels increased ($p \leq 0.05$) DS substantially (Table 4-2), as the dough with 1.5 g β-glucan/serving exhibited DS nearly five times that of the control. Because longer DS times indicate stronger flours (Shuey 1984), it would appear that the BBG conc confers a beneficial strengthening effect upon its addition to bread dough. Hanh and Rasper (1974) reported that the addition of

water-soluble, non-starch polysaccharide fractions from yam, sorghum, and millet to dough also showed a strengthening effect by increasing DS, though the addition of brewers' spent grain decreased DS, indicating a reduction in the quality of the resultant dough (Prentice and D'Appolonia 1977). Sudha et al (2007) and Mohamed et al (2008) also reported a reduction in DS upon incorporation of increasing levels of barley bran into biscuit batter and a β-glucan extract from oats into bread, respectively. Therefore, incorporating BBG conc into bakery product formulations may serve as a beneficial alternative due to the improvement in dough mixing characteristics, in addition to the increase in fibre content. Gluten addition also greatly increased ($p \leq 0.05$) the stability of the doughs fortified with β-glucan (Table 4-2), with DS values ranging from 20.5 to 35 min. Previous work by Preston and Tipples (1980) also showed an increased DS upon the addition of gluten to wheat dough.

The mixing tolerance index (MTI) and time to breakdown (TTB) measure the dough's resistance to the destructive forces of the farinograph's revolving blades (Watanabe et al 1992). In general, flours with good tolerance to mixing have low MTI values, while higher MTI values are indicative of weaker flours (Shuey 1984). The MTI decreased ($p \leq 0.05$) upon β-glucan addition at all levels (Table 4-2). The control dough showed a MTI of 40 BU, which is similar to that reported by Rosell et al (2001), while the addition of β-glucan at the highest level produced a MTI of 0 BU. According to Sudha et al (2007), incorporating increasing levels of barley bran into biscuit batter to increase its fibre content resulted in an opposite effect by increasing the MTI, indicating a weakening

effect. The addition of gluten decreased ($p \leq 0.05$) the MTI of dough with 0.75 g β-glucan/serving to 5 BU, but remained similar at 5 and 0 BU for the doughs with 1.0 and 1.5 g β-glucan/serving, respectively. The addition of a β-glucan extract from oat to bread dough was shown to increase the mixing tolerance index, despite the addition of gluten (Mohamed et al 2008). Therefore, due to the improvement in dough MTI, the use of BBG conc may be a more advantageous way of fortifying bread with β-glucan and/or increasing the fibre content of bakery products. In terms of TTB, the control dough had the lowest ($p \leq 0.05$) TTB at 8.8 min. The addition of β-glucan at each level increased ($p \leq 0.05$) TTB up to 30.5 min for the highest level of β-glucan addition (Table 4-2). Gluten addition increased ($p \leq 0.05$) TTB of the β-glucan-fortified dough further to 50 min for the dough with 1.5 g β-glucan/serving.

4.3.3. SMALL AMPLITUDE OSCILLATORY MEASUREMENTS

4.3.3.1. STRAIN SWEEP TEST

The % strain at which the LVE region of each experimental dough was exceeded is presented in Figure 4-1. The control dough was able to withstand the highest ($p \leq 0.05$) % strain with a LVE limit of 1.47%. This is higher than that reported previously (Phan-Thien and Safari-Ardi 1998, Safari-Ardi and Phan-Thien 1998). However, such studies cannot serve as a direct comparison as the strain limits were determined on flour-water doughs and thus were not formulated with all the ingredients required to produce the bread dough in this study, which has a characteristically strong and well developed gluten network. Similarly, Hayta and Schofield (2005) determined that 1% strain was still in the LVE region

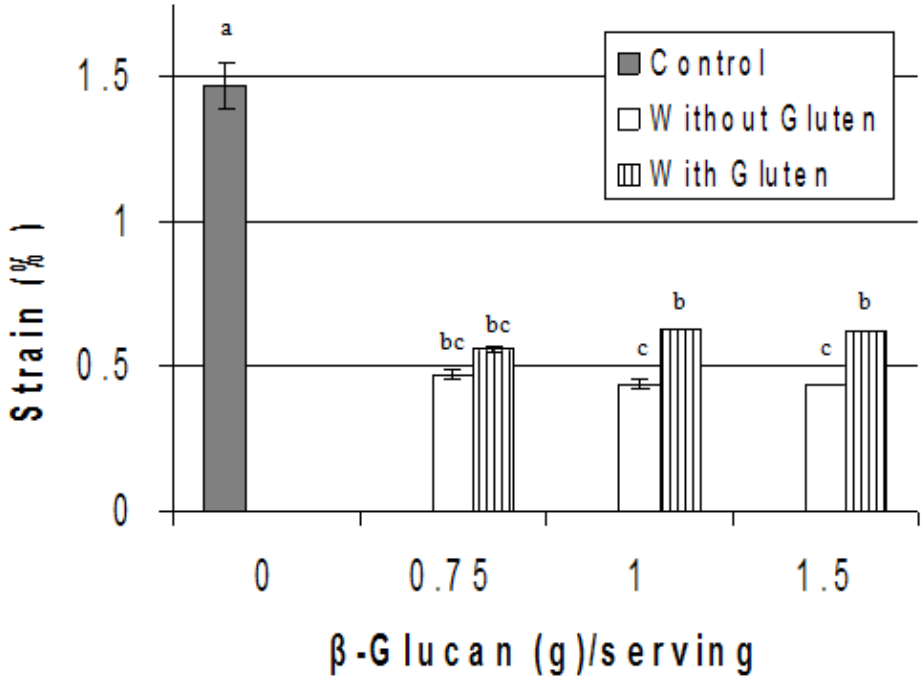


Fig. 4-1. Linear Viscoelastic (LVE) limit of bread dough with 0, 0.75, 1.0 and 1.5 g β-glucan/serving, without and with vital gluten addition. [a-c]Columns with the same letter are not significantly different ($p>0.05$) in LVE.

of wheat gluten, while Sivaramakrishnah et al (2004) performed a frequency sweep on wheat dough at 1% strain. Despite these observations, Khatkar and Schofield (2002) state that the critical deformation identifying the limit of the LVE region for wheat flour is difficult to characterize and is not often reported.

β-Glucan addition decreased the LVE limit ($p\leq0.05$) to 0.47, 0.44, and 0.44% for the doughs with 0.75, 1.0, and 1.5 g β-glucan/serving, respectively. The doughs fortified with 1.0 and 1.5 g β-glucan/serving with gluten exhibited LVE limits of 0.63 and 0.62%, respectively, which were higher ($p\leq0.05$) than the LVE limits of their corresponding doughs without gluten. The dough with 0.75 g β-glucan/serving with vital gluten had a LVE limit of 0.56%, which was similar

($p>0.05$) to that of its corresponding dough without gluten. Deviations from linearity occur when the sample is strained to a point at which certain weak physical bonds of the aggregated network structure are destroyed (Dickinson and Merino 2002).

The reduction in the LVE limit upon β-glucan addition may indicate that the presence of β-glucan decreases the strength of some of the network bonds, possibly by diluting the native flour gluten and/or interfering with gluten network formation either by binding water or simply due to its physical presence. In addition, the presence of β-glucan within the dough may have facilitated the formation of a tighter, less flexible network of shorter range through hydrogen bonding between the β-glucan molecules and the other dough components. This has been shown to reduce the LVE limit (Dickinson and Merino 2002). The addition of vital gluten to the dough with β-glucan would have facilitated the formation of a more prominent gluten network and thus interrupted the tighter, less flexible network resulting from interactions between β-glucan and the dough components. Mezger (2006) reported that higher maximum deformation is possible in an elastic, more loosely meshed network, such as that exhibited by an elastic gluten network bound primarily through disulphide linkages, while the maximum deformation is lower in a more tightly meshed network (Mezger 2006), such as that exhibited by the dough with β-glucan bound primarily through hydrogen bonds. This behaviour would explain the increase in the LVE limits when higher amounts of gluten were added to the doughs fortified with 1.0 and 1.5 g β-glucan/serving (Fig. 4-1).

4.3.3.2. FREQUENCY SWEEP TEST

Frequency sweep tests provide information regarding the time required for polymer entanglements to form or break within the variable periods of oscillations (Lazaridou et al 2003). The results of the frequency sweeps are presented in Figures 4-2 and 4-3 for the β-glucan-enriched dough without and with gluten addition, respectively. For all the dough samples, G' values were higher than G'' values, indicating the presence of a network structure. Stathopoulos et al (2006) reported that the plateau region in the frequency curve of a polymer, where G'>G'' and G' is approximately constant with frequency, reflects the degree of polymer entanglement and the ability of such polymers to move freely past each other, with a higher plateau reflecting a greater extent of polymer cross-linking or entanglement (Mezger 2006).

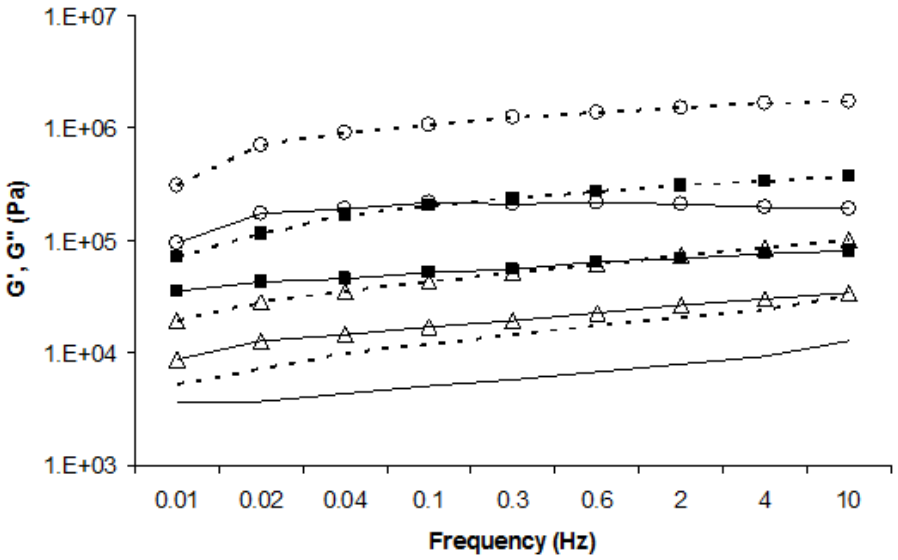


Fig. 4-2. Frequency sweeps of G' and G'' at 25°C of bread dough with 0 (G' - - - , G'' ——), 0.75 (G'- - △- - , G'' —△—), 1.0 (G'- - ■- - , G'' —■—), and 1.5 (G'- - ○- - , G'' —○—) g β-glucan/serving without added gluten.

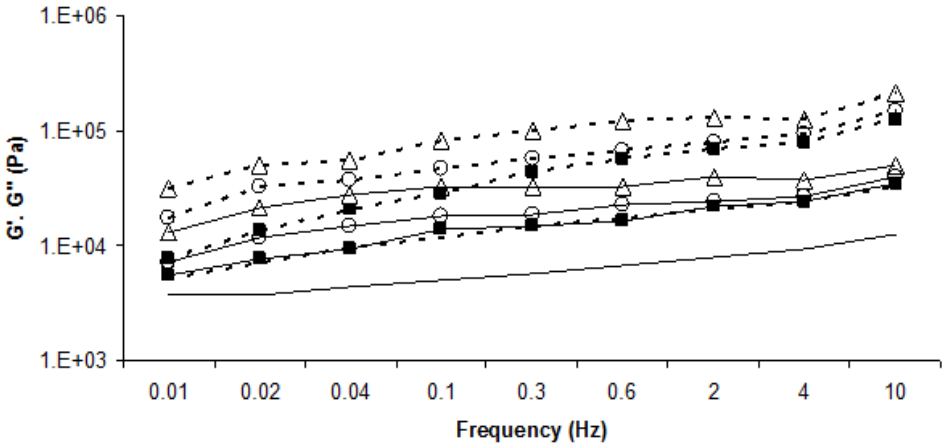


Fig. 4-3. Frequency sweeps of G' and G'' at 25°C of bread dough with 0 (G' - - - , G'' ——), 0.75 (G'- - △- - , G'' —△—), 1.0 (G'- - ■- - , G'' —■—), and 1.5 (G'- - ○- - , G'' —○—) g β-glucan/serving with added gluten.

The frequency sweeps of the doughs with 0, 0.75, 1.0, and 1.5 g β-glucan/serving (Fig. 4-2) show that the height of the plateau increases with increasing levels of β-glucan, reflecting a higher degree of entanglement (Mezger 2006). Modulus values were also shown to increase upon the incorporation of increasing levels of Oatrim® into solution, which contains β-glucan (Byars et al 2003). Higher G' values may indicate a firmer test material (Angioloni et al 2006), higher material rigidity and inflexibility (Mezger 2006), and/or a higher degree of cross-linking (Stathopoulos et al 2006). However, Watanabe et al (1992) indicated that higher modulus values may also indicate the presence of more rheologically active components and not necessarily a more solid dough. As discussed previously, the doughs with β-glucan showed lower LVE range limits (Fig. 4-1), indicative of a structure exhibiting a tighter, less flexible network of

shorter range, which may have resulted from interactions, primarily hydrogen bonds, between the β-glucan molecules and the dough components. This would result in a more rigid dough structure at the molecular level, as indicated by the higher G' values (Mezger 2006), as these measurements were performed within the LVE region. Because β-glucan is able to tightly bind large amounts of water (Wang et al 1998) within the dough system, less would have been available for the hydration of gluten, resulting in an underdeveloped gluten network (Symons and Brennan 2004). Though the reduction in gluten interactions alone would decrease modulus values, this was not the case in this study as the dough water was adjusted to facilitate the increase in water absorption seen upon the addition of β-glucan. An increase in interactions between β-glucan and the dough components would explain the increase in the G' and G'' values upon increasing levels of β-glucan addition. Lazaridou et al (2007) also reported an increase in G' values upon increasing levels of β-glucan addition, though this effect was studied in a gluten-free dough system.

The addition of vital gluten to the doughs with β-glucan produced modulus values (Fig. 4-3) that were also higher than those of the control dough. The dough with 0.75 g β-glucan/serving with added gluten showed the highest plateau value. The doughs fortified with 1.0 and 1.5 g β-glucan/serving with added vital gluten showed similar, but slightly lower values. The larger amount of vital gluten added to the dough with 1.0 and 1.5 g β-glucan/serving may have facilitated an increase in cross-linking between gluten molecules that interfered with the tighter, less flexible network between the β-glucan and dough components discussed

previously. This would result in lower modulus values, as lower modulus values are reflective of a reduction in system interactions (Salvador et al 2006). This is also supported by Keentok et al (2002) who compared doughs made from flours of differing protein contents and reported a reduction in modulus values upon an increase in the strength of the gluten network. The dough with 0.75 g β-glucan/serving exhibited the highest curve, indicating that the lower level of vital gluten added to this dough was not sufficient to facilitate the same extent of gluten cross-linking present in the doughs with 1.0 and 1.5 g β-glucan/serving and thus was not as effective in disturbing the tighter, less flexible network facilitated by β-glucan. These observations are also supported by the LVE range limits for these doughs (Fig. 4-1). It must be noted, however, that the rheological measurements were performed within the LVE range of the doughs, and thus the dough behaviour and consistency will likely be different at strains outside this LVE limit, such as those attributed to mixing and molding.

Overall, the rheological measurements indicate that β-glucan may be interfering with the gluten network, which would suggest a reduction in the loaf volume of the final product (Cavallero et al 2002). On the other hand, the results of the farinograph measurements indicate an improvement in the quality parameters of the dough (Preston and Kilborn 1984, Shuey 1984). Analysis of the mixing characteristics upon interpretation of the rheological measurements indicates that the increases in DS, DDT, and TTB and reduction in the MTI may have simply resulted from an underdeveloped/interrupted gluten network. Despite the difference in shear rates employed in each of these tests, it appears

that rheological measurements are very important in interpreting the nature of the exhibited mixing characteristics.

4.3.4. FLUORESCENCE MICROSCOPY

Figure 4-4 presents the microstructure of the experimental dough fortified with 0 g and 1.5 g of β-glucan/serving, without and with gluten, after staining with Safranin O. Attempts were made to use Calcofluor for fluorescence microscopy of the dough and bread since Calcofluor has been used extensively for staining β-glucan in the past. However, the images obtained exhibited continuous fluorescence and did not allow for distinction of structures. Even though Safranin O is not as specific for β-glucan, it did allow for better distinction of structure in the dough and bread. The image of the control dough (Fig. 4-4a) shows a well developed gluten network enveloping the fairly evenly distributed fluoresced areas that correspond to the starch granules and any cell wall polysaccharides present in the original dough formulation. Upon the addition of β-glucan to the dough (1.5 g β-glucan/serving) (Fig. 4-4b), the well-developed gluten network is no longer visible. The bright areas of the image have increased due to β-glucan addition, which appear to be evenly distributed throughout the dough matrix. The close proximity and uniform distribution of the fluoresced areas support the LVE range limit and the results of the frequency sweep, which indicate the presence of a tighter, less flexible network and numerous interactions between the experimental dough's rheologically active components. Also, because β-glucan is able to tightly bind large amounts of water (Wang et al 1998), less may have been available for the development of the gluten network (Symons and Brennan 2004).

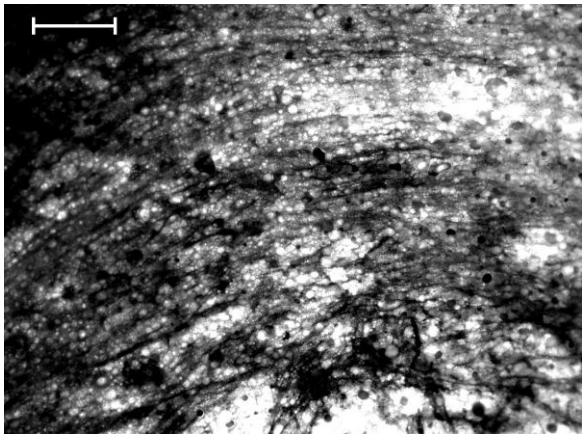
In the absence of water, gluten proteins tend to hydrogen bond together and form a dense mass (Belton 1999), which may explain the dark blotches evident in the fluorescence image (Fig. 4-4b) of the β-glucan-fortified dough. However, the similar shapes of the frequency sweep curves for the dough with 1.5 g β-glucan/serving, with and without added gluten indicate that perhaps a weak gluten network is present, though the bright fluorescence exuded by the β-glucan is sufficient to mask this dim feature (Fig. 4-4b).

Upon the addition of vital gluten to the dough with 1.5 g β-glucan/serving (Fig. 4-4c), the uniform distribution of the fluoresced area is still apparent; however, coarse gluten strands appear to have formed and have loosely enveloped the dough components. The formation of these gluten strands appeared to interfere with the tighter, less flexible network facilitated by the β-glucan. The interruption of the fluoresced areas by the coarse gluten strands supports this dough's LVE limit, which indicates the presence of a looser, more elastic network, and the results of the frequency sweep, which reflect a reduction in the interactions within the dough system (Salvador et al 2006).

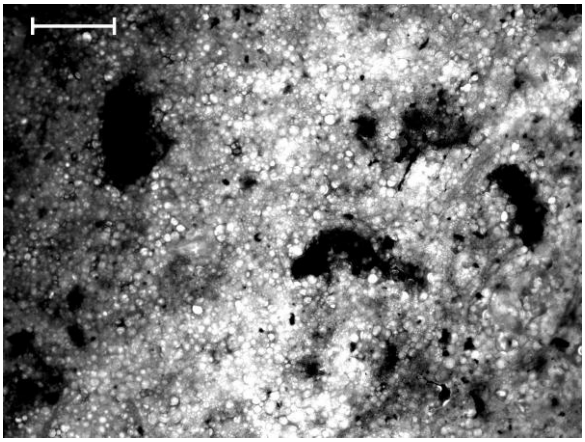
4.4. CONCLUSIONS

Water addition, dough development time, dough stability, and time to break down all increased, while the mixing tolerance index decreased, following incorporation of β-glucan into the dough system. This typically indicates an improvement in the quality parameters of the dough. The addition of vital gluten to the doughs with β-glucan resulted in further improvements in these dough

(a)



(b)



(c)

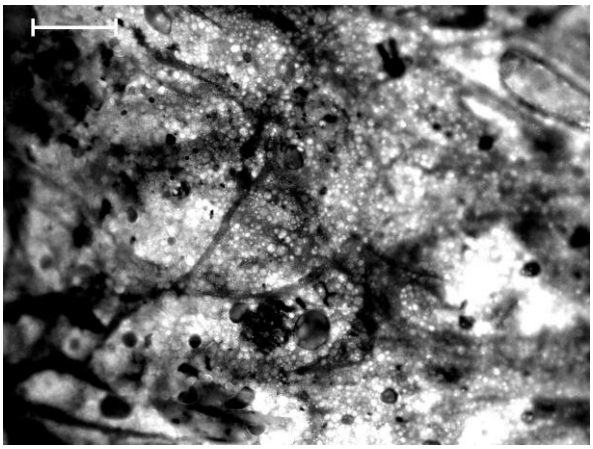


Fig. 4-4. Fluorescence image of bread dough with 0 g (a) and 1.5 g β-glucan/serving, without (b) and with added gluten (c), stained with Safranin 0 with x10 objective at excitation and emission wavelengths of 470 ± 40 nm and 525 ± 50 nm, respectively. Scale bar corresponds to 20 µm.

quality parameters. The results of the strain sweep test indicated that the addition of β-glucan to the bread dough resulted in a decrease in the LVE strain limit, while the addition of sufficient amounts of vital gluten increased this strain limit, though not to the level exhibited by the control dough. This indicates that the dough with β-glucan exhibits a tighter, less flexible network, while the increase in the LVE region limit upon the addition of vital gluten reflects a structure exhibiting a looser and more elastic meshed network. The results of the frequency sweep tests also indicated that incorporating β-glucan into the dough facilitated the formation of a tighter, less flexible network between the β-glucan and the dough components, likely through hydrogen bonding, which diluted and disturbed the formation of an adequate gluten network. Upon incorporation of increasing amounts of vital gluten, this tight network appeared to be disturbed by the formation of coarse gluten strands as indicated by the rheological measurements. These observations were also reflected in the microstructures of the bread.

The farinograph results indicated that β-glucan addition to bread dough improved dough mixing characteristics; however, the rheological measurements and fluorescence images demonstrated that β-glucan actually interfered with the formation of an adequate gluten network, which may be detrimental to the quality of the dough and the resultant bread. Therefore, in addition to the determination of mixing characteristics utilizing farinograph measurements, it is also essential to perform rheological measurements and study the microstructure of the system in

order to better understand and interpret the behaviour of bread dough upon incorporation of experimental ingredients.

4.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 88-04. The Association: St. Paul, MN.

Angioloni, A., Romani, S., Pinnavaia, G.G., & Rosa, M.D. 2006. Characteristics of bread making doughs: influence of sourdough fermentation on the fundamental rheological properties. Eur Food Res Technol. 222:54-57.

Belton, P.S. 1999. On the elasticity of wheat gluten. J Cereal Sci. 29:103-107.

Bhatty, R.S. 1993. Physicochemical properties of roller-milled barley bran and flour. Cereal Chem.70(4):397-402.

Bhatty, R.S. 1992. β-Glucan content and viscosities of barleys and their roller-milled flour and bran products. Cereal Chem. 69:469-471.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr. 69:55-63.

Burkus, Z., Temelli, F. 2005. Rheological properties of barley β-glucan. Carb Polym. 59:459-465.

Byars, J.A., Carriere, C.J., Inglett, G.E. 2003. Constitutive modeling of β-glucan/amylodextrin blends. Carb Polym. 52:243-252.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Chen, H., Rubenthaler, G.L., & Schanus, E.G. 1988. Effect of apple fiber and cellulose on the physical properties of wheat flour. J Food Sci. 53(1):304-305.

Dhingra, S., Jood, S. 2004. Effect of flour blending on functional, baking, and organoleptic characteristics of bread. Int J Food Sci Technol. 39:213-222.

Dickinson, E., Merino, L.M. 2002. Effect of sugars on the rheological properties of acid caseinate-stabilized emulsions gels. Food Hydrocoll. 16:321-333.

Gallaher, D.D., Locket, P.L., & Gallaher, C.M. 1992. Bile acid metabolism in rats fed two levels of corn oil and brans of oat, rye, and barley and sugar beet fiber. J Nutr. 122:473-481.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Hanh, P.P., Rasper, V. 1974. The effect of non-starchy polysaccharides from yam, sorghum and millet flours on the rheological behavior of wheat doughs. Cereal Chem. 51:734-750.

Haraszi, R., Gras, P.W., Tomoskozi, S., Salgo, A., Bekes, F. 2004. Application of micro Z-arm mixer to characterize mixing properties and water absorption of wheat flour. Cereal Chem. 81(5):555-560.

Hayta, M., Schofield, D. 2005. Dynamic rheological behaviour of wheat gluten during heating. J Sci Food Agric. 85(12):1992-1998.

Jackson, K.A., Suter, D.A.I., Topping, D.L. 1994. Oat bran, barley, and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J Nutr. 124:1678-1684.

Jelaca, S.L., Hlynka, I. 1971. Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem. 48:211-222.

Keentok, M., Newberry, M.P., Gras, P., Bekes, F., Tanner, R.I. 2002. The rheology of bread dough made from four commercial flours. Rheol Acta. 41:173-179.

Khatkar, B. S., and Schofield, J. D. 2002. Dynamic rheology of wheat flour dough. II. Assessment of dough strength and bread-making quality. J Sci Food Agr. 82:823-826.

Lazaridou, A., Biliaderis, C.G., Izydorczyk, M.S. 2003. Molecular size effects on rheological properties of oat β-glucans in solutions and gels. Food Hydrocoll. 17:693-712.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., Biliaderis, C.G. 2007. Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. J Food Eng. 79:1033-1047.

Markley, M.C., Bailey, C.H. 1938. The colloidal behavior of flour doughs. II. A study of the effects of varying the flour-water ratio. Cereal Chem.15:317-322.

Martinez, V.M., Newman, R.K., Newman, C.W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. J Nutr. 122:1070-1076.

Mezger, T.G. 2006. The Rheology Handbook. Hannover: Vincentz Network.

Mohamed, A., Rayas-Duarte, P., Xu, J. 2008. Hard red spring wheat/C-TRIM 20 bread: Formulation, processing and texture analysis. Food Chem. 107:516-524.

Moore, M.M, Schober, T.J., Dockery, P., Arendt, E.K. 2004. Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. Cereal Chem. 81(5):567-575.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., Hofer, P.J. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Phan-Thien, N., Safari-Ardi, M. 1998. Linear viscoelastic properties of flour–water doughs at different water concentrations. J Non-Newtonian Fluid Mechanics. 74:137-150.

Prentice, N., D'Appolonia, B.L. 1977. High-fiber bread containing brewer's spent grain. Cereal Chem. 54:1084-1095.

Prentice, N., Burger, W.C., D'Appolonia, B.L. 1979. Rolled high-lysine barley in breakfast cereal, cookies and bread. Cereal Chem. 56:413-416.

Preston, K.R., Kilborn, R.H. 1984. Dough rheology and the farinograph, The farinograph handbook (3rd Ed.) (pp. 38-42). Minnesota; MN: American Association of Cereal Chemists, Inc.

Preston, K.R., Tipples, K.H. 1980. Effects of acid-soluble and acid-insoluble gluten proteins and baking properties of wheat flours. Cereal Chem. 57:314-320.

Rosell, C.M., Rojas, J.A., Benedito de Barber, C. 2001. Influence of hydrocolloids on dough rheology and bread quality. Food Hydrocoll. 15:75-81.

Safari-Ardi, M., Phan-Thien, N. 1998. Stress relaxation and oscillatory tests to distinguish between doughs prepared from wheat flours of different varietal origin. Cereal Chem. 75:80-84.

Salvador, A., Sanz, T., Fiszman, S.H. 2006. Dynamic rheological characteristics of wheat flour-water doughs. Effect of adding NaCl, sucrose, and yeast. Food Hydrocoll. 20:780-786.

Shuey, W.C. 1984. Interpretation of the farinogram. In B.L. D'Appolonia, & W.H. Kunerth, The farinograph handbook (3rd Ed.) (pp. 31-32). Minnesota; MN: American Association of Cereal Chemists, Inc.

Sivaramakrishnah, H.P., Senge, B., Chattopadhyay, P.K. 2004. Rheological properties of rice dough for making rice bread. J Food Eng. 62:36-45.

Sloan, A.E. 2005. Healthy vending and other emerging trends. Food Technol. 59:26-35.

Stathopoulos, C.E., Tsiami, A.A., Dobraszczyk, B.J., Schofield, D.J. 2006. Effect of heat on rheology of gluten fractions from flours with different bread-making quality. J Cereal Sci. 43:322-330.

Sudha, M.L., Vetrimani, R., Leelavathi, K. 2007. Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chem. 100:1365-1370.

Symons, L.J., Brennan, C.S. 2004. The effect of barley β-glucan fiber fractions on starch gelatinization and pasting characteristics. J Food Sci. 69(4):C257-C261.

Trogh, I., Courtin, C.M., Goesaert, H., Delcour, J.A., Andersson, A.A, Aman, P., Fredriksson, H., Pyle, D.L., Sorensen, J.F. 2005. From hull-less barley and wheat to soluble dietary fibre-enriched bread. Cereal Foods World. 50:253-260.

Wang, L., Behr, S.R., Newman, R.K., Newman, C.V. 1997. Comparative cholesterol-lowering effects of barley β-glucan and barley oil in golden Syrian hamsters. Nutr Res. 17(1):77-88.

Wang, L., Miller, R.A., Hoseney, R.C. 1998. Effects of (1→3)(1→4)-β-D-glucans of wheat flour on breadmaking. Cereal Chem. 75(5):629-633.

Watanabe, E., Bell, A.E., Brockway, Brockway, B.E. 1992. Rheological studies on wheat flour dough. I. Empirical and fundamental methods. Food Control. 3(2):97-101.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnlund, J.R., Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74(3):293-296.

Chapter 5

SOLUBILITY AND VISCOSITY OF β-GLUCAN EXTRACTED FROM BREAD AND EVALUATION OF LOAF CHARACTERISTICS[3]

5.1. INTRODUCTION

β-Glucan is found in appreciable amounts in both barley (3-11%) and oats (3-7%) (Bhatty 1992, Lee et al 1992, Skendi et al 2003). Incorporation of large amounts of barley or oat flour needed to fortify bakery products with a sufficient amount of β-glucan to meet the guidelines set out by the FDA (2005) for the risk reduction claim for heart disease can lead to quality defects often due to gluten dilution, particularly in bread. Therefore, different commercial technologies (Vasanthan and Temelli 2008), described in Chapter 2, have been developed to obtain more concentrated sources of β-glucan and thus help reduce or even eliminate any negative effects on quality. However, the common use of wet separation processes to hydrate and solubilize β-glucan from the cell walls of barley or oats has many associated disadvantages including high costs and high susceptibility of the solubilized β-glucan to enzymatic degradation by contaminant enzymes and shear induced molecular fragmentation during mixing and centrifugation steps (Vasanthan et al 2004). Both enzymatic degradation and shear fragmentation would lead to a reduction in β-glucan molecular weight and, as a result, its viscosity. This is unfortunate as β-glucan's primary health benefits

[2] A version of this chapter is to be submitted to the Journal of Agricultural and Food Chemistry for consideration for publication.

are related to its viscosity. Fortification of bakery products, particularly bread products, with β-glucan extracts obtained via this method results in a further reduction in β-glucan viscosity and its health benefits (Gallaher et al 1993, Wood et al 1994, Wood et al 2000, Frank et al 2004, Pins and Kaur 2006). As such, it has been suggested that the use of β-glucan in bread products be avoided (Cavallero et al 2002).

Recently, Vasanthan and Temelli (2009) introduced a new technique that does not require solubilization of β-glucan, instead an aqueous ethanol based enzymatic process is employed to concentrate β-glucan in its native form. Because β-glucan is not solubilized and remains intact within the cell walls, it is protected from shear fragmentation and enzymatic degradation, exhibiting higher molecular weight and solution viscosity (Vasanthan and Temelli 2009). Despite these advantages however, β-glucan obtained via this process has not been tested in bread products and, due to its unique properties including relatively lower solubility under physiological conditions, may hold the key to effectively fortifying bread with the powerful health benefits elicited by β-glucan. Therefore, the main objective of this study was to investigate the effects of β-glucan concentration and gluten addition on the physicochemical properties of bread and the viscosity and solubility of the β-glucan following incorporation of low solubility β-glucan concentrate at levels corresponding to 0.75, 1.0, and 1.5 g β-glucan/serving of bread. The effect of baking temperature and prior β-glucan solubilization on the viscosity and solubility of β-glucan was also evaluated, as

well as the effect of β-glucan and gluten addition on the microstructure of the bread.

5.2. MATERIALS AND METHODS

5.2.1. BREAD PREPARATION

The materials, level of β-glucan and gluten addition, and bread dough preparation and fermentation regimes are consistent with the methods described in Sections 3.2.1 and 3.2.2. Following the 235 min fermentation step the dough was baked in an oven for 25 min at 220 ± 8^{o}C and then cooled on wire racks for 1 hr. To determine the effect of baking temperature, the bread dough was prepared utilizing the recipe for 1.5 g of β-glucan/serving without the addition of gluten (Table 3-1) and baked for 25 min at 110 ± 8 ^{o}C instead of the recommended 220 ± 8^{o}C. Additionally, to determine the effect of solubilizing the β-glucan prior to dough formulation, bread dough was prepared utilizing the recipe for 0.75 g of β-glucan/serving without the addition of gluten (Table 3-1). In this case, deviations from the original dough recipe included solubilizing the β-glucan concentrate in the water required for formulation of the dough at 85^{o}C for 1 hr. Evaporative losses were corrected for. Following solubilization, the cooled solution was added to the dry ingredients and the dough was prepared, fermented, and baked according to the procedure discussed previously.

5.2.2. LOAF CHARACTERIZATION

Loaf volume of the experimental breads was determined according to AACC Method 10-05 (2000). The loaf was put into a container of known volume

and the container was filled with rapeseeds. The volume of remaining seeds was measured using a graduated cylinder. The volume of the loaf was determined as the difference between the container volume and remaining seed volume.

Loaf height was determined using calibrated calipers and reported in mm with measurements taken from the center of each loaf. The colour of the loaves was analyzed using a Hunter Colorimeter (Model LSXE/UNI, Hunter Associates Laboratory, Inc., Reston, VA) that measured *L*, *a*, and *b* values. Crumb firmness was determined on fresh loaves after 1 hr of cooling at ambient temperature by performing compression tests using an Instron Universal Testing Machine (Model 4201, Instron Corp, Canton, MA). A 20 mm long bread crumb cylinder (28 mm diameter) was cut out from each bread and longitudinally compressed once to 50% of its original height at a crosshead speed of 100 mm/min. Compression force (kgF) for each sample was recorded as a function of displacement as a measure of firmness and the data were processed using Instron series IX software.

5.2.3. PHYSIOLOGICAL EXTRACTION OF β-GLUCAN

Physiological extraction methods are consistent with those described in Section 3.2.2., with the exception that bread samples containing 1/10 of the barley β-glucan originally incorporated into the whole dough were initially mixed with 75 mL of 20 m*M* sodium phosphate buffer (pH 6.9) containing 10 m*M* NaCl versus 50 mL due to buffer uptake by the baked bread. The physiological extract of the original BBG conc was also obtained using this method.

5.2.4. SOLUBILITY AND VISCOSITY DETERMINATION

Determination of β-glucan solubility and extract viscosity are consistent with the methods described in Sections 3.2.5 and 3.2.6. However, the following equation was used, accounting for the increase in the amount of buffer added during the physiological extraction, to determine the amount of β-glucan solubilized in the physiological extract as a percentage of the total amount of β-glucan in the original sample:

Physiological solubilization (%) = ΔA*F*150*1/1000*100/W*162/180*79.25/3

where ΔA is the difference between the absorbance reading of the sample and the sample blank, F is 100/average absorbance of 100 μg of glucose, W is the amount of β-glucan in the sample, and 150 x (79.25/3) is the dilution factor.

5.2.5. FLUORESCENCE MICROSCOPY

The structure of the experimental bread was examined by fluorescence microscopy. The Safranin O dye was added to the dough using the method described in Section 4.2.6. The dough was then prepared, baked, and cooled for 1 hr, as described previously (Section 5.2.1), and thin (1 mm) slices of the fresh samples were placed on a microscope slide and covered uniformly by a glass coverslip. A fluorescence microscope (Axio Imager M1m, Zeiss, Göttingen, Germany) with x10 objective was used to obtain the bread images using excitation and emission wavelengths of 470 ± 40 nm and 525 ± 50 nm, respectively.

5.2.6. STATISTICAL ANALYSIS

Bread samples were prepared with two complete replications. All measurements for each replication were performed in duplicate. Analysis of variance was performed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Means were compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$.

5.3. RESULTS AND DISCUSSION

5.3.1. LOAF CHARACTERIZATION

The loaf characteristics of the experimental bread are presented in Table 5-1. The volume of the bread decreased ($p \leq 0.05$) upon β-glucan addition at all levels. The control bread exhibited a volume of 710 mL, while the addition of β-glucan at levels of 1.0 and 1.5 g β-glucan/serving produced the bread with the lowest volume at 563 and 510 mL, respectively. A similar trend in loaf height was also seen. The addition of β-glucan decreased ($p \leq 0.05$) the height of the loaves from 77.5 mm for the control to 61.8 mm for the highest level of β-glucan addition. These observations are supported by previous studies. Cavallero et al (2002) and Trogh et al (2005) reported that the addition of barley flour and high β-glucan fractions from barley decreased the volume of breads in comparison to a wheat control. Interestingly, Cleary et al (2007) reported that the greatest loss in bread quality resulted from the addition of high molecular weight β-glucan to bread due to its ability to bind large amounts of water as opposed to the addition

of low molecular weight β-glucan. Increasing the amount of water-extracted β-glucan and barley flour added to bread resulted in a progressive decrease in loaf volume (Gill et al 2002, Jacobs et al 2008). Reductions in loaf volume and height upon the addition of β-glucan may have occurred due to binding of the water needed for gluten development by β-glucan (Gill et al 2002) or competition between β-glucan and starch for water during the baking step, resulting in starch granule retention (Symons and Brennan 2004). In addition, steam is also an important leavening agent and, due to β-glucan's high affinity for water, the amount of steam generated was likely reduced (Gill et al 2002).

TABLE 5-1
Characteristics of Bread with Varied Levels of β-Glucan and With or Without Vital Gluten Addition[1]

	β-Glucan (g)/serving of bread	Height (mm)	Volume (mL)	Firmness (kgF)	Colour		
					L	*a*	*b*
Control	0	77.5^{c}	710^{b}	0.19^{d}	78.9^{a}	1.1^{e}	19.8^{a}
Without Gluten	0.75	68.0^{d}	598^{cd}	0.33^{c}	72.8^{b}	2.5^{d}	19.6^{a}
	1.0	63.5^{de}	563de	0.48^{b}	70.1^{c}	3.1^{bc}	20.2^{a}
	1.5	61.8^{e}	510^{e}	0.64^{a}	68.6^{c}	3.5^{a}	19.7^{a}
With Gluten[2]	0.75	74.0^{c}	638^{c}	0.32^{c}	73.7^{b}	2.5^{d}	19.6^{a}
	1.0	82.5^{b}	750^{ab}	0.19^{d}	69.4^{c}	3.0^{c}	19.9^{a}
	1.5	88.3^{a}	790^{a}	0.19^{d}	68.5^{c}	3.3^{ab}	19.7^{a}

[a-e]Means within the same column with the same letter are not significantly different ($p > 0.05$).
[1]Standard deviation was less than 5% for all values presented.
[2]Gluten added at levels of 0 or 2.18 g per g of added β-glucan.

The addition of gluten to the breads with β-glucan did not affect ($p>0.05$) loaf volume at the lowest level of β-glucan addition (0.75 g β-glucan/serving). However, the bread with 1.0 g β-glucan/serving exhibited a volume similar

($p>0.05$) to that of the control, while the addition of gluten to the bread with the highest level of β-glucan (1.5 g/serving) produced a loaf with volume higher ($p\leq0.05$) than that of the control, at 790 mL. Gujral et al (2003) and Mohamed et al (2008) also experienced a reduction in loaf volume upon the addition of barley flour and β-glucan to bread; however, this was corrected upon the addition of gluten. A similar trend in loaf height was also seen as the addition of gluten at all levels increased ($p\leq0.05$) the height of the loaves fortified with the BBG conc. Moreover, the 1.0 and 1.5 g β-glucan/serving breads showed heights greater than that of the control. This is exciting as β-glucan alone usually produces unacceptably shrunken loaves, and the majority of previous studies did not include additional gluten in their bread formulations (Knuckles et al 1997, Cavallero et al 2002, Gill et al 2002, Symons and Brennan 2004).

Additionally, the longer dough development time (DDT) needed to produce β-glucan-fortified dough of optimal consistency is usually viewed as unacceptable due to the high power consumption associated with long mixing times (Chapter 4). This is because the DDT as determined by the farinograph testing for dough fortified with the highest level of β-glucan (1.5 g/serving) without and with gluten was between 21 and 45 min, respectively, as opposed to only 7 min for the control dough (Table 4-2). However, the mixing time actually applied for all of the experimental dough in this study was only 7 min, which indicates that loaf volume exceeding that of the control can be achieved while using the highest level of β-glucan addition and a mixing time equivalent to that of the control, thus easing manufacturer concerns of excessive power usage and loss of loaf volume.

The firmness of the bread increased ($p \leq 0.05$) upon the addition of β-glucan at each level, as the bread with the highest level of β-glucan addition exhibited firmness values nearly 3 times that of the control (Table 5-1). Jacobs et al (2008) also reported that the addition of a β-glucan-rich flour fraction increased firmness. Gill et al (2003) reported a similar trend upon the addition of barley flour to bread, which was attributed to β-glucan binding the water needed for gluten development and steam generation, thus resulting in a reduction in loaf volume and a subsequent increase in firmness. Results in Chapter 4 also indicated that the addition of β-glucan to dough dilutes and disturbs the formation of an adequate gluten network, which would indicate a reduction in loaf volume. In addition, β-glucan may compete with the starch for water and thus reduce gelatinization of the starch granules (Symons and Brennan 2004).

The addition of gluten to the breads with β-glucan did not affect ($p > 0.05$) firmness at the lowest level of β-glucan addition (0.75 g β-glucan/serving). However, the addition of gluten to the breads with 1.0 and 1.5 g β-glucan/serving produced breads with firmness values similar ($p > 0.05$) to that of the control. Gujral et al (2003) indicated that the addition of wet gluten to wheat breads with added barley flour decreased firmness. This likely occurred because the increased amount of gluten and water added to these formulations allowed for the formation of a more prominent gluten network, as discussed in Chapter 4, and hydration of the starch allowing the granules to more fully gelatinize (Symons and Brennan 2004).

In terms of colour, the control bread had the whitest ($p \leq 0.05$) crumb, while the addition of β-glucan caused the bread to darken ($p \leq 0.05$), based on the *L* values reported in Table 5-1. Gill et al (2002) and Trough et al (2005) also indicated that the addition of barley flour to bread caused it to darken. The 1.0 g β-glucan/serving bread was darker ($p \leq 0.05$) than the 0.75 g β-glucan/serving bread, but was similar ($p > 0.05$) in darkness to the 1.5 g β-glucan/serving bread. The *a* values indicated that the addition of β-glucan caused the bread to become redder ($p \leq 0.05$) at each level of addition, while there was no effect on the *b* values. Gill et al (2002) showed an increase in both *a* and *b* values upon the addition of barley flour to bread. The addition of gluten did not affect ($p > 0.05$) any of the colour parameters at each level of β-glucan. Therefore, gluten can be added to beneficially increase the loaf volume without changing the colour.

5.3.2. SOLUBILITY OF β-GLUCAN UNDER APPROXIMATED PHYSIOLOGICAL CONDITIONS

5.3.2.1. EFFECTS OF β-GLUCAN LEVEL, GLUTEN ADDITION AND BAKING ON β-GLUCAN SOLUBILITY

The results of the solubility test are presented in Figure 5-1 as percentage of β-glucan solubilized under approximated physiological conditions. Following baking, the solubility of the β-glucan within the breads fortified at different levels was similar ($p > 0.05$), ranging between 57.6-59.7%. The β-glucan solubility after baking was substantially higher than the 9% solubility reported previously from bread dough (Fig. 3-1) and the 12-33% from bran and rolled oat samples (Beer 1997). The addition of gluten to the 0.75 g β-glucan/serving bread did not affect

(p>0.05) the solubility of the β-glucan. However, the addition of gluten to the 1.0 and 1.5 g β-glucan/serving breads increased (p≤0.05) β-glucan solubility to 68.6 and 66.6%, respectively, which were similar (p>0.05).

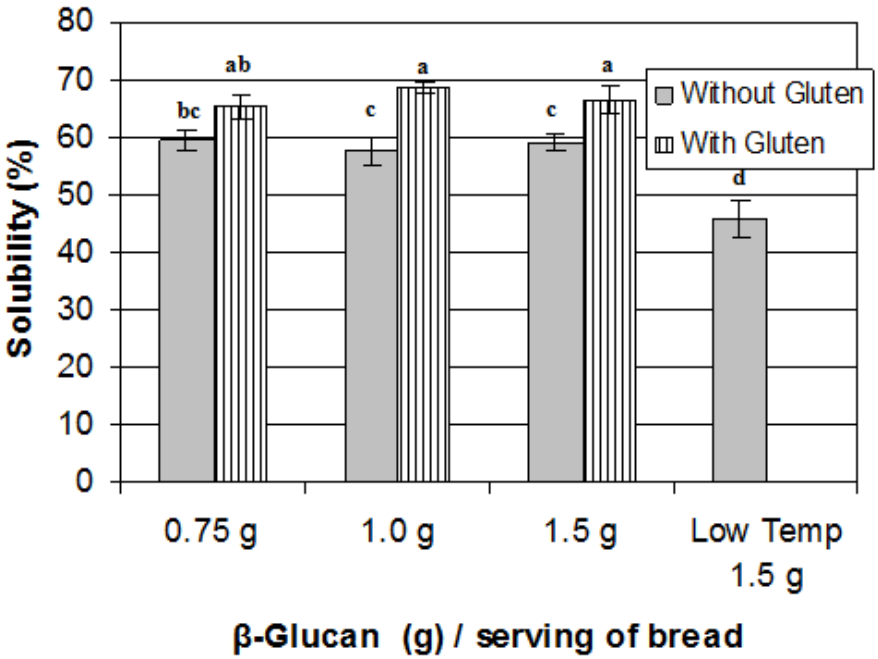


Fig. 5-1. Solubility (%) of β-glucan upon incorporation into bread at various levels. `Low temp` refers to baking temperature of 110°C as opposed to 220°C for the others. [a-d]Columns with the same letter are not significantly different (p>0.05) in solubility.

Because the amount of water added progressively increased upon the addition of β-glucan and gluten in order to form a dough of appropriate consistency, the higher water content may have allowed a greater amount of β-glucan to solubilize. In addition, a less dense bread structure would allow for the enzymes used for physiological extraction to more easily penetrate the bread structure and access their target substrates (primarily starch or protein). Thus, any

insoluble complexes that may have formed between β-glucan and these components, described in Chapter 3, would be broken upon digestion. Conversely, the breads with β-glucan, but without added gluten, required a lower level of water addition, had lower loaf volume, a more dense structure, and lower β-glucan solubility. Juntunen et al (2003) reported that the firmer, less porous structure of rye bread resulted in lower hydrolysis of starch *in vitro*. This was attributed to the bread's denser structure and larger particle size, which slowed the rate of hydrolysis. Additionally, Andersson et al (2004) reported that following fermentation the extraction yield of β-glucan from bread using hot water was similar or slightly lower than that from the corresponding doughs. Therefore, by utilizing the BBG conc obtained via the aqueous-ethanol based enzymatic process that escapes the enzymatic degradation associated with the mixing and fermentation steps, but becomes soluble following the baking step, β-glucan-fortified bread with superior final β-glucan quality may be achieved.

5.3.2.2. EFFECTS OF BAKING TEMPERATURE AND SOLUBILIZATION OF β-GLUCAN CONCENTRATE ON SOLUBILITY

The effect of baking temperature on the solubility of β-glucan was revealed upon baking the bread at 110^{o}C versus the recommended 220^{o}C. The results are presented in Figure 5-1. Baking the bread at the reduced temperature decreased ($p \leq 0.05$) β-glucan solubility to only 45.8%, as opposed to 59% when the bread was baked at the appropriate temperature. Temelli (1997) also reported that the extraction efficiency of β-glucan increased with temperature. Therefore, it is

essential to bake at the highest temperature possible during the short time of baking without compromising the quality of the product when using a low solubility β-glucan concentrate.

The effect of solubilizing the BBG conc prior to dough making on the solubility of the β-glucan in the final bread product was also investigated. This was done to simulate β-glucan addition using a readily soluble extract obtained via the conventional method of extraction. Due to the high solution viscosity that results from solubilizing such a high concentration of β-glucan, only the lowest level of β-glucan addition (0.75 g/serving) was investigated. Solubilizing the β-glucan prior to dough formulation decreased ($p \leq 0.05$) β-glucan solubility to only 20.8% versus 59.7% achieved by adding β-glucan concentrate in its original form. Solubilizing β-glucan prior to dough formulation causes it to be readily susceptible to enzymatic degradation (Vasanthan and Temelli 2008). Reductions in solubility may have occurred due to self association between degraded β-glucan strands (Fincher and Stone 1986, Böhm and Kulicke 1999, Lazaridou et al 2004, Tosh et al 2004) or interaction with the other bread components, which may be comparable to interactions described previously (Stainsby 1980, Sarker et al 1998, Izydorczyk and MacGregor 2000, Wesley and Blakeney 2001), though the presence of such complexes in β-glucan-fortified bread has not yet been investigated. Therefore, despite the fact that solubilizing β-glucan prior to dough formulation produced a bread with similar ($p > 0.05$) volume, height, and firmness to the 0.75 g β-glucan/serving bread formulated without solubilizing the β-glucan concentrate (results not shown), the detrimental impact on the β-glucan solubility

warrants the aversion of adding readily solubilized β-glucan extracts to fortify bread products. For this reason, it is essential that β-glucan extracts with initially low solubility be used for fortification of breads and bakery products.

5.3.3. VISCOSITY OF PHYSIOLOGICAL EXTRACTS

5.3.3.1. EFFECTS OF β-GLUCAN LEVEL, GLUTEN ADDITION AND BAKING ON VISCOSITY

As described in Chapter 2, β-glucan's cholesterol lowering and glucose regulating effects are primarily related to its viscosity. Measuring changes in viscosity is viewed as a sensitive indicator of small changes in β-glucan MW (Wood et al 1991). The impact of baking, β-glucan concentration, and gluten addition on the viscosity of the breads' physiological extracts obtained at 12.9 sec^{-1} is presented in Figure 5-2. The control bread produced an extract with a viscosity of 1.0 mPa·sec, while the addition of β-glucan to the breads caused the viscosity of the extracts to increase ($p \leq 0.05$), which supports the solubility values discussed previously (Fig. 5-1). The 0.75 and 1.0 g β-glucan/serving breads resulted in extracts with similar ($p > 0.05$) viscosities ranging from 3.0-4.2 mPa·sec, while the 1.5 g β-glucan/serving bread produced an extract with viscosity nearly three times higher ($p \leq 0.05$) at 11.1 mPa·sec. In comparison to the viscosity values obtained for dough fortified with the same BBG conc discussed in Chapter 3, it can be seen that baking substantially increases the viscosity of the physiological extracts. In previous studies, utilization of barley flour or soluble β-glucan extracts in bread resulted in a reduction in β-glucan viscosity (Gallaher et al 1993, Wood et al 1994, Wood et al 2000, Frank et al 2004, Pins and Kaur

2006), though Andersson et al (2004) reported that there was no significant difference in the molecular weights of β-glucan between the final dough and the baked bread. This indicates that the increase in extract viscosity seen in the present study is primarily the result of an increased amount of β-glucan solubilized upon each level of addition following the baking step, despite similar level of solubility on a percentage basis (Fig. 5-1).

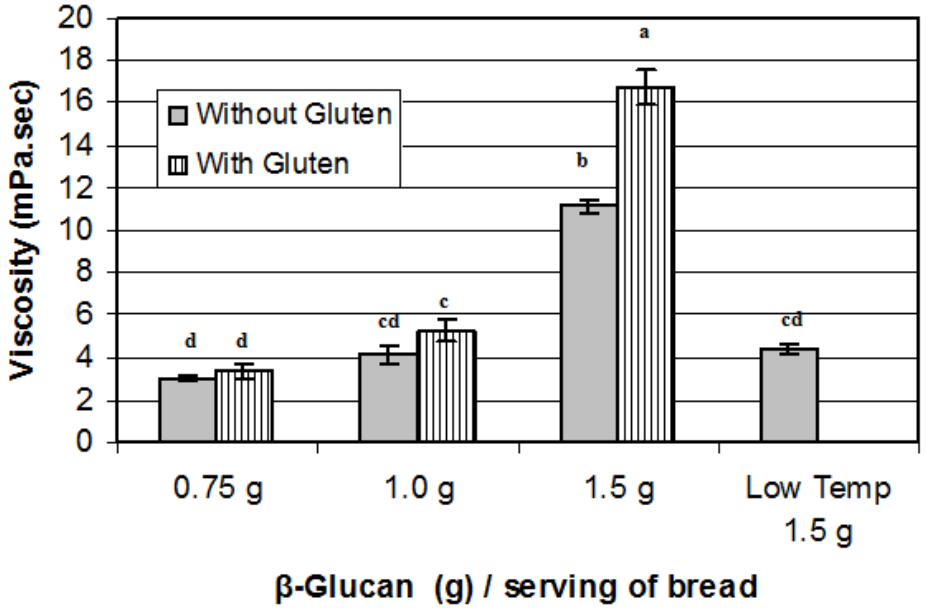


Fig. 5-2. Viscosity (mPa·sec) at 12.9 sec^{-1} and 37°C of the physiological extracts of bread fortified with various levels of β-glucan. `Low temp` refers to baking temperature of 110°C as opposed to 220°C for the others. [a-d]Columns with the same letter are not significantly different ($p>0.05$) in solubility.

In comparison to its corresponding bread without gluten, the addition of gluten did not affect ($p>0.05$) the viscosity of the extract from the 0.75 or 1.0 g β-glucan/serving bread. However, the addition of gluten to the bread with the highest level of β-glucan (1.5 g/serving) significantly increased ($p\leq0.05$) the

viscosity of the extract to 16.8 mPa·sec. Studies investigating the effect of gluten addition to β-glucan-fortified bread on the viscosity of the physiological extract are lacking. This bread's high loaf volume and height and, consequently, less dense structure may have allowed for greater amounts of β-glucan to be solubilized due to heat upon baking, in comparison to its corresponding dough without gluten. This porous structure may have also allowed for greater penetration of the digestive enzymes involved in the *in vitro* extraction technique. Therefore, more of the β-glucan may have been released from the bread structure and thus increased the amount of β-glucan in solution, which is reflected in the increase in β-glucan solubility (Fig. 5-1). The higher amount of water required to produce this dough may have also allowed more β-glucan to solubilize and thus resulted in greater solution viscosity.

5.3.3.2. EFFECTS OF BAKING TEMPERATURE AND SOLUBILIZATION OF β-GLUCAN CONCENTRATE ON VISCOSITY

The impact of baking temperature on the viscosity of the extract was revealed upon baking the bread at 110^{o}C versus the recommended 220^{o}C, as presented in 5-2. Baking the bread at the reduced temperature decreased ($p \leq 0.05$) extract viscosity to less than half (4.4 mPa·sec) of that produced when the bread was baked at the recommended temperature (11.1 mPa·sec). This reduction in viscosity is likely the result of the decrease in the amount of β-glucan solubilized within this bread (Fig. 5-2), as viscosity is highly dependent on β-glucan MW and the amount solubilized (Wood 2000). However, a reduction in heating rate due to

the lower oven temperature would have also allowed for the bread to remain in the temperature zone of high enzymatic activity for a longer period of time (Stear 1990), resulting in β-glucan degradation.

The effect of solubilizing β-glucan prior to dough making and bread baking on the viscosity of the bread's physiological extract was also investigated. Again, this was done to simulate incorporation of a readily soluble β-glucan extract into bread, as was previously the norm. Solubilizing the β-glucan prior to dough formulation significantly decreased ($p \leq 0.05$) the extract viscosity to only 0.87 mPa·sec, which was less than one-third of the viscosity achieved by adding the β-glucan concentrate in its original, low solubility form to the bread. Thus, the use of a highly soluble β-glucan extract would warrant the initial recommendation to abandon the use of β-glucan in yeast-leavened bread in order to avoid the substantial reduction in viscosity and, consequently, in the desired health benefits (Andersson et al 2004). Due to the improved performance of the low solubility β-glucan concentrate obtained through the aqueous-ethanol based enzymatic process (Vasanthan and Temelli 2009), it appears that β-glucan extract in this form is well suited for bakery applications where enzymatic degradation from native/exogenous flour enzymes is expected and where the heating step and added water are sufficient to solubilize the β-glucan in addition to inactivating enzymes. Thus, the use of β-glucan in yeast leavened bread as a method of conferring health benefits need not be avoided, provided a β-glucan concentrate with these physicochemical properties is utilized.

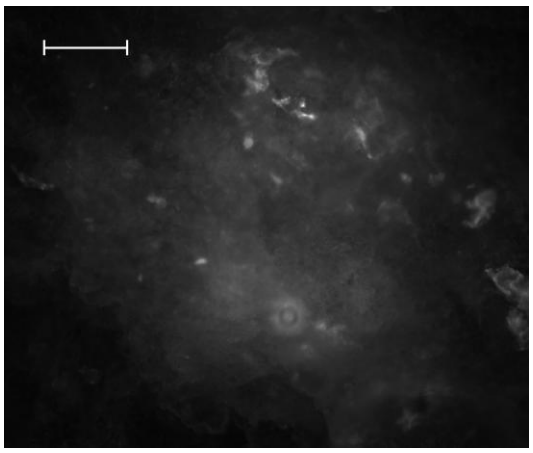
5.3.4. FLUORESCENCE MICROSCOPY

Safranin O was used to stain the starch and polysaccharides in the cell walls (Dürrenberger et al 2001). Figure 5-3 presents fluorescence microscopy images of the experimental bread fortified with 0 g and 1.5 g β-glucan/serving, without and with added gluten, after staining with Safranin O. The image of the control bread (Fig. 5-3a) is the least fluoresced, showing a small amount of cell wall polysaccharides and amylose that has leached from the starch granules. Upon the addition of β-glucan, the image (Fig. 5-3b) shows more fluorescence, indicating a greater presence and solubilization of polysaccharides, primarily β-glucan. The native, honey comb structure of the β-glucan concentrate is also readily apparent. The β-glucan-fortified bread with gluten (Fig. 5-3c) exhibits the greatest fluorescence. The greater fluorescence of this bread, indicates enhanced β-glucan solubility and also supports the viscosity and solubility values discussed previously. However, some of this fluorescence may also be due to the leaching of amylose from the starch granule as Autio and Salmenkallio-Marttila (2001) indicated that in the presence of higher amounts of water starch granules are swollen to a greater extent and more amylose leaches out from the granule.

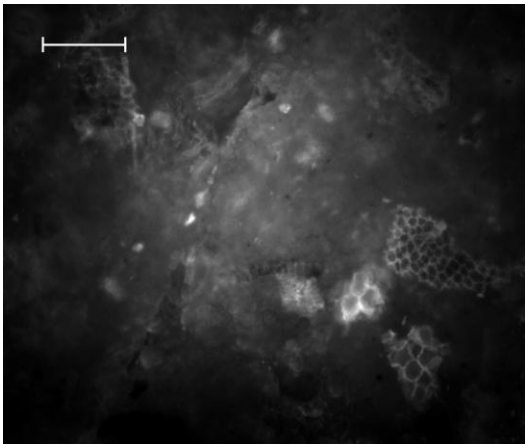
5.4. CONCLUSIONS

β-Glucan addition to bread decreased loaf volume and height, increased firmness, and resulted in darker and redder bread. The addition of gluten to the loaves with the highest level of β-glucan (1.5 g/serving) had no effect on colour;

(a)



(b)



(c)

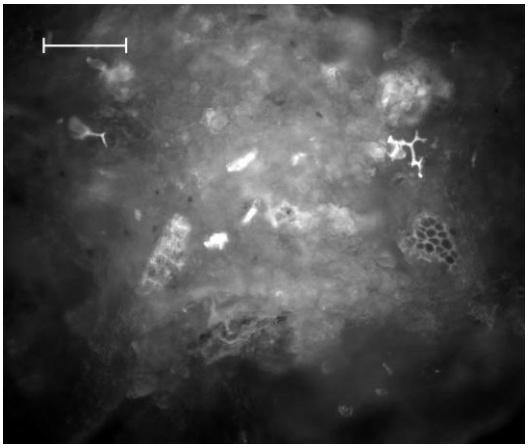


Fig. 5-3. Fluorescence image of bread with 0 g (a) and 1.5 g β-glucan/serving, without (b) and with added gluten (c), stained with Safranin 0 with x10 objective at excitation and emission wavelengths 470 ± 40 nm and 525 ± 50 nm, respectively. Scale bar corresponds to 200 µm.

however, it did decrease firmness and caused an increase in loaf height and volume to values similar to or exceeding those for the control.

β-Glucan was solubilized to a greater extent after baking, which was substantially higher than that previously reported for β-glucan in bread dough. The addition of gluten to the bread with the highest level of β-glucan (1.5 g/serving) increased solubility, as well as the viscosity of the physiological extracts, further. These results were supported by the fluorescence images of the bread microstructure. Baking the bread at the reduced temperature, however, decreased β-glucan solubility and extract viscosity, emphasizing the importance of baking at the highest temperature possible to ensure adequate β-glucan solubility when utilizing an extract of low initial solubility. Solubilizing the β-glucan prior to dough formulation also decreased β-glucan solubilization from bread and extract viscosity.

5.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 44-15A. The Association: St. Paul, MN.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Autio, K., Salmenkallio-Marttila, M. 2001. Light microscopic investigations of cereal grains, doughs and breads. Lebensmitt Wiss-u Technol. 34:18-22.

Beer, M.U., Wood, P.J., Weisz, J., Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-glucan extracted

from oat products by an *in vitro* digestion system. Cereal Chem. 74(6):705-709.

Bhatty, R.S. 1992. β-glucan content and viscosities of barleys and their roller-milled flour and bran products. Cereal Chem. 69:469-471.

Bohme, N., Kulicke, W.M. 1999. Rheological studies of barley (1→3)(1→4)-β-glucan concentrated solution: mechanistic and kinetic investigation of the gel formation. Carb Res. 315:302-311.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chem. 102:889-897.

Dürrenberger, M.B.; Handschin, S.; Conde-Petit, B.; Escher, F. Visualization of food structure by confocal laser scanning microscopy (CLSM). Lebensm Wiss-u Technol. 2001. 34, 11-17.

Fincher, G. B., Stone, B.A., 1986. Cell walls and their components in cereal grain technology, Vol 8. Pomeranz, Y. (ed.) Am Assoc Cereal Chem, St. Paul, MN, USA. pp:206-207.

Food and Drug Administration. 2005. Food labeling: health claims; soluble dietary fibre from certain foods and coronary heart disease; Final Rule Federal Register Doc. 2004P-0512, filed 12-23-05.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. J Nutr. 123:244-252.

Gill, S., Vasanthan, T., Ooraikul, B., Rossnagel, B. 2002. Wheat bread quality as influenced by substitution of waxy and regular barley flours in their native and extruded forms. J Cereal Sci. 36:219-237.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Izydorczyk, M.S., MacGregor, A.W. 2000. Evidence of intermolecular interactions of β-glucan and arabinoxylans. Carb Polym. 41:417-420.

Jacobs, M.S., Izydorczyk, M.S., Preston, K.R., Dexter, J.E. 2008. Evaluation of baking procedures for incorporation of barley roller milling fractions containing high levels of dietary fibre into bread. J Sci Food Agric. 88:558-556.

Juntunen, K.S., Laaksonen, D.E., Autio, K., Niskanen, L.K., Holst, J.J., Savolainen, K.E., Liukkonen, K., Poutanen, K.S., Mykkanen, H.M. 2003. Structural differences between rye and wheat breads may explain the lower postprandial insulin response to rye bread. Am J Clin Nutr. 78:957-964.

Knuckles, B.E., Hudson, C.A., Chiu, M.M. 1997. Effect of β-glucan barley fractions in high-fibre bread and pasta. Cereal Foods World. 42:94–99.

Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M., Steele, B.R. 2004. A comparative study on structure-function relations of mixed-linkage (1→3), (1→4) linear β-D-glucans. Food Hydrocoll. 18:837-855.

Lee, S.C., Prosky, L., deVries, J.W. 1992. Determination of total soluble, and insoluble dietary fibre in foods-enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. J AOAC Int. 75:395-402.

Mohamed, A., Rayas-Duarte, P., Xu, J. 2008. Hard Red Spring wheat/C-TRIM 20 bread: formulation, processing and texture analysis. Food Chem. 107:516-524.

Pins, J.J., Kaur, H. 2006. A review of the effects of barley β-glucan on cardiovascular and diabetes risk. Cereal Foods World. 51(1):8-11.

Sarker, D.K., Wilde, P.J., Clark, D.C. 1998. Enhancement of protein foam stability by formation of wheat arabinoxylan-protein crosslinks. Cereal Chem. 75(4):493-499.

Skendi, A., Biliaderis, C.G., Lazaridou, A., Izydorczyk, M.S. 2003. Structure and rheological properties of water soluble β-glucans from oat cultivars of Avena sativa and Avena bysantina, J Cereal Sci. 38:15-31.

Stainsby, G. 1980. Proteinaceous gelling systems and their complexes with polysaccharides. Food Chem. 6:3-14.

Stear, C. 1990. Handbook of Breadmaking Technology. Elsevier Applied Science. London and New York. Ch 1.

Symons, L.J., Brennan, C.S. 2004. The effect of barley β-glucan fiber fractions on starch gelatinization and pasting characteristics. J Food Sci. 69(4):C257-C261.

Temelli, F., 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J Food Sci. 62:1194-1201.

Tosh, S., Brummer, Y., Wood, P.J., Wang, Q., Weisz, J. 2004. Evaluation of structure in the formation of gels by structurally diverse (1→3)(1→4)-β-D-glucans from four cereal and one lichen species. Carb Polym. 57(3):249-259.

Trogh, I., Courtin, C.M., Goesaert, H., Delcour, J.A., Andersson, A.A, Aman, P., Fredriksson, H., Pyle, D.L., Sorensen, J.F. 2005. From hull-less barley and wheat to soluble dietary fibre-enriched bread. Cereal Foods World. 50:253-260.

Vasanthan, T., Temelli, F., Burkus, Z. 2004. Preparation of high viscosity β-glucan concentrates. Patent application, International Publication Number WO/2004/085484. International publication date-2004 July.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Vasanthan, T., Temelli, F. 2008. Grain fractionation technologies for cereal β-glucan concentration. Food Res Int. 876-881.

Wesley, I.J., Blakeney, A.B. 2001. Investigation of starch-protein-water mixtures using dynamic near infrared spectroscopy. J Near Infr Spec. 9(3):211-220.

Wood, P.J., Beer, M.U., Butler, G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Chapter 6

EFFECT OF DIFFERENT STORAGE CONDITIONS ON THE SOLUBILITY AND VISCOSITY OF β-GLUCAN EXTRACTED FROM BREAD UNDER *IN VITRO* CONDITIONS[4]

6.1. INTRODUCTION

Certain storage conditions have been shown to negatively impact the viscosity and solubility of β-glucan in muffins and reduce β-glucan's physiological effects (Beer et al 1997, Lan-Pidhainy et al 2007), though research within this important area of interest is still in its infancy. Reduction in β-glucan quality due to certain storage conditions may partly explain why 12 of the 33 clinical trials evaluated by the FDA prior to the approval of the health claim for the association between the consumption of products containing β-glucan and a reduction in the risk of heart disease showed no change in serum cholesterol upon consumption (Wood et al 2000). The impact of storage conditions on the solubility and viscosity of β-glucan in bread products has not yet been investigated. This research is necessary in order to make recommendations on the best storage conditions for β-glucan-fortified bread in an effort to maintain its efficacy. Therefore, the main objective of this study was to investigate the effect of storage temperature and time similar to those seen in the market place and also in clinical studies (23^{o}C for 1, 4, and 7 days, 4^{o}C for 4, 7, and 14 days, and -20^{o}C

[4] A version of this chapter is to be submitted to the Journal of Food Science for consideration for publication.

for 1, 4, and 8 weeks) on the solubility and viscosity of β-glucan upon incorporation into bread at levels corresponding to 0 or 1.5 g β-glucan/serving, with or without vital gluten addition. The firmness and moisture content of bread following each storage treatment were also evaluated.

6.2. MATERIALS AND METHODS

6.2.1. BREAD PREPARATION AND STORAGE CONDITIONS

The materials, level of β-glucan and gluten addition, and the bread dough preparation and fermentation regime for the control bread and the 1.5 g β-glucan/serving bread, with and without added gluten, are consistent with the methods described in Sections 3.2.1 and 3.2.2. Following the 235 min fermentation step the dough was baked in an oven for 25 min at 220 ± 8^{o}C and then cooled on wire racks for 1 hr. Once cooled, the breads were packed into polyethylene bags. Bread loaves were stored at room temperature (23^{o}C for 1, 4, and 7 days), refrigerated temperature (4^{o}C for 4, 7, and 14 days), and frozen (-20^{o}C for 1, 4, and 8 weeks) conditions. Prior to evaluation, the frozen bread was allowed to thaw at room temperature for 2 hr.

6.2.2. FIRMNESS AND MOISTURE DETERMINATION

Crumb firmness was determined after completion of the bread storage treatment at different temperatures by performing compression tests using an Instron Universal Testing Machine (Model 4201, Instron Corp, Canton, MA). A 20 mm long bread crumb cylinder (28 mm diameter) was cut out from the center of each bread and longitudinally compressed once to 50% of its original height at

a crosshead speed of 100 mm/min. Compression force (kgF) for each sample was recorded as a function of displacement as a measure of firmness and the data were processed using Instron series IX software. The moisture content (%) of the breads was determined according to AACC Method 44-15A (2000).

6.2.3. PHYSIOLOGICAL EXTRACTION OF B-GLUCAN AND DETERMINATION OF SOLUBILITY AND VISCOSITY

Physiological extraction (Section 5.2.3) and β-glucan solubility and viscosity (Section 5.2.4.) were determined according to the methods described previously in Chapter 5.

6.2.4. STATISTICAL ANALYSIS

Bread samples were prepared with two complete replications. All measurements for each replication were performed in duplicate. Analysis of variance was performed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Means were compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$.

6.3. RESULTS AND DISCUSSION

6.3.1. BREAD MOISTURE AND FIRMNESS

Bread staling refers to changes that take place after baking other than spoilage by microorganisms (Bechtel et al 1953). During storage the most pronounced changes are related to losses in moisture content and hardening of the crumb in bread (Guarda et al 2004). Firmness measurements are often used as a

method to determine the degree of staling in bread, though measurements of moisture loss may also be important (Stear 1990, Mohamed et al 2008, Kalinga and Mishra 2009).

6.3.1.1. ROOM TEMPERATURE STORAGE

Initial moisture values on fresh bread (Table 6-1) revealed that the 1.5 g β-glucan/serving bread with added gluten had the highest ($p \leq 0.05$) moisture content, followed by the 1.5 g β-glucan/serving bread, which is reflective of the higher amount of water added to the initial dough formulations. Control bread had the lowest moisture content, which decreased ($p \leq 0.05$) upon increases in storage time at each temperature. The moisture content of the 1.5 g β-glucan/serving bread decreased upon 1 and 4 days of room temperature storage to similar ($p > 0.05$) values, then decreased further ($p \leq 0.05$) after 7 days. The moisture content of the 1.5 g β-glucan/serving bread with added gluten after 1 day was similar ($p > 0.05$) to its corresponding fresh bread. Moisture levels decreased ($p \leq 0.05$) upon each increase in storage time for 4 and 7 days. The progressive loss of moisture at room temperature occurs due to diffusion from the crumb to the crust (Stear 1990). Previous research by Guarda et al (2004) also showed that hydrocolloids can reduce the rate of crumb dehydration in bread during storage.

Initial measurements on the fresh bread revealed that the 1.5 g β-glucan/serving bread was the firmest ($p \leq 0.05$), while the control and the 1.5 g β-glucan/serving bread with added gluten were similar ($p > 0.05$) (Table 6-2). The firmness of the control bread increased after 1 day of storage at room temperature

TABLE 6-1
Moisture[1] Content of Bread with 0 g or 1.5 g β-Glucan/Serving, With and Without Vital Gluten Addition, Stored Under Various Conditions[2]

Storage Treatment[3]	Time (days)	Amount of β-glucan/serving		
		0 g	1.5 g	1.5 g & vital gluten
Room Temperature	0	47.5[a]	58.3[a]	60.0[a]
	1	45.9[b]	55.1[b]	60.1[a]
	4	42.3[c]	54.1[b]	57.0[b]
	7	38.9[d]	51.5[c]	54.2[c]
Refrigerated	4	44.5[a]	53.0[a]	56.1[a]
	7	39.5[b]	53.5[a]	54.8[a]
	14	41.8[b]	53.4[a]	55.8[a]
Frozen	4	44.7[a]	53.9[a]	54.9[a]
	7	46.5[ab]	54.9[ab]	57.2[b]
	14	47.1[b]	55.8[b]	58.4[b]
	28	45.5[ab]	52.6[c]	55.1[c]
	56	44.7[c]	53.6[c]	57.0[c]

[1]Moisture values are reported as % moisture (w/w).
[2]Room, refrigeration and frozen storage temperatures were 23, 4 and -20°C.
[3]Standard deviation was less than 5% for all values presented.
[a-c]Means of each bread within the same storage treatment with the same letter are not significantly different ($p > 0.05$).

and increased further after 7 days. The 1.5 g β-glucan/serving bread stored for 1 and 4 days was similar ($p>0.05$) in firmness to the fresh bread, though storage for 7 days increased ($p\leq0.05$) firmness. Room temperature storage of the 1.5 g β-glucan/serving bread with added gluten for 7 days increased ($p\leq0.05$) its firmness, though storage for up to 4 days did not affect ($p>0.05$) firmness. Gujral et al (2003) and Mohamed et al (2008) also showed a delay in firming upon ambient storage of bread with added β-glucan and gluten, in comparison to a control. In addition, various hydrocolloids have also been shown to reduce the rate of firmness increase during bread storage (Davidou et al 1996, Guarda et al 2004).

The delayed rate of firming seen in this study is also in agreement with He and Hoseney (1990) who showed that breads with higher moisture contents firm at a slower rate.

TABLE 6-2
Firmness[1] of Bread with 0 g or 1.5 g β-Glucan/Serving, With and Without Vital Gluten Addition, Stored Under Various Conditions[2]

Storage Treatment[3]	Time (days)	Amount of β-glucan/serving		
		0 g	1.5 g	1.5 g & vital gluten
Room Temperature	0	0.17[a]	0.76[a]	0.25[a]
	1	0.49[b]	0.72[a]	0.40[a]
	4	0.70[bc]	0.89[a]	0.42[a]
	7	0.76[c]	1.19[b]	0.48[b]
Refrigerated	4	0.80[a]	1.59[a]	0.82[a]
	7	0.90[a]	1.63[a]	0.78[a]
	14	1.48[b]	2.0[b]	0.88[a]
Frozen	4	0.19[a]	0.68[a]	0.37[a]
	7	0.23[ab]	0.70[a]	0.42[a]
	14	0.29[ab]	0.75[ab]	0.46[a]
	28	0.42[b]	0.83[ab]	0.44[a]
	56	0.43[b]	0.91[b]	0.91[b]

[1]Firmess values are reported in kgF.
[2]Room, refrigeration and frozen storage temperatures were 23, 4 and -20°C.
[3]Standard deviation was less than 10% for all values presented.
[a-c]Means of each bread within the same storage treatment with the same letter are not significantly different ($p > 0.05$).

6.3.1.2. REFRIGERATED STORAGE

During refrigerated storage for 4 days, the moisture content (Table 6-1) and firmness values (Table 6-2) for each bread were lower ($p \leq 0.05$) than those for the corresponding fresh bread. The moisture content of the control bread decreased further ($p \leq 0.05$) after 7 days, though did not change after 14 days. Storing the

1.5 g β-glucan/serving bread without and with added gluten at refrigeration temperature for 4-14 days did not significantly affect bread moisture content. The reason for the stability in moisture values exhibited by the breads with β-glucan may have been due to β-glucan's ability as a hydrocolloid to tightly bind appreciable amounts of water and thus reduce crumb dehydration.

During refrigerated storage, the firmness values (Table 6-2) for each bread were higher ($p \leq 0.05$) than that of its corresponding fresh bread. The control bread had similar ($p > 0.05$) firmness values at 4 and 7 days refrigerated storage, though firmness increased ($p \leq 0.05$) after 14 days. The increase in the control bread's firmness corresponds to the decrease in its moisture content upon storage. The 1.5 g β-glucan/serving bread also showed similar ($p > 0.05$) firmness values at 4 and 7 days, but firmness increased after 14 days. However, this increase in bread firmness did not correspond with a loss of moisture for this bread upon refrigerated storage. The 1.5 g β-glucan/serving bread with added vital gluten did not increase ($p > 0.05$) in firmness when stored for 4-14 days. The stability in the moisture content for the breads fortified with β-glucan and firmness values for the β-glucan bread with added gluten upon prolonged refrigerated storage was unexpected, as this temperature range is highly conducive to bread staling (Stear 1990). Mohamed et al (2008) also reported stability in firmness values under similar storage conditions upon incorporation of β-glucan and gluten into bread. This was thought to have partially resulted from an alteration in water migration attributable to the increased protein and β-glucan content of the breads (Mohamed

et al 2008), though the change in bread moisture content upon storage was not measured.

6.3.1.3. FROZEN STORAGE

Under frozen storage, the moisture content of each bread was lower ($p \leq 0.05$) than that of its corresponding fresh bread (Table 6-1). Moisture content of the control bread decreased ($p \leq 0.05$) after 56 days of frozen storage. The moisture content of the 1.5 g β-glucan/serving bread was unaffected ($p > 0.05$) after 14 days, but decreased after 28 days. The moisture content of the 1.5 g β-glucan/serving bread with added gluten also decreased ($p \leq 0.05$) after 28 days. A reduction in bread moisture upon frozen storage for 2 weeks was also reported by Fik and Macura (2001).

When stored frozen, the firmness of the control bread on day 4 was lower ($p \leq 0.05$) than that for the bread stored for 28 and 56 days (Table 6-2). Bárcenas and Rosell (2006) also showed an increase in crumb firmness with frozen storage. Firmness of the 1.5 g β-glucan/serving bread, with and without gluten, was unaffected ($p > 0.05$) during 4 to 28 day storage, though prolonged storage for 56 days increased firmness. It has been long known that freezing as a preservation method can retard the staling of bread primarily due to reduced movement of molecules within the bread system and improved moisture retention (Stear 1990). Stear (1990) reported that crumb softness remained relatively constant for one month when bread was stored at -18^{o}C, though progressively increased in firmness after this.

Upon comparison of the effect of the different storage conditions on the breads' moisture and firmness values at days 4 and 7, where overlapping observations can be made, the results indicate that after 4 days of storage the lowest ($p \leq 0.05$) moisture losses were seen upon frozen storage for the control bread, at room temperature storage for the 1.5 g β-glucan/serving bread with added gluten, and at either room temperature or frozen storage for the 1.5 g β-glucan/serving bread. In terms of firmness, 4 day frozen storage showed the lowest ($p \leq 0.05$) firmness values for all of the breads, though both of the breads fortified with β-glucan could also be stored at room temperature to achieve firmness values similar ($p > 0.05$) to that of frozen storage. Furthermore, for 7 days storage, freezing the breads resulted in the lowest ($p \leq 0.05$) moisture losses and firmness values. This staling trend is characteristic to bread (Stear 1990, Kent and Evers 1994). The 1.5 g β-glucan/serving bread with added gluten could also be stored at room temperature for 7 days to achieve firmness values similar ($p > 0.05$) to that of frozen storage. Mohamed et al (2008) showed that frozen storage of bread with added β-glucan and gluten over one week also produced the lowest firmness values when compared to storage at ambient and refrigeration temperatures. In addition, Kalinga and Mishra (2009) reported that frozen storage of low-fat cakes with β-glucan produced the least change in hardness over a 21 day storage period, in comparison to ambient and refrigeration temperatures.

6.3.2. β-GLUCAN SOLUBILITY AND VISCOSITY

Measurement of β-glucan solubility for the fresh breads showed that the 1.5 g β-glucan/serving bread with added gluten exhibited higher ($p \leq 0.05$) solubility

(62.1%) than the 1.5 g β-glucan/serving bread without added gluten (55.3%), which is consistent with the findings in Chapter 5. Upon 1 day storage at room temperature, this value decreased ($p \leq 0.05$) by almost half to 26.3% for the 1.5 g β-glucan/serving bread and to 29.6% for the 1.5 g β-glucan/serving bread with added gluten. Interestingly, this reduction corresponds to the >50% decrease in soluble amylose in bread crumb upon storage for one day at room temperature (Kim and D'Appolonia 1977), which is thought to result from associations with itself and/or other carbohydrate chains present in the interstitial aqueous phase (Stear 1990). Therefore, such interactions with β-glucan chains may be possible, which would result in a reduction in the solubility of β-glucan to a similar extent, though interactions between amylose and β-glucan have not been studied. Solubility of β-glucan decreased further ($p \leq 0.05$) at 4 days room temperature storage, to similar ($p > 0.05$) values for both breads, ranging from 9.8-10.9%. Reductions in solubility may have also resulted from complexing between β-glucan molecules over time, which was also thought to occur between β-glucan molecules within bread dough during fermentation, as discussed in Chapter 3. This self association was also thought to be the mechanism responsible for the reduction in β-glucan solubility in oat bran muffins upon long-term frozen storage by Beer et al (1997) and upon increases in the number of freeze-thaw cycles (Lan-Pidhainy et al 2007). However, reductions in β-glucan solubility upon room temperature storage of bakery products have not been investigated previously.

The results of the viscosity measurements are presented at a shear rate of 12.9 sec^{-1}. Viscosity determination of the physiological extracts from the fresh

breads indicated that the control bread had the lowest ($p \leq 0.05$) viscosity, while the 1.5 g β-glucan/serving bread with added gluten showed the highest ($p \leq 0.05$). The viscosity of the physiological extract from the control was similar ($p \leq 0.05$) throughout all of the storage treatments ranging from 0.65-0.75 mPa·sec. For the 1.5 g β-glucan/serving bread, the viscosity of the physiological extract for the fresh bread was highest ($p \leq 0.05$) at 8.8 mPa·sec, though it decreased substantially to 1.5 mPa·sec upon 1 day of storage and further ($p \leq 0.05$), to 0.7, upon 4 days storage at room temperature. The 1.5 g β-glucan/serving bread with added gluten showed a similar trend. The viscosity of the physiological extract for the fresh bread was the highest ($p \leq 0.05$) at 14.4, though it also decreased ($p \leq 0.05$) substantially upon 1 day room temperature storage to 1.5 mPa·sec and further ($p \leq 0.05$), to 0.7 mPa·sec, at 4 days. The progressive reduction in viscosity for these breads corresponds with the progressive reduction in β-glucan solubility. As shown in Chapter 3, the amount of β-glucan solubilized has a large impact on the viscosity of the extract.

To identify the storage conditions that most effectively maintain β-glucan quality, the results for the storage treatment times that overlapped were compared. The solubility and viscosity results for the 1.5 g β-glucan/serving bread without and with added gluten stored at 23, 4 and -20^{o}C for 4 and 7 days are presented in Figures 6-1 and 6-2, respectively. At 4 days, the frozen bread had the highest ($p \leq 0.05$) β-glucan solubility, ranging from 44.6-45% for both β-glucan containing breads. Storage at room temperature and refrigeration temperature produced similar ($p \leq 0.05$) values, ranging from 9.8-10.9% for both β-glucan containing

breads. The viscosity values also reflected this as the bread that was frozen had the highest ($p \leq 0.05$) extract viscosity with values ranging from 2.3-2.8 mPa·sec for both breads. Storage at room temperature and refrigeration temperature produced similar ($p > 0.05$) values, ranging from 0.7-0.95 mPa·sec for both breads.

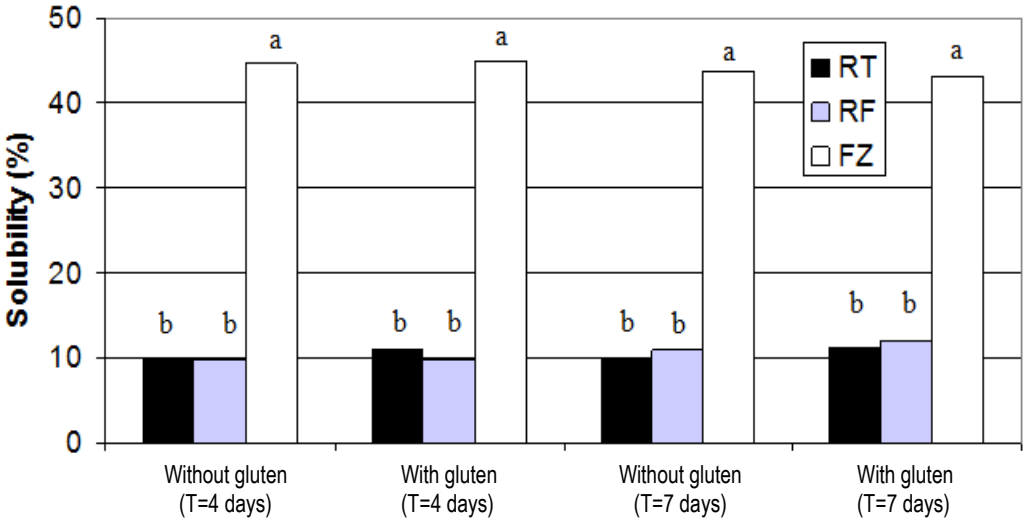


Fig. 6-1. Solubility (%) of β-glucan extracted from 1.5 g β-glucan/serving bread, without and with added gluten, stored for 4 or 7 days at room temperature (RT, 23°C), refrigerated storage (RF, 4°C) or frozen (FZ, -20°C) storage. Standard deviation was less than 5% for all values presented.

Storage for 7 days produced similar results, as frozen bread had the highest ($p \leq 0.05$) β-glucan solubility, ranging from 43-43.6% for both breads. Seven day storage at room temperature and refrigeration temperature produced similar ($p \leq 0.05$) values, ranging from 10.9-11.9% for both breads. The viscosity values also reflect this as the bread that was frozen had the highest ($p \leq 0.05$) extract viscosity, ranging from 2.7-2.8 mPa·sec for both breads. Storage at room temperature and refrigeration temperature produced similar ($p \leq 0.05$) values, ranging from 0.65-0.95 mPa·sec. Because frozen storage is efficient in retarding

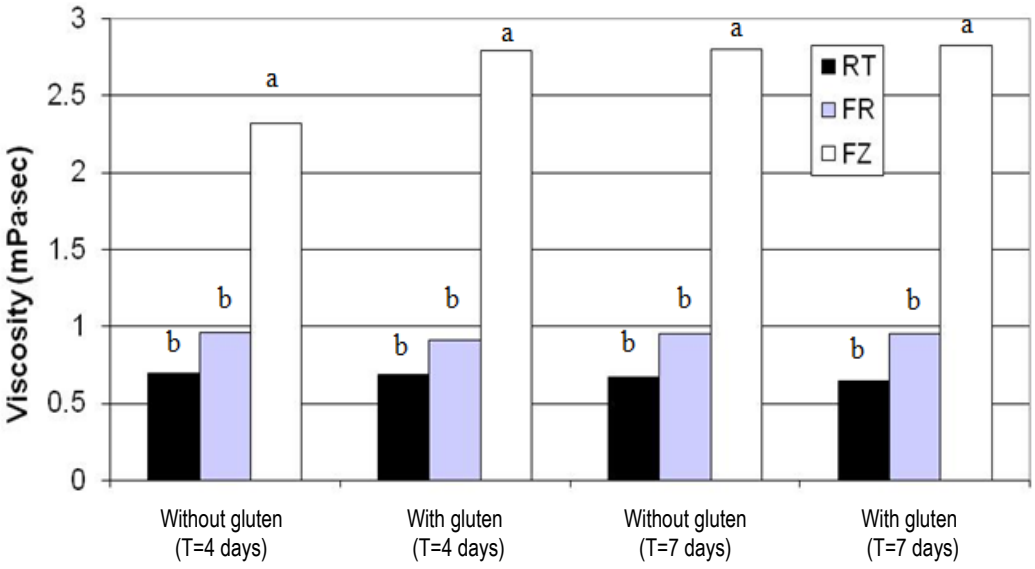


Fig. 6-2. Viscosity (mPa·sec) of β-glucan extracted from 1.5 g β-glucan/serving bread, without and with added gluten, stored for 4 or 7 days at room temperature (RT, 23°C), refrigerated storage (RF, 4°C) or frozen (FZ, -20°C) storage. Standard deviation was less than 10% for all values presented.

the staling process and the movement of molecules within the bread system (Stear 1990), it appears that it may also prevent the movement and complexing of β-glucan with other molecules or amongst itself, allowing it to remain more soluble within the bread system. In addition, the behaviour of β-glucan in the frozen dough system may be similar to that of the β-glucan concentrate product that is obtained by freezing the solubilized β-glucan extract, i.e. Glucagel® (Vasanthan and Temelli 2008). This process yields a β-glucan-based gel upon thawing. If a similar gel is formed in the bread system upon thawing, this may allow for a greater amount of β-glucan to be solubilized, though further research is required.

Prolonging the refrigerated storage of the 1.5 g β-glucan/serving bread, with and without added gluten, from 7 to 14 days did not ($p>0.05$) decrease β-glucan solubility or the viscosity of these extracts further. However, prolonged frozen

storage of the 1.5 g β-glucan/serving bread reduced ($p \leq 0.05$) β-glucan solubility to 38.7% for 14 days and further ($p \leq 0.05$) for 28 days, which was similar ($p > 0.05$) to solubility at 56 days of storage, ranging from 26.3-27.1%. Extract viscosity also followed a similar trend and decreased ($p \leq 0.05$) to 2.1 mPa·sec for 14 days and then further ($p \leq 0.05$) for 28 days, which was similar ($p > 0.05$) to viscosity at 56 days of storage, ranging from 1.5-1.6 mPa·sec. The 1.5 g β-glucan/serving bread with added gluten also showed a progressive reduction ($p \leq 0.05$) in β-glucan solubility to 41% for 14 days, and decreased further at 28 days, which was again similar to solubility at 56 days of storage, ranging from 27.2-29.2%. Extract viscosity also followed a similar trend, with viscosity of 2.7 mPa·sec for 14 day storage and 1.4-1.7 mPa·sec for 28 and 56 days.

There are very few reports on the effect of storage conditions on β-glucan solubility and viscosity in food products. Lan-Pidhainy et al (2007) showed that subjecting β-glucan-fortified muffins to 4 freeze-thaw cycles reduced β-glucan solubility from 30–40% in fresh muffins to just 10%. Following consumption, the blood glucose response was significantly lower for fresh muffins than that after consumption of muffins treated with 4 freeze-thaw cycles. In addition, Beer et al (1997) reported that frozen storage of oat bran muffins decrease extractability of β-glucan by 30-50% upon frozen storage for 8 weeks. Despite the reduction in β-glucan solubility and viscosity upon storage in bread products, it is still unclear as to what extent the quality of β-glucan can be degraded and still maintain its physiological effects attributable to its ability to increase viscosity within the gut (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004,

Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008). It is apparent that further research within this area is necessary.

6.4. CONCLUSIONS

In order to maintain the highest bread quality, in terms of moisture and firmness values, bread fortified with β-glucan should be consumed fresh. However, if that is not possible, appreciable bread quality can still be maintained upon room temperature storage of the 1.5 g β-glucan/serving bread with added gluten and at either room temperature or frozen storage for the 1.5 g β-glucan/serving bread over 4 days. If it is desirable to store bread for 7 days or more, frozen storage should be utilized in order to best maintain bread moisture and firmness levels.

Though these storage conditions are optimal for the retention of bread firmness and moisture values, the impact of the storage conditions on the solubility and viscosity of β-glucan must also be taken into account. Based on the results of this study, β-glucan-fortified bread should be consumed fresh for greatest β-glucan solubility and viscosity, which would also allow the consumer to enjoy the highest level of bread quality. However, if that is not possible, β-glucan solubility of approximately 40% is still achievable upon frozen storage of the bread for up to two weeks. It is still unclear, however, as to what extent reductions in the solubility and viscosity of β-glucan lower its physiological effectiveness.

6.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 44-15A. The Association: St. Paul, MN.

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Bárcenas, M. E., Rosell, C.M.2006. Effect of frozen storage time on the bread crumb and aging of par-baked bread. Food Chem. 95(3):438-445.

Bechtel, W.G., Meisner, D. F., Bradley, W. B. 1953. The effect of the crust on the staling of bread. Cereal Chem. 30:160-168.

Beer, M.U., Wood, P.J., Weisz, J., Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by an *in vitro* digestion system. Cereal Chem. 76:705-709.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chem. 102:889-897.

Davidou, S., Le Meste, M., Debever, E., Bekaert, D. 1996. A contribution of the study of staling of white bread: Effect of water and hydrocolloid. Food Hydrocoll. 10:375-383.

Fik, M., Macura, R. 2001. Quality changes during frozen storage and thawing of mixed bread. Nahrung/Food. 45(2):138-142.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not

differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

Guarda, A., Rosell, C.M., Benedito, C., Galotto, J. 2004. Different hydrocolloids as bread improvers and antistaling agents. Food Hydrocoll. 18(2):241-247.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

He, H., Hoseney, R.C. 1990. Changes in bread firmness and moisture during long-term storage. Cereal Chem. 67(6):603-605.

Kalinga, D., Mishra, V.K. 2009. Rheological and physical properties of low fat cakes produced by addition of cereal β-glucan concentrates. J Food Proc Preser. 33:384-400.

Kent, N.L., Evers, A.D. 1994. Technology of Cereals, 4th Ed., Pergamon, Elsevier Science, Ltd, Oxford, UK.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Kim, S.K., D'Appolonia, B.L. 1977. Bread staling studies. II. Effect of protein content and storage temperature on the role of starch. Cereal Chem. 54:216-224.

Lan-Pidhainy, X., Brummer, Y., Tosh, S., Wolever, T.M., Wood, P. 2007. Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. Cereal Chem. 84(5):512-517.

Mohamed, A., Rayas-Duarte, P., Xu, J. Hard Red Spring wheat/C-TRIM 20 bread:Formulation, processing and texture analysis. Food Chem. 107:516-524.

Stear, C. 1990. Handbook of Breadmaking Technology. Elsevier Applied Science. London and New York. pp:649-708.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Wood PJ, Beer MU, Butler G. 2000. Evaluation of role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on

plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Vasanthan, T., Temelli, F. 2008. Grain fractionation technologies for cereal beta-glucan concentration. Food Res Int. 41(9):876-881.

Yokoyama, W.H., Knuckles, B.E., Stafford, A., Inglett, G. 1998. Raw and processed oat ingredients lower plasma cholesterol in the hamster. J Food Sci. 63(4):713-715.

Chapter 7

GLYCEMIC RESPONSE, SATIETY RATINGS AND REDUCING SUGAR RELEASE FOR BREAD FORTIFIED WITH β-GLUCAN CONCENTRATE OF LOW SOLUBILITY AND GLUTEN[5]

7.1. INTRODUCTION

β-Glucan, a soluble fibre present in barley and oats, has blood glucose regulating (Wood et al 1994, Yokoyama et al 1997, Bourdon et al 1999, Kim et al 2009) and cholesterol lowering effects (Gallaher et al 1992, Martinez et al 1992, Newman et al 1992, Jackson et al 1994, Wang et al 1997, Bourdon et al 1999). Due to its soluble nature, β-glucan may also possess the ability to slow gastric emptying and increase feelings of satiety (Lupton and Turner 2000, Lyly et al 2009, Stevenson and Inglett 2009, Willis et al 2009), which is important as greater than two-thirds of consumers desire a fibre that is able to reduce hunger and help control appetite (Witwer 2005).

Traditionally, highly soluble β-glucan extracts have been used to achieve appropriate levels of fortification, though, due to their soluble nature, these β-glucan extracts are also highly susceptible to enzymatic degradation in bakery products due to native flour enzymes, as described in Chapter 2. Previous studies have shown that the beneficial effects of β-glucan may be decreased when incorporated into the bread system (Kerckhoffs et al 2003, Åman et al 2004,

[5] A version of this chapter is to be submitted to the Journal of the American Dietetic Association for consideration for publication.

Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008) and many have incorporated β-glucan at levels that are substantially higher (Cavallero et al 2002, Andersson et al 2004, Frank et al 2004, Cleary et al 2007) than those likely to be adopted by the food industry. However, β-glucan concentrate extracted in its native form using a new technology (Vasanthan and Temelli 2009) exhibiting lower solubility under physiological conditions is more protected from enzymatic degradation during the mixing and fermentation steps compared to high solubility β-glucan extracts and may hold the key to effectively fortifying bread products to achieve the health benefits attributed to β-glucan. However, the effect of β-glucan fortification at realistic levels using low solubility β-glucan concentrate has not been investigated. Therefore, the main objective of this study was to investigate the effects of β-glucan and gluten addition on perceived satiety, glycemic response and *in vitro* reducing sugar release following incorporation of low solubility β-glucan concentrate (BBG conc) at a level corresponding to 1.5 g β-glucan/serving of bread with and without the addition of vital gluten.

7.2. MATERIALS AND METHODS

7.2.1. MATERIALS

The materials incorporated into the bread dough are consistent with those described in Section 3.2.1.

7.2.2. BREAD PREPARATION

Bread dough was prepared using AACC Method 10-09 (2000) with modifications as described in Section 3.2.2. For this study, two different bread recipes were employed for use in satiety and glycemic response measurements. Bread doughs for the satiety measurement and the corresponding reducing sugar release were prepared as shown in Table 3-1. Bread doughs for the glycemic response measurement and the corresponding reducing sugar release were prepared according to Table 7-1 to ensure the available carbohydrates supplied by each bread were equal. For both recipes, a control batch and 2 dough treatments were formulated with BBG conc at 7.5 g/100 g of flour via direct (w/w) flour substitution, without or with vital gluten addition at 2.18 g/g of β-glucan. This corresponded to breads that would supply 0 or 1.5 g β-glucan/serving of bread, without and with vital gluten. Similar to the previous studies (Chapters 3-6), the actual levels of BBG conc inclusion were calculated to reflect the amount of β-glucan required to produce a 15-slice commercial loaf with approximately 7.5 bread servings providing the desired amount of β-glucan per serving.

Following preparation and fermentation (Section 3.2.2.) the dough was baked in a conventional oven (Bakers' Pride, Model X300, Bakers Pride Canada, Inc., Ft. Washington, PA) for 25 min at 220 ± 8^{o}C and then cooled on wire racks for 1 hr. Fresh bread samples (≤12 hr old) were used for the determination of satiety measures, glycemic response, and reducing sugar release.

TABLE 7-1
Recipes for Bread Dough for Glycemic Response Measures and Related Reducing Sugar Release

Ingredient (g)	Amount of β-glucan/ serving		
	0 g	**1.5 g**	**1.5 g & vital gluten**
Yeast[a]	3.0	3.0	3.0
Wheat Flour	100.0	92.5	92.5
Vital Gluten	0	0	8.2
Sugar	6.7	11.45	10.7
Salt	1.0	1.0	1.0
Distilled Water	68.0	86.0	99.0
β-Glucan Concentrate	0	7.5	7.5

7.2.3. SATIETY MEASURES

Participants (n=23) were recruited at the University of Alberta (Edmonton, AB) campus by way of poster and word of mouth. The participants were instructed not to eat after 9:00pm on the evening before each test session to establish overnight fasting time of 10-12 hr. The order of bread presentation was balanced between participants. As a measure of perceived satiety, the Satiety Labeled Intensity Magnitude (SLIM) scale has been established as a sensitive, reliable, and easy-to-use scale (Cardello et al 2005). On three separate mornings, each participant marked their feeling of fullness on a 100 mm SLIM scale (Fig. 7-1) (Cardello et al 2005) before and following the consumption of one of three bread servings supplying 0 g or 1.5 g β-glucan, with or without added gluten (Table 3-1). Satiety measurements were taken 6 times during a 2-hr period, just prior to bread consumption and 15, 30, 60, 90 and 120 min after bread consumption. Each evaluation of fullness was performed on a new scale sheet.

Data from the scales were obtained by taking metric measurements along the line scales and then determining the arithmetic means of the ratings from all participants for each measurement time. Participants were provided with 500 mL of water to consume throughout the test. Following the test, information on consumer gender, age, whether or not he/she likes and regularly consumes bread, and frequency of bread consumption was collected. This protocol was approved by the Faculty of Agricultural, Life and Environmental Sciences Research Ethics Board.

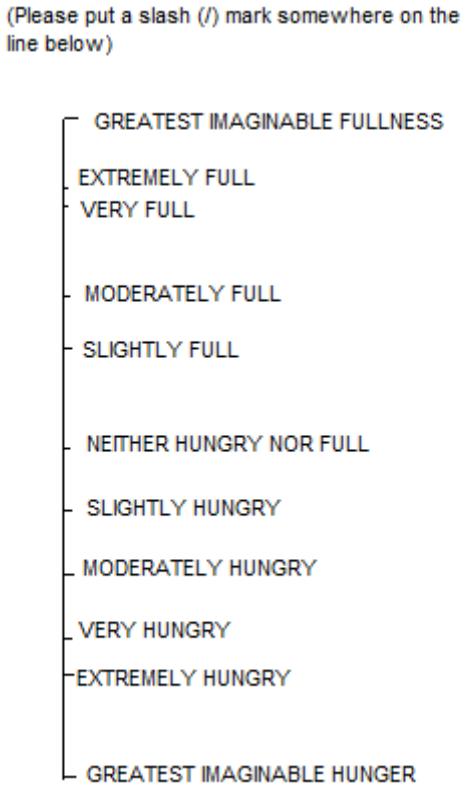


Fig. 7-1. Satiety labeled intensity magnitude (SLIM) scale used for the measurement of perceived satiety (Adapted from Cardello et al 2005)

7.2.4. GLYCEMIC RESPONSE MEASUREMENT

Participants were recruited at the University of Alberta (Edmonton, AB) by way of poster and word of mouth. A total of 14 people participated in the study, which was shown to be an adequate number for sufficient power to detect significant differences at $p \leq 0.05$ (Nazare et al 2009). Following a 12-hr overnight fast, participants, with no previous history of diabetes mellitus, gastrointestinal disease, abdominal surgery, or blood clotting disorders, consumed one of three bread servings supplying 0 g or 1.5 g β-glucan, with or without added gluten (Table 7-1). Each bread serving supplied the same amount of available carbohydrates as the control (29.3 g). Fingerprick capillary blood analysis was carried out 15 min prior to bread consumption to obtain the fasting blood glucose and at 15, 30, 45, 60, 90 and 120 min after starting to eat using a Precision Xtra (Abbott Diagnostics, Abbott Park, IL) amperometric glucose monitor, which was calibrated to give plasma-equivalent glucose results. Participants were provided with 500 mL of water to consume throughout the test and requested to refrain from intensive physical activity the day before. Additionally, the participants were told to maintain their diet, body weight and regular living habits throughout the study. The area under the plasma glucose response curve (AUC) was calculated for each subject, ignoring the area below the fasting (0 min) concentration. This protocol was approved by the Faculty of Agricultural, Life and Environmental Sciences Research Ethics Board.

7.2.5. REDUCING SUGAR RELEASE

To determine the extent of reducing sugar release at different times upon *in vitro* digestion, bread samples from each of the experimental treatments (Tables 3-1 and 7-1) were subjected to the *in vitro* digestion procedure outlined by Beer et al (1997) with modifications to accommodate the limitations of a bread system. A bread sample containing 1/10 of the barley β-glucan originally incorporated into the whole dough, was broken into 1 cm^3 pieces by hand and mixed in an Erlenmeyer flask with 75 mL of 20 m*M* sodium phosphate buffer (pH 6.9) containing 10 m*M* NaCl. The solution was stirred slowly for 15 min at 37^oC, and then 500 μL of human salivary α-amylase solution (5 mg/mL in 3.6 m*M* $CaCl_2$; A-1031, Sigma-Aldrich, St. Louis, MO) was added. The mixture was stirred for another 15 min. Following this, the pH was adjusted to 2.0 (±0.1) with 4 *M* HCl and 1250 μL of porcine pepsin solution (0.5 mg/mL in 0.9% NaCl, P7012, Sigma-Aldrich) was added. The mixture was stirred further for 30 min at 37^oC. The pH was then neutralized to pH 6.9 ($\pm$0.1) using 4 *M* NaOH and 2500 μL of the pancreatin solution was added (0.5 mg/mL in 20 m*M* sodium phosphate buffer containing 10 m*M* NaCl, P-7545, Sigma-Aldrich). The solution was then incubated and 1 mL aliquots of the solution were taken at 0, 15, 45 and 75 min and centrifuged (2200 x *g*, 10 min). Aliquots of the mixture were taken at these times because with the neutralization of pH and the addition of pancreatin this step is meant to represent the "small intestine phase" of the approximated digestion process, where absorption of sugars takes place in the body.

The supernatant was analyzed for reducing sugars by the 3, 5-dinitrosalicylic acid method (Bruner 1964). Reducing sugar released (RSR) is expressed in maltose equivalents as a percentage of total available carbohydrates present in the sample using the following equation (Brighneti et al 1995):

$$RSR=A_{sample} \times 79.25 \times 0.95/A_{maltose} \times SS \times 100$$

where A_{sample} is the absorbance at 540 nm; $A_{maltose}$ is the absorbance of a solution containing 1 mg of pure maltose per mL/phosphate buffer; SS is the amount (mg) of starch plus sugars contained in the sample; 79.25 is the total volume; and 0.95 is the conversion from maltose to starch. This value was then converted to glucose equivalents and reported as such.

7.2.6. STATISTICAL ANALYSIS

The results of the satiety measures and the blood glucose values were analyzed using SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000) and means were compared using paired comparisons (two tailed *t*-test) with significance defined at $p \leq 0.05$. The reducing sugar release values were analyzed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Means were compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$. The reducing sugar release procedure was replicated and analysis was performed in duplicate for each sample.

7.3. RESULTS AND DISCUSSION

7.3.1. SATIETY MEASURES

The ratings for the satiety measures are presented in Table 7-2. Just prior to bread consumption the panelists were asked to rate their initial feeling of satiety. Interestingly, the participants rated themselves as feeling more ($p \leq 0.05$) satiated prior to the consumption of the 1.5 g β-glucan/serving bread. Previous results (Table 5-1) indicated that the 1.5 g β-glucan/serving bread was darker and redder than the control and had lower loaf volume when compared to the 1.5 g β-glucan/serving bread with added gluten and the control bread. Perhaps the appearance of the 1.5 g β-glucan/serving bread was not as appealing as that of the control or the 1.5 g β-glucan/serving bread with added gluten and the panelists were not as inclined to consume it. This mechanism may be important in the creation of diet aids that assist in reducing food intake. It has been reported that colour and appearance can have a halo effect that can modify food acceptability and flavour perception (Kostyla and Clydesdale 1978, Hutchings 1994). However, little work has been done on food appearance and its impact on initial feelings of satiety. Results of the satiety measures 15, 30, 45 and 60 min after bread consumption were similar ($p > 0.05$) between all of the breads at each of these times. However, at 90 min and 120 min the 1.5 g β-glucan/serving bread had the highest ($p \leq 0.05$) satiety ratings, which were similar to those for bread with gluten addition, indicating that these breads kept panelists full, longer. The satiety ratings between the control and the 1.5 g β-glucan/serving bread with gluten did not differ ($p > 0.05$).

TABLE 7-2
Satiety Rating[1] for Bread with Varied Levels of β-glucan and With or Without Vital Gluten Addition

	β-Glucan (g)/serving of bread	Time (min) 0	15	30	45	60	90	120
Control	0	35.8[b]	62.3[a]	63.0[a]	61.3[a]	59.0[a]	52.8[b]	47.4[b]
Without Gluten	1.5	40.4[a]	66.7[a]	65.0[a]	64.0[a]	61.2[a]	58.0[a]	54.0[a]
With Gluten[2]	1.5	36.7[ab]	63.7[a]	63.0[a]	62.1[a]	58.6[a]	54.8[ab]	53.0[ab]

[a-e]Means within the same column with the same letter are not significantly different ($p > 0.05$).
[1]Satiety ratings (n=23) reported as average rating on a 100 mm SLIM scale (Cavallero, 2005).
[2]Gluten added at levels of 0 or 2.18 g per g of added β-glucan.

7.3.2. REDUCING SUGAR RELEASE FROM BREAD USED FOR SATIETY MEASURES

Reducing sugar release values for the breads were obtained at 0, 15, 45 and 75 min, which proceeded the addition of pancreatin to the *in vitro* digestion system (Section 7.2.5.). The amount of available carbohydrates among the bread samples was highest for the control (29.3 g), then the 1.5 g β-glucan/serving bread with added gluten (27.7 g), and then the 1.5 g β-glucan/serving bread without added gluten (27.4 g). Reducing sugar release values for the breads used in the satiety panel (Fig. 7-2) indicated that at 0, 15, 45 and 75 min the 1.5 g β-glucan/serving bread with added gluten produced the least ($p \leq 0.05$) glucose equivalents, at 8.3, 11.5, 12.5, and 14.1 g, respectively, indicating a lower degree of hydrolysis. The values obtained at 0, 15, 45 and 75 min were similar ($p > 0.05$) for the control and the 1.5 g β-glucan/serving bread at each of these times, ranging

from 9.3-9.6, 12.3-12.6, 13.5-13.7, and 14.9-15.2 g glucose equivalents, respectively.

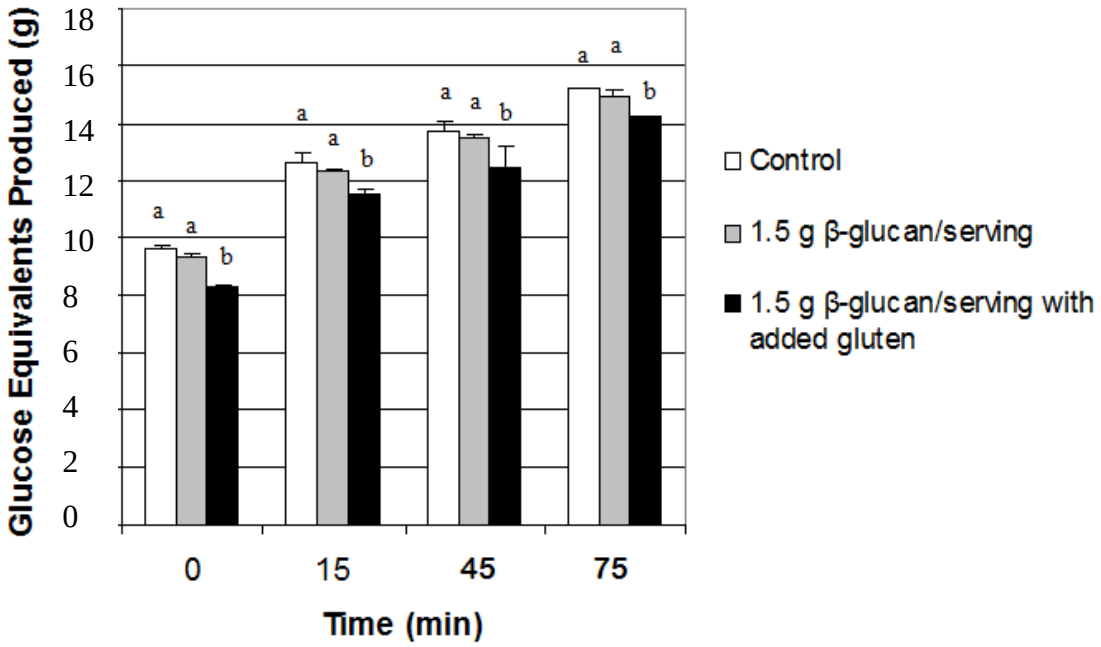


Fig. 7-2. Comparison of the reducing sugar release for the control and 1.5 g β-glucan/serving bread, without and with added gluten used for the satiety ratings. Values are reported in glucose equivalents (g). [a-b]Columns with the same letter for each time are not significantly different ($p>0.05$).

7.3.3. GLYCEMIC RESPONSE

The results of the blood glucose readings for the glycemic response measures (Fig. 7-3) indicated that at 15 min after bread consumption the average blood glucose levels for all of the breads were similar ($p>0.05$), ranging from 5.1-5.3 mmol/L. This was also the case at 30 min, with values ranging from 6.2-6.7 mmol/L. At 45 min, all of the experimental breads again elicited the same ($p>0.05$) average blood glucose response of 6.9 mmol/L. However, at 60 min the

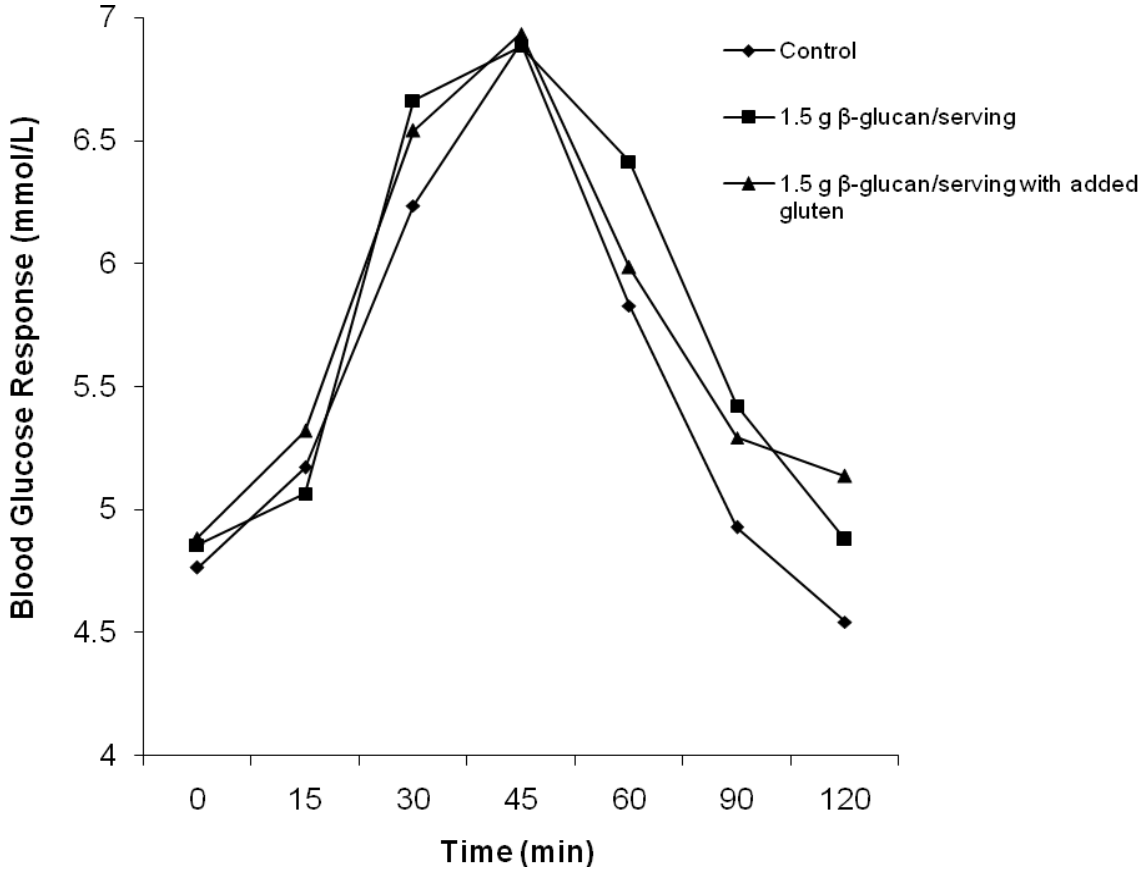


Fig. 7-3. Comparison of incremental changes in postprandial blood glucose from subjects given the control and 1.5 g β-glucan/serving bread, without and with added gluten (n=14)

1.5 g β-glucan/serving bread elicited a blood sugar response that was higher ($p \leq 0.05$) than that of the control, at 6.4 mmol/L versus 5.8 mmol/L, respectively, which is still within the normal range for postprandial blood glucose levels (CDA 2009). The 1.5 g β-glucan/serving bread with added gluten elicited a blood sugar response of 6.0 mmol/L, which was similar ($p > 0.05$) to that of the other breads. At 90 min, all of the experimental breads again elicited similar ($p > 0.05$) average blood glucose responses, ranging from 4.9 to 5.3 mmol/L. However, at 120 min, the 1.5 g β-glucan/serving bread with added gluten elicited an average blood sugar

response that was higher ($p \leq 0.05$) than that of the control, at 5.1 mmol/L versus 4.5 mmol/L, respectively. The 1.5 g β-glucan/serving bread without added gluten elicited a blood sugar response of 4.9 mmol/L, which was similar ($p > 0.05$) to that of the other breads.

Analysis of the area under the curve (AUC) for the blood glucose measurements indicated that there were no ($p > 0.05$) differences in AUC between the breads (Fig. 7-4). This is due to the fact that the increases in the blood sugar response elicited by each of the breads at different times ultimately resulted in blood glucose curves with similar areas. Similar findings were reported by Kim et al (2009) upon consumption of cooked barley cereal containing 10 g of β-glucan, though this study involved obese women at increased risk for insulin resistance. Kim et al (2006) showed that consumption of cooked cereal products containing 2 g of β-glucan significantly reduced postprandial glucose responses (peak glucose response and 2 hr glucose AUC), in comparison to a control, though consumption of 1 g β-glucan did not. A decrease in AUC was also seen by Lan-Pidhainy et al (2007) following consumption of muffins with 8 and 12 g β-glucan/serving, while Tosh et al (2008) only showed a reduction in AUC upon the consumption of 8 g, but not 4 g, of β-glucan incorporated into bread, despite reductions in peak blood glucose response. Cavallero et al (2002) only showed a reduction in AUC upon fortification of bread products with 6.7% β-glucan, while fortification levels from 2.4-4.3% were similar to the control. Perhaps slightly more β-glucan is necessary to produce lower blood glucose values in bread products or the use of more available carbohydrates in the test meal in order to see

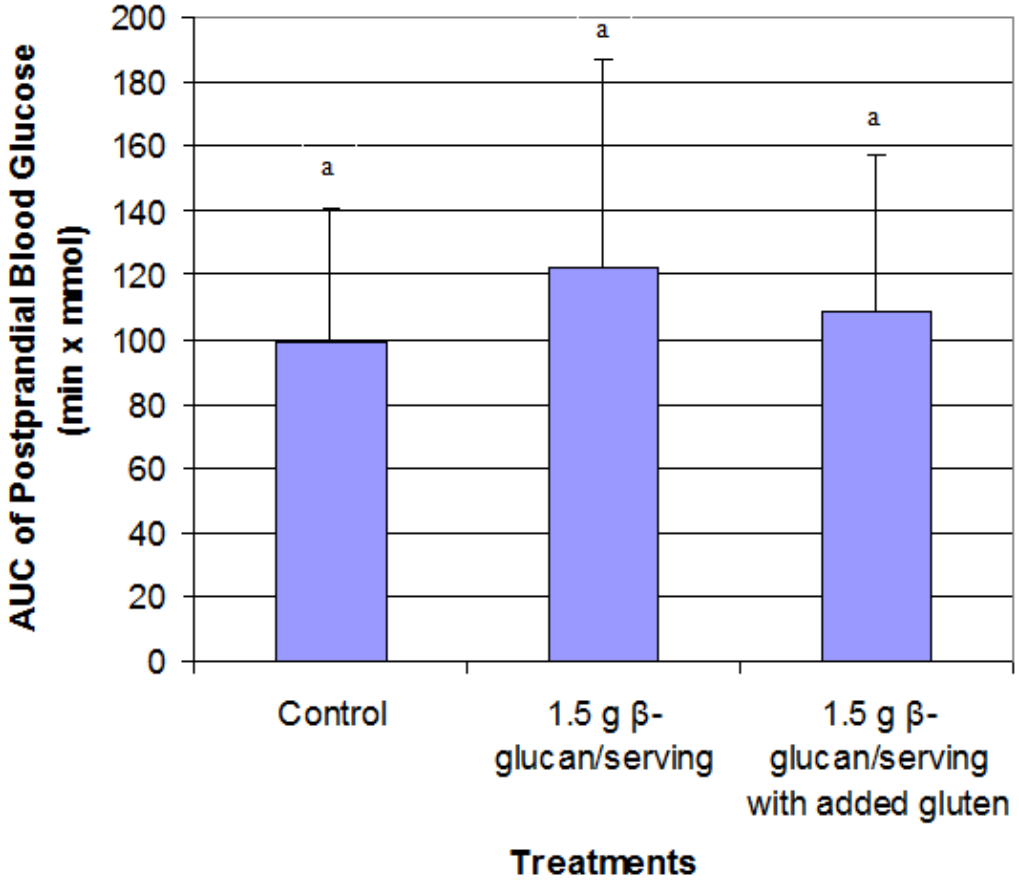


Fig. 7-4. Comparison of area under the blood glucose curve (AUC) from subjects given the control and 1.5 g β-glucan/serving bread, without and with added gluten (n=14). Bars with similar letters are not significantly ($p>0.05$) different.

differences between the test breads and the control. However, the amount of available carbohydrates in our study was chosen to reflect the average bread serving size of 2 slices that would be consumed by the general public.

7.3.4. REDUCING SUGAR RELEASE FROM BREADS USED FOR GLYCEMIC RESPONSE

Reducing sugar release values for the breads were obtained at 0, 15, 45 and 75 min, following the addition of pancreatin to the *in vitro* digestion system (Section 7.2.5). This time frame was thought to most closely correspond with the

progression of digestion and absorption over the 2-hr glycemic response analysis period. Reducing sugar release values (Fig. 7-5) at 0 min were similar ($p>0.05$) for all of the breads, ranging from 8.1-9.9 g glucose equivalents. This trend continued 15 min into the *in vitro* digestion process with similar ($p>0.05$) values ranging from 11.3-12.4 g. However at 45 min, the 1.5 g β-glucan/serving bread produced more ($p\leq0.05$) glucose equivalents (13.6 g) than the control (11.7 g). Reducing sugar release from the 1.5 g β-glucan/serving bread with added gluten at 45 min (12.7 g) was similar ($p>0.05$) to the other breads. At 75 min, the values were all similar ($p>0.05$), ranging from 13.1-14.4 g. However, if the reducing sugar release was measured over a slightly longer period of time, the 1.5 g β-glucan/serving bread with added gluten may have shown an increase in reducing sugar release values, which would support the increase in blood glucose readings exhibited by this bread at 120 min (Fig. 7-3). Symons and Brennan (2004) investigated bread with 2.5 or 5% β-glucan rich extracts from barley (70% β-glucan) and showed that 5% inclusion significantly decreased reducing sugar release during a 300 min digestion process compared with the control, though substitution at 2.5% did not have an effect. Cleary et al (2007) reported that breads with β-glucan (4.5% β-glucan with ~11 g of total dietary fibre) exhibit attenuated reducing sugar release 90 min into a 300 min *in vitro* digestion process in comparison to a control. However, the decrease in reducing sugar release in both of these studies may have simply been due to a reduction in starch gelatinization and thus its availability for digestion that resulted from not

correcting (Symons and Brennan 2004) or only slightly correcting (Cleary et al 2007) for the increases in dough water absorption upon the addition of β-glucan.

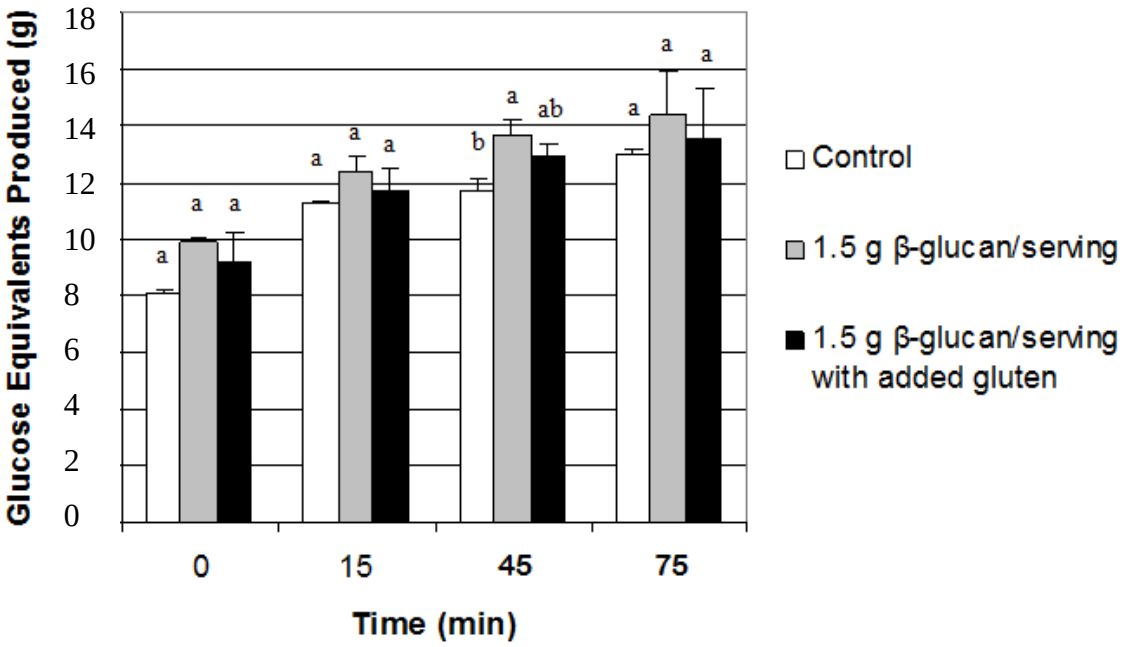


Fig. 7-5. Comparison of the reducing sugar release from the control and 1.5 g β-glucan/serving bread, without and with added gluten, used for the glycemic response tests. Values are reported in glucose equivalents (g). [a-b]Columns with the same letter for each time are not significantly different ($p>0.05$).

7.3.5. IMPLICATIONS

Previous results (Fig. 5-2) indicated that the 1.5 g β-glucan/serving bread with added gluten had greater physiological extract viscosity compared to that of the same bread without added gluten. Thus, the β-glucan bread with added gluten was expected to elicit the greatest feelings of satiety due to anticipated prolonged gastric distention based on increasing digesta viscosity and decreasing gastric emptying rate (French and Read 1994, Lupton and Turner 2000). However, the results of this study may imply that digesta that is too viscous to allow for an

adequate gastric emptying rate or the release of sufficient amounts of glucose for absorption by the small intestine may be disadvantageous to feelings of satiety. Previous reports on β-glucan's impact on satiety may support this. For example, Peters et al (2009) showed that the addition of β-glucan, fructooligosaccharides, or a combination of both did not affect appetite ratings or food intake when panelists were supplied meal replacement bars with 0.3-0.9 g β-glucan from oatmeal and whole grain barley flour, even though the addition of β-glucan to the bars was shown to double gastric viscosity under *in vitro* conditions. Additionally, Juvonen et al (2009) showed that an oat bran beverage with low viscosity produced greater feelings of satiety and faster gastric emptying than a high viscosity oat bran beverage. As well, the low-viscosity beverage was also shown to increase plasma glucose to levels greater than the high-viscosity oat bran beverage (Juvonen et al 2009). Similarly, Kim et al (2006) and Hlebowicz et al (2008) showed that the consumption of 2 g of β-glucan in the form of a cooked cereal from barley or muesli with 4 g oat β-glucan, respectively, were able to lower glycemic response, though they did not impact satiety ratings. Previous studies have also suggested that an increase in blood glucose concentrations results in increased feelings of satiety, whereas a drop in blood glucose concentrations has the opposite effect (Mayer 1955, Chaput and Tremblay 2009). Because the 1.5 g β-glucan/serving bread was able to increase feelings of satiety among test subjects, it appears that an appropriate balance between these factors may have been achieved with bread utilizing low-solubility β-glucan concentrate.

The reducing sugar release values (Fig. 7-2) may offer further insight into why the 1.5 g β-glucan/serving bread kept panelists full, longer. Though the control and the 1.5 g β-glucan/serving bread showed similar reducing sugar release values, indicating a similar degree of hydrolysis, and hypothetically a similar amount of sugar available for breakdown and/or absorption within the intestine, human physiology must be taken into account when comparing *in vitro* and *in vivo* findings. Foods with soluble fibre have been shown to slow gastric emptying and increase feelings of fullness due to gastric distention (Lupton and Turner 2000). However, foods low in fibre and high in carbohydrates, such as white bread, are broken down quickly and are rapidly absorbed within the intestine. Therefore, the 1.5 g β-glucan/serving bread may have exited the stomach and entered the intestine more slowly than the control, prolonging feelings of fullness due to stomach distention, and, upon gradual delivery to the intestine, may have presented a steady supply of reducing sugars for hydrolysis and absorption. Additionally, because a variety of hormones influence feelings of satiety and appetite, including insulin, glucagon, cholecystokinin, leptin and ghrehlin (Holt et al 1992, Schwartz 2000, Tschop et al 2000, Wren and Bloom 2007), it is important to see if the differences in satiety are related to changes in the concentrations of these hormones.

The 1.5 g β-glucan/serving bread with added gluten showed significantly ($p \leq 0.05$) lower reducing sugar release upon *in vitro* digestion compared to the other two breads possibly due to increased viscosity of this bread's physiological extract (Fig. 5-2), which may have impeded the access of the digestive enzymes to

the starch resulting in a reduction of starch digestion products and/or a reduction in the diffusion of the products from the digest (Flourie et al 1984, Lupton and Turner 2000). Similar decreases in reducing sugar release were seen by Symons and Brennan (2004) upon increasing levels of β-glucan incorporation, which would have also likely resulted in an increase in extract viscosity. However, the decreases in reducing sugar release seen by Symons and Brennan (2004) may have also been the result of inadequate starch gelatinization, as discussed previously. The decrease in reducing sugar release from the 1.5 g β-glucan/serving bread with added gluten may have also been due to the production of a stronger protein network, as described in Section 4.3. This effect may be similar to the action of the well-formed protein–starch matrix in pasta that allows the protein strands to entrap large starch granules and thus reduce starch accessibility to enzymatic degradation, reducing sugar release (Cleary and Brennan 2006). Regardless of the method of action, decreases in reducing sugar release may have positive implications for Type 2 diabetics who require a reduction in blood glucose levels primarily due to insulin resistance and a subsequent rise in blood sugar levels above normal range (Watford et al 2000). However, further research is warranted to characterize the impact of this bread on glycemic response, particularly in diabetic populations, and gastric emptying so accurate recommendations for health can be established.

For the glycemic response, despite the increase in blood sugar response elicited by the β-glucan fortified breads in comparison to the control at 60 and 120 min, these values are still within the normal range, if not a little low, for

postprandial blood glucose measurements (CDA 2009). Though the lack of effect shown in this study was unexpected, it may have occurred for a variety of reasons. Östman et al (2006) showed that consumption of approximately 8 or 12 g of β-glucan in barley bread lowered blood glucose response in comparison to a white wheat bread. A high correlation was also found between the viscosity of the *in vitro* digests and the glycemic responses of the bread products (Östman et al 2006). Therefore, because the addition of low-solubility β-glucan to bread increases extract viscosity (Fig. 5-2), perhaps this bread, upon slight increases in β-glucan content, may be effective in reducing blood sugar values, though more research is required. Additionally, the 1.5 g β-glucan/serving bread appeared to produce the most leveled blood glucose curve of all of the breads (Fig. 7-3). Foods that elicit more uniform blood glucose curves are recommended in weight loss due to improvements in appetite control (Holt et al 1992) by sustaining energy needs over a greater period of time. Therefore, consumption of 1.5 g β-glucan/serving bread may have positive implications for the greater than 65% of Americans and nearly 60% of Canadians who are overweight or obese (Tjepkema 2005, Ogden et al 2006), though more research is required.

In addition, further research is warranted to see the effect of this bread on gastric emptying. Because there was not a significant reduction in blood sugar values within the initial stages of the glycemic response measurements, this may imply that the gastric emptying rates were similar among all of the breads. However, it would also be benefial to measure the panelists' insulin response, as this may have masked differences in blood sugar values. Furthermore, the role of

β-glucan concentrate in bread in relation to other aspects of health, including stimulation of the immune system (Yun et al 1997, Yun et al 1998, Davis et al 2007) and its potential as a prebiotic (Snart et al 2006), should not be ignored and requires investigation within this system.

7.4. CONCLUSIONS

Satiety measures indicated that the 1.5 g β-glucan/serving bread without added gluten allowed panelists to feel full, longer, with results similar to the bread with added gluten. In terms of reducing sugar release, the 1.5 g β-glucan/serving bread and the control bread exhibited similar results, while the 1.5 g β-glucan bread with added gluten released the least. Taken together, these results may imply that satiety ratings rely on an appropriate balance between the ability of the test food to increase digesta viscosity and its ability to release adequate amounts of reducing sugars for digestion and absorption. Glycemic response measures indicated that blood sugar values higher than the control, but still within normal range, were achieved at 60 min for the 1.5 g β-glucan/serving bread and at 120 min the 1.5 g β-glucan/serving bread with added gluten. Measures of the reducing sugar release for the experimental breads used in the glycemic response tests indicated that the 1.5 g β-glucan/serving bread produced the most glucose equivalents at 45 min, though was similar to the other breads throughout the rest of the digestion period. The 2-hr blood glucose AUC for all of the breads were similar; however, the 1.5 g β-glucan/serving bread appeared to produce the blood glucose curve with the most leveled appearance.

7.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 44-15A. The Association: St. Paul, MN.

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Beer, M.U. Wood, P.J. Weisz, J. Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-glucan extracted from oat products by an *in vitro* digestion system. Cereal Chem. 74:705-709.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr. 69:55-63.

Braaten, J.T., Wood, P.J., Scott, F.W., Wolynetz, M.S., Lowe, M.K., Bradley-White, P., Collins, M.W. 1994. Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J Clin Nutr. 48:465-474.

Canadian Diabetes Association. 2009. Managing your blood glucose. Accessed July 31, 2009 at http://www.diabetes.ca/about-diabetes/living/management/manage-glucose/.

Cardello, A., Schultz, H.G., Lesher, L.L., Merrill, E. 2005. Development and testing of a labeled magnitude scale of perceived satiety. Appetite. 44:1-13.

Cavallero, A., Empilli, S., Brighenti, F., Stanco, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59.

Chaput, J.P., Tremblay, A. 2009. The glucostatic theory of appetite control and the risk of obesity and diabetes. Int J Obesity. 33:46-53.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and in vitro digestion. Food Chem. 102:889-897.

Cleary, L., Brennan, C. 2006. The influence of a (1→3)(1→4)- β-d-glucan rich fraction from barley on the physico-chemical properties and in vitro reducing sugars release of durum wheat pasta. Int J Food Sci Technol. 41(8):910-918.

Davis, J.M., Murphy, E.A., Brown, A.S., Carmichael, M.D., Ghaffar, A., Mayer, E.P. 2004. Effects of moderate exercise and oat ß-glucan on innate immune function and susceptibility to respiratory infection. Am J Physiol Regul Integr Comp Physiol. 286:R366-R372.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Flourie B, Vidon, N., Florent, C.H., Bernier, J.J. 1984. Effect of Pectin on jejuna glucose absorption and unstirred layer thickness in normal man. Gut. 25:936-941.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

French, S.J., Read, N.W. 1994. Effect of guar gum on hunger and satiety after meals of differing fat content: relationship with gastric emptying. Am J Clin Nutr. 59:87-91.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M. 1993. Viscosity and fermentability as attributes of dietary fibre responsible for hypocholesterolemic effect in hamsters. J Nutr. 123:244-252.

Hlebowicz, J., Darwiche, G., Björgell, O., Almér, L. 2008. Effect of muesli with 4 g oat β-glucan on postprandial blood glucose, gastric emptying and satiety in healthy subjects: a randomized crossover trial. J Am College Nutr. 27(4):470-475.

Holt, S., Brand, J., Soveny, C., Hansky, J. 1992. Relationship of satiety to postprandial glycemic, insulin and cholecystokinin responses. Appetite. 18:129-141

Hutchings, J.B. 1994. Sensory assessment of appearance – methodology. In: Food Colour and Appearance. J.B. Hutchings (ed.), Aspen Publishers, Inc., Gaithersburg, MD. pp:105-41.

Jackson, K.A., Suter, D.A.I., Topping, D.L. 1994. Oat bran, barley, and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J Nutr. 124:1678-1684.

Juvonen, K., Purhonen, A., Salmenkallio-Marttila, M., Lähteenmäki, L., Laaksonen, D., Herzig, K., Uusitupa, M., Poutanen, K., Karhunen, L. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J Nutr. 139(3):461-466.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Kim, H., Behall, K.M., Vinyard, B., Conway, J. 2006. Short-term satiety and glycemic response after consumption of whole grains with various amounts of β-glucan. Cereal Foods World 51:29–33.

Kim, H., Stokes, K.S., Behall, K.M., Spears, K., Vinyard, B., Conway, J.M. 2009. Glucose and insulin responses to wholegrain breakfasts varying in soluble fibre, β-glucan. Eur J Nutr. 48(3):170-175.

Kostyla, A.S, Clydesdale, F.M. 1978. The psychophysical relationships between colour and flavour. Food Sci Nutr. 10:303-19.

Lan-Pidhainy, X., Brummer, Y., Tosh, S., Wolever, T.M., Wood, P. 2007. Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. Cereal Chem. 84(5):512-517.

Lupton, J.R., Turner, N.D. 2000. Dietary Fibre. In: Biochemical and Physiological Aspects of Human Nutrition. M.H. Stipanuk (ed.), W.B. Saunders Co. Philadelphia, PA, pp. 143-154.

Lyly, M., Liukkonen, K.H., Salmenkallio-Marttila, M., Karhunen, L., Poutanen, K., Lahteenmaki, L. 2009. Fibre in beverages can enhance perceived satiety. Eur J Nutr. 48(4):251-258.

Martinez, V.M., Newman, R.K., Newman, C.W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. J Nutr. 122:1070-1076.

Mayer, J. 1955. Regulation of energy intake and the body weight, the glucostatic theory and the lipostatic hypothesis. Ann NY Acad Sci. 63:15–43.

Nazare, J., Normand, S., Triantafyllou, A.O., de la Perrière, A.B., Desage, M., Laville, M. 2009. Modulation of the postprandial phase by β-glucan in

overweight subjects: Effects on glucose and insulin kinetics. Mol Nutr Food Res. 53:361-369.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., Hofer, P.J. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Ogden, C.L., Carroll, M.D., Curtin, L.R., McDowell, M.A., Tabak, C.J., Flegal, K.M. 2006. Prevalence of overweight and obesity in the United States, 1999-2004. J Am Med Assoc. 295:1549-1555.

Östman, E., Rossi, E., Larsson, H., Brighenti, F., Björck, I. 2006. Glucose and insulin responses in healthy men to barley bread with different levels of (1→3, 1→4)-β-glucans; predictions using fluidity measurements of *in vitro* enzyme digests. J Cereal Sci. 43:230-235.

Peters, H., Boers, H., Haddeman, E., Melnikov, S.M., Qvyjt, F. 2009. No effect of added b-glucan or of fructooligosaccharide on appetite or energy intake. Am J Clin Nutr. 89:58-63.

Schwartz, G.J. 2000. The role of gastrointestinal vagal afferents in the control of food intake: current prospects. Nutr. 16:866–873.

Snart, J., Bibiloni, R., Grayson, T., Lay, C., Zhang, H., Allison, G., Laverdiere, J.K., Temelli, F., Vasanthan, T., Bell, R., Tannock, G.W. 2006. Supplementation of the diet with high-viscosity β-glucan results in enrichment for Lactobacilli in the rat cecum. Appl Env Micro. 72(3):1925-1931.

Stevenson D.G., Inglett, G.E. 2009. Cereal β-glucans. In: Handbook of Hydrocolloids. G.O. Phillips and P.A. Williams (eds.), Woodhead Publishing Ltd. Boca Raton, FL, 615-639.

Symons, L.J., Brennan, C.S. 2004. The influence of (1→3)(1→4)-β-D-glucan-rich fractions from barley on the physicochemical properties and *in vitro* reducing sugar release of white wheat breads. J Food Sci. 69(4):C463-C467.

Tjepkema, M. 2005. Adult obesity in Canada: measured height and weight. Statistics Canada. Accessed July 31, 2009: http://www.statcan.gc.ca/pub/82-620-m/2005001/article/adults-adultes/../../pdf/4224906-eng.pdf (PDF Version, 300 kb)

Tosh, S., Brummer, Y., Wolever T.M., Wood, P.J. 2008. Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. Cereal Chem. 85(2):211-217.

Tschop, M., Smiley, D.L., Heiman, M.L. 2000. Ghrelin induces adiposity in rodents. Nature. 407:908–913.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Wang, L., Behr, S.R., Newman, R.K., Newman, C.V. 1997. Comparative cholesterol-lowering effects of barley β-glucan and barley oil in golden Syrian hamsters. Nutr Res. 17(1):77-88.

Watford, M., Phil, D., Goodridge, A.G. 2000. Regulation of fuel utilization. In: Biochemical and Physiological Aspects of Human Nutrition. M.H. Stipanuk (ed.), W.B. Saunders Co. Philadelphia, PA, pp. 384-407.

Willis, H.J., Eldridge, A.L., Beiselgel, J., Thomas, W., Slavin, J.L. 2009. Greater satiety response with resistant starch and corn bran in human subjects. Nutr Res. 29(2):100-105.

Witwer, R. 2005. Understanding glycemic impact. Food Technol. 59(11):22-29.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Wren, A.M,, Bloom, S.R. 2007. Gut hormones and appetite control. Gastroenterology. 132:2116-2130.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnlund, J.R., Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74(3):293-296.

Yun, C.H., Estrada, A., van Kessel, A., Gajadhar, A., Redmond, M., Laarveld, B. 1997. β-(1-3, 1-4) oat ß-glucan enhances resistance to Eimeria vermiformis infection in immunosuppressed mice. Int J Parasit. 27: 329-337

Yun, C.H., Estrada, A., van Kessel, A., Gajadhar, A., Redmond, M., Laarveld, B. 1998. Immunomodulatory effects of oat ß-glucan administered intragastrically or parenterally on mice infected with *Eimeria vermiformis*. Microbial Immunol. 42:457-465.

Chapter 8

EFFECT OF HEALTH INFORMATION ON THE CONSUMER ACCEPTABILITY OF BREAD FORTIFIED WITH β-GLUCAN[6]

8.1. INTRODUCTION

Food choice is highly influenced by consumer perception of the product's sensory quality, though previous research has shown that food choice is also affected by presentation context (Shepherd 1989, Kahkonen et al 1997). The presence of nutrition information is very common in retail market settings and may or may not have a large impact on consumer acceptability. Previous studies have reported that the provision of nutritional information during sensory evaluation may increase, decrease, or simply not affect consumer acceptability depending on the food product (Wansink et al 2004, van Kleef et al 2005, Goerlitz and Delwiche 2004). For bakery products, Baixauli et al (2008) found that the liking of whole-wheat muffins increased when information about the dietary fibre content was provided, though Mialon et al (2002) reported that liking of whole-wheat bread did not differ. Ginon et al (2009) reported that consumers' willingness to pay for French baguettes increased if the baguette was considered a 'source of fibre'. The effect of providing nutritional information on the consumer acceptability of β-glucan-fortified bread has not yet been investigated. It is essential to determine if the health information provided by the FDA health claim

[6] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

(FDA 2005) has an effect on consumer liking and purchase intent for bread fortified with β-glucan. Therefore, the main objective of this study was to investigate the consumer acceptability and purchase intent of bread fortified with a β-glucan concentrate exhibiting low solubility under physiological conditions at levels corresponding to 0, 0.75 and 1.5 g β-glucan/serving of bread in relation to the provision of health information, gender and whole-wheat bread consumption. The effect of β-glucan concentration on the physical properties of the bread produced under pilot plant settings was also investigated.

8.2. MATERIALS AND METHODS

8.2.1. MATERIALS

The materials incorporated into the bread dough are consistent with those described in Section 3.2.1.

8.2.2. BREAD PREPARATION

Bread dough was prepared using AACC Method 10-09 (2000) with modifications. As shown in Table 3-1, a control batch and 2 dough treatments were formulated with BBG conc at 3.75 and 7.5 g/100 g of flour, which corresponded to 0.75 or 1.5 g β-glucan/serving of bread, via direct (w/w) substitution of the bread flour. Gluten was added at 2.18 g added vital gluten per g of β-glucan for each level of β-glucan addition.

Bread was prepared at a pilot plant setting at the Food Processing Development Center in Leduc, AB. All dry ingredients were sifted together immediately prior to dough formulation. The amount of distilled water used to

optimally hydrate the sifted dry ingredients was first determined using a farinograph (Brabender Farino/Resistograph, Model FA/R-2, C.W. Brabender Instruments Inc., South Hackensack, NJ) equipped with a 50 g mixing bowl. The temperature was maintained at 30^{o}C with a circulating water bath (MGW Lauda, Model RM6, Konigshofen, Germany) and the optimal amount of distilled water addition was determined as the point at which the consistency of the sample reached 500 BU. However, upon scale up, reductions in the water content of the doughs with β-glucan was required to achieve optimal consistency. Following sifting of the dry ingredients, all of the ingredients were combined and mixed in an electric mixer (Model DD-80DT, Blakeslee and Co., Scarborough, ON) with a bread hook for 7 min until an adequate dough formed. The prepared dough was split into 500 g, 545 g, and 590 g pieces for the 0, 0.75 and 1.5 g β-glucan/serving bread, respectively. This was done to ensure each batch produced 17 loaves with the appropriate ratio of ingredients and to ensure adequate loaf height for the consumer panel samples. The loaves were then fermented for 235 min within a fermentation cabinet (Model ESL-4CA, Plantinous Sterling Series, ESPEC North America, Hudsonville, MI) maintained at 30^{o}C and 85% relative humidity. Following fermentation the dough was baked in an oven (Baxter rotating rack oven, Baxter Mfg. Co. Inc., Ortina, WA) for 25 min at 220 ± 8^{o}C and then cooled on wire racks for 1 hr.

8.2.3. LOAF CHARACTERIZATION

Loaf volume of the experimental dough was determined according to AACC Method 10-05 (2000). Loaf height was determined using calibrated

calipers and reported in cm with measurements taken from the center of each loaf. The colour of the loaves were analyzed using a Hunter Colorimeter (Model LSXE/UNI, Hunter Associates Laboratory, Inc., Reston, VA) in terms of *L*, *a*, and *b* values. Crumb firmness was determined on fresh loaves after 1 hr of cooling at room temperature by performing compression tests using an Instron Universal Testing Machine (Model 4201, Instron Corp, Canton, MA) according to the methods described in Section 5.2.2.

8.2.4. CONSUMER ACCEPTABILITY EVALUATION

Fresh bread samples (≤24 hr old) were evaluated by consumers and standard consumer panel protocols were followed (Stone and Sidel 1993, Resurreccion 1998). Participants were recruited at the University of Alberta (Edmonton, AB) campus by way of poster and word of mouth. An e-mail was also sent out to all the staff and students of the Department of Agricultural, Food and Nutritional Science. Consumers (n=122) evaluated liking of appearance, flavour, texture, and overall liking of three bread samples formulated with 0, 0.75 and 1.5 g β-glucan/serving on a 9-point hedonic scale. The verbal anchors on the 9-point hedonic scale ranged from 'dislike extremely' (1), to 'neither like nor dislike' (5) as a midpoint, to 'like extremely' (9). Purchase intent of the three bread samples was determined using a 5-point scale with anchors "definitely would not buy", "probably would not buy", "maybe/maybe not", "probably would buy" and "definitely would buy", from left to right. Half of the panelists were presented with the identity of the breads and were instructed to read the health information regarding the relationship between the consumption of barley β-glucan and a reduction in the risk of heart disease prior to

evaluating the breads. The paragraph on the front page of the evaluation form read "Each of these three breads has been made with either 0 g, 0.75 g or 1.5 g of barley β-glucan, a soluble fibre. Soluble fibre from foods, such as β-glucan, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease". The other half of the consumers evaluated blinded samples and no health information was provided. In addition, demographic information regarding consumer gender, age, whether or not he/she likes and regularly consumes bread, frequency of bread consumption, whether or not he/she consumes whole-wheat bread, and, if the subject consumes whole-wheat bread, the primary reason for whole-wheat bread consumption was collected.

Bread samples (6.4 cm x 6.4 cm x 1.3 cm) void of crust were presented on a tray in appropriately labeled baggies. Panelists were provided the appropriate paper ballot, napkin, pencil, and water in a plastic cup for rinsing. The order of sample and ballot presentation was balanced and randomized. The test location was the sensory evaluation laboratory on the University of Alberta campus where panelists were assigned to individual booths. The panel room was illuminated with white incandescent lighting for evaluation. The sensory evaluation protocol for this study was approved by the Faculty of Agricultural, Life and Environmental Sciences Research Ethics Board.

8.2.5. STATISTICAL ANALYSIS

All physical measurements were replicated and analysis was performed in duplicate. Results were analyzed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000). Means were

compared using the least significant difference (LSD) test with significance defined at $p \leq 0.05$. The results of the consumer panel were also analyzed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, Inc. 2000), though means were compared using Tukey's test with significance defined at $p \leq 0.05$.

8.3. RESULTS AND DISCUSSION

8.3.1. LOAF CHARACTERIZATION

The loaf characteristics of the bread produced at a pilot plant are presented in Table 8-1. The height of the control (11.6 cm) exceeded ($p \leq 0.05$) that of the 1.5 g β-glucan/serving bread (10.8 cm), while the height of the 0.75 g β-glucan/serving bread (11.1 cm) was similar to both ($p > 0.05$). Similar trends were seen in terms of loaf volume. The control bread had the highest volume ($p \leq 0.05$), while the volume of the 0.75 g β-glucan/serving bread (1605 mL) and the 1.5 g/serving bread (1595 mL) were similar ($p > 0.05$). Both β-glucan-fortified breads with gluten were more firm ($p \leq 0.05$) than the control bread. Possible reasoning for the reduction in loaf volume and increase in firmness was discussed in Section 5.3.1. However, bread volume and firmness values were shown to be better than that of the control upon addition of gluten to bread containing 1.5 g β-glucan/serving previously (Section 5.3.1), though the bread was produced within a lab setting utilizing a small batch size. In addition, reducing the amount of water added to the β-glucan-fortified dough would have also reduced loaf volume. Therefore, to achieve a final product with comparable quality attributes to control

bread within a pilot plant setting, perhaps slight modifications to vital gluten and water addition are necessary.

TABLE 8-1
Characteristics of Bread with Varied Levels of β-Glucan[1]

	β-Glucan (g)/ serving	Height (cm)	Volume (mL)	Firmness (kgF)	Colour		
					L	***a***	***b***
Control	0	11.6[a]	1675[a]	0.24[a]	84.6[a]	-1.7[c]	21.4[ab]
With β-Glucan[2]	0.75	11.1[ab]	1605[b]	0.27[b]	76.4[b]	0.8[b]	21.9[a]
	1.5	10.8[b]	1595[b]	0.28[b]	68.7[c]	2.3[a]	20.6[b]

[a-c]Means within the same column with the same letter are not significantly different ($p > 0.05$)
[1]Standard deviation was less than 5% for all values presented
[2]Breads contain 2.18 g vital gluten/g β-glucan

In terms of the colour, the bread darkened ($p \leq 0.05$) upon each increase in β-glucan level. Gill et al (2002) and Trogh et al (2005) also indicated that the addition of barley flour to bread caused it to darken. The *a* value indicated that the bread also became redder ($p \leq 0.05$) upon each increase in β-glucan level. These changes in colour are similar to those reported in Section 5.3.1 for the bread produced in a laboratory setting. In terms of the *b* value, the 1.5 g β-glucan/serving bread was more blue ($p \leq 0.05$) than the 0.75 g/serving bread, while the control was similar ($p > 0.05$) to the other two. Gill et al (2002) showed an increase in *a* and *b* values upon the addition of barley flour to bread.

8.3.2. CONSUMER ACCEPTABILITY OF β-GLUCAN-FORTIFIED BREAD

A total of 122 panelists took part in the consumer evaluation. Of the 122, 40% were male and 60% were female. In terms of age, 54% were 18-25 years old, 30% were 26-35 years old, 8% were 36-45 years old, and 6% were 46-55 years old. All of the participants liked bread and consumed it regularly. In addition, 89% liked and consumed whole-wheat bread regularly, with 60% of this total doing it primarily for the perceived health benefits of whole-wheat bread consumption.

8.3.2.1. EFFECT OF HEALTH INFORMATION ON CONSUMER ACCEPTABILITY

The results of the consumer evaluation for those who were blinded and, thus, not informed of the health benefits of β-glucan prior to bread evaluation are presented in Figure 8-1. Panelists liked the appearance of all the breads similarly ($p>0.05$) with a score of 6.3-6.6, corresponding to a rating between 'like slightly' and 'like moderately'. However, the control and 0.75 g β-glucan/serving breads were liked more ($p\leq0.05$) than the 1.5 g β-glucan/serving bread in terms of flavour, 6.3-6.5 versus 5.3, texture, 6.7-6.9 versus 6.1, and overall acceptability, 6.5 versus 5.4, respectively. Cavallero et al (2002) reported that substitution of 20% wheat flour with a water-extracted β-glucan concentrate (33% w/w β-glucan) received similar sensory ratings to those of the control. However, Cavallero et al's (2002) consumer panel consisted only of 10 laboratory workers and this bread contained added fat. Purchase intent followed a similar trend (3.3-3.4 versus 2.8)

with consumers rating the control and 0.75 g β-glucan/serving breads between "maybe/maybe not" and "probably would buy", while the purchase intent of the 1.5 g β-glucan/serving bread was rated between "maybe/maybe not" and "probably would not buy". This supports the observation of Bower et al (2003), who reported higher purchase intent by consumers for the product that was liked more. Therefore, because the attributes of the 0.75 g β-glucan/serving bread and purchase intent were rated similarly ($p>0.05$) to the control, this bread may see success within the food industry regardless of whether health information is provided or not. However, the amount of β-glucan incorporated into this bread also meets the minimum level of incorporation specified by the FDA (2005) for the approved health claim.

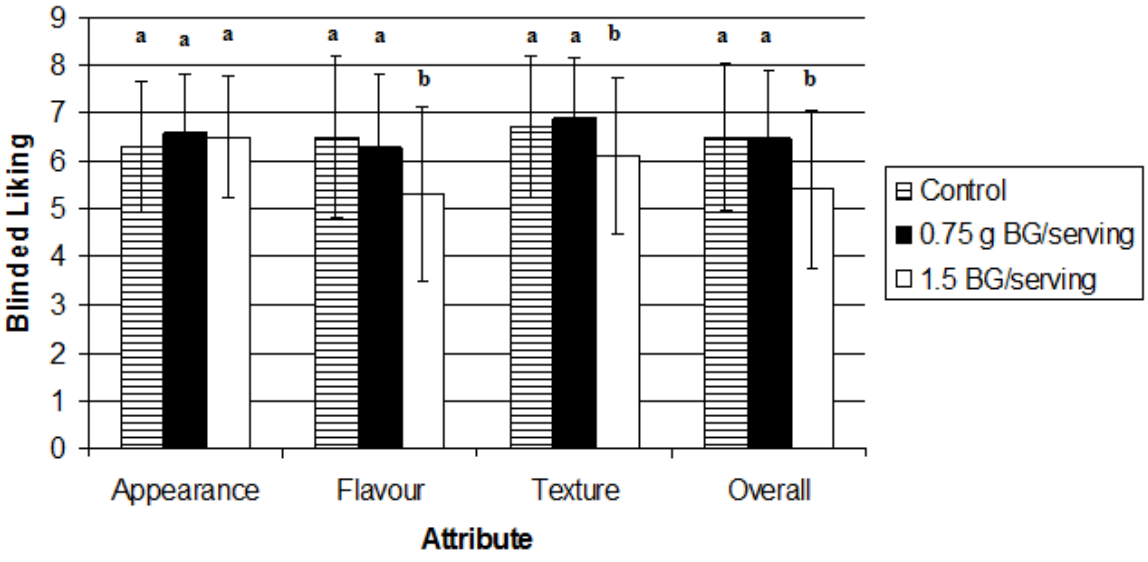


Fig. 8-1. Blinded consumer evaluation of appearance, flavour, texture and overall acceptability of bread fortified with 0, 0.75 and 1.5 g β-glucan/serving

The results of the consumer evaluation of the breads following the provision of β-glucan health information and revealing the identity of the breads is presented in Figure 8-2. Health information did have a positive effect ($p \leq 0.05$) on consumer liking of the appearance of the 1.5 g β-glucan/serving bread in comparison to the others. The 1.5 g β-glucan/serving bread received a rating of "like moderately" or 7, which was a higher rating ($p \leq 0.05$) than the control at 6.2. The liking of the 0.75 g β-glucan/serving bread appearance did not differ ($p > 0.05$) from that of the other two breads. These results indicate that when fortifying bread with higher amounts of β-glucan, health information may increase liking of appearance when evaluated in comparison to other breads. This may be due to the provision of the health benefits and/or an association of the bread's colour with an appearance similar to that of whole-wheat bread, which is perceived to be linked to improved health benefits (Mialon et al 2002), as the 1.5 g β-glucan/serving bread was darker, redder, and bluer than the control (Table 8-1).

When the identities of the samples and the β-glucan health information were provided, liking of the 1.5 g β-glucan/serving bread was rated similarly ($p > 0.05$) to the control and the 0.75 g/serving breads (Fig. 8-2), which were previously rated higher in terms of flavour, texture and overall acceptability under blind evaluation (Fig. 8-1). The liking of the flavour, texture and overall acceptability of all the breads ranged from 6-6.3, 6.2-6.5, and 6.2-6.4, respectively. Due to the similarity in liking values, it appears that the pairing of the 1.5 g β-glucan/serving read with the health claim is preferable, which is in line with the findings of van Kleef et al (2005) that consumers tend to prefer functional food concepts that

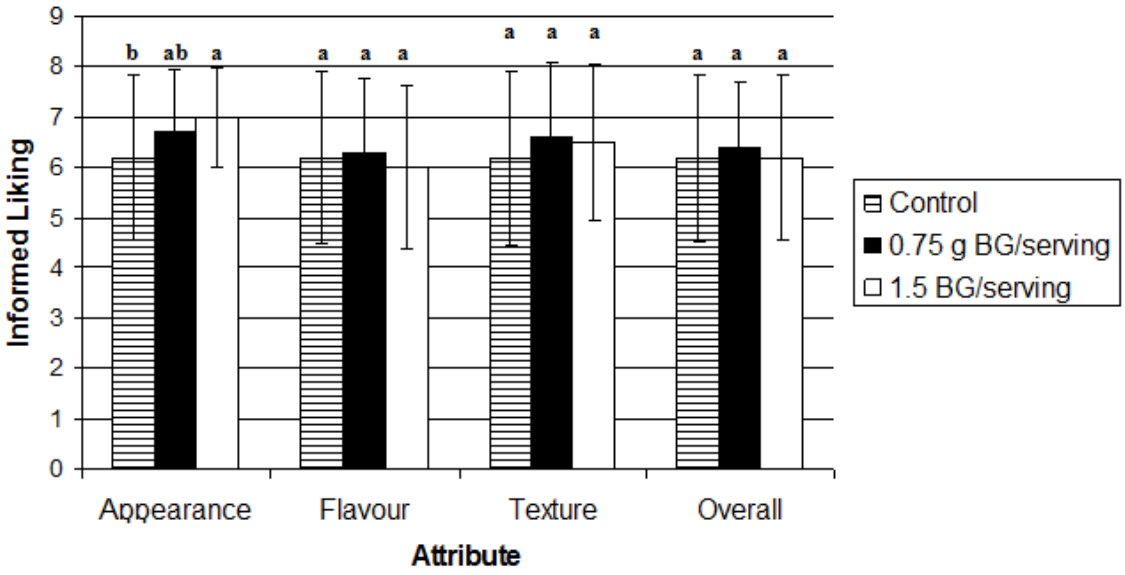


Fig. 8-2. Consumer evaluation of appearance, flavour, texture and overall acceptability of bread fortified with 0, 0.75 and 1.5 g β-glucan/serving after information on β-glucan health benefits was given and the identities of the bread samples were revealed

communicate disease-related health benefits in carriers with a healthy image. Furthermore, disconfirmation, which is the difference between the expected acceptability of a product and the blind acceptability of the same product before being affected by expectations (Caporale and Monteleone 2004), may have played a role in the improved liking of the 1.5 g β-glucan/serving bread in comparison to the other breads upon the provision of health information and the bread's identity. When the product is better than expected, the disconfirmation is positive (Caporale and Monteleone 2004). However, when the product is worse than expected, the disconfirmation is negative. Therefore, it appears that upon the provision of health information and the identity of the bread, consumers did not expect to like the 1.5 g β-glucan/serving bread and were positively disconfirmed

as this bread was liked more than expected. Similarly, in a study by Mialon et al (2002) comparing the effect of dietary fibre information on consumer responses to whole-wheat bread, Australian consumers also expected to like the control bread more than the whole-wheat bread. Contrary to our results, however, Mialon et al (2002) reported that the consumers rated the control bread higher in actual liking than the whole-wheat. Wansink et al (2004) stated that for health or diet labels to have a positive impact the label needs to create a diminished expectation of the food and there needs to be a very positive disconfirmation. This seems to be the case with the 1.5 g β-glucan/serving bread. This type of disconfirmation is rare when the foods are not highly favorable (Wansink et al 2004), though Oliver (1980) suggests that when such foods are presented to consumers, the resulting disconfirmation of expectation would result in a more favorable rating compared with the rating of an unlabeled version of the same food.

Purchase intent of the breads was rated similarly ($p>0.05$) as well, ranging from 3.1-3.2, which is associated with a purchase intent just above "maybe/maybe not". Ginon et al (2009) reported that labeling French baguettes with "source of fibre" increased intent to purchase, though additional information on the health benefits of fibre did not have an effect. The findings of this study demonstrate that in addition to the liking of flavour, texture and overall acceptability, health information may cause purchase intent of 1.5 g β-glucan/serving bread to be rated similarly to other breads when evaluation is performed in comparison to a variety of breads, as is often the case when shopping in a grocery store.

To determine if the provision of health information had an effect on the liking of each bread individually, statistical comparison of consumers' liking of each bread's attributes when blinded versus when the consumers were informed of the bread's identity and health benefits was performed. The results (Table 8-2) indicate that providing health information and revealing the identity of the control and 0.75 g β-glucan/serving breads did not ($p>0.05$) affect liking of any of the evaluated attributes or purchase intent. Health information did improve ($p\leq0.05$) liking of the 1.5 g β-glucan/serving bread's appearance, flavour, and overall acceptability, in addition to its purchase intent. Consumers liked the appearance (7 versus 6.5), flavour (6 versus 5.3), and overall acceptability (6.2 versus 5.4) more ($p\leq0.05$) and purchase intent was higher (2.8 versus 3.1) when the health information and the identity of the bread was provided versus when the panelists were blinded, respectively. Liking of the texture remained the same (6.6-6.9) ($p>0.05$). Carneiro et al (2005), who explored buying intention of soybean oil, reported that all consumers had their buying intention affected by price. Therefore, more information on the consumer purchase intent of bread fortified with β-glucan may be achieved if differences in price in addition to the provision of health information are investigated.

TABLE 8-2
Consumer Evaluation of Bread with 0, 0.75, and 1.5 g β-Glucan/serving With or Without the Provision of Health Information Prior to Evaluation[1]

Bread		Appearance	Flavour	Texture	Overall	Purchase Intent
Control	**Blinded**	6.3^{a} (±1.4)	6.5^{a} (±1.7)	6.7^{a} (±1.5)	6.5^{a} (±1.5)	3.4^{a} (±1.0)
	Informed	6.2^{a} (±1.6)	6.2^{a} (±1.7)	6.2^{a} (±1.7)	6.2^{a} (±1.7)	3.2^{a} (±1.0)
0.75 g β-glucan /serving	**Blinded**	6.6^{a} (±1.2)	6.3^{a} (±1.5)	6.9^{a} (±1.3)	6.5^{a} (±1.4)	3.3^{a} (±1.1)
	Informed	6.7^{a} (±1.2)	6.3^{a} (±1.5)	6.6^{a} (±1.5)	6.4^{a} (±1.3)	3.2^{a} (±1.0)
1.5 g β-glucan /serving	**Blinded**	6.5^{b} (±1.3)	5.3^{b} (±1.8)	6.1^{a} (±1.6)	5.4^{b} (±1.7)	2.8^{b} (±0.9)
	Informed	7^{a} (±1.0)	6^{a} (±1.6)	6.5^{a} (±1.5)	6.2^{a} (±1.6)	3.1^{a} (±1.1)

[1]Means of determinations are reported (n=61 blinded, n=61 informed). Mean score on 9-point hedonic scale (1: like extremely, 5: neither like nor dislike, 9: dislike extremely). Each bread evaluated individually comparing liking of each attribute with or without the provision of health information prior to consumption.
[a-b]Means ± standard deviation within the same category with the same letter are not significantly different ($p > 0.05$)

8.3.2.2. EFFECT OF GENDER AND THE PROVISION OF HEALTH INFORMATION ON CONSUMER ACCEPTABILITY AND PURCHASE INTENT

Analyzing consumer liking of the breads individually on the basis of gender revealed that there were no ($p>0.05$) differences in the liking of the control bread or the 0.75 g β-glucan/serving bread in any of the attributes evaluated or purchase intent between men and women, regardless of whether they were blinded or informed. There were also no differences ($p>0.05$) in the liking of the flavour, texture, overall acceptability or purchase intent for the 1.5 g/serving bread between men and women, regardless of whether they were blinded or informed. When blinded, men and women liked the appearance of the 1.5 g β-glucan/serving bread similarly ($p>0.05$); however, when informed women liked the appearance of the 1.5 g β-glucan/serving bread (7.3) more ($p\leq0.05$) than the men (6.6). Further analysis indicated that upon the provision of health information women's

liking of the appearance of the 1.5 g β-glucan/serving actually exceeded ($p \leq 0.05$) that of the control. This may have positive implications within grocery stores as appearance is a primary attribute that consumers rely on when evaluating quality and purchasing groceries (von Alvensleben and Meier 1990, Wansink et al 2004) as they generally do not get to taste the products in store. Increased liking of appearance in relation to the provision of health information may be due to the fact that women are regarded as having more concern for health (Towler and Shepherd 1992, Bower and Saadat 1998). Furthermore, because the flavour liking of all the breads was rated similarly ($p > 0.05$) this would also encourage a repeat purchase as taste is still the most important product attribute that influences food choice (Wandel and Bugge 1997, Glanz et al 1998, Chryssohoidis and Krystallis 2005, Radder and la Roux 2005). In addition, because women do the majority of the shopping for their families (ERS 2005) this may increase purchases in an actual grocery store setting and allow greater amounts of β-glucan-fortified bread to be brought home. However, more research is required on the impact of health information on liking of appearance and purchase intent, in the absence of tasting the product, to more fully understand the impact of such variables in a situation that more fully reflects a grocery shopping setting.

8.3.2.3. EFFECT OF REGULAR WHOLE-WHEAT CONSUMPTION AND THE PROVISION OF HEALTH INFORMATION ON CONSUMER ACCEPTABILITY

Because the β-glucan-fortified bread is similar to whole-wheat type bread, it is logical to see if regular consumption of whole-wheat bread and the provision of

health information had an effect on consumer liking of β-glucan-fortified bread. Of the 122 consumers, 53% stated that they ate whole-wheat bread primarily for the perceived health benefits. Evaluation of each of the breads individually for this subset of consumers revealed a similar ($p>0.05$) liking of appearance, flavour, texture, overall acceptability, and purchase intent of both the control and the 0.75 g β-glucan/serving bread between the consumers who were blinded and who were informed. Liking of the flavour (5-5.7), texture (6.1-6.3), overall acceptability (5.2-5.9) and purchase intent (2.7-3) were also similar ($p>0.05$) for the 1.5 g β-glucan/serving bread, however liking of this bread's appearance improved ($p\leq0.05$) from 6.4 to 7 upon the provision of health information. Because this subset of consumers likely already associated the β-glucan bread's appearance with that of bread with added fibre (Mialon et al 2002), this food may have already been perceived as "healthy". Mialon et al (2002) reported that in the absence of product information, consumers perceived a whole-wheat type bread as healthier than a white control bread. According to Wansink et al (2004), health labels have little impact on the perceived taste of foods that are typically expected to already be healthy.

8.4. CONCLUSIONS

Measurement of the breads' physical characteristics upon processing under pilot-plant conditions indicated that β-glucan addition decreased loaf volume, increased firmness, and resulted in darker, redder bread, while fortification at the 1.5 g β-glucan/serving level decreased height, as well. Consumer evaluation

revealed that when consumers were not informed of β-glucan health benefits, they rated the liking of the flavour, texture, overall acceptability and purchase intent of the 0.75 g β-glucan/serving bread and the control higher than the 1.5 g β-glucan/serving bread. However, when health information was provided liking of the 1.5 g β-glucan/serving bread in all evaluated attributes and purchase intent was similar to or exceeded the control. Health information improved the liking of the 1.5 g β-glucan/serving bread's appearance, flavour and overall acceptability, although it had no effect on the liking of the 0.75 g β-glucan/serving bread or the control.

Men and women rated the breads similarly in purchase intent and all attributes, with the exception of the appearance of the 1.5g β-glucan/serving bread. Women were more positively affected by health information in this aspect as they rated the liking of this bread's appearance higher than men, which may have positive implications for purchase within grocery stores. Consumers who regularly ate whole-wheat bread due to the perceived health benefits also showed an increase in the liking of the 1.5 g β-glucan/serving bread's appearance upon the provision of health information and the identity of the bread.

8.5. REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 10-09 and 44-15A. The Association: St. Paul, MN.

Baixauli, R., Salvador, A., Hough, G., Fiszman, S.M. 2008. How information about fibre (traditional and resistant starch) influences consumer acceptance of muffins. Food Qual Pref. 19(7):628-635.

Bower, J.A., Saadat, M.A. 1998. Consumer preference for retail fat spreads: an olive oil based product compared with market dominant brands. Food Qual Pref. 9(5):367-376.

Bower, J.A., Saadat, M.A., Whitten, C. 2003. Effect of liking, information and consumer characteristics on purchase intention and willingness to pay more for a fat spread with a proven health benefit. Food Qual Pref. 14:65-74.

Caporale, G., Monteleone, E. 2004. Influence of information about manufacturing process on beer acceptability. Food Qual Pref. 15:271-278.

Carneiro, J.D., Minim, V.P., Deliza, R., Silva, C.H., Carneiro, J.C., Leao, F.P. 2005. Labeling effects on consumer intention to purchase for soybean oil. Food Qual Pref. 16:275-282.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Chryssohoidis, G.M., Krystallis, A. 2005. Organic consumers' personal values research: Testing and validating the list of values (LOV) scale and implementing a value-based segmentation task. Food Qual Pref. 16:585-599.

Economic Research Service. 2005. A healthy, well-nourished population: food, nutrition, and time use patterns. United States Department of Agriculture. Accessed on June 4, 2009: http://www.ers.usda.gov/emphases/healthy/atus/.

Food and Drug Administration. 2005. Food labeling: health claims; soluble dietary fibre from certain foods and coronary heart disease; Final Rule Federal Register Doc. 2004P-0512, filed 12-23-05.

Gill, S., Vasanthan, T., Ooraikul, B., Rossnagel, B. 2002. Wheat bread quality as influenced by substitution of waxy and regular barley flours in their native and extruded forms. J Cereal Sci. 36:219-237.

Ginon, E., Loheac, Y., Martic, C., Combris, P., Issanchou, S. 2009. Effect of fibre information on consumer willingness to pay for French baguettes. Food Qual Pref. 20(5):343-352.

Glanz, K., Basil, M., Maibach, E., Goldberg, J., Snyder, D. 1998. Why Americans eat what they do: Taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. J Am Diet Assoc. 98(10):1118-1126.

Goerlitz, C.D., Delwiche, J.F. 2004. Impact of label information on consumer assessment of soy-enhanced tomato juice. J Food Sci. 69(9):S376-S379.

Kahkonen, P., Tuorila, H., Lawless, H. 1997. Lack of effect of taste and nutrition claims on sensory and hedonic responses to a fat-free yogurt. Food Qual Pref. 8(2):125-130.

Mialon, V.S., Clark, M.R., Leppard, P.I. Cox, D.N. 2002. The effect of dietary fibre information on consumer responses to breads and "English" muffins: a cross-cultural study. Food Qual Pref. 13(1):1-12

Oliver, R.L. 1980. A cognitive model of the antecedents and consequences of satisfaction decisions. J Market Res. 17:460-469.

Radder, L., le Roux R. 2005. Factors affecting food choice in relation to venison: A South Africa example. Meat Sci. 71:583-589.

Resurreccion, A. 1998. Consumer Sensory Testing for Product Development. Aspen Publishers, Gaithersburg, MD.

Shepherd, R. 1989. Factors influencing food preferences and choices. In: Handbook of the Psychology of Human Eating. Shepherd, R. (ed). John Wiley and Sons Inc, Chichester, UK. pp. 3-24.

Stone, H., Sidel, J. 1993. Sensory Evaluation Practices. Academic Press, San Diego, CA.

Towler, G., Shepherd, R. 1992. Application of Fishbein and Ajzen's expectancy-value model to understanding fat intake. Appetite. 18:15-27.

Trogh, I., Courtin, C.M., Goesaert, H., Delcour, J.A., Andersson, A.A.M., Aman, P., Fredriksson, H., Pyle, D.L., Sorensen, J.F. 2005. From hull-less barley and wheat to soluble dietary fibre-enriched bread. Cereal Foods World. 50:253-260.

Van Kleef, E., Van Trijp, H.C., Luning, P. 2005. Functional foods: health claim-food product compatibility and the impact of health claim framing on consumer evaluation. Appetite. 44:299-308.

Von Alvensleben, R., Meier, T. 1990. The influence of origin and variety on consumer perception. Acta Horticulturae. 259:151-161.

Wandel, M., Bugge, A. 1997. Environmental concern in consumer evaluation of food quality. Food Qual Pref. 8(1):19-26.

Wansink, B., van Ittersum, K., Painter, J.E. 2004. How diet and health labels influence taste and satiation. J Food Sci. 69(9):S340-S346.

Chapter 9

CONCLUSIONS AND RECOMMENDATIONS

β-Glucan, a soluble dietary fibre, has been shown to allow for better regulation of blood glucose levels (Wood et al 1994, Yokoyama et al 1997, Bourdon et al 1999, Panahi et al 2007, Kim et al 2009) and also to lower blood cholesterol (Gallaher et al 1992, Martinez et al 1992, Newman et al 1992, Jackson et al 1994, Wang et al 1997, Bourdon et al 1999, Karmally et al 2005, Naumann et al 2006, Queenan et al 2007). β-Glucan's cholesterol lowering and blood glucose regulating effects are primarily related to its viscosity, which can be negatively influenced by certain processing and storage conditions. Previous studies have indicated that the beneficial effects of β-glucan are decreased when incorporated into the bread system (Kerckhoffs et al 2003, Åman et al 2004, Andersson et al 2004, Frank et al 2004, Trogh et al 2004, Cleary et al 2007, Flander et al 2007, Andersson et al 2008) and that β-glucan addition may be detrimental to bread loaf volume (Cavallero et al 2002, Gujral et al 2003, Trogh et al 2004). This thesis examined the journey of β-glucan through the mixing, fermenting, baking, and storage of bread at levels of addition most likely to be presented to consumers. The changes in β-glucan throughout the various bread making steps were evaluated in terms of solubility (or extractability) of β-glucan under approximated physiological conditions and the viscosity of the extracts, considering that viscosity has been identified as the primary mechanism of health benefits even though other possible mechanisms exist, involving different

parameters. In addition, the quality and consumer acceptability of bread fortified with β-glucan and the effect of health information on acceptability were studied.

Investigation of the effect of formulation and processing treatments on the solubility and viscosity of β-glucan in bread dough (Chapter 3) indicated that the viscosity of the physiological extract from bread fortified with β-glucan was impacted by the level of β-glucan addition, fermentation time, and the flour's endogenous enzymes. Fermentation decreased β-glucan solubility indicating the presence of interactions between β-glucan through self association and/or with other dough components. As such, decreases in viscosity that have been primarily attributed to reductions in β-glucan molecular weight in the past may also be highly dependent on decreased solubility. The importance of using extraction techniques approximating physiological conditions when evaluating the characteristics of products targeted for human consumption was also emphasized, as some traditional experimental methods (hot water extraction) yield less applicable results.

Evaluation of the impact of β-glucan on the mixing characteristics, rheological properties, and microstructure of bread dough (Chapter 4) emphasized the importance of simultaneously studying dough rheological characteristics and microstructure in addition to mixing characteristics to better understand and interpret the behaviour of bread dough upon incorporation of experimental ingredients. The rheological measurements and fluorescence images indicated that β-glucan likely interferes with the formation of an adequate gluten network, though the addition of vital gluten may help to correct this detrimental effect on

dough quality. Measurement of the bread's physical properties (Chapter 5) supported these observations as the addition of β-glucan decreased loaf volume and height and increased firmness, while the addition of gluten corrected these defects to values similar to or better than that of the control.

Baking the bread solubilized β-glucan to substantially higher values (58-60%) than in dough (9%) (Chapter 5). The addition of gluten to the bread increased β-glucan solubility further (67-68%). Similar trends were seen for extract viscosity and were supported by the fluorescence microscopy images of the bread microstructure. Such results have not been reported in the past and are encouraging as an increase in β-glucan solubility and viscosity may indicate an increase in the ability of the β-glucan-fortified bread to produce health benefits. Past studies have utilized β-glucan extracts of high solubility obtained mostly by aqueous extraction technologies. However, in this thesis research barley β-glucan concentrate of low solubility under approximated physiological conditions was used, which was obtained by using a newly patented technology (Vasanthan and Temelli 2009) based on an aqueous-alcohol enzymatic process. It was demonstrated that low solubility β-glucan concentrate is well suited for bakery applications where enzymatic degradation from native/exogenous flour enzymes is expected and where the heating step and added water are sufficient to solubilize the β-glucan in addition to inactivating enzymes. Thus, the use of β-glucan in yeast-leavened bread as a method of conferring health benefits need not be avoided provided a β-glucan concentrate with these physicochemical properties is utilized. The barley β-glucan concentrate utilized in this thesis research contained

50% β-glucan. Even though other polysaccharides such as arabinoxylans were also present in this concentrated ingredient, their contribution to the viscosity of the extracts was expected to be minimal since arabinoxylans are mainly soluble in alkali and would not be solubilized to any appreciable extent under the applied physiological conditions.

The effect of β-glucan addition on the quality of bread subjected to various storage times and conditions (Chapter 6) indicated that it is optimal to consume bread fortified with β-glucan fresh in order to maintain the highest quality, in terms of moisture content and firmness. However, appreciable bread quality can still be maintained upon room temperature storage of β-glucan-fortified bread with added gluten and at either room temperature or frozen storage for β-glucan-fortified bread without gluten for 4 days. If it is desirable to store bread for 7 days or more, frozen storage should be utilized in order to best maintain bread moisture and firmness levels.

Though these storage conditions are optimal for the retention of bread quality, the impact of the storage conditions on the solubility and viscosity of β-glucan must also be taken into account. It is recommended that β-glucan-fortified bread be consumed fresh for greatest β-glucan solubility and viscosity, though β-glucan solubility of approximately 40% is still achievable upon frozen storage of the bread for up to two weeks. It is still unclear, however, as to what extent reductions in the solubility and viscosity of β-glucan lower its physiological effectiveness. It is apparent that more work within this important area of research is necessary in order to understand the impact of storage on the physiological

effectiveness of β-glucan in bread. Despite the reduction in β-glucan solubility over storage, however, β-glucan would still exhibit health benefits attributable to insoluble dietary fibre, such as increased fecal bulk and decreased transit time, or may elicit a response similar to resistant starch, such as providing additional substrate for colonic fermentation (Slavin 2003).

The impact of incorporating β-glucan concentrate into the bread system on its physiological effectiveness, including measures of perceived satiety, glycemic response and *in vitro* reducing sugar release was studied (Chapter 7). Bread with β-glucan was able to keep panelists full, longer. Bread reducing sugar release values implied that satiety ratings may rely on an appropriate balance between gastric viscosity, gastric emptying and the ability of the test food to cause an increase in blood glucose levels. Glycemic response measures showed that the bread with β-glucan appeared to produce the most leveled blood glucose response, though there were no differences in the 2 hr postprandial area under the blood glucose curve, which was unexpected. Further investigation of bread fortified with β-glucan concentrate is warranted to identify its physiological benefits in different patient populations, including those with Type 2 diabetes, hypercholesterolemia, and those who are obese, as this bread may have positive implications for management of such conditions. In addition, the effect of bread fortified with β-glucan concentrate on other measures of human health, including various hormone levels, cholesterol levels, immune response and prebiotic effects, is warranted in future studies. Nonetheless, commercialization of bread with 1.5 g β-glucan/serving may be beneficial to society as it appears to elicit a more leveled

blood glucose response curve and a prolonged feeling of fullness upon consumption, which may be important for weight loss and general health.

Consumer acceptability and purchase intent of bread fortified with β-glucan concentrate was determined in the presence and absence of health information (Chapter 8). When consumers were not informed of β-glucan health benefits, they rated the liking of the flavour, texture, overall acceptability and purchase intent of the 0.75 g β-glucan/serving bread and the control higher than the 1.5 g β-glucan/serving bread. However, when health information was provided liking of the 1.5 g β-glucan/serving bread in all evaluated attributes and purchase intent was improved to values similar to those of the control. Women and those who regularly consumed whole-wheat bread for its perceived health benefits were most positively affected by health information.

The 0.75 g β-glucan/serving bread not only meets the currently approved β-glucan content necessary by the FDA (2005) for use of the health claim, but it was also rated similarly to the control in all aspects, regardless of whether or not health information was provided to consumers. Therefore, a successful bread product fortified with 0.75 g β-glucan/serving is possible within the food industry regardless of whether or not the health benefits of β-glucan are permitted on the product's packaging. Though not yet approved in Canada, the United States has approved the use of the FDA health claim on packaging for products containing at least 0.75 g β-glucan/serving. Therefore, the 1.5 g β-glucan/serving bread may also see success within this food market. This would provide consumers with even higher levels of β-glucan due to the positive impact of health information on

the ratings of this bread. Providing consumers with an additional source of dietary fibre in the food market may allow them to come closer to the 21-38 g recommended daily intake of dietary fibre, as consumers currently receive less than half of this recommendation (Heart and Stroke Foundation 2009).

Although FDA (2005) has approved a health claim for barley β-glucan for reducing the risk of heart disease there are still challenges for the delivery of efficacious products to the consumers. Despite the comprehensive approach to characterizing the role of barley β-glucan concentrate in bread, future work is warranted to better understand the interactions among numerous variables. For example, genetic and environmental factors have a major effect on the content and molecular weight distribution of β-glucan in cereal grains, which in turn affect its physicochemical properties. As well, different processing techniques applied for the concentration of β-glucan from the grain also impact β-glucan properties. Scale-up from laboratory to commercial scale is challenging to achieve a consistent end product. Incorporation of the β-glucan concentrate into various food formulations requires a good understanding of the interactions between β-glucan and other components.

Because β-glucan's health benefits have been primarily attributed to its ability to increase viscosity within the gut, it is important to compare it to other soluble fibres that would elicit similar viscosity effects to establish mechanisms and other physiological markers that may be unique to β-glucan. Other soluble fibres, such as psyllium, guar gum, xanthan gum, as well as other sources of β-glucan, such as oat β-glucan and β-glucan concentrates produced using traditional

methods should be tested in order to better understand the effects of barley β-glucan concentrate in bread relative to these seemingly similar products. Evaluation of the other components present in the β-glucan concentrate is also warranted to identify the extent of their contributions to the increase in extract viscosity observed upon increases in the amount of β-glucan concentrate incorporated into the system as well as any synergistic, neutral or detrimental effect on solution viscosity.

Furthermore, upon consumption of β-glucan-containing products the impact of variability within the human population, in terms of consumer acceptability and physiological responses, cannot be ignored. Therefore, additional studies are needed to understand the effect of other food systems on the quality of β-glucan as well as the impact of β-glucan concentrate in specific patient/consumer populations. This thesis research evaluated only the satiety effect and glucose response as physiological outcomes with marginal results; however, it is also important to investigate other health parameters, including modulation of immune response, prebiotic potential, and β-glucan's impact on hormonal markers, among other things, in order to identify potential health benefiting mechanisms unique to β-glucan.

Overall, characterization of β-glucan's journey through the entire dough making and eventually bread baking process under realistic conditions throughout this thesis research allowed for identification of the key areas within commercial production that can reduce β-glucan viscosity and potentially its health benefits. To the best of this author's knowledge, such a comprehensive approach to the

fortification of bread with β-glucan has not been done before. By recognizing factors that may degrade β-glucan and finding solutions to maintain β-glucan viscosity, such as the use of low solubility β-glucan concentrate in the fortification of bakery products, the overwhelming potential of β-glucan to create functional foods may finally be recognized. The role of low solubility β-glucan concentrate in bread and bakery products as an effective and reliable means of lowering blood cholesterol and attenuating blood glucose levels for the millions of North American's at risk for or suffering from heart disease and Type 2 diabetes is a new and promising area of research requiring further investigation and scale-up for commercialization.

9.1. REFERENCES

Åman, P., Rimsten, L., Andersson, R. 2004. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

Andersson, A.A., Rüegg, N., Åman, P. 2008. Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. 2004. Molecular weight and structure units of (1→3, 1→4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., Schneeman, B.O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr. 69:55-63.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1→3, 1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci. 36:59-66.

Cleary, L.J., Andersson, R., Brennan, C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chem. 102:889-897.

Flander, L., Salmenkallio-Marttila, M., Suortti, T., Autio, K. 2007. Optimization of ingredients and baking process for improved wholemeal oat bread quality. Lebesmitt Wiss-u Technol. 40:860-870.

Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. 2004. Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

Gallaher, D.D., Locket, P.L., Gallaher, C.M. 1992. Bile acid metabolism in rats fed two levels of corn oil and brans of oat, rye, and barley and sugar beet fibre. J Nutr. 122:473-481.

Gujral, H.S., Gaur, S., Rosell, C.M. 2003. Effect of barley flour, wet gluten, and ascorbic acid on bread crumb texture. Food Sci Tech Int. 9(1):17-21.

Heart and Stroke Foundation. 2009. Fibre, whole grains and carbohydrates. Accessed August 21, 2009. http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3484239/k.E1F9/Healthy_living__Fibre_whole_grains_and_carbohydrates.htm

Jackson, K.A., Suter, D.A.I., Topping, D.L. 1994. Oat bran, barley, and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J Nutr. 124:1678-1684.

Karmally W, Montez MG, Palmas W. 2005. Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. J Am Diet Assoc. 105:967–70.

Kerckhoffs, D., Hornstra, G., Mensink, R.P. 2003. Cholesterol-lowering effect of β-glucans from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr. 78:221-227.

Kim, H., Stokes, K.S., Behall, K.M., Spears, K., Vinyard, B., Conway, J.M. 2009. Glucose and insulin responses to wholegrain breakfasts varying in soluble fibre, β-glucan. Eur J Nutr. 48(3):170-175.

Martinez, V.M., Newman, R.K., Newman, C.W. 1992. Barley diets with different fat sources have hypocholesterolemic effects in chicks. J Nutr. 122:1070-1076.

Naumann, E., van Rees, A.B., Onning, G., Oste, R., Wydra, M., Mensink, P. 2006. β-Glucan incorporated into a fruit drink effectively lowers serum LDL-cholesterol concentrations. Am J Clin Nutr. 83:601-605.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., Hofer, P.J. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Panahi, S., Ezatagha, A., Temelli, F., Vasanthan, T., Vuksan, V. 2007. β-Glucan from two sources of oat concentrates affect postprandial glycemia in relation to level of viscosity. J Am College Nutr. 26(6):639-644.

Queenan, K.M., Stewart, M.L., Smith, K.N., Thomas, W., Fulcher, R.G., Slavin, J.L. 2007. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. Nutr J. 6:6.

Slavin, J. 2003. Impact of the proposed definition of dietary fibre on nutrient databases. J Food Comp Anal. 16:287–291.

Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. 2004. The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

Vasanthan, T., Temelli, F. 2009. Grain fractionation methods and products. U.S. Patent 7,566,470 B2.

Wang, L., Behr, S.R., Newman, R.K., Newman, C.V. 1997. Comparative cholesterol-lowering effects of barley β-glucan and barley oil in golden Syrian hamsters. Nutr Res. 17(1):77-88.

Wood, P.J., Beer, M.U., Butler, G. 2000. Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

Yokoyama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M.M., Sayre, R.N., Turnlund, J.R., Schneeman, B.O. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74(3):293-296.