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Chemical and sensory characterization of oat bran from experimental oat lines with varying amounts of total beta-glucan

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Chemical and sensory characterization of oat bran from experimental oat lines with varying amounts of total beta-glucan

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Pamela White, Major Professor
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Ames, Iowa

2007

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To my loving father, mother, and sister

Table of Contents

List of Nomenclature.....	v
Acknowledgments.....	vi
Abstract.....	vii
General Introduction.....	1
Literature Review.....	3
History of Oats.....	3
Health Benefits.....	6
Methods of Measuring Dietary Fiber.....	9
Beta-Glucan.....	9
Sources.....	10
Uses.....	11
Health Implications.....	13
Oats as Food Products.....	17
Sensory Evaluation for Food Products.....	18
Instrumental Methods to Measure Physical Attributes.....	19
Literature Cited.....	23
Characterization, Bile Acid Binding, and Short-Chain Fatty Acid Production of Oat Bran from Oat Lines with Typical and High Amounts of Beta-Glucan	33
Abstract.....	34
Introduction.....	34
Materials and Methods.....	36
Results and Discussion.....	40
General Conclusions.....	45
Acknowledgments.....	45
Literature Cited.....	46
Tables and Figures.....	49
Sensory and Instrumental analysis of Porridge and Muffins made from Oat Bran with Varying Amounts of Total Beta-Glucan.....	55
Abstract.....	56
Introduction.....	56
Materials and Methods.....	58
Results and Discussion.....	64
General Conclusions.....	71
Acknowledgments.....	72
Literature Cited.....	72

Tables and Figures.....	75
General Conclusions.....	81
Appendix I.....	85
Appendix II.....	86
Appendix III.....	87
Appendix IV.....	88
Appendix V.....	89

List of Nomenclature

AACC – American Association of Cereal Chemists, International

AOAC – Association of Analytical Chemists

β – beta

BA – Bile Acid

BAB – Bile Acid Binding

CHD – Coronary Heart Disease

FDA – Food and Drug Administration (United States)

IA95 – IA95111; Experimental Oat Line by Iowa State University

ISU – Iowa State University

Jim – public cultivar developed by the University of Minnesota, Twin Cities

LDL-cholesterol – Low Density Lipoprotein Cholesterol

N979 – N979-5-2-4; Experimental Oat Line by Iowa State University

NASS – National Agriculture Statistics Service

Paul – public naked oat cultivar

QDA – Quantitative Descriptive Analysis

SCFA – Short-Chain Fatty Acids

TPA – Texture Profile Analysis

USDA – United States Department of Agriculture

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Abstract

Oat bran from experimental oat lines (*Avena sativa*) with increased β -glucan content, N979-5-2-4 and IA95111, had a greater amount of total β -glucan, water absorption and water solubility indices than bran from public oat cultivars, 'Paul' and 'Jim,' or retail oat products, Oat Bran and Old Fashioned Quick Oats (both, Quaker Oats Company); with positive correlations between total β -glucan and water absorption index ($r = 0.853$), and water solubility index ($r = 0.820$). N979 and IA95 brans bound 99% of bile acids, and had the greatest Rapid Visco Analyser values for peak viscosity, final viscosity, trough, and breakdown. Jim produced the most gas and short-chain fatty-acids, but had the lowest pH during fermentation. By sensory evaluation, N979 and IA95 brans: 1) had larger particle sizes, less creaminess and mouth-coating in porridge; and 2) were more crumbly and gritty, but less moist and cohesive, in muffins than public cultivars.

General Introduction

Oats are recognized as a whole grain cereal crop, highly recommended as an important part of the daily diet. In particular, the dietary fiber in oats, β -glucan, is credited with imparting several nutritional benefits, including: inducing satiety, aiding in blood glucose metabolism, reducing serum cholesterol, and improving gastrointestinal health.

Coronary heart disease (CHD) is currently the leading cause of death in the United States. The risk of developing this disease can be reduced through several life-style changes, including diet. In January of 1997, the United States Food and Drug Administration (FDA) passed a Code of Federal Regulations which allowed a health claim for soluble fiber from certain foods to be placed on food labels. Soluble fiber, along with a diet low in saturated fat, may lower the blood's level of low density lipoprotein (LDL) cholesterol, diminishing the risk of CHD. The list of foods published by the FDA that contain soluble fiber included oat bran. Oats, and oat fractions, are common food ingredients easily incorporated into a variety of food products. The FDA recommends consuming 3 g of soluble fiber (β -glucan) daily to lower the risk of developing heart disease.

For several years, faculty members in the departments of Agronomy and Food Science and Human Nutrition at Iowa State University have worked together to produce oat lines with a higher amount of total β -glucan than found in the traditional oat cultivars currently available to the public. The experimental oat lines with an increased amount of β -glucan used in this study are N979-5-2-4 (N979) and IA95111 (IA95).

The general objectives of this study were to evaluate the brans from two experimental oat lines (N979 and IA95), two publicly available oat cultivars ('Paul' and 'Jim'), and two retail oat products (Quaker Oats Company Quick Oats and Oat Bran), for proximate

composition, for soluble and insoluble β -glucan amounts, for water absorption and solubility indices, for bile acid binding, and for short-chain fatty-acid production. In addition, the brans were tested in two food products, porridge and muffins. Trained sensory panelists evaluated the finished products for textual characteristics. Viscosity of the porridge was measured by using a Rapid Visco Analyser, and muffins were measured for tenderness by texture profile analysis by using a Texture Analyzer.

Literature Review

Oat production ranked in the top five for grain crop production in 2006 (NASS 2007). The growing recognition of oats as a healthy part in a balanced diet of whole grains and fiber has increased their popularity among the general public (Anderson and Chen 1979; Jennings et al 1988; Anderson 1990; Wood 1993; Burley and Blunder 1995; Brown et al 1999; Pomeroy et al 2001; FDA 2003). Included in this literature review is a brief history of oats as a human food, the general health benefits of oats, methods for measuring the total, soluble and insoluble, and β -glucan fiber concentrations in oats, current uses of oats in food products, and sensory and instrumental means of evaluating the quality of food products containing oats.

Oats

The presence of oats in society dates back as far as 2000 B.C. (Schrickel 1986). Some archeologists determined that one of the first oat harvests was in the Middle Eastern region of the Mediterranean Sea, for reasons of rich soil and ease of access to water for irrigation, yet other historians believe that harvests previous to the ones in the Middle East were in Egypt and even further north among the ancient lake dwellers (Schrickel 1986). As the oat grain has evolved, so have different cultivars of oats that can be grown and bred for unique processing, manufacturing and even nutritional qualities. More than 75% of the total cultivated oat production in the world is of the species, *Avena sativa*, with the remaining 25% consisting primarily of *A. byzantina* (Coffman 1961). *A. sativa* is most commonly known as

a white oat, used for planting in the spring and the type of oat consumers expect from the retail shelves.

In the 20th Century, technology advances in farm practices, such as the ease in planting and harvesting soybeans and corn, had a major impact on the amount of oats planted in the United States, causing almost a 40% decline in oat production (NASS USDA 2006). Since 2000-2001, the world production of oats has stabilized at around 25 million tons (Mt; Asgarali 2006), ending a 40 year decline in output. The 1970s represented an end in peak production, 50 Mt, a level that had been constant for over 50 years (Schrickel 1986). Because oats are a cool weather crop, the majority of production occurs in the Northern Hemisphere. The European Union (EU)-25 is the world's largest oat producing region followed by Russia, Canada, the US, and Australia (Asgarali 2006). Although Russia produces 20% of world production, Canada is the largest exporter (Asgarali 2006). In 2004-2006 the north-central plains states of the United States (Minnesota, North Dakota, South Dakota, Wisconsin, and Montana) contributed a large percentage of the overall U.S. production of oats (NAAS USDA 2007). Farmers in the southern states of the United States plant a considerable amount of oats acre-wise, but use oats as a forage crop rather than for grain production. The environmental conditions affect different composition components in the oat seed. Beta-glucan especially is affected by environmental factors including soil nitrogen level and precipitation (Brunner and Freed 1994; Humphreys et al 1994; Peterson 1991; Peterson et al 1995; Welch et al 1991, Lim et al 1992; Cervantes-Martinez 2001).

The oat grain is usually comprised of 70-75% kernel (groat) and 25-30% hull (Leonard & Martin 1963). Naked or hull-less oat grains are not frequently grown for general use because the seed yield decreases, future hull-less expression is not guaranteed, and loss in

germination capacity can occur if the seed is stored incorrectly. The whole grain composition of the oat cultivar *Avena sativa* is 13.4% protein, 4.7% fat, 13.3% crude fiber, 63.6 % carbohydrate, and 3.9% ash, on a dry weight basis (db; Leonard & Martin 1963). Composition of the oat groat (the oat seed with the hull removed) is 17.6 % protein, 6.2% fat, 1.3% crude fiber, 73.4% carbohydrates, and 2.1% ash, on a dry weight basis (Leonard & Martin 1963). Oats are a good source of vitamins and minerals, particularly thiamine, niacin, panthothenic acid, and phosphorus (Rupp 1955).

Overall, the oat kernel is as complex as other cereal grains (wheat, barley, rice, and corn). All cereal grains are members of the Gramineae (grass) family, and organized according to a similar structural pattern (Fulcher 1986). Various processing techniques yield a range of proximate composition in oats. Proximate composition of oat fractions processed through a roller mill at 12 % moisture range from 47.8-84.0% starch, 10.1-26.6% protein, 4.02-5.81% lipid, 0.56-5.10% ash, and 1.26-11.0% beta-glucan (Doehlert and Moore 1997). The screen size used in a Retsch ZM-1 Ultracentrifugal mill affects the bran yield and proximate composition percentages of the oats. As the screen size increases in the ultracentrifugal mill, so does the starch content and overall yield, the lipid content remains the same, but protein, beta-glucan and ash varied by screen size (Doehlert and Moore 1997). A pearling mill results in a lower yield in protein, beta-glucan concentration, and ash than the roller-milled bran (Doehlert and Moore 1997). The pearling mill is beneficial because it isolates the outer layers of the groat directly, but because of the low beta-glucan composition in the groat, it does not meet the American Association of Cereal Chemists' definition of oat bran (AACC Definitions 1989; Doehlert and Moore 1997).

Oats have evolved as a multi-purpose crop. Seventy-eight percent of the world production of oats is used for livestock feed, 18 % for human food, and the remaining 4 % for industrial, seed, and export reasons (Schrickel 1986). In addition to using oats as food and feed, farmers might also use the stalks for oat straw and small animal bedding, or may plant oats to break cycles in fields to reduce the soil-borne insects and diseases.

In the processing of oats for human food, the hulls are removed from the seed and the interior portion (groat) is consumed as a whole grain. Oats are naturally the highest protein- and fat-containing cereal grain, with significant amounts of carbohydrates and fiber to aid in digestion (Schrickel 1986). Many of the vitamins and minerals are found in the bran and germ portion of the oat seed, so removal of these nutrient-rich parts during processing is a concern. As the current trend of grain consumption continues to shift to greater amounts of whole grain products, the nutritional benefits of whole grain oats likely will become more recognized.

Health Benefits

Whole grains are highly recommended as an important part of the daily diet. Considerable epidemiological evidence and clinical studies indicate whole-grain foods reduce the risk for certain cancers, coronary heart disease, and all-cause mortality. In July 1999, the Food and Drug Administration approved a nutritional health claim for use on food product labels that contain at least 51% whole grain by weight and are low in total fat and cholesterol (Cleveland 2000). Still, the definition on how to measure whole grains for product labeling is being discussed. A commonly used definition states: whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical

components - the starchy endosperm, germ and bran - are present in the same relative proportions as they exist in the intact caryopsis (AACC Definitions, 1999). Four oat products – whole oats, oat cereals (regular, quick, instant), oat flour, and oat bran (raw) – are considered to be whole grain because they are high in fiber, even though some are not purely whole grain products (USDA database 1997).

Increasing dietary fiber consumption has been recommended as a safe and practical approach for improving the overall health of Americans. Dietary fiber is a collective term for a variety of plant substances that are resistant to digestion by human gastrointestinal enzymes (Eastwood & Passmore 1983). Dietary fibers can be classified into two major groups according to their solubility. In humans, the structural or matrix fibers (lignin, cellulose, and some hemicelluloses) are insoluble, whereas the natural gel-forming fibers (pectin, gums, mucilages, beta-glucan, and the remainder of the hemicelluloses) are mainly soluble (Brown et al 1999).

Soluble and insoluble dietary fibers can have quite different physiological effects. The potential value of oat bran as a source of dietary fiber, particularly soluble dietary fiber, was first recognized by Anderson and Chen (1979). Kirby et al (1981) noted that in addition to being a valuable component of a high-carbohydrate, high-fiber diet useful to diabetics for control of glucose metabolism, oat bran brought about a selective decrease in serum low-density lipoprotein (LDL) cholesterol levels. Since then, more scientists have added to the findings that consumption of the soluble fiber in oat bran, beta-glucan, is indeed connected to a decrease in LDL-cholesterol concentrations in the blood (Shinnick 1988; Krauss 2000; Ridker et al 2001), by bile acid binding (Colleoni-Sirghie 2004b; Sayar et al 2006; Sayar et al 2007). As research proved the continued health benefits provided by oats, official statements

regarding the health benefits from oats were implemented to claim oats as a multi-purpose food.

On January 23, 1997, the Food and Drug Administration released the statement claiming: “Soluble fiber from foods such as oats or oat bran, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease” (FDA Code of Federal Regulations 2003). More recently, a definition of dietary fiber has been written and accepted by the American Association of Cereal Chemists. Their definition reads: “dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine; dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plants substances; dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” (AACC definitions 2003).

As the food industry delivers more cereal-based products, there will be increasing questions regarding the impact of processing on nutritional content of the food. However, processing does not always lead to nutritional loss, as often assumed by consumers. For example, Shinnick et al (1988) reported that processing did not reduce the hypocholesterolemic effect of fiber supplements made from various types and percentages of oat fiber, consumed by rats, but in fact tended to produce a greater hypocholesterolemic effect than unprocessed fiber. A possible explanation for this phenomenon is that processing increased the accessibility to soluble β -glucan and neutral sugars in the food product, or that the increase in total soluble fiber in the diet fed to the rats may have been insufficient to cause the overall effect on hypercholesterolemia. In other work, the baking process of different pastry products affected the distribution of bond formation in the dietary fiber

components between the soluble and insoluble fractions (Vollendorf et al 1994). Total amounts of all soluble and insoluble dietary fiber components, except lignin, were predictable based on any dietary fiber analysis method (Vollendorf et al 1994), but the solubility of the fiber was not predictable after baking.

Methods for Dietary Fiber Analysis

The quest to find a standardized method for measuring the amount of total, soluble, and insoluble dietary fiber in a food source is ongoing. Two primary professional organizations have published individual methods of measuring dietary fiber - AOAC (Association of Analytical Communities) International and AACC (American Association of Cereal Chemists) International, with a private company, Megazyme International Ireland Ltd. (Wicklow, Ireland), providing an enzymatic kit for the analyses. The Food and Drug Administration (FDA) has also stated guidelines and regulations as to what food components are considered dietary fiber, and recommends that 25 g of fiber be consumed each day on a 2,000 calorie diet (FDA 2005).

Beta-Glucan

Overall Structure: Beta-glucans constitute the most abundant class of naturally occurring polysaccharides because of the wide occurrence of the 1,4- β -glucan polymer, cellulose (Marquardt 1997). β -glucans are homopolymers of D-glucose linked in a beta configuration. Some glucans are relatively simple molecules consisting of linear chains of glycosyl residues joined by a single linkage type, whereas others consist of a variety of linkages in either linear or branched chains.

Sources: Cereal (1→3)(1→4)-β-D-glucan, commonly referred to as β-glucan, occurs in the sub-aleurone and endosperm cell walls of the grains. β-glucan is found in the cell walls of oat (2.5-6.5%), barley (*Hordeum vulgare*; 2.0-10.7%), wheat (*Triticum aestivum*; 0.3-1.4%), rye (*Secale cereale*; 1.9-2.9%), rice (*Oryza sativa*; 0.13%), sorghum (*Sorghum bicolor*; 1.0%), and triticale (x *Triticosecale*; 0.3-1.2%) (Fincher & Stone 1986). Purified β-glucan from oats is a linear, un-branched polysaccharide primarily composed of cellotriose and cellotetraose blocks separated by (1→3)-linkages, but there are also minor amounts of sequences of (1→4)-linkages longer than the tetraose type (Johansson 2000). See Figure 1.

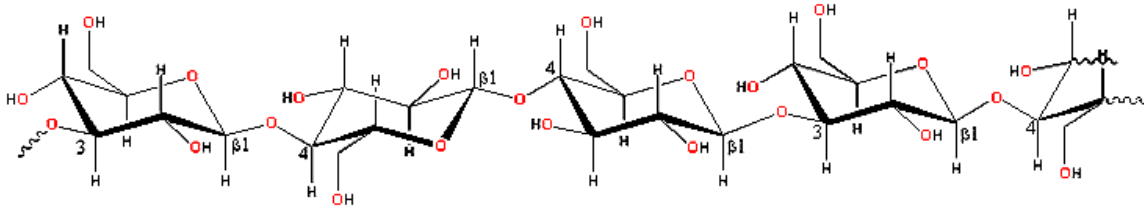


Figure 1. Linear Structure of Oat (1→3), (1→4) β-glucan

The (1→4)-linkages of cellulose make it a rigid, highly crystalline and non-soluble structure, whereas the (1→3)-linkages of the cereal β-glucan make it a soluble and flexible structure (Anderson & Bridges; Johansson 2000). Other sources of β-glucan include fungi, microbes, and brewers' and bakers' yeasts. As in cereal grains, β-glucans are the major structural polymers of fungal cell walls (Olson et al 1996). The β-glucans from microbial sources fulfill a variety of biological and immuno-pharmacological activities (Wasser 2002). For example, unexpected applications for (1,3)- β-D-glucans from bacteria and fungi lies in their potential as biomedical drugs against viral or bacterial infections and in their anti-tumor activity (Sutherland 1998). Yeast cell walls are primarily formed of (1,3)- β-glucan linkages

along with some (1,6)- β -glucan linkages that form a highly fibrous network (Lipke and Ovalle 1998). β -glucan extracted from spent brewer's yeast is useful as a thickener, water-holding agent, oil-binding agent, or emulsifying stabilizer in food products such as soups, sauces, desserts and salad dressings (Thammakiti 2004).

Oats and barley are the most common sources of β -glucans. Similar to oats, barley contains (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucan as a major component of the cell walls in the endosperm (Böhm & Kulicke 1999). There are structural differences between barley and oat β -glucans. The ratio of oligosaccharides with degrees of polymerization 3 to 4 (DP3:DP4) is greater for barley than for oats (Johansson et al 2004). This signifies a greater number of celotriose (DP3) segments found in the barley β -glucan fractions, and almost no areas of branching in oats. Even with differing DP arrangements, molar masses were the same for both soluble and insoluble β -glucan fractions in both oats and barley, but there was a greater amount of soluble than insoluble fractions in both grains (Johansson et al 2004).

Uses: Oat β -glucan's nutritional and functional value lies in its classification as a viscous gum, or soluble dietary fiber. Levels of β -glucan are influenced by both genetic and environmental effects, but genetic factors seem to be more important (Stuart et al 1988; Peterson 1991; Miller et al 1993). Between years 1989 and 1990 Lim et al (1992) reported a highly significant genetic inheritance ($p < 0.0001$) of β -glucan content among oat lines ($n=102$), suggesting that oat varieties with high or low β -glucan content could be developed as desired. In addition, Lee et al (1997) found that the ratio of soluble to total β -glucan content of oat groats was not significantly ($p=0.05$) influenced by the environment. The potential association of oat β -glucan content with agronomic characteristics and other grain

quality traits is important to consider, because selection for greater β -glucan content might also change other traits as a correlated response (Cervantes-Martinez 2002).

The viscosity of polymer solutions depends on molecular weight (MW), concentration, and polymer solubility; thus, the amount of β -glucan solubilized in a food system is important for rheological studies. β -glucans purified from certain high- β -glucan oat lines were significantly more viscous than β -glucans purified from a traditional oat variety (lower β -glucan amount), when measured at the same β -glucan concentration (Colleoni-Sirghie et al 2003). Several different methods have been reported in the literature for extracting β -glucan from oats (Ajithkumar 2005, Åman et al 1985, Åman et al 1987, Beer et al 1997, Wood et al 1991). The extraction methods include enzymatic, alkaline, or water modes with various temperatures, concentrations, and incubation times. So far, complete extraction of β -glucan without degradation has not been accomplished (Ajithkumar et al 2005).

β -glucan polymers tend to have high molecular weights and therefore form highly viscous solutions, but both endogenous enzymes, as well as shear forces and heat treatment, may reduce the molecular weight in isolated fractions or products (Wood 1993). These changes in MW influence the physical properties and must be controlled during processing and in oat-containing novel foods (Luharoo et al 1998). Processing procedures can produce substantial fragmentation of β -glucan with subsequent effects on the physiological response, though a loss in MW can be compensated for by an increase in the concentration of β -glucan (Colleoni-Sirghie et al 2004a). Long-term frozen storage of cooked products could also lead to a reduction in the solubility of β -glucan via a change in the molecular organization and crystallinity of the food matrix (Beer et al 1997). β -glucan solubility may also be influenced

by drying methods as well as with the removal of water by heat or electronic waves. Other processing steps, such as baking, fermentation, fresh pasta preparation, and mixing of pancake batter all caused extensive degradation of oat β -glucan by interfering with the chemical structure and bonding between the glucose molecules that determine the structure of β -glucan (Åman et al 2004). One means of reducing the amount of β -glucan degradation during baking of yeast-leavened bread is to use large oat bran particles and a short fermentation time (Åman et al 2004). Minimal loss in solubility and an even distribution of high and low MW β -glucan structures are a contributing factor for nutritional value. In non-food applications, β -glucan was found to be a promising film-forming hydrocolloid, which could be potentially useful as biodegradable edible food packaging material (Skendi et al 2003).

Health Implications: Oats exhibit a number of health benefits beyond basic nutrition, enabling it to be called a functional food. Oat bran and other whole-grain oat products are a tasty, convenient, and economical source of nutrients that can provide proven health benefits. Such health benefits include inducing satiety, aiding in blood glucose metabolism, cholesterol reduction, and improving gastrointestinal health.

Oats contain both protein and fiber, two macronutrients shown to enhance the feeling of satiety. Protein-rich foods and rich amino acid formulas consistently produced stronger and more sustained feelings of fullness and decreased subsequent food intake than foods high in sugar or fat (Holt et al 1995), and high-fiber foods were more satiating than refined foods (Burley et al 1995). Dietary fiber has a variety of functions in a diet. The unique physical and chemical properties of fiber aid in early signals of satiation and enhanced or prolonged signals of satiety, leading to better energy intake control and reduced risk for development of

obesity (Burton-Freeman 2000). Satiety is an integral process controlling food intake and feeding behavior. Fiber-rich diets employ a reduced energy density, defined as number of kilojoules per unit weight of food, compared with high-fat or high-calorie diets, reducing the amount of energy intake.

Dietary fiber can affect satiety, satiety, and promote weight loss by several mechanisms. In 1990, Anderson proposed these mechanisms for dietary fiber: 1) it takes longer to eat, increasing satiety and satisfaction; 2) it slows gastric emptying, contributing to a feeling of fullness; 3) it lowers serum insulin, thus decreasing food intake because insulin stimulates hunger; 4) it decreases absorption of nutrients, providing less energy/calories than comparable low-fiber foods; 5) it may increase rates of dietary thermogenesis compared to low-fiber foods; 6) its fermentation products, such as gas and short chain fatty acids may act to decrease food intake; 7) it may stimulate the release of peptides that could modify feeding behavior; and 8) it may enhance adherence to diet.

Human studies confirm that β -glucan is the active component in oats that inhibits the postprandial rise in glucose and insulin (Kapica 2001). The Coronary Artery Risk Development in Young Adults (CARDIA) Study found that fiber consumption predicted insulin levels, weight gain, and other cardiovascular disease risk factors more strongly than did the total of saturated fat consumption and concluded that high-fiber diets may protect against obesity and cardiovascular disease by lowering insulin levels (Ludwig et al 1999). The mean peak value of glucose occurred for oat gum (formed from oat β -glucans) and commercial guar gums 30-40 minutes after the meal (Wood et al 1990). Both guar gum and oat gum significantly and similarly reduced the postprandial glucose rise. Native cell wall fiber of oat bran and isolated oat gum, when incorporated into a meal, acted similarly by

lowering postprandial plasma glucose and insulin levels (Braaten et al 1994). Therefore, a diet rich in β -glucan may be beneficial in the regulation of postprandial plasma glucose levels in subjects with Type 2 diabetes.

Hypercholesterolemia has long been known to be a major risk factor for coronary heart disease (CHD). In 1963, DeGroot, Luyken, and Pikear were the first to report that the addition of an oat product to the diet of humans resulted in lowered blood cholesterol levels. In that trial, 21 male volunteers substituted bread made with 140 g of oatmeal for their traditional bread. After three weeks, the total blood cholesterol level was reduced by 11%. Since then, more analyses supported the hypothesis that incorporating oat products into the diet causes a modest reduction in blood cholesterol level. Rats fed a prepared 66% oat gum (β -glucan) solution showed that food intake decreased by 11% as well as slightly decreasing serum cholesterol levels (Chen et al 1981). Jennings et al (1988) affirmed the findings by Chen et al (1981) in a study where rats were fed a 4% β -glucan diet and decreased both food intake and serum cholesterol levels. A human feeding study determined a daily intake of 3 g of soluble fiber from oat was needed to reduce cholesterol by 0.13-0.16 mg/dL (Ripsin et al 1992), and the cholesterol reduction was greater in those individuals with initially higher blood cholesterol levels.

Cholesterol is removed from blood circulation by being converted to bile acids in the liver. The soluble fiber fraction consisting of β -glucans is believed to bind cholesterol-bearing bile acids, preventing bile acid resorption and stimulating conversion of serum and liver cholesterol to additional bile acids (Camire et al 1993). Oat-gum soluble fiber (β -glucan) exerted a greater hypocholesterolemic effect than several other fibers tested and was similar to that of cholestyramine (Pomeroy et al 2001). Cholestyramine is a strongly basic

resin that forms complexes with bile acids and is used to lower cholesterol levels in patients with high amounts of serum cholesterol. β -glucan decreased plasma and LDL-cholesterol in a group of free-living, or non-monitored, hypercholesterolemic individuals (Pomeroy et al 2001). Several different oat fractions were characterized for their bile acid binding capabilities (Sayar et al 2006). Among oat flours and oat flour fractions tested, *in vitro* bile acid binding values were greatest for bran, binding 1.5-2 times more bile acids than the flours (Sayar et al 2006). Ideally, if the synthesis of the bile acids in the liver could be increased, more cholesterol would be bound, thus lowering the levels of cholesterol in the blood. A 1% reduction in the serum cholesterol level has been estimated to reduce heart disease morality in the United States by two percent (Lipid Research Clinics Program I and II 1984).

Certain beneficial effects of fiber in the human diet may also be mediated by short-chain fatty acids (SCFA) produced during anaerobic fermentation in the colon. The dietary fiber reaching the colon contains mainly β -glucan, cellulose, hemicellulose, pectin, and some plant gums. However, the main substrates for fermentation are the non-cellulosic polysaccharides, which may degrade by up to 80% in the colon (Mortensen et al 1988). Fiber fermentation results in the production of SCFA, the fermentation end products that most benefits physiological properties in humans. Many of the valuable effects of fiber are mediated by the SCFA - acetate, propionate, and butyrate - produced by the microflora of the large intestine (Titgemeyer et al 1991). Uniquely, each is metabolized by an individual source; the muscle (acetic), colonic epithelium (propionic), and liver (butyric) (Cummings & Macfarlane, 1997). Intestinal microflora degraded 95-97% of total polyphenols, 30-32% of dietary fiber, and 60-70% of protein in grape seed and peel (Goñi et al 2005). Substrates from oat flours with the greatest amount of β -glucan also tended to produce the greatest

percent of propionate and butyrate, the SCFA believed to have the greatest potential to reduce the risk of tumorigenesis and hypercholesterolemia (Goñi and Martín-Carrón 1998).

Oats as Food Products

In 2006, just under 1500 Mt of oats were produced in the United States, with a 59.8 bushel yield (NASS 2007), compared to just under 300,000 Mt production and 149 bushel yield for corn, just under 96,000 Mt production and 43 bushel yield for soybeans, and just over 54,000 Mt production and 39 bushel yield for wheat (NASS 2007). Although only about 20% of the total oat harvest is used for applications in food products, they still play an important part of the diet.

In Britain, oats are occasionally brewed into beer, generally stout (Jackson 1999). More commonly, oats are de-hulled and flaked to make old-fashioned oatmeal flakes (Quaker Oats Company). These flakes can be further processed by fine-grinding to produce whole oat flour, or coarsely ground and separated into oat bran and fine oat flour fractions (Quaker Oats Company). Typically, the old-fashioned oatmeal flakes are heated with water to make a porridge, whereas the bran is likely to be added in a baking system such as cookies, cakes, or muffins (Quaker Oats Company). The whole oat flour can be found in cereal products and breakfast bars (Quaker Oats Company).

Since the early 1990's, researchers at Iowa State University have developed oat lines with high amounts of total β -glucan, in an effort to even further improve this crop's nutritional value. In addition, they are interested in evaluating the oats for incorporation into a wide range of food products.

Sensory Evaluation for Food Products

Texture, appearance, and flavor are the three major components of food acceptability. Sensory evaluation is the measurement of a product's quality based on information received from human senses, and offers the opportunity to obtain a complete analysis of the sensory properties of a food. Humans employ a range of senses in perceiving food quality such as: vision, gustitation, olfaction, chemical/trigeminal, touch, and hearing (Kilcast 1999). Different means of testing the senses have been developed into numerous sensory testing procedures, fitting under two categories: hedonic or analytical tests (Kilcast 1999). Hedonic tests include preference, acceptability, or relative-to-ideal. The participants in hedonic testing should not be given any training for evaluating the products, but large numbers of participants (100 persons or more) are needed to improve the confidence level of the data generated. Analytical tests are further sub-divided into either difference or quantitative tests. Difference, or discrimination, tests are perceived as one of the easiest classes of sensory testing to apply in an industrial environment and are consequently heavily used (Kilcast 1999). These tests can be used in one of two ways: to determine if there is a difference between two samples, or to determine whether one sample has more or less of a specific attribute than the other. Examples of difference or discrimination tests include paired comparison, duo-trio, triangle, or R-index. Quantitative descriptive analysis (QDA) is a comprehensive system covering sample selection, assessor screening, vocabulary development, testing, and data analysis (Stone & Sidel 1993). QDA uses small numbers of highly trained panelists, typically 6-15 people. The three steps of analysis: development of vocabulary, quantification of sensory characteristics, and statistical analysis of the results; lead to a strong and accountable means for sensory evaluation.

Correlating measurements of sensory assessments and physical properties of texture are important in the development of food products. The most difficult question is how to define texture to encompass both sensory and physical testing. In 1992, the International Organization for Standardization defined texture as: Texture (noun): All the mechanical (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors (Bourne 2002). Sensory and instrumental measures of food texture can interrelate, and texture can be described from multiple stimuli, but instrumental measurements tend to concentrate on one property of the food product.

Instrumental Methods to Measure Physical Attributes

Instrumental methods for texture measurement have been divided into three classes, including fundamental, empirical, and imitative tests (Szczesniak 1963).. An example of a fundamental test for measuring the physical attributes of a food product is viscosity, or the resistance of a substance to flow. Viscosity can be measured by line spread, which involves retaining a fluid sample in a uniform cylinder in the center of a concentric circle outline with known distances, then removing the cylinder for a stated time, allowing the fluid food to spread (Penfield and Campbell 1990). Determining the distance the product traveled can be related to the viscosity of the food. A second instrument, the Brookfield viscometer, measures the viscosity of liquid or semi-solid food at room temperature via measuring the resistance exerted by the food source on a spinning spindle (Penfield and Campbell 1990). A third instrument to measure viscosity is a Rapid Visco Analyser (RVA). This machine measures viscosity of a small sample, typically at 8 % dry weight basis (db), also allowing

the controller to determine the spin rate (rpm), temperature changes, and time of measurement. The sample is stirred and heated at a constant rate, allowing thickening to occur (Newport Scientific 2002). A fourth instrument used to measure the viscosity of starch-water suspensions for pastry making is a Brabender visco-amylograph (Penfield and Campbell 1990).

An example of an empirical test is measuring the volume of a food product. Volume of a product can be measured by seed displacement or index to volume. Seed displacement measures the volume of a seed source (usually rape seeds) taken by a food product from a known volume (Penfield and Campbell 1990), whereas index to volume is the mathematical average of the height of the product from five locations from either a traced copy or photocopy of the food using a planimeter (Penfield and Campbell 1990).

Texture characteristics of a food can be measured with a Penetrometer. Here, a food product is placed under a probe with either a cone- or disc-shaped attachment on the end. The probe is calibrated with a known weight (usually 5 g) and dropped downward into the food. A meter on the front measures the distance the probe penetrated into the food source and is used as a gauge of the firmness of the food source. The further the probe penetrates, the softer the food's texture. Other instruments used to measure texture are an Instron UTM and a texture analyzer. These automated machines employ the same principles as a penetrometer, but are also capable of measuring the fracturability, cohesiveness, adhesiveness, springiness, gumminess, and chewiness of a food source with multiple probe attachments (Penfield and Campbell 1990). These instruments, along with a previous model termed a Texturometer, initiated an era of study, Texture Profile Analysis.

Texture profile analysis (TPA) is an imitative test pioneered to compress a bite-size piece of food two times in a reciprocating motion to imitate the action of the jaw, and create parameters that correlate well with sensory evaluation of the same parameters. Developed at General Foods in the 1960s (now part of Kraft Foods), the Texturometer revolutionized the instrumental analysis of food texture. The texturometer uses a small flat-faced cylinder to compress a bite-sized piece of food to one-quarter of its original height, or 75% compression (Bourne 1978). From the analysis of the force-time curve made by the texturometer, seven textural parameters have been defined and refined. These parameters include (Szczesniak 1975):

Fracturability (originally called brittleness): the force at the first significant break in the curve

Hardness: the peak force during the first compression cycle (“first bite”)

Cohesiveness: the ratio of the positive force area during the second compression to that during the first compression (A_2/A_1)

Adhesiveness: the negative force area for the first bite, representing the work necessary to pull the compressing plunger away from the sample

Springiness (originally called elasticity): the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite

Gumminess: the product of hardness x cohesiveness

Chewiness: the product of gumminess x springiness (also equal to hardness x cohesiveness x springiness).

Each of the textural parameters identified by the General Foods group gave excellent correlations with sensory ratings (Szczesniak 1963). The development of TPA has proved to be a valuable aid to assessing food texture. However, care should be exercised in accepting the results for purposes other than comparative evaluation. The TPA has also been the basis for other texture instruments such as the Instron Universal Testing Machine or the Stable Micro Systems TA-XT2 texture analyzer. Szczesniak and Hall (1975) noted that the proper use of the Texturometer is still much of an art since the operator must supply the thinking of which the instrument is not capable.

Porridge (oat bran cooked in water) and muffins are relatively simple products in which to measure potential differences in function of oat bran types. Measurements generated by a RVA for porridge can display the differences in viscosity from oat brans with various molecular weights by determining the amount of adhesiveness. Muffins are an example of a baked product that can be evaluated for differences in TPA caused by the different oat bran types.

The overall objectives of this research project are to characterize the composition, food product applications, and potential selected nutritional benefits of oat bran from two experimental oat lines (*Avena sativa*; IA95111 and N979-5-2-4) developed by Iowa State University to have increased levels of β -glucan, two publicly available cultivars ('Jim' and 'Paul') with typical levels of β -glucan, and two retail oat products (Quaker Oats Company Traditional Quick Oats and Oat Bran). We hypothesize there will be differences in composition, specifically in total β -glucan and water absorption capacities, of the experimental oat lines, and that there will be minimal differences in food products made from all types of oat bran.

The remainder of this thesis is divided into four sections. The next two sections are research papers prepared for submission to the Cereal Chemistry Journal. The first manuscript covers the oat bran composition, bile-acid binding potential, and short-chain fatty-acid production of five oat bran materials and traditional flaked oat material used in this study. The second manuscript discusses the characteristics of two food products, porridge and muffins, made from the oat brans, as evaluated by a trained sensory panel and by instrumental and physical tests. Within each section, there is an individual abstract, introduction, listing of materials and methods used, results and discussion, conclusions, literature cited, and tables and figures corresponding to each report. Finally, general conclusions for the overall project and referenced appendices conclude the thesis.

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Characterization, Bile Acid Binding, and Short-Chain Fatty-Acid Production of Oat Bran from Oat Lines with Typical and High Amounts of Beta-Glucan

A paper to be submitted to Cereal Chemistry

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Running title: CHARACTERIZATION OF OAT BRANS WITH VARYING BETA-
GLUCAN

Abstract

Bran from two publicly available oat cultivars (*Avena sativa*), 'Paul' and 'Jim'; two experimental oats lines developed at Iowa State University to have increased concentrations of β -glucan, N979-5-2-4 (N979) and IA95111 (IA95); and two retail oat products, Quaker Oats Company Old Fashioned Oats (flakes) and Oat Bran, were evaluated for composition, bile acid binding (BAB) as an *in vitro* indicator of cholesterol binding, and short-chain fatty-acid (SCFA) production as an *in vitro* indicator of the brans acting as prebiotics. Total β -glucan amounts differed as follows: Quaker Oats flakes, 4.2% < Quaker Oats bran, 6.2% < Jim, 6.4% < Paul, 7.5% < IA95, 8.9% < N979, 10.8%. IA95 had 7.0% soluble β -glucan and N979 with 8.6% soluble β -glucan, greater percentages of than all other treatments and retail products. Both the amount of total β -glucan and water absorption index ($r = 0.853$) and total β -glucan and water solubility index ($r = 0.820$) were positively correlated. There were no differences among the six oat types in the $\mu\text{mol}/100\text{ mg}$ of bile acids bound. Gas production (8.5 psi), pH (6.5), and total production of SCFA (134 $\mu\text{mol}/\text{mg}$) was consistently higher in the Jim bran compared with all other oat types.

Introduction

The presence of oat (*Avena sativa*) as a grain for use in civilization dates back as far as 2000 B.C. (Schrickel 1986). More recently, oats have been found to have a positive role in a heart-healthy diet. In 1997, the Food and Drug Administration (FDA) implemented a Code of Federal Regulations to allow a health claim on food labels of products that contain oats (FDA 2003). It was determined that a diet low in saturated fat, along with 3 grams of the soluble fiber β -glucan daily, may lower the risk of heart disease (FDA 2003).

Whole oats contain approximately 13% fiber (Leonard and Martin 1963), part of which is soluble fiber. β -glucan is a homopolymer of glucose, linked primarily by β -(1,4) bonds, with some β -(1,3) branches. β -glucan is found in the sub-aleurone and endosperm cell walls of the oat grain, making processing techniques an important step in maintaining the β -glucan structure (Wood 1993). Oat bran is defined as the food produced by grinding clean oat groats (the oat seed with the hull removed) or rolled oats and separating the resulting oat flour by sieving, bolting, and/or other suitable means into fractions, such that the oat bran fraction is not more than 50% of the original starting material has a total β -glucan concentration of at least 5.5% (dry weight basis; db), a total dietary fiber concentration of at least 16.0% (db), and at least one-third of the total dietary fiber is soluble (AACC Definitions, AACC 1989).

Health benefits of consuming dietary fiber in oats include increased satiety, control of blood glucose metabolism, cholesterol reduction, and improved gastrointestinal health. The unique physical and chemical properties of fiber aid in early signals of satiation and enhanced or prolonged signals of satiety, leading to better control of energy intake and a consequent reduced risk for development of obesity (Burton-Freeman 2000). DeGroot et al (1963) was the first to report that the addition of an oat product to the diet of humans resulted in lowered blood cholesterol levels. Cholesterol is removed from blood circulation by being converted to bile acids (BA) in the liver (LaRusso 1983). It is thought that oat β -glucan binds BA, thus inhibiting the reabsorption of BA by the small intestine, but also increasing the viscosity of ileum fluids which also reduces BA reabsorption and impacts colon health (Anderson 1990). Binding between oat β -glucan and micelles consisting of bile and fatty acids from the small intestine, along with removal of the complexes from the body, warrants

the need for increased synthesis of BA, in turn consuming more cholesterol (Bowels et al 1996; Wood et al 2002). Among oat fractions tested (flour, bran, protein concentrate, starch, and the layer above starch) *in vitro* BA binding values were greatest for bran (Sayar et al 2006). Beneficial effects of β -glucan in the human diet may also be mediated by short-chain fatty acids (SCFA) produced during anaerobic fermentation in the colon with the β -glucan acting as a prebiotic. Among the three SCFA produced (acetate, propionate, and butyrate), propionate and butyrate are the fermentation end products that most benefit physiological properties, such as reducing the risk of tumorigenesis and hypercholesterolemia in humans (Goñi and Martín-Carron 1998). Oat fractions with the greatest amount of β -glucan also tended to produce the greatest percentage of these two SCFA, propionate and butyrate (Sayar et al 2006).

The aim of this work was to characterize the physical, chemical, and physiological properties of oat bran separated from whole oats harvested from two experimental oat lines with high amounts of total β -glucan, two traditional oat cultivars with normal amounts of β -glucan, and two retail oat products. Understanding the changes in bran composition resulting from an overall increase in the β -glucan of the oat, and evaluating the impact of these changes will aid in further understanding the mechanisms by which β -glucan mediates BA binding and SCFA production, and will aid in the ability to continue the development of a superior oat.

Materials and Methods

Oat Material: Four oat lines were evaluated. Two publicly available certified oat lines: ‘Jim’, developed by the University of Minnesota, Twin Cities, and ‘Paul’, a naked oat,

were selected as traditional cultivars containing a typical concentration of total β -glucan in the groat and bran. Two experimental oat lines, IA 95111 (IA95; Cervantes-Martinez 2001) and N979-5-2-4 (N979; Cervantes-Martinez 2001), being developed at Iowa State University with a high level of β -glucan, also were analyzed. All four oat lines were planted in 2005 and grown in two field replicates at the Agronomy and Agricultural Engineering Field Research Center in a randomized complete block field design. All fields were located in Ames, Iowa, Story County. Retail purchased samples, including Quaker Old Fashioned Oats (whole oat flakes) and Quaker Oat Bran (Hy-Vee grocery store, Ames, IA) served as control materials in the study.

The harvested oat kernels were dried, and stored in plastic bags at 4° C with relative humidity of 40-50% until being sent to Quaker Oats Company processing plant (Cedar Rapids, Iowa) where they were de-hulled by a Buhler Aspirator, steamed for 1 min at 80° C and rolled to a flake thickness of 0.61 mm (Yao et al 2006). The bran was processed in the exact means as retail products. This process starts by running the flakes over a Hammer Mill fitted with a 0.56 mm screen, followed by sifting through a 36-mesh, stainless-steel, bolting cloth, which allowed the flour, termed de-branned flour, to fall through. The bran was collected and stored at -40 ° C in resealable storage bags within a sealed Rubbermaid® container. Before analyses, the bran was warmed to room temperature and then either ground in an ultra-centrifugal mill (ZM-1, Retch GmbH&Co, Haan, Germany) fitted with a 0.5 mm sieve, or directly weighed and used.

Oat Composition: All oat brans were analyzed in triplicate and values converted to a dry weight basis (db). Proximate analyses followed AACC procedures (2000): moisture 44-15, crude protein 46-12 (nitrogen conversion factor of 6.25), crude fat 30-25, starch 76-13,

and ash 08-05. Total β -glucan was measured by using method 32-23 and total fiber by using method 32-21 (AACC 2000). Water absorption index (WAI) and water solubility index (WSI) were measured as described by Anderson et al (1969). Soluble and insoluble β -glucan fractions were determined by a method in Åman and Graham (1987). Pentosan concentration in the bran was measured by using concentrated acids, a method by Douglas (1980).

In Vitro Digestion, Short-Chain Fatty Acid Production and Bile-Acid Binding: An *in-vitro* digestion (Sayar et al 2005) was followed by analysis of short-chain fatty-acid (SCFA) production (Sayar et al 2007) and bile-acid binding (BAB) (Sayar et al 2006). In brief, oat samples were digested by hydrating and cooking in a boiling water bath. To the cooled samples, sodium phosphate buffer was added and used to neutralize the bran before adding human salivary α -amylase enzyme. After allowing the enzyme to penetrate, the reaction was stopped by adjusting the pH to 2.0. Pepsin was added next, allowed to penetrate using heat, then the mixture was neutralized to pH 6.9. A 1.41 mM/L bile acid mixture, composed of 35% sodium cholate, 35% sodium deoxycholate, 15% sodium glycocholate, and 15% sodium taurocholate and 1.25 mL pancreatin were added, allowed to penetrate in a heated water bath.. Materials were centrifuged and the pellets were evaluated for SCFA production, whereas the supernatant was used to test for BAB.

The SCFA method was conducted as described by Sayar et al (2007). Briefly, the pellets were batch fermented under anaerobic conditions for 24 hr with a fresh fecal sample as inoculum. Fermentation was stopped by adding 0.1 mL of saturated mercury chloride. The materials were tested at selected hour intervals for pH and gas production for a representative of changes over time.

A 1-mL aliquot from the supernatant of the centrifuged fermented material was mixed with 100 μ L of 2-ethylbutyric acid (the internal standard), concentrated hydrochloric acid, and diethyl ether. The hydrochloric acid protonizes the SCFA so they can be extracted into the diethyl ether. The diethyl ether layer was allowed to separate, then pipetted into a different container. One mL of the ether layer from each aliquot was put in individual 1.8 mL vials then derivatized by using 100 μ L of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) to silylate the extracted SCFA (Schooley et al 1985). The vials were sealed by placing them in a water bath at 80° C for 20 min, removed and allowed to finish derivatizing for 24 hr in the dark at 25° C.

The SCFA (acetate, propionate, and butyrate) were analyzed by using a gas chromatograph after being transformed into their silyl derivatives. Derivatized samples were injected onto a SPB-5 gas chromatography column (30 m x 1.0mm; Supelco, Inc.), in 2 μ L injections, with He₂ as the carrier gas. The column temperature was held at 70° C for 4 min, and programmed to rise to 160° C in 7° C per min. The injector and detector temperatures were held at 220° C and 250° C, respectively. To quantify peak sizes, acetic acid, propionic acid, and butyric acid standards were derivatized and injected onto the gas chromatography (GC) column to determine the peak response per amount injected. In addition, the peak size of the internal standard of each injection was used to adjust peak quantities as a ratio of the actual peak response.

To measure BAB, an *in vitro* digestion, followed by application of a Bile Acid Diagnostic Kit (Trinity Biotech plc, Bray Co., Wicklow, Ireland) was used as noted in Sayar et al (2006). Briefly, bile acid standards A and B were reconstituted as directed then warmed to 37° C. An aliquot of 0.2 mL of each digested oat material was dispensed into test tubes,

followed by addition of either test reagent or blank reagent. The solutions were incubated in a water bath at 37° C for 5 min. The reaction was stopped with a solution of 2% phosphoric acid and absorbance of the solutions was read at 530 nm via a spectrophotometer. Sample readings were applied to a standard curve for determination of the amount of BAB.

Statistical Analyses: All analyses were conducted in triplicate per each field replicate unless otherwise noted. Comparison of means was conducted by analysis of variance (ANOVA) then least significant difference (LSD) at a significance level (α) of $p < 0.05$. A computerized statistical analysis program software system was used to calculate differences and correlation analyses (SAS v 9.1 2002-2003; Cary, NC).

Results and Discussion

Composition of Oat Bran:

The IA95 bran had the greatest percent protein with 17.3%, followed by N979 (16.6%), retail bran (15.7%), Jim (14.7%), Paul (13.5%), and retail oats (10.7%) (Table 1). Crude fat percent of Paul (8.5%), N979 (8.3%), Jim (7.8%), and IA95 (7.5%) were greater than the percent crude fat in the retail bran (6.6%) and retail oats (6.3%) (Table 1). Total starch values were greater for the retail oats (40.9%), Jim (37.1%), and Paul (36.5%) than for the retail bran (32.3%), IA95 (32.0%), and N979 (30.7%) (Table 1). No differences in total dietary fiber or ash were found between any of the treatments (Table 1). Only small amounts of pentosans were detected in all treatments ($<0.01\%$), thus results are not reported.

All oat bran types and retail oats were different from each other in total β -glucan percent, except Jim bran and retail bran were similar in percentage. Total β -glucan percentages were in the following order: N979, 10.8% > IA95, 8.9% > Paul, 7.5% > Jim,

6.4% = Retail Bran, 6.2% > Retail Oats, 4.2% (Table 1). Water absorption index (WAI) was highest among the experimental brans (IA95, 3.0 g/mL and N979, 2.9 g/mL), with Paul (2.6 g/mL) being similar to N979, but Jim (2.2 g/mL), retail bran (2.2 g/mL), and retail oats (2.2 g/mL) being different from the other treatments and similar among each other (Table 1). Water solubility index (WSI) was again highest among experimental brans (IA95 8.8% and N979 8.7%), next highest among public cultivars (Paul 7.3% and Jim 7.0%), and least among retail products (oats 3.7% and bran 3.0%) (Table 1). The amount of total β -glucan was positively correlated to both WAI ($r = 0.853$) and WSI ($r = 0.820$) with a significance level of $p < 0.05$ (Table 1).

Figure 1 depicts the separation of total β -glucan into soluble and insoluble β -glucan fractions. N979 (8.7%) and IA95 (7.0%) had the highest amount of soluble β -glucan and were similar to each other (Figure 1). The public cultivars, Paul (5.8%) and Jim (5.7%), were different from N979 in the amount of soluble β -glucan, although similar to IA95 (Figure 1). Retail products, bran (1.8%) and oats (1.6%) had the least amount of soluble β -glucan, and were different from all other treatments (Figure 1). The retail bran (4.4%) had more insoluble β -glucan than did the retail oats (2.7%) or bran from the experimental lines and public cultivars (Figure 1), but there were no differences among the experimental lines or public cultivars. The amount of soluble β -glucan is important, because of its positive link to health. It was first recognized by DeGroot (1963) that the hypocholesterolemic effect of oats may be related in part to their soluble fiber content. Since then, other researchers have solidified the importance for the incorporation of soluble fiber from oats into the diet for many health benefits (Anderson and Chen 1979; Kirby et al 1981; Shinnick et al 1988; Krauss et al 2000; Ridker et al 2001).

Few publications have analyzed just the bran fraction of the oat groat (oat seed without the hull). Other findings show that the composition of oat groats can range from protein 12.1-20.8%, lipids 6.2-10.7%, starch 54.4-73.4%, fiber 1.3-13.0%, β -glucan 1.3-11.0%, and ash 2.1-2.5% (Leonard and Martin 1963; Doehlert and Moore 1997; Sayar et al 2005; Yao et al 2007). In the current study, bran is similar to other published data in protein, lipid, fiber and ash content, but lower in starch because bran lacks the high starch concentration from the endosperm of the oat groat. The amount of insoluble (0.6-4.4%) and soluble (1.6-8.7%) β -glucan in the oat bran treatments in this study were higher than in the amount found by Åman and Graham (1987; insoluble 0.65%), but the amount of total β -glucan (4.2-10.8%) was also higher in the treatments in this study than in the Åman and Graham study (3.0%).

Bile Acid Binding:

Because the percentages of BAB were all above 99%, it would be useful to use a higher concentration of bile acids, or a smaller amount of bran sample, to find more conclusive differences between the bran treatments. An ideal concentration of oat material is around 2% db for optimum binding of bile acids (Yao, unpublished data). Previously, bran fractions bound greater amounts of bile acids than did other fractions from oats such as flour, protein concentrate, starch, or the layer above starch (Sayar et al 2006). Therefore, it would be beneficial to find more accurate concentrations of either the bile acid mixture or bran concentration to better determine differences between the binding capabilities of the different oat bran materials.

In vitro production of short-chain fatty-acids (SCFA):

During fermentation of *in vitro* digestion, the gas pressure, pH, and SCFA were measured. Differences in gas pressure were found at all five time points (2 hr, 4 hr, 8 hr, 12 hr, and 24 hr) with retail oats consistently being the lowest of the treatments (Figure 2). At 2 hr, Paul (7.8 psi), N979 (7.8 psi), Jim (7.6 psi), and IA95 (7.5 psi) were all similar with the most gas production. Retail bran (7.1 psi) was similar only to IA95 at 2 hr. Retail oats were the lowest of the treatments at 2 hr with 6.1 psi. At 4 hr, Jim was the highest at 8.2 psi, with IA95 (8.1 psi) and Paul (8.1 psi) being similar. N979 (8.0 psi) and retail bran (8.0 psi) were similar only to IA95 and Paul at 4 hr. Again, retail oats were the lowest in gas pressure at 4 hr with 6.3 psi. At 8 hr, Jim was the highest in gas pressure with 8.3 psi. IA95 (7.8 psi), Paul (7.7 psi), and N979 (7.6 psi) were similar at 8hr for gas pressure. At 8 hr, both retail bran (7.0 psi) and oats (6.9 psi) were similar with the lowest production. At 12 hr, Jim (8.5 psi), IA95 (8.5 psi), and Paul (8.2 psi) had the greatest gas pressure. N979 (6.9 psi) and retail oats (6.7 psi) were similar, while retail oats were also similar to retail bran (6.4) at 12 hr. At 24 hr, N979 (8.6 psi), Paul (8.5 psi), IA95 (8.5 psi) and Jim (8.3 psi) all had the highest gas pressures, while retail oats (6.9 psi) and bran (6.8 psi) were similar to each other. At the 2 hr and 24 hr time points, both lactulose (positive gas pressure control) and the blank (negative gas pressure control) were different than all treatments including the retail products (Figure 2).

No differences in pH of the treatments were found at the zero hour (pH 6.51) or at 2 hr (Figure 3). At 4 hr, only the retail oats (pH 6.64) were greater than the other treatments including retail bran (all pH 6.60). At 8 hr, retail oats were still greater (6.71), with retail bran (6.65), Jim (6.64), N979 (6.64), and Paul (6.63) being similar, and IA95 (6.61) being

lower than all treatments. Again at 12 hr, all treatments were similar with a pH between 6.55-6.59. At 24 hr, retail bran had a higher pH (6.74) than all other treatments (6.61-6.58). At 2 hr and 24 hr, lactulose and the blank were different from all treatments, including retail products (Figure 3).

No differences in the total amount of SCFA produced during fermentation were found at 2 hr, 12 hr, or 24 hr (Figure 4). At 4 hr, Jim had the greatest SCFA production with 134 μg . IA95 (128 μg) and N979 (126 μg) were similar, with Paul (123 μg) and retail bran (123 μg) being similar to N979. Retail oats had the least amount of SCFA at 4 hr with 107 μg . At 8 hr, Jim had the greatest SCFA production with 125 μg . Retail oats (116 μg), Paul (112 μg), IA95 (107 μg), and N979 (106 μg) were similar at 8 hr, with retail bran (104 μg) being similar to N979.

Wood et al (2002) also reported a higher volume of gas production from purified oat β -glucan (oat gum) than from oat bran subjected to a digestion process, noting that gas production correlated with the amounts of soluble dietary fiber. A previous study in our laboratory, also using human fecal inoculation, found that Paul had a lower gas production than N979, IA95, Jim, purified oat starch, and purified oat β -glucan, perhaps due to a difference in insoluble dietary fibers (Sayar et al 2007). Lowering of the pH is beneficial for colon health because lower pH is thought to slow the conversion rate of primary to secondary bile acids and lower their carcinogenic potential (Nugent 2005). Also, a low intestinal pH may protect against pathogenic bacteria and aid in the absorption of minerals crucial to health, such as calcium and magnesium (Cummings 1981). A minimal decrease in pH from zero to 24 hr also was noted by Wood et al (2002), although in that study the lowest pH was reached at 4 hr, compared with 12 hr in the current study. IA95 also had low pH values in the study

by Sayar et al (2007), although Jim also tended to be lower in the current study. In both Wood et al (2002) and Sayar et al (2007) the amount of SCFA production increased as fermentation time proceeded, contrary to this study where SCFA production decreased initially (4 hr) then increased gradually as time proceeded (Figure 4).

Conclusions

All five oat bran treatments and the retail oat differed from each other in chemical composition. N979 was highest in protein, lipid and total β -glucan content, followed by IA95, Paul, Jim, and the retail bran. Retail oats were lowest in protein, lipid and β -glucan content. N979 and IA95 were the highest in both WAI and WSI, followed by Paul, and Jim, with the retail bran and oats having the lowest WAI and WSI. N979 and IA95 were higher in soluble β -glucan percentages, with retail bran having the greatest amount of insoluble β -glucan. Retail bran was the highest in BAB, with all other treatments being similar. Differences in gas production and pH varied over a 24 hr time period, with Jim tending to be the greatest over the other treatments. SCFA production did not differ at the 2 hr, 12 hr, and 24 hr time points, but N979, IA95, and Jim were the highest SCFA producers at 4 hr and 8 hr.

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Tables and Figures

Table 1. Chemical Analysis of Oat Bran and an Oat Flake from Oat Lines¹

Oat Type	Protein %	Lipid %	Starch %	TDF %	Ash %	β -glucan %	WAI ² g/mL	WSI ² %
Jim	14.7 d	7.8 ab	37.1 b	4.0 a	2.0 a	6.4 d	2.2 c	7.0 b
Paul	13.5 e	8.5 a	36.5 b	4.8 a	1.9 a	7.5 c	2.6 b	7.3 b
IA95	17.3 a	7.5 b	32.0 c	4.3 a	2.1 a	8.9 b	3.0 a	8.8 a
N979	16.6 b	8.3 ab	30.7 c	5.3 a	2.1 a	10.8 a	2.9 ab	8.7 a
Retail Bran	15.7 c	6.6 c	32.3 c	5.4 a	2.2 a	6.2 d	2.2 c	3.0 c
Retail Oats	10.7 f	6.3 c	40.9 a	4.5 a	1.8 a	4.2 e	2.2 c	3.7 c

¹ = All values are mean of triplicate analysis of two field replications and reported on a dry weight basis (db)

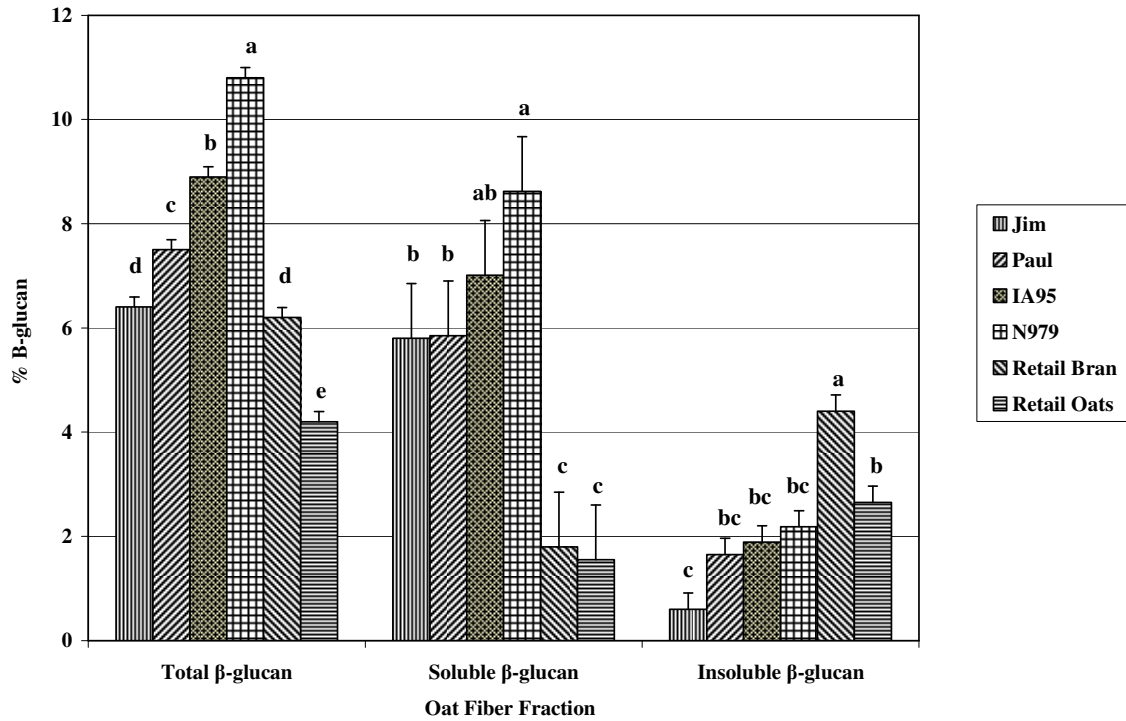
= Remaining composition to equal 100% estimated to be lignin and cellulose

= Values followed by the same letter in a column are not significantly different ($p < 0.05$)

² = Water Absorption Index; Water Solubility Index

Note – Significant correlation value between percent β -glucan and g/mL WAI: $r = 0.853$

Significant correlation value between percent β -glucan and percent WSI: $r = 0.820$

Figure 1. Total, Soluble, and Insoluble β -glucan Fractions of Oat Brans and Oat Flake¹

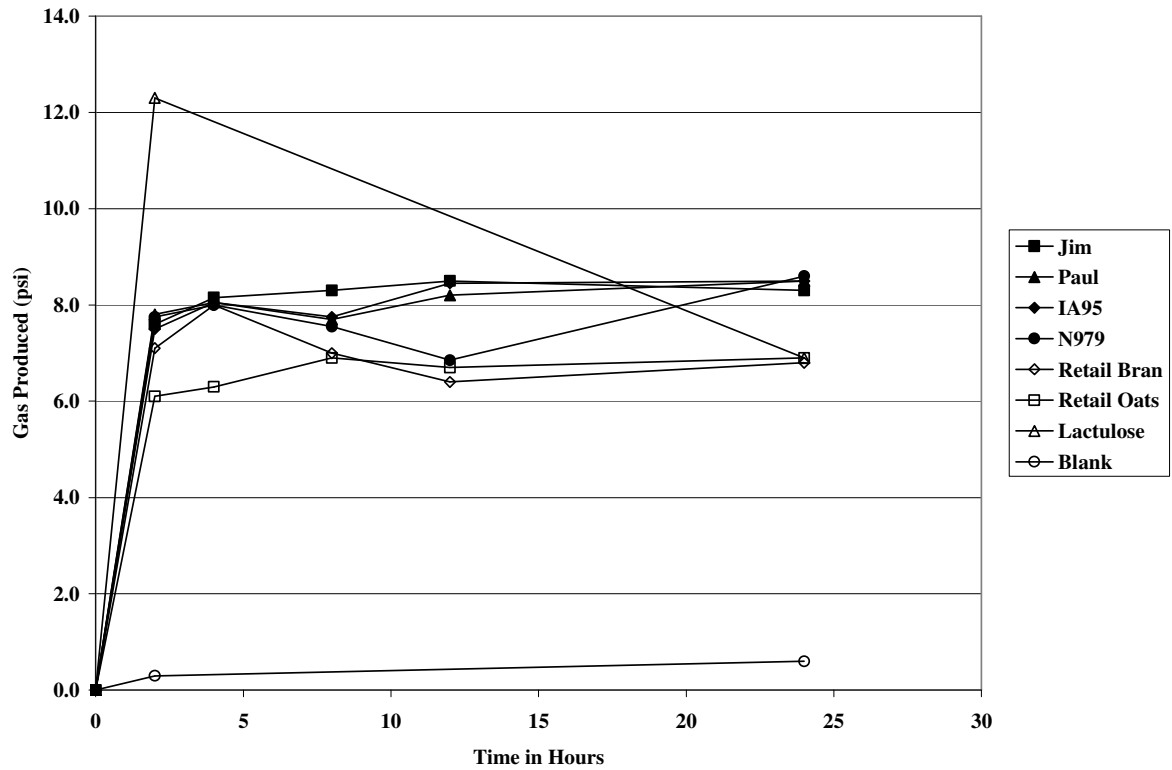
- ¹ = Soluble β -glucan + Insoluble β -glucan = Total β -glucan
 = Each bar represents mean of triplicate analysis of two field replications on a dry weight Basis (db)
 = Bars within each oat fiber fraction with the same letter are not significantly different ($p < 0.05$)

Table 2. Amount of Bile Acids¹ Bound During Digestion²

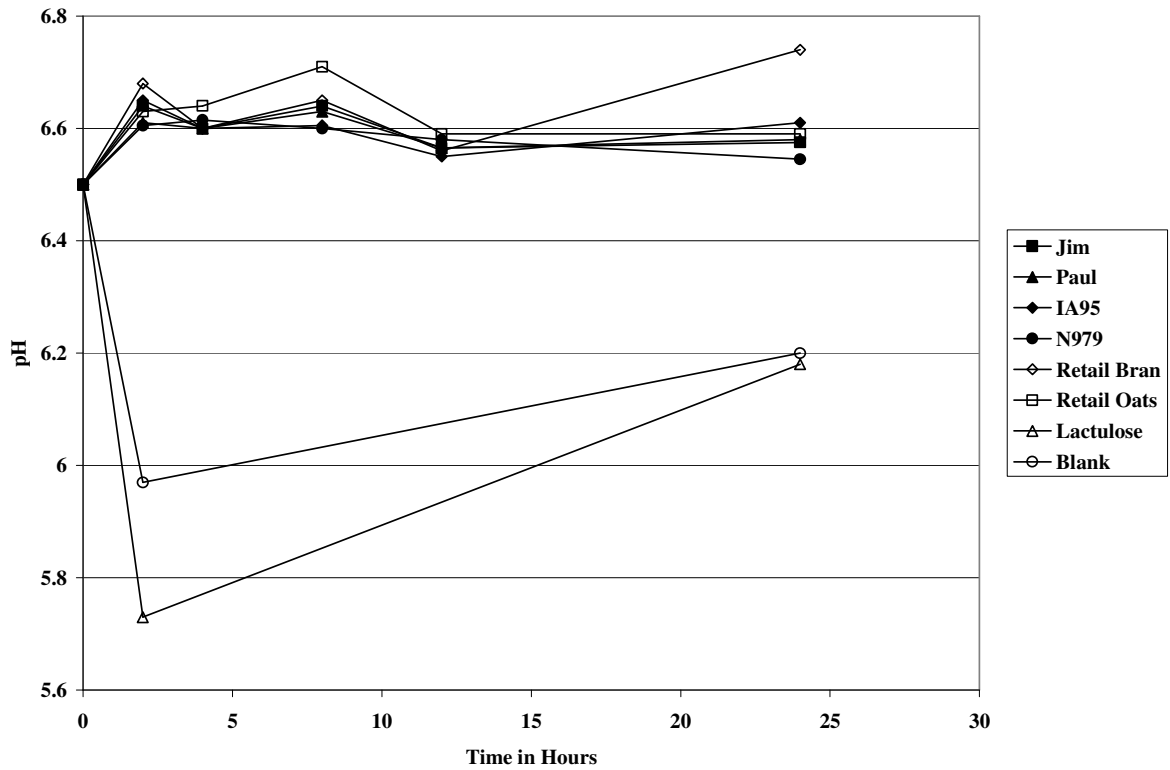
Oat Type	Bile Acids Bound μ mol/100 mg	Bile Acids Bound %
Jim	3.52 a	99.80 b
Paul	3.52 a	99.79 b
IA95	3.52 a	99.81 b
N979	3.52 a	99.80 b
Retail Bran	3.52 a	99.86 a
Retail Oats	3.52 a	99.79 b

¹ = Concentration of Bile Acid Solution = 1.41 mM/L

² = All values are mean of triplicate analysis of two field replications. Values within a column with the same letter are not significantly different ($p < 0.05$).

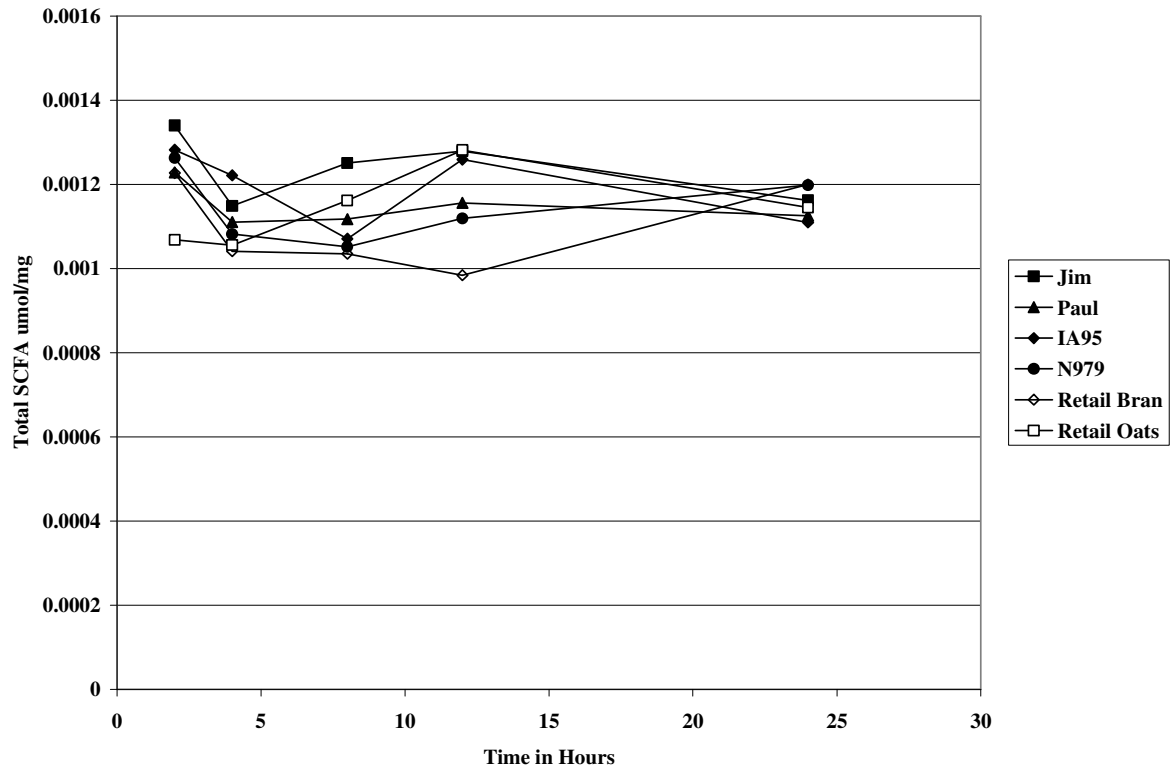
Figure 2. Gas Production (psi) During Fermentation of Oat Bran¹

¹ = Each value represents the mean of two field replications. Error bars not depicted since < 0.05 . Significance between times and samples stated in text.

Figure 3. pH During Fermentation of Oat Bran¹

¹ = Each value represents the mean of two field replications. Error bars not depicted since < 0.03 . Significance between times and samples stated in text.

Figure 4. Amount of Short-Chain Fatty-Acids Produced During Fermentation of Oat Bran¹



¹ = Each value represents the mean of two field replications. Error bars not depicted since < 0.05 . Significance between times and samples stated in text.

Sensory and Instrumental analysis of Porridge and Muffins made from Oat Bran with Varying Amounts of Total Beta-Glucan

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Running title: SENSORY AND INSTRUMENTAL ANALYSIS OF HIGH BETA-GLUCAN OAT BRAN

Abstract

Brans from oat lines (*Avena sativa*) with different amounts of β -glucan were evaluated for potential use in food products. The brans came from two oat cultivars with typical concentrations of β -glucan, 'Jim,' 6.4%; and 'Paul,' 7.5 %, and two experimental oat lines with high levels of β -glucan, IA95111 (IA95, 8.9%), and N979-5-2-4 (N979, 10.8%). Porridge (oat bran cooked in water) and a muffin were tested by 13 trained human subjects in a Quantitative Descriptive Analysis (QDA) sensory panel. Panelists evaluated the two products on a 15-centimeter line. A standard product made from retail oat bran was provided as a reference for the panelists in each analysis but was not judged. Differences between the porridges indicated that experimental bran was less creamy and mouth-coating, but had larger particle sizes than the public bran. Differences between the muffins indicated experimental bran had more dome top, coarse surface texture, crumbliness, and cohesiveness, but lower moistness and grittiness than the public bran. Experimental brans had more water absorption (2.05-2.17 g/mL) but less water solubility (20.2-20.5 %) after baking, but were more viscous than public brans as a raw product. IA95, Jim, and the retail bran were the hardest (65.9-77.0 g force) and most springy (61.5-71.8 g force) of the muffins as measured by a Texture Analyzer, but the retail bran had the highest cohesiveness (0.93) and gumminess (71.8 g). These differences likely were not great enough to alter acceptability of either of the food products.

Introduction

Oats exhibit a number of health benefits beyond basic nutrition, enabling them to be categorized as a functional food. In recent years, the market has driven the continued interest

in developing foods containing functional ingredients; therefore, the interest in oats has also expanded. The fraction in oats most known to have an impact on health is the fiber, (1,3)(1,4) β -D-glucan, known as β -glucan. In 1997, the United States Food and Drug Administration (FDA) passed a Code of Federal Regulations allowing a health claim to be placed on the food label of products containing oats. The claim must read that eating three grams of the soluble fiber, β -glucan, from oats daily may aid in reducing the development of heart disease (FDA 2003). Oat bran, the fraction from the sub-aleurone and endosperm walls in oats, is required to provide at least 5.5% dry weight basis (db) of total β -glucan (AACC 1989), and is also on the list of foods that can label the health claim.

Other documented health benefits of oats include promoting satiation (Burley et al 1995), inducing satiety (Holt et al 1995), aiding in blood glucose metabolism (Kapica 2001), reducing serum cholesterol (Pomeroy et al 2001; Camire et al 1993), and improving gastrointestinal health (Titgemeyer et al 1991; Sayar et al 2007). Human studies confirm β -glucan from oats as the food component attributed to instigating these health effects (Kapica 2001).

Processing and cooking may change the physicochemical properties of oat β -glucan through fragmentation (Yao et al 2006) and modify its physiological properties (Beer et al 1997). However, isolated β -glucan fractions form highly viscous, shear-thinning solutions shown to be stable against pH, salt, and heat (Autio et al 1992). Viscosity of polymer solutions depends on molecular weight (MW), concentration, and polymer solubility; thus the amount of β -glucan solubilized in a food system is important for rheological studies. A study examining the extractability of β -glucan from food products showed that oat bran made into porridge did not greatly change the amount of extractable β -glucan or MW amounts

compared to uncooked bran, yet the baking process in a muffin led to lower MW and increased solubility of β -glucan (Beer et al 1997).

The aim of this work was to evaluate oat lines developed at Iowa State University to have high amounts of total β -glucan and oat cultivars with typical amounts of β -glucan for their potential use in food products. Porridge (oat bran cooked in hot water) and muffins, simple food products which can be used to measure potential differences in function of oat bran types, were evaluated by trained panelists in a sensory panel. The brans also were characterized by chemical and instrumental tests to mimic the attributes chosen by the panel. Understanding the effects of β -glucan concentration in oat brans on sensory characteristics will help guide future breeding of oat lines with high β -glucan concentrations for food uses.

Materials and Methods

Materials:

Oat Brans: Materials in this study included brans from two experimental oat lines (*Avena sativa*) with high amounts of β -glucan, N979-5-2-4 (N979) and IA95111 (IA95), developed by Iowa State University, and from two commercial oat cultivars with typical amounts of β -glucan, 'Jim,' a traditional oat from the University of Minnesota, Twin Cities, and 'Paul,' a naked oat (hull-less). All oats were grown in two field replicates and harvested in 2005 at the Iowa State University (ISU) Agronomy and Agricultural Engineering Field Research Center in Ames, Iowa. The two replicates of the four oat types grown at ISU were milled at the Quaker Oats Company facility in Cedar Rapids, IA by using a hammer mill fitted with a 0.56 mm screen to break the flakes. The broken oats were sifted through a 36-mesh, stainless-steel bolting cloth to separate the bran from the oat flour. The milling

process is the same as that used to produce retail bran. The oat brans were stored in sealed plastic bags within an air-tight Rubbermaid® container at -40° C until used. A retail oat bran (Quaker Oat Bran, Hy-Vee grocery, Ames, IA) served as a control in chemical, physical, and instrumental tests, and as a reference standard in sensory tests.

Other Food Materials: Other baking materials used in the study also were purchased from Hy-Vee stores. The materials, all Hy-Vee brand, included: light pure cane brown sugar, double acting baking powder, iodized salt, clover honey, 100% pure vegetable oil (soybean), 1% fat milk, and Grade A large, whole chicken eggs. Muffin pans held twelve muffins each with cup size of 3 cm diameter bottom x 2 cm tall x 5 cm diameter top. For baking, muffin pans were lined with 4.1 cm designer baking cups (Reynolds® brand, Henrietta NY).

Products:

Oat Brans: The brans from the two field replicates for each oat type were pooled in equal amounts, only for sensory testing, to provide four bran treatments. The retail oat bran was purchased at one time and contents from all four boxes were pooled.

Porridge: To prepare the porridge, 80 g of oat bran and 1.25 L of water were brought to a light boil on the stove top and cooked for 1 min. Immediately, the porridge was spooned into 59 mL translucent plastic soufflé cups (ProPak, Independent Marketing Alliance, Houston TX). The order of cooking was randomized. All cups were cooled to room temperature on a cooling rack before being tested by sensory panelists.

Muffins: To prepare the muffins, the formula in Table 1 was used. Dry ingredients were stirred to create a homogeneous mixture prior to adding the wet ingredients. After adding wet ingredients, the batter was stirred for 20 strokes. The batter was weighed into the paper-lined muffin pans, at 15 g batter per muffin. Muffins were baked in a conventional

oven at 400° F (204.4° C) for 10-12 min. Muffins were done when a toothpick inserted into three muffins from the batch was clean when removed. The muffins were cooled in the pan for 30 sec to 1 min, then were removed from the tin and cooled on a cooling rack for 60 min, and packaged in freezer quality resealable storage bags (Hy-Vee, Ames, IA) bags, and held at room temperature until needed for evaluations. Muffins were prepared 12 hr prior to testing, and the order of preparation was randomized.

Sensory Panels:

The testing of food products with human subjects was approved by the International Review Board, approval number 04-348. . Thirteen panelists were trained during four, 1-hr sessions each for the porridge and muffins, for a total of eight training sessions. Each product was tested by the 13 panelists on three separate occasions to provide three replicates. Products made with retail bran were used as reference standards in all training and testing sessions.

An adapted quantitative descriptive analysis (QDA) method was used in this study (Kilcast 1999). During training, panelists agreed upon descriptive words to characterize each product and practiced evaluating the chosen attributes on a 15-cm line scale, with 0-cm (left-hand side) being a low amount of the attribute and 15-cm (right-hand side) being a high amount of the attribute. Products with different amounts of each attribute were provided and discussed so that agreement on intensity of attributes could be made. Some examples include:

- Porridge: excess/minimal water added for shininess; Quick oats for high creaminess; different ratios of oat flours for cohesiveness; no stirring or

different water levels for particle size; corn and potato starch for mouth coating (Meilgaard et al 1991)

- Muffin: over mixing of batter for peaked top; Quick oats, corn meal, and potato flakes for surface texture differences; water differences for crumbliness and moistness; oat flours with different concentrations of β -glucan for tenderness, cohesiveness and grittiness (Meilgaard et al 1991)

During the actual test sessions, panelists evaluated the porridge or muffin treatments in individual, fully lighted booths and were provided a score-sheet, pencil, napkin, eating utensils, water, expectorant cup, and soda crackers. Averages of each attribute for all panelists combined, per testing session, were used for statistical analyses. Panelists each selected a personal code allowing the researchers to be unaware of panelist responses during testing. Porridges and muffins were individually labeled with a random 3-digit code only known to the researchers.

Attribute Description:

Panelists generated descriptive words for each porridge and muffin products, then as a group selected the descriptors which they felt most important in describing the product precisely and accurately. The descriptors chosen for each product are:

Porridge – Visual: Shininess (evaluated after peeling the top skin of the porridge back); In-mouth: Creaminess (determined by tongue); Cohesiveness(force required to separate porridge when pressing tongue against roof of mouth); Particle Size (determined during cohesion press); Mouth-Coating (feeling of residue left in mouth after sample is swallowed or expectorated). Score sheet for porridge is demonstrated in Appendix II.

Muffin – Visual: Dome (sunken or risen shape of muffin top); Surface Texture (the perception of what the texture might be); Crumbliness (evaluated during cutting of muffin into equal halves); In-mouth: Moistness (wet feeling of muffin); Tenderness (degree of firmness felt while compressing muffin with jaw); Cohesiveness (ability of muffin to remain together as a food matrix during initial chew); and Grittiness (feeling of the number of individual particles before swallowed or expectorated). Score sheet for muffin is demonstrated in Appendix III.

Chemical and Instrumental Measurements:

Bran: Proximate analysis of the brans was performed according to AACC accepted methods. They include: moisture 44-15, crude protein 46-12 (nitrogen conversion factor of 6.25), crude fat 30-25, starch 76-13, and ash 08-05 (AACC 2000). Total β -glucan concentration was measured by using AACC method 32-23 (AACC 2000). Water absorption index (WAI) and water solubility index (WSI) were completed (Anderson et al 1969).

Porridge: The pasting properties of the oat bran porridge were analyzed by using a Rapid Visco Analyser (RVA; Model 4, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) and an STD2 temperature profile equipped with ThermoLine for Windows software version 1.2. Triplicate RVA profiles conducted on a 28-g sample measured at 8% db were obtained for each of the four bran treatments, plus the retail oat bran, with mean results for treatment replicates being analyzed. The time-temperature profile is briefly described as: heat the oat bran/water mixture for 1 min at 50° C to equilibrate the temperature. Allow the temperature to rise to 95° C in 7.5 min, and hold at that temperature for 5 min. After the 95° C holding time, decrease the temperature to 50° C in 7.5 min and hold for 2 min (Jane et al 1999). Data collected included initial peak viscosity (PV; the first

viscosity peak before heating), trough (T; holding strength during heating), breakdown (BD; difference between PV and T), final viscosity (FV; highest viscosity reached at end of heating), and setback (SB; difference between FV and T). A RVA profile is depicted in Appendix IV.

Muffins: Muffins from each treatment were evaluated fresh and after storage in resealable storage bags (Hy-Vee, Ames IA) at room temperature for 48 hr and 72 hr.

Moisture level (AACC 44-15) and total β -glucan content (AACC 32-23) of each bran type were measured (AACC 2000) on the baked product. WAI and WSI were measured for the fresh and 48 hr sample (Anderson et al 1969).

Muffin volume of three muffins from each treatment was measured by using seed displacement (AACC 10-05; AACC 2000) at each of the storage times (fresh, 48 h, and 72 h), and the data of the three measurements were averaged.

Texture attributes of hardness, cohesiveness, springiness, and chewiness were measured on a texture analyzer (Stable Micro Systems TA-XT2, Texture Technologies Corp., Scarsdale, NY) with Texture Expert for Windows software (V1.11). A method based off AACC method 74-09 for bread firmness (Stable Micro Systems, Ltd 1997) was developed for the oat bran muffins in this study. Briefly, the top of a muffin was cut off to leave a height of 20 mm. This 20 mm piece was placed under the TA-212, 11 mm cylinder probe. The probe was pressed into the muffin at 1.0 mm per second, to a depth of 5.0 mm, or 25% of its height. The up and down motion was repeated three times at the same location on the muffin, recording the force required for each descent into the muffin. This protocol was repeated two additional times in different locations on the muffin. A total of three muffins were tested in each oat bran type. A texture profile from muffins is depicted in Appendix V.

Statistical Analysis: All tests were repeated three times unless otherwise reported. Sensory panel data were analyzed by using the generalized linear models analysis of variance for randomized blocks. Chemical and instrumental measures were analyzed by LSD. All programs were run by using a computerized statistical program (SAS v 9.1 2002-2003; Cary, NC). Differences among treatments were compared at a significance level (α) of <0.05 .

Results and Discussion

Sensory evaluation:

Porridge – Sensory panelists were a significant source of variance in all attributes for the porridge. This finding is common for most sensory panels and therefore is considered acceptable in descriptive profiling measures (Lapvetelainen and Rannikko 2000). More specifically, among the treatments tested in the panel, shininess and cohesiveness were not different, thus the data is not further discussed. Creaminess, particle size and mouth coating were different as judged by the panelists and is discussed (Figure 1).

IA95 was less creamy than N979, Jim, and Paul porridges, which were not different from each other (Figure 1). For particle size, IA95 was different than all other treatments, and N979 and Jim were not different from each other (Figure 1). Paul was different from all brans except Jim in particle size (Figure 1). Paul, Jim, and N979 were not different in mouth-coating, but IA95 had a lower value (Figure 1). Creaminess and particle size of the oat bran porridge is likely related to the water holding capacity of the oat bran particles. All treatments scored lower than the agreed upon value for the bran which was provided as a reference for the panelists, except for IA95 bran, which scored the highest on the 15-cm scale for particle size, signifying that the cooked bran held water and agglomerated with other bran

particles more than the other bran treatments. In other work, all oat fractions (whole meal, bran, and flour) exhibited a higher water absorption capacity than wheat, corn, or rice fractions, with oat bran having the greatest (Chang and Sosulski 1985; Paton and Lenz 1993).

Previously, the β -glucan molecular weight (MW) of groats from the four oat lines evaluated in the current study had been determined. Specifically, N979 and IA95 had greater peak MW values than did the β -glucan of Jim and Paul during all the 2002-2004 growing years (Yao et al 2007). In other work, the extractable β -glucan content was found to be a heritable trait, but MW of the β -glucan in oats depended more on environmental factors during growing (Ajithkumar et al 2005), therefore not all characteristics of oats can be determined through breeding. A combination of MW, one of the most important characteristics of β -glucan, and extractability may have influenced the particle size of the porridge. It can be thought that as the MW so would the particle size since there would be likelihood for an increase in viscous properties.

The solubility of the β -glucan from bran types during cooking likely influenced the mouth-coating values for porridge. β -glucan from oat bran was 44% soluble at 100°C (Carr et al 1990), and β -glucan in ready-to-eat oat products was 75-80% soluble (Wood 1993). These values are higher than the solubility indices in this study as will be discussed in a later section of the results. Oat β -glucan is commonly classified as a viscous gum or soluble dietary fiber (Wood 1993), lending to its viability to coat surfaces, such as the interior of the mouth.

Muffins – Panelists were a significant source of variation in all oat bran muffin attributes. All attributes, except for tenderness, were different among treatments (Figure 2). The dome shape was greatest in IA95 and N979, followed by Jim and Paul treatments (Figure 2). IA95

and N979 were closest to the reference bran muffin, previously agreed by the panelists to have a value of 11.25 cm. Surface texture was more coarse in IA95 and N979, followed by Paul and Jim (Figure 2). Crumbliness was highest for IA95 and N979, with N979 and Jim not different from each other (Figure 2). Paul was the least crumbly out of the four treatments (Figure 2). Jim and Paul were the most moist and cohesive, followed by N979 and IA95 (Figure 2). IA95 and N979 had the highest grittiness values and Paul had the lowest, but Jim and N979 were not different from each other (Figure 2).

The hygroscopic nature of the β -glucan could create differences among muffins as a result of their percent β -glucan. Indeed, IA95 and N979, with greater percent β -glucan, had greater domes and more coarse surface texture (Figure 2). Crumbliness should be inversely related to the amount of cohesion between the muffin crumb. Also, the gum-like characteristics of β -glucan should increase the moistness and cohesion and decrease the crumbliness, but the findings did not support this assumption, and in fact, tended to be the reverse (Figure 2). Although, the greater amounts of β -glucan in N979 and IA95 brans did not give greater values for moistness and cohesiveness, all bran types scored above the value of 7.5, previously determined for the reference muffin. The β -glucan content of IA95 and N979 brans in this study was likely to be of high MW (Yao et al 2007). Viscosity is directly related to MW (Beer et al 1997), but perhaps there is an optimum MW within this food system, with higher MW reducing viscosity.

Chemical and Instrumental Measurements:

Bran – Protein content differed among all bran treatments with IA95 (17.3 %), > N979 (16.6 %), > retail bran (15.7 %), > Jim (14.7%), > Paul (13.5 %) (Table 2a). Lipid percentage was greatest among Paul (8.5 %), \geq N979 (8.3 %), = Jim (7.8 %), = IA95 (7.5 %)

(Table 2a). Retail bran had the lowest lipid content at 6.6 % (Table 2). Starch content was different among all bran treatments: Jim (37.1 %), = Paul (36.5 %), > retail bran (32.3 %), = IA95 (32.0 %), = N979 (30.7 %) (Table 2a). As starch percentage decreased, total β -glucan content increased. Total β -glucan values differed among all treatments, except for Jim and retail bran which were not different (Table 2a). The β -glucan values were: N979 (10.8 %), > IA95 (8.9 %), > Paul (7.5 %), > Jim (6.4 %), = retail bran (6.2 %). WAI was greatest in IA95 (3.0 g/mL), \geq N979 (2.9 g/mL), = Paul (2.6 g/mL), > Jim (2.2 g/mL) = retail bran (2.2 g/mL) (Table 2a). WSI was greatest for IA95 (8.8 %), = N979 (8.7 %), > Paul (7.3 %), = Jim (7.0 %), > retail bran (3.0 %) (Table 2a).

Porridge: RVA measurements – All four treatments, and the retail bran, were different in the RVA measurement of PV, with the following values: N979 (569 cP), > IA95 (485 cP), > Paul (360 cP), > Jim (241 cP), = retail bran (232 cP) (Table 3). RVA T values were: N979 (463 cP), = IA95 (414 cP), > Paul (299 cP), > Jim (210 cP), = retail bran (199 cP) (Table 3). The BD was greatest in N979 (106 cP), > IA95 (71 cP), \geq Paul (61 cP), \geq retail bran (34 cP), = Jim (31 cP) (Table 3). Final viscosity (FV) followed a different pattern, with N979 (3601 cP), \geq Jim (2954 cP), \geq IA95 (2812 cP), \geq Paul (2508 cP), > retail bran, which was the lowest at 1440 cP (Table 3). The SB followed the same pattern as BD with the following values: N979 (3138cP) \geq Jim (2743 cP) \geq IA95 (2398 cP) \geq Paul (2209 cP) > retail bran (1242 cP). The low values of the retail bran for FV and SB are attributed to the low β -glucan percentage compared to the treatment brans. Previously as well as in this study, an increase of starch coupled with a decrease of β -glucan amounts was negatively related to PV, T, FV, and SB (Yao et al 2007). Although starch can contribute, β -glucan is the major component responsible for the development of viscosity, with a significant correlation existing between

the total and extractable β -glucan in bran (Luharoo et al 1998). In fact, the measurement of viscosity can be a useful screening method for selecting breeding lines (Colleoni-Sirghie et al 2004; Chernyshova et al 2007).

Muffins: volume, moisture, and β -glucan – There were differences among the fresh muffin volumes. N979 (32.1 cc³) \geq IA95 (30.9 cc³) = Paul (29.9 cc³) > Jim (26.7 cc³) = retail bran (26.7 cc³) (Table 2b). No differences among the treatments were found in muffin volume at 48 hr (Table 2b). At 72 hr, IA95 (31.0 cc³) \geq N979 (29.9 cc³) \geq Jim (29.9 cc³) but N979 and Jim were not different from the retail bran (28.9 cc³) (Table 2b). At 72 hr, retail bran and Paul (27.8 cc³) were not different (Table 2b). Muffin treatments did not differ in percent moisture at any of the three storage times (Table 2b). The total β -glucan percentages differed between treatment brans and retail bran at all times. Specifically, while fresh, Paul (14.0 %) \geq N979 (13.0 %) \geq IA95 (12.0 %) \geq Jim (12.0 %), but N979 = IA95 = Jim = retail bran (Table 2b). At 48 hr, Paul (16.0 %) \geq N979 (15.0 %) \geq IA95 (15.0 %) \geq Jim (13.0 %) but only Jim was not different from the retail bran (12.0 %) (Table 2b). At 72 hr there were fewer differences, showing that N979 (18.0 %) = IA95 (18.0 %) = Paul (17.0 %), while Jim was different at 14.0 %, and retail bran different from all at 11.0 % total β -glucan (Table 2b). The total percent β -glucan of the baked muffins was generally greater than the total percent β -glucan in the raw bran, despite also a dilution of the percent β -glucan with other ingredients. Heat and ingredient interactions presumably allowed the β -glucan to solubilize and become more easily detected using the AACC method 32-23 (AACC 2000), a phenomenon also noted by Beer et al (1997) in a baking experiment with muffins having different percentages of total β -glucan.

WAI and WSI: The WAI tended to be greatest for the retail bran in fresh muffins (2.45 g/mL) and in those stored for 48 hr (2.49 g/mL) (Table 2). Specifically, while fresh, retail bran (2.5 g/mL) = IA95 (2.1 g/mL) = N979 (2.1 g/mL) = Jim (2.0 g/mL), but none of the treatment brans were different from each other (IA95 = N979 = Jim = Paul (1.8 g/mL)) (Table 2). At 48 hr, retail bran (2.5 g/mL) = N979 (2.1 g/mL) = IA95 (1.9 g/mL) and again, treatment brans did not differ from each other (N979 = IA95 = Jim (1.7 g/mL) = Paul (1.5 g/mL)) (Table 2). For WSI, Paul was highest at the fresh point with 26.0 % solubility (Table 2). Paul was different from Jim (23.0 %) \geq retail bran (21.8 %) = N979 (20.5 %) = IA95 (20.2 %) (Table 2). At 48 hr, Paul (27.2 %) was \geq Jim (24.2 %) \geq IA95 (22.4%) \geq N979 (20.9 %) = retail bran (19.7 %) (Table 2). The β -glucan percentage in the muffins tended to be inversely related to the WAI values (the higher the β -glucan, the lower the WAI value), but followed the same trends as WSI (the higher the β -glucan, the higher the WSI). This relationship suggests that in a baking system, such as muffins, β -glucan becomes more soluble, which could lead to better health benefits. Previously, a different study found that the process of baking muffins increased the extractability of the β -glucan from the food matrix (Beer et al 1997). The study went on to demonstrate that differences in viscosity in the intestine might occur with different β -glucan concentrations consumed.

Texture Analyzer: Hardness differed among the treatment brans and the retail bran at all three times of fresh, 48 hr, and 72 hr as measured by a Texture Analyzer (Table 4). For the fresh muffins, retail bran was the hardest (77.0 g) \geq Jim (73.0 g) \geq IA95 (65.9 g) = N979 (59.2 g) = Paul (58.9 g) (Table 4). At 48 hr, the retail bran (100.2 g) was different from the four treatment brans (Table 4). Following retail bran were: Jim (61.7 g, \geq IA95 (54.4 g, \geq N979 (51.1 g) = Paul (35.6 g) (Table 4). At 72 hr, retail bran was the hardest at 97.9 g force,

while IA95 (45.5 g), N979 (43.3 g), Jim (43.3 g), and Paul (41.4 g) were not different from each other (Table 4).

Springiness followed the same pattern as hardness, but with smaller values as it took less force to compress the muffin a second time (Table 4). Springiness values of the fresh muffins were: retail bran (71.8 g) \geq Jim (65.4 g) \geq IA95 (61.5 g) = N979 (55.6 g) = Paul (55.1 g) (Table 4). At 48 hr, retail bran (93.3 g) $>$ Jim (55.1 g) \geq IA95 (48.9 g) \geq N979 (46.0 g) = Paul (31.0 g) (Table 4). At 72 hr, the retail bran (90.8 g) was different from the treatment brans, where Paul (38.5 g) = IA95 (38.1 g) = N979 (38.0 g) = Jim (37.9 g) (Table 4).

There were no differences in cohesiveness in the fresh muffins (N979 (0.94), = Paul (0.94) = IA95 (0.93) = retail bran (0.93) = Jim (0.90)) (Table 4). At 48 hr, the retail bran had the most cohesiveness with 0.93 $>$ N979 (0.90) = IA95 (0.90) = Jim (0.89) $>$ Paul (0.87) (Table 4). At 72 hr, retail bran (0.93) = N979 (0.90) = Paul (0.90) = Jim (0.88), but IA95 (0.84) was different from only the retail bran (Table 4).

Gumminess of the fresh muffins was greatest for retail bran (71.8 g) \geq Jim (65.4 g) \geq IA95 (61.5 g) = N979 (55.6 g) = and Paul (55.1 g) (Table 4). At 48 hr, retail bran was the most gummy at 93.3 g, while all treatments were differed as follows: Jim (55.1 g), = IA95 (48.9 g) = and N979 (46.0 g) = Paul (31.0 g) (Table 4). At 72 hr, retail bran was the most gummy with 90.8 g, and treatments did not differ from each other: (Paul (38.5 g) = IA95 (38.1 g) = N979 (38.0 g) = Jim (37.9 g)) (Table 4). Gumminess values mirrored the values of hardness and springiness.

Correlations:

Correlations between sensory, instrumental, physical, and chemical measures were examined, with only one significant correlation being found. There was a correlation of $r = 0.952$ ($p < 0.05$) between the shininess attribute in the sensory panel and the amount of water absorption of the bran. Relationships among the sensory, instrumental, physical, and chemical measures can be observed, but correlations give a better understanding between the various analyses.

Conclusions

Oat brans separated from oats varying in β -glucan concentrations were evaluated for their potential use in foods. The bran types had β -glucan concentrations as follows: N979 (10.8 %) > IA95 (8.9 %) > 'Paul' (7.5 %) > 'Jim' (6.4 %) = retail bran (6.2 %). Sensory panelists evaluated attributes of two food products scored on a 15-cm line. Porridges differed in the amount of creaminess, particle size, and mouth-coating among treatments. Muffins differed in dome shape, surface texture, crumbliness, moistness, cohesiveness, and grittiness among treatments. Raw brans from N979 and IA95 were the highest in protein, lipid, total β -glucan, WAI, and WSI. Muffin treatments did not differ in moisture content. Paul muffins had the highest total β -glucan concentration in the fresh and 48 hr stored muffin, when and WSI percentage at all storage times, and the retail bran muffins had the highest WAI in the fresh and 48 hr stored muffin. In fresh, 48 hr and 72 hr muffins, retail bran had the greatest hardness, springiness, cohesiveness, and gumminess. The N979 bran had the greatest values for all attributes measured by RVA, including PV, T, BD, FV, and SB.

Acknowledgments

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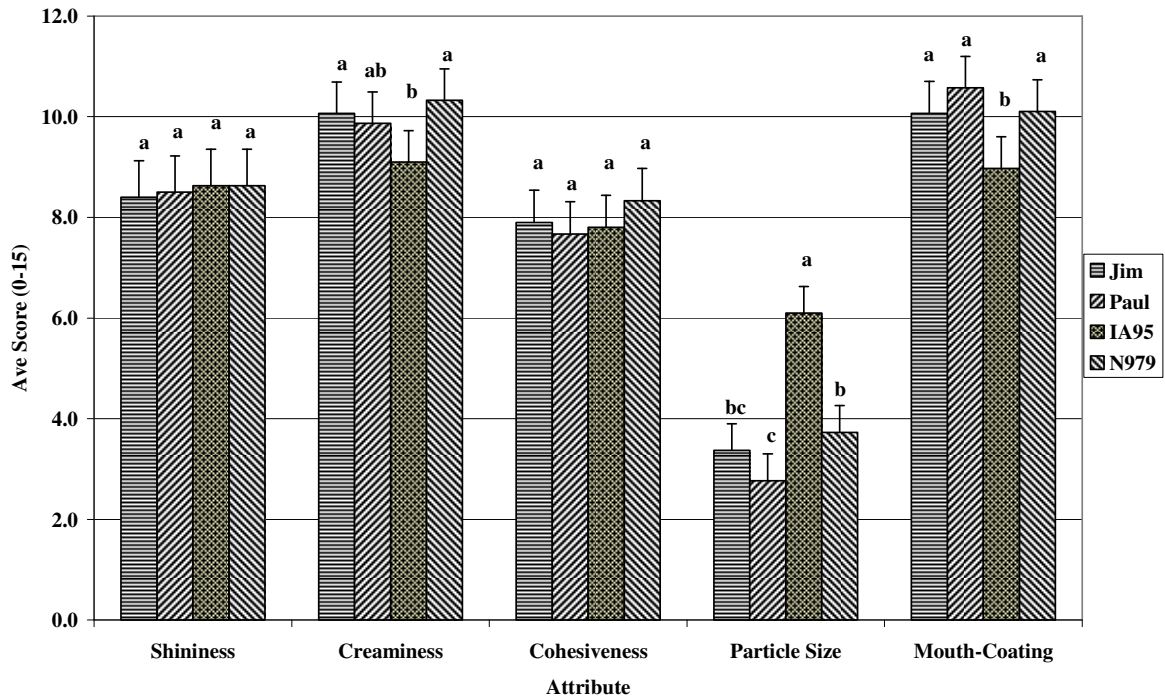
Tables and Figures

Table 1. Formula for 100% Oat Bran Muffin

Sample type	grams
Oat bran	100
Light Brown Sugar	39.0
Baking Powder	5.0
Salt	0.5
Honey	26.5
Vegetable Oil	13.0
1% fat Milk	151
Egg	~55
Total	390

Muffin weight (batter) = 15.0 grams

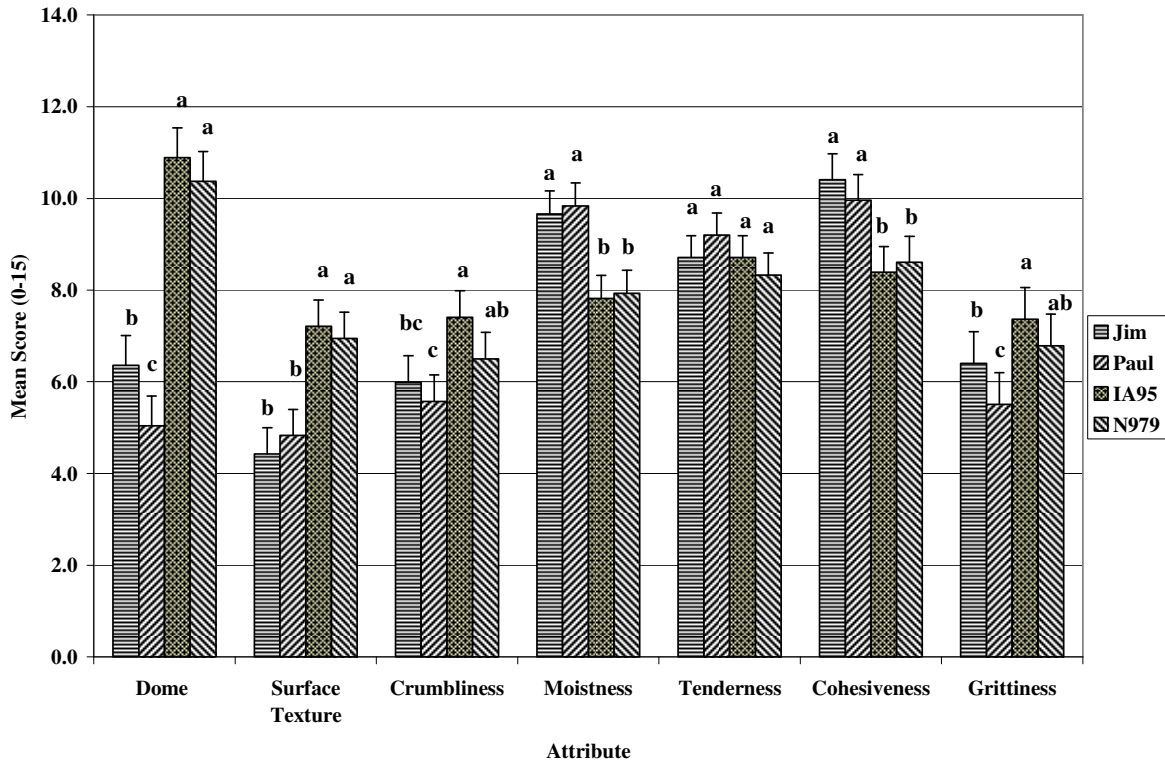
Muffin weight (cooked) = 13.2 grams

Figure 1. Mean Scores² for Sensory Characteristics of Oat Bran Porridge¹³

¹ = Reference value for porridge is 7.5 for all attributes; Bar value represents mean score of three replications

² = Score sheet values are; 0 (low) to 15 (high)

³ = Bars within each attribute with same letter are not significantly different ($p < 0.05$)

Figure 2. Mean Scores² for Sensory Characteristics of Fresh Oat Bran Muffins¹³

¹ = Reference value for muffins is 7.5 for all attributes except 'Dome' where reference muffin is 11.25; Bar value represents mean score of three replications

² = Score sheet values are; 0 (low) to 15 (high)

³ = Bars within each attribute with same letter are not significantly different ($p < 0.05$)

Table 2a. Chemical Analysis¹ of Oat Bran, Porridge, and Muffins

Oat Type	Raw Bran / Porridge					
	Protein %	Lipids %	Starch %	β -glucan %	WAI ² g/mL	WSI ³ %
Fresh						
Jim	14.7 d	7.8 ab	37.1 a	6.4 d	2.2 c	7.0 b
Paul	13.5 e	8.5 a	36.5 a	7.5 c	2.6 b	7.3 b
IA95	17.3 a	7.5 b	32.0 b	8.9 b	3.0 a	8.8 a
N979	16.6 b	8.3 ab	30.7 b	10.8 a	2.9 ab	8.7 a
Retail Bran	15.7 c	6.6 c	32.3 b	6.2 d	2.2 c	3.0 c
48 hr						
Jim						
Paul						
IA95						
N979						
Retail Bran						
Not measured for 48 hr or 72 hr						
72 hr						
Jim						
Paul						
IA95						
N979						
Retail Bran						

¹ = All values are mean of triplicate analysis of two field replications and reported on a dry weight basis (db); Values followed by the same letter in a column are not significantly different ($p < 0.05$)

² = Water Absorption Index

³ = Water Solubility Index

Table 2b. Chemical Analysis¹ of Oat Bran, Porridge, and Muffins

Oat Type	Muffin				
	Volume cc ³	Moisture %	β -glucan %	WAI ² g/mL	WSI ³ %
Fresh					
Jim	26.7 c	38.8 a	12.0 ab	2.0 ab	23.0 b
Paul	29.9 b	38.9 a	14.0 a	1.8 b	26.0 a
IA95	30.9 ab	39.9 a	12.0 ab	2.2 ab	20.2 c
N979	32.1 a	39.9 a	13.0 ab	2.1 ab	20.5 c
Retail Bran	26.7 c	39.0 a	9.0 b	2.5 a	21.8 bc
48 hr					
Jim	29.1 a	40.1 a	13.0 bc	1.7 b	24.2 ab
Paul	27.8 a	40.4 a	16.0 a	1.5 b	27.2 a
IA95	29.3 a	40.3 a	15.0 ab	1.9 ab	22.4 bc
N979	29.5 a	39.6 a	15.0 ab	2.1 ab	20.9 bc
Retail Bran	29.0 a	39.8 a	12.0 c	2.5 a	19.7 c
72 hr					
Jim	29.9 ab	39.7 a	14.0 b		
Paul	27.8 c	39.5 a	17.0 a		
IA95	31.0 a	39.4 a	18.0 a		
N979	29.9 ab	39.7 a	18.0 a		
Retail Bran	28.9 bc	40.3 a	11.0 c		

¹ = All values are mean of triplicate analysis of two field replications and reported on a dry weight basis (db); Values followed by the same letter in a column are not significantly different (p<0.05)

² = Water Absorption Index

³ = Water Solubility Index

Table 3. Viscosity Measurements Using a Rapid Visco Analyser (RVA)²

Oat Type	Peak Viscosity cP ¹	Trough cP	Breakdown cP	Final Viscosity cP	Setback cP
Jim	241 d	210 c	31 d	2954 ab	2743 ab
Paul	360 c	299 b	61 bc	2508 c	2209 c
IA95	485 b	414 a	71 b	2812 bc	2398 bc
N979	569 a	463 a	106 a	3601 a	3138 a
Retail Bran	232 d	199 c	34 cd	1440 d	1242 d

¹ = cP = Centipoise; 12 cP is equal to 1 RVU (Rapid Visco Unit)

² = All values are mean of triplicate analysis of two field replications; Values followed by the same letter in a column are not significantly different ($p < 0.05$)

Table 4. Texture Profile Analysis of Oat Bran Muffin¹

Time	Oat Type	One-bite			
		Hardness g	springiness g	Cohesiveness ² g	
Fresh					
	Jim	73.0 ab	65.4 ab	0.90 a	65.4 ab
	Paul	58.9 b	55.1 b	0.94 a	55.1 b
	IA95	65.9 ab	61.5 ab	0.93 a	61.5 ab
	N979	59.2 b	55.6 b	0.94 a	55.6 b
	Retail Bran	77.0 a	71.8 a	0.93 a	71.8 a
48 h					
	Jim	61.7 b	55.1 b	0.89 ab	55.1 b
	Paul	35.6 c	31.0 c	0.87 c	31.0 c
	IA95	54.4 bc	48.9 bc	0.90 b	48.9 bc
	N979	51.1 bc	46.0 bc	0.90 b	46.0 bc
	Retail Bran	100.2 a	93.3 a	0.93 a	93.3 a
72 h					
	Jim	43.2 b	37.9 b	0.88 ab	37.9 b
	Paul	41.4 b	38.5 b	0.90 ab	38.5 b
	IA95	45.5 b	38.1 b	0.84 b	38.1 b
	N979	43.2 b	38.0 b	0.90 ab	38.0 b
	Retail Bran	97.9 a	90.8 a	0.93 a	90.8 a

¹ = Values in each attribute column, of each time section, with the same letter are not significantly different (p<0.05)

² = Cohesiveness = One-bite Springiness / Hardness

³ = Gumminess = Hardness x Cohesiveness

General Conclusions

This study demonstrated that the bran from experimental oat lines developed at Iowa State University have greater than normal concentrations of total β -glucan, also had greater amounts of total β -glucan than bran from the typical oat genotypes, 'Paul' and 'Jim', and than bran and oat flakes purchased in the retail market. Bran from N979 had a β -glucan content of 10.8 % db and bran from IA95 had a β -glucan content of 8.9 % db. In addition, the amounts of soluble β -glucan in N979 and IA95 were higher than the amounts of soluble β -glucan in Paul, Jim, retail bran and retail oat flakes. Insoluble β -glucan amounts for the high- β -glucan brans were not different than that found in the other brans.

The values for WAI and WSI were positively correlated to the total β -glucan content. The correlation coefficients were $r = 0.853$ for β -glucan and WAI, and $r = 0.820$ for β -glucan and WSI. When the bile acid concentration of 1.41 mM/L was added before the pancreatic digestion step of the *in vitro* digestion, the percentage of bile acids bound was 99% or higher for all brans. Even though all brans bound at least 99% of bile acids, retail bran was different (higher) than the other brans or oat flakes, binding 99.86% of bile acids. Gas production and pH levels during fermentation by fecal inoculum differed among treatments over a 24-hr time period, generally with bran from Jim having the greatest gas production. The pH values did not vary much among treatments, but Jim bran was generally lowest. SCFA production (acetate, propionate, and butyrate) was not consistently greater for any one or group of treatments, but Jim bran tended to be highest at most times and the retail bran tended to be lowest, except at 2 hr. The retail oats tended to be low at the beginning. In general, N979 tended to produce less SCFA than the other non-retail brans.

Sensory evaluation showed some differences in porridge and muffins made from the four treatment brans (N979, IA95, Paul and Jim). In the porridge, bran types were not different in shininess and cohesiveness compared to the publicly available oat brans, but IA95 and N979 brans were different from the publicly available brans in creaminess, particle size, and mouth-coating. For the muffins, panelists found differences in the dome shape, surface texture, crumbliness, moistness, cohesiveness and grittiness in all brans, but no difference in the tenderness with brans from IA95 and N979 compared to the publicly available oat brans.

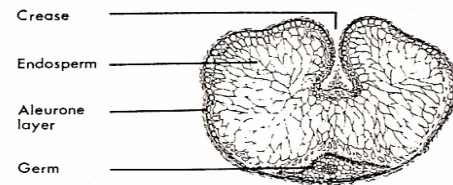
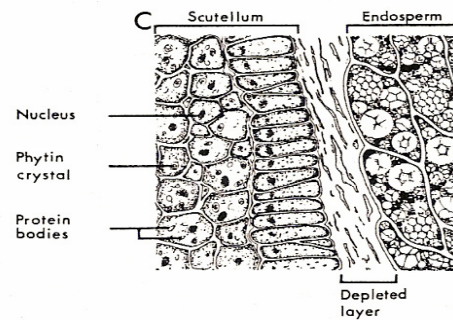
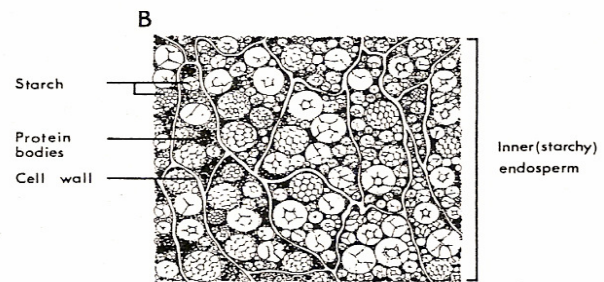
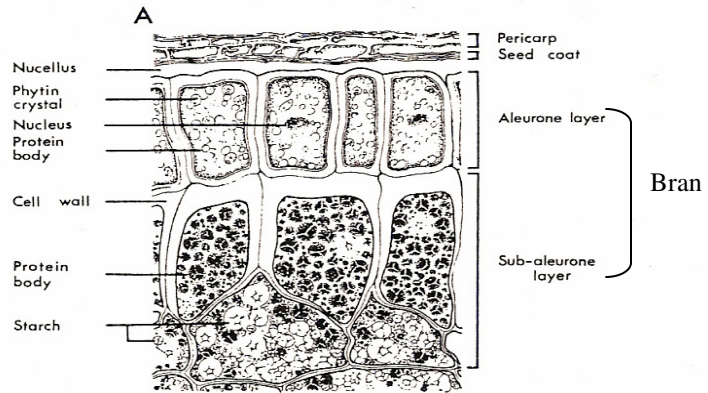
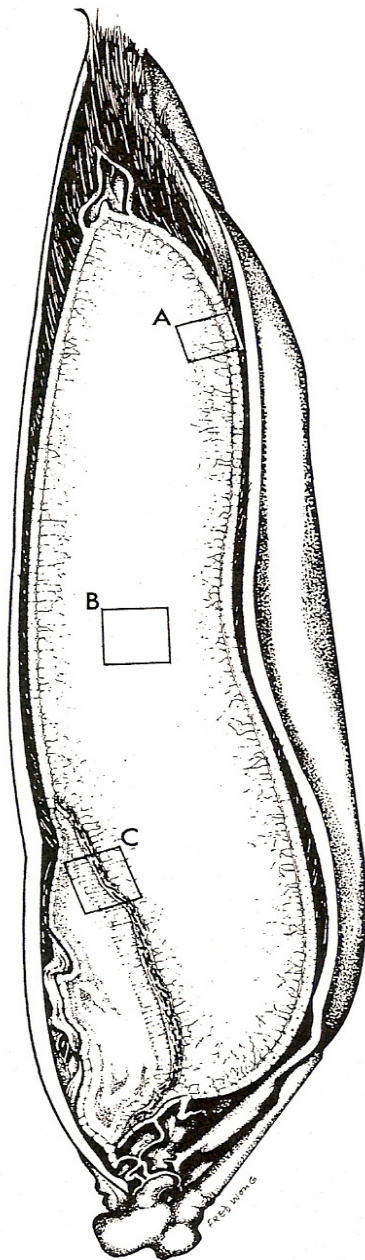
In general, muffins made from N979 and IA95 brans had greater volumes than all other treatments when fresh, but few differences were found in at 48 and 72 hr. There were no differences in muffin moisture content at any of the time points. Total β -glucan concentration increased in percentage from the raw bran to the muffin samples in all bran types. In the muffins, total percent β -glucan of N979, IA95, and Paul brans tended to be highest at 48 and 72 hr, but when fresh, these amounts were not different from that found in Jim muffins. WAI was highest in the muffins with the lowest total β -glucan (retail bran), but WSI was highest in the muffins with the highest total β -glucan (Paul). By using a slurry of bran and water, IA95 and N979 brans had the highest values of peak viscosity and trough among all treatments measured by the Rapid Visco Analyser. N979 bran had the greatest values for breakdown and final viscosity. Texture profiles, measured by a Texture Analyzer, showed no differences between muffins made with IA95 and N979 bran in hardness, one-bite springiness, cohesiveness, or gumminess.

The continued development of oat lines with high amounts of the dietary fiber, β -glucan, will be crucial in the future because of their importance in digestive and heart health.

This study has demonstrated that the experimental oat lines, IA95 and N979, are chemically similar to publicly available oat cultivars, but with a higher concentration of the β -glucan, and are usable in two food products.

Appendix I

Diagram of oat grain.



Appendix II

Panelist ID # _____

Oat Bran Sensory Panel - Porridge

Please place a vertical dash on the line appropriate to where you think the sample best fits the attribute listed. Please view the line as: left = low, right = high. After placing the vertical dash mark, put the corresponding sample number above the dash. Thank you!

**Please rinse your mouth with water between samples.*

VISUAL

Peel back top skin on porridge cup with spoon. Then observe the following visual attribute:

Attribute: *Shininess*

_____ | R _____
 Low High

IN MOUTH

Scoop a dime-sized amount of porridge on tip of spoon. Analyze the following attributes with the sample, repeat as needed.

Attribute: *Creaminess*

_____ | R _____
 None High

Attribute: *Cohesiveness (force required to separate porridge when pressing tongue against roof of mouth – push once)*

_____ | R _____
 None High

Attribute: *Particle Size*

_____ | R _____
 Small Large

Attribute: *Mouth-coating (feeling left in mouth after sample is swallowed)*

_____ | R _____
 None High

Appendix III

Panelist ID # _____

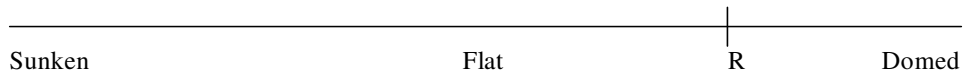
Oat Bran Sensory Panel - Muffin

Please place a vertical dash on the line appropriate to where you think the sample best fits the attribute listed. Please view the line as: left = low, right = high. After placing the vertical dash mark, put the corresponding sample number above the dash. Thank you!

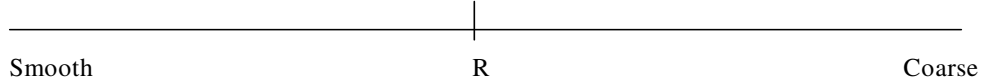
VISUAL

Look at top of muffin before taking paper off and record first attribute.

Attribute: *Dome (shape)*

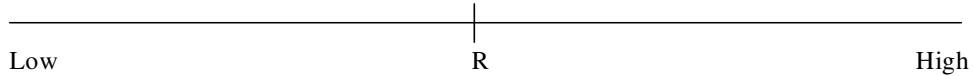


Attribute: *Surface Texture*



Remove paper from muffin. Cut muffin in half. Analyze this attribute while cutting.

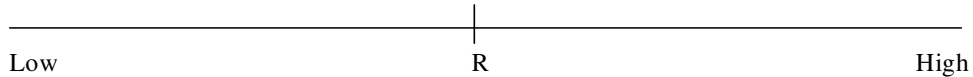
Attribute: *Crumbliness*



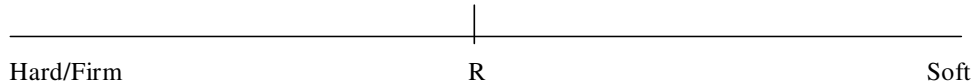
IN MOUTH

Cut one of muffin halves in half again (for ¼ of the total muffin). Place whole ¼ of muffin in mouth and analyze the following attributes. Repeat with ¼ size muffin pieces as needed.

Attribute: *Moistness*



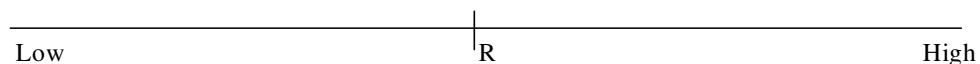
Attribute: *Tenderness (1st bite)*



Attribute: *Cohesiveness (ability to remain together as a food system during initial chewing)*

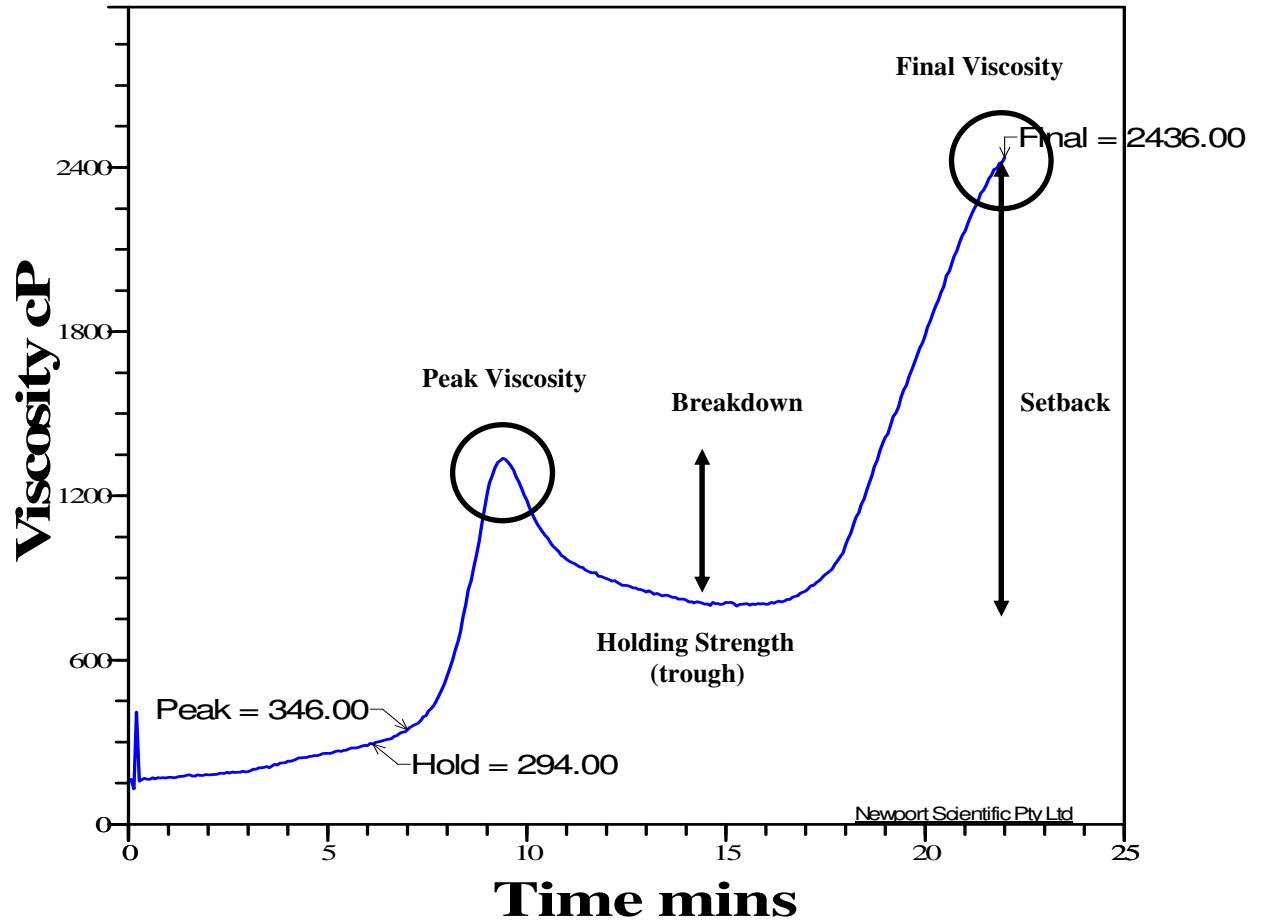


Attribute: *Grittiness (feeling of individual particles before swallowing)*



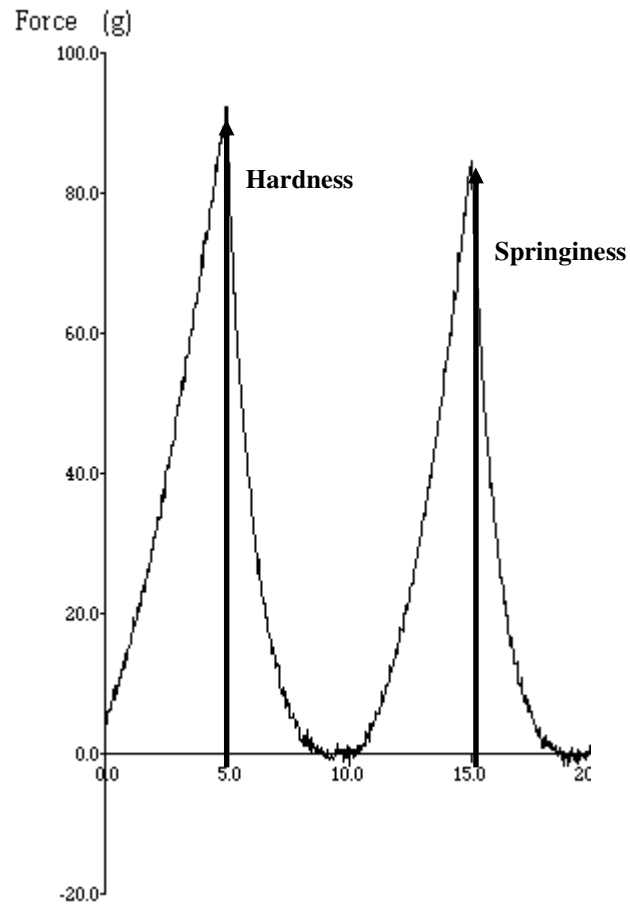
Appendix IV

Data curve from a Rapid Visco Analyser



Appendix V

Data curve from a Texture Analyzer (TA-XT2) reading



Cohesiveness = Hardness x Springiness

Gumminess = Hardness x Cohesiveness