MONTANA
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Chemical characterization and metabolic function of soluble dietary fiber from select milling fractions of a hull-less barley and its waxy starch mutant
by Theodore Mori

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
Two hull-less barley cultivars; AZ-76-19-7 (AZ) (non-waxy) and AZ-76-19-7-WX (AZWX) (waxy) were milled through a MIAG multomat 8-roller experimental mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA 99164) . Barleys were tempered to 13% moisture for 12 h before milling. Feed rate was 900 g*h'1. Streams were collected and 2nd break flour through 4th middlings were combined and labeled flour. Fractions were analyzed for insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total β-glucans, starch, molecular weight (MW) of extractable dietary fiber, viscosity, ether extract, ash, and crude protein (N*6.25). Soluble dietary fiber and total /3-glucans were concentrated in break shorts, red dog, and bran in both barleys. The red dog fractions of AZ and AZWX were highest in SDF, IDF, viscosity and total β-glucans. The waxy cultivar and its milling fractions were higher in SDF compared to those of the non-waxy cultivar. Distributions of MW of the SDF fractions extracted in water were similar between barleys. Ether extract, ash, and protein from 1st break flour and flour were lower compared to the high fiber fractions from both barleys.

Weanling male rats were fed diets containing high fiber milling fractions (red dog) from the AZ and AZWX barleys with and without /3-glucanase and a corn control diet containing alphacel. All diets were balanced for 20% total dietary fiber, 16% protein, and essential amino acids. Rats were provided unlimited access to feed and water. Rats fed the red dog diets with or without /3-glucanase had no differences (P>0.1) in average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (FG). Rats fed the corn control diet were not different (P>0.1) from the red dog diets in ADG, but were higher (P<0.05) in ADFI and FG. Rats fed the control diet were higher (P<.01) in plasma total cholesterol, triacylglycerides, and LDL-cholesterol. Fecal dry matter was (P<0.05) higher on the control diet, while fecal ether extract was lower (P<0.05). These data demonstrate that barley soluble dietary fiber has a marked effect on lipid metabolism and fecal components compared to insoluble dietary fiber.

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DIETARY FIBER FROM SELECT MILLING FRACTIONS OF A

HULL-LESS BARLEY AND ITS WAXY STARCH MUTANT

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Theodore Mori

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APPROVAL

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Theodore Mori

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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___July 31, 1990___   _____
Date   Graduate Dean

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ABSTRACT

Two hull-less barley cultivars; AZ-76-19-7 (AZ) (non-waxy) and AZ-76-19-7-WX (AZWX) (waxy) were milled through a MIAG multomat 8-roller experimental mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA 99164). Barleys were tempered to 13% moisture for 12 h before milling. Feed rate was 900 g*h^{-1}. Streams were collected and 2nd break flour through 4th middlings were combined and labeled flour. Fractions were analyzed for insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total β-glucans, starch, molecular weight (MW) of extractable dietary fiber, viscosity, ether extract, ash, and crude protein (N*6.25). Soluble dietary fiber and total β-glucans were concentrated in break shorts, red dog, and bran in both barleys. The red dog fractions of AZ and AZWX were highest in SDF, IDF, viscosity and total β-glucans. The waxy cultivar and its milling fractions were higher in SDF compared to those of the non-waxy cultivar. Distributions of MW of the SDF fractions extracted in water were similar between barleys. Ether extract, ash, and protein from 1st break flour and flour were lower compared to the high fiber fractions from both barleys.

Weanling male rats were fed diets containing high fiber milling fractions (red dog) from the AZ and AZWX barleys with and without β-glucanase and a corn control diet containing alphacel. All diets were balanced for 20% total dietary fiber, 16% protein, and essential amino acids. Rats were provided unlimited access to feed and water. Rats fed the red dog diets with or without β-glucanase had no differences (P>0.1) in average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (FG). Rats fed the corn control diet were not different (P>0.1) from the red dog diets in ADG, but were higher (P<0.05) in ADFI and FG. Rats fed the control diet were higher (P<.01) in plasma total cholesterol, triacylglycerides, and LDL-cholesterol. Fecal dry matter was (P<0.05) higher on the control diet, while fecal ether extract was lower (P<0.05). These data demonstrate that barley soluble dietary fiber has a marked effect on lipid metabolism and fecal components compared to insoluble dietary fiber.

CHAPTER 1

INTRODUCTION

Montana is the second largest barley producing state in the U.S. following North Dakota. Barley is one of the most widely grown cereal grain crops in the world because of its: broad ecological adaptation, utility as a feed and food grain, and superiority for use in brewing (Poehlman, 1985). Barley comprises about 12 percent of the world's total cereal production, ranking fourth in importance behind wheat, rice, and maize (MacKey, 1981). At present the major uses for barley in the U.S. are animal feed and malt in the brewing industry. A small amount of barley and barley malt are used for human food production with the majority being in breakfast cereals. In other parts of the world such as southeast Asia and northern Europe barley is a very important animal feed and human food crop. With the introduction of specially bred barleys, such as waxy hull-less cultivars, the potential for increased human consumption is great.

With the development of food barleys greater amounts of by-products will be produced from the milling industry. Some of these products may be used as human food, but the majority will probably be utilized in animal feeds as is the case with corn gluten meal and wheat bran. Barley milling products are

relatively unknown as to nutrient content, nutritional value and end use, either in livestock feeding or in the food industry.

The purpose of this research was to determine the feasibility of milling waxy and non-waxy starch hull-less barleys in a wheat type roller mill. Composition of the milling fractions was determined to identify a high fiber fraction for possible use in food products. Red dog, the fraction highest in fiber, was chosen and evaluated in a metabolism study using cholesterol-fed weanling rats. The objective was to determine if lipid metabolism could be altered by different amounts of soluble and insoluble dietary fiber supplied by red dog from a non-waxy and waxy starch hull-less barleys.

CHAPTER 2

LITERATURE REVIEW

Hull-less and Waxy Hull-less Barley

The introduction and production of hull-less barley cultivars has many positive benefits for food. Processing costs are reduced since barley does not require dehulling and pearling to become a food product. Removal of the hull, seed coat, and aleurone layers of the kernel that occur during the pearling process decreases the amount of dietary fiber in the finished product. Chung (1982) demonstrated that the highest extract viscosity and dietary fiber concentration was obtained in the pearlings when ≈30% of the original kernel weight was removed by pearling.

Genetic removal of the hull decreases grain yield to the farmer but at least 50% of that loss can be accounted for by the fact that the hull is left in the field after combining. Concentration of nutrients such as protein, starch and β-glucans increases while cellulose decreases in hull-less barley due to the removal of the hull and a dilution effect (Hockett, 1981).

Insertion or mutation of barley to include the waxy gene on chromosome 1 has been shown to improve many of the

desirable qualities preferred in food barley. One difference between non-waxy and waxy barley is starch composition. Normal barley starch contains ≈30:70 ratio of amylose to amylopectin compared to waxy starch barley which contains up to 100% amylopectin (Goering and Eslick, 1976). Other compositional changes have been noted in barley cultivars with the waxy gene (C.W. Newman, unpublished data) such as an increase in fiber components, mainly β-glucans, and a decrease in starch. The decrease in starch is compensated by an increase in free sugars, especially maltose and sucrose. Studies performed in our laboratory showed waxy cultivars to be much more efficient in lowering plasma total cholesterol and LDL-cholesterol than non-waxy cultivars (Fadel et al., 1987). Other studies have shown that milling these waxy hull-less barleys produces flour, shorts, and bran fractions, with the shorts and bran fractions containing relatively high levels of soluble dietary fiber. Considerable effort is now being expended by research and development departments of several major cereal and milling companies to incorporate these into human food products (R.K. Newman, unpublished data). Thus these barleys may be the immediate choice by the pearling and milling industry for production of human food products.

Fiber and Lipid Metabolism

Coronary heart disease (CHD) is one of the major causes of death in the industrial nations of the world. One of the risk factors associated with CHD is elevated plasma LDL-cholesterol levels (LRCP, 1984). Research on the control of plasma cholesterol levels is extensive. Many different forms of controls have been investigated from dietary intervention to drug therapy (NHLBI, 1986). Dietary intervention of various types has been reported, from reduction of total and saturated fats to increased intake of soluble dietary fiber. The American Heart Association recommends that people reduce their fat intake to 30% of total calories and increase their dietary fiber intake to 30 $g*d^{-1}$. This recommendation of 30 $g*d^{-1}$ of dietary fiber does not describe the type of fiber.

Epidemiological studies have shown a relationship between inadequate consumption of total dietary fiber and cancer of the colon, hypercholesterolemia, atherosclerosis, and complications of diabetes, diverticulosis, constipation, hypertension, gallstones and obesity. Recently increased emphasis has been placed on the importance of soluble dietary fiber and its role in nutrition. To date, scientific evaluation of the efficacy of soluble dietary fiber in reducing the incidence of CHD and in explaining how total dietary fiber contributes to overall good health is still incomplete.

One component of the soluble dietary fiber issue is its influence on lipid metabolism. Soluble dietary fiber appears to be involved in regulating lipid metabolism in several different ways. The objective of this literature review is to give a overall description of the hypotheses of regulation of lipid metabolism by soluble dietary fiber.

Description

Dietary fiber has been defined as plant material unavailable for hydrolysis by the digestive enzymes of mammals. More recently, Theander (1989) defined dietary fiber as non-starch polysaccharides plus lignin. The understanding of the chemical structure of fiber and the relationship of its structure to function and effect on animal metabolism is far from complete.

Classification

In this review, total dietary fiber will be divided into: structural polysaccharides, structural nonpolysaccharides (lignin) and nonstructural polysaccharides. Total dietary fiber may also be classified as water soluble and insoluble dietary fiber under given conditions of the extraction procedure used in the assay. Insoluble fiber is made up mainly of structural polysaccharides and structural nonpolysaccharides. Soluble fiber is comprised mainly of

nonstructural polysaccharides and some structural nonpolysaccharides (Southgate, 1982).

Structural Polysaccharides

These are polysaccharides associated with the cell wall of cereal grains. Cellulose and a portion of the hemicelluloses are usually classified as insoluble dietary fiber. Wheat bran is ≈100% insoluble dietary fiber and is mainly cellulose. Fiber sources high in structural polysaccharides are usually associated with increases in fecal bulking (Jenkins et al., 1979; Kay and Truswell, 1977a, 1977b; Kirby et al., 1981; Miettinen and Tarpila, 1977; and Wrick et al., 1983). A portion of the hemicellulose in cereals is soluble and contributes to extract viscosity especially in rye (Fengler et al., 1990) and possibly barley (A.I. Fengler, unpublished data).

Structural Nonpolysaccharides

This grouping is made up of predominantly lignins and other phenolic compounds. These compounds are considered very inert, insoluble and resistant to enzymatic or other hydrolyses.

Nonstructural Polysaccharides

This class of dietary fiber includes gums, pectins, arabinoxylans, mucilages, and mixed-linked $(1\rightarrow3),(1\rightarrow4)-\beta-D-$glucans [CAS# 55965-23-6]. These fibers are soluble in water and are believed to be the active compounds decreasing plasma cholesterol, nutrient uptake, enzyme activity, and postprandial glycemic response (Klopfenstein, 1988; Reiser, 1979). Barley contains very high amounts of nonstructural polysaccharides, especially mixed linked β-glucans (Åman and Newman, 1986).

Important Physical Characteristics

Dietary fiber exhibits four major physical properties that are unique to its function (Kay, 1982). They are susceptible to bacterial degradation, water-holding capacity, adsorption of organic material, and cation exchange. These properties and their influence on physiologic function in animals will be considered in the following discussion.

Bacterial Degradation

Mammalian enzymes are unable to degrade dietary fiber, but the microflora in the large intestine and to some extent in the small intestine hydrolyze polysaccharides to varying degrees depending on the polysaccharide. Cellulose is only partially degraded; whereas, the more soluble nonstructural

polysaccharides are almost totally digested by the microbes. Microbial degradation leads to the formation of volatile short chain fatty acids (VFAs)(Pomare et al.,1985). The possible mechanism of action of VFAs will be discussed later in more detail. Bacterial cells can account for a significant portion of fecal weight and contribute to fecal bulk.

Water-Holding Capacity

This physical property is enhanced in polysaccharides by the presence of sugar residues with free polar groups. Hydration of these fibers forms a gel matrix which can increase intestinal viscosity. Increase in viscosity decreases gastric emptying rate (Schwartz et al., 1982) and nutrient absorption by reducing diffusion through the unstirred water layer to the absorptive surfaces of the small intestine (Schneeman, 1982).

Adsorption of Organic Molecules

Important organic molecules have been shown to adsorb to fiber in vitro (e.g. bile acids, bile salts, cholesterol, and some toxic compounds). In vivo studies with animals have shown a fiber induced increase in fecal bile acids and steroid excretion (Anderson and Chen, 1986). This is one of the possible mechanisms by which fiber may reduce plasma cholesterol.

Cation Exchange

The number of free carboxyl groups and uronic acid content of polysaccharides appears to be related to its ability to bind minerals and make them unavailable for absorption. This mechanism will not be considered in detail in this thesis.

Physiologic Effects

Gastrointestinal Effects

Soluble fiber decreases the rate of absorption and/or availability of minerals, proteins, and lipids (Imaizumi and Sugano, 1986). Increases in GI tract viscosity may reduce enzyme ability to interact with substrate, reduce the chances of nutrients coming in contact with the unstirred water layer and being absorbed, and impair micelle formation, which would decrease lipid absorption and bile acid recirculation. Soluble fiber may influence the absorption of lipids by mechanisms other than viscosity effects on the convective movements in the gut. These mechanisms include reduction of bile acid recirculation, digestive enzyme concentration, α-amylase activity, and impaired chylomicron formation (Vahouny and Cassidy, 1986). Long-term effects of soluble fiber may include changes in small intestine morphology (Cassidy et al., 1981). Diets supplemented with pectin resulted in flattening

of the villus structure in the rat and a decrease in brush border enzyme concentration. Soluble fiber may also alter the rate and site of absorption of lipids from the gastrointestinal tract. This hypothesis was supported by the data of Schneeman et al. (1984) where rats fed oat bran and guar gum showed increases in the percentage of plasma HDL-associated apoprotein (apo A-1), compared to rats fed wheat bran.

In vitro bile acid adsorption by dietary fiber has been reported for many types of fiber (Story, 1986). These findings along with in vivo studies, show that an increase in soluble fiber consumption results in a greater fecal bile acid and neutral steroid excretion (Anderson and Chen, 1986). This fecal loss reduces efficiency of enterohepatic re-circulation of bile acids and places a drain on hepatic cholesterol to meet the need for de novo bile acid synthesis. The amount of cholesterol available for hepatic lipoprotein synthesis is therefore decreased. Another hypothesis is that the reduction of hepatic cholesterol, resulting from increased fecal bile acid excretion, may lead to an increase in hepatic LDL receptors with resultant facilitated removal of circulating cholesterol by the liver (Shepard et al., 1980).

Colon-Related Effects

Dietary fiber was considered for a long period of time to be an inert component of the diet that passed through the digestive tract unaltered. It is now recognized that the lower gastrointestinal tract is an effective fermentation compartment, inhabited by a mixed anaerobic microbial population. Bacteroides species in the colon have significant fermenting ability for soluble fiber substrates (Salyers et al., 1977a). Human anaerobic bacteria also appear to ferment various gums, including xylans, noncellulosic glucans, pectins, galactomannans, and arabino-galactans (Salyers et al., 1977b). Anaerobic fermentation of these polysaccharides yields primarily VFAs - acetic, propionic, and butyric (Smith and Bryant, 1979). Volatile fatty acids produced in the colon can be absorbed from the colon and enter the portal circulation (Cummings, 1981). Propionate is reported to be an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), the rate limiting enzyme in hepatic cholesterol synthesis (Ide et al., 1978), and has been shown to reduce serum cholesterol when fed to rats (Chen et al., 1984).

CHAPTER 3

HIGH FIBER MILLING FRACTIONS

Summary

Two hull-less barley cultivars; AZ-76-19-7 (AZ) (non-waxy) and AZ-76-19-7-WX (AZWX) (waxy) were milled through a MIAG multomat 8-roller experimental mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA 99164). All barleys were tempered to 13% moisture for 12 h prior to milling. Feed rate for all barleys was 900 $g*h^{-1}$. Streams were collected and 2nd break flour through 4th middlings were combined and labeled flour (2b-4m). Fractions were analyzed for insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total β-glucans, starch, molecular weight (MW) of extractable dietary fiber, viscosity, ether extract, ash, and crude protein (N*6.25). Soluble dietary fiber and total β-glucans were concentrated in break shorts, red dog, and bran in both barleys. The red dog fraction of AZ and AZWX were highest in SDF (7.28 and 12.48%), IDF (12.82 and 18.61%), viscosity (7.13 and 24.5cP) and total β-glucans (9.58 and 13.4%), respectively. The waxy starch barley cultivar and its milling fractions were higher in SDF compared to the non-waxy cultivar and its comparable milling fractions. Molecular

weight distributions of the SDF fractions extracted in water at 37° C were similar between AZ and AZWX barleys. Ether extract, ash, and crude protein from 1st break flour and flour (2b-4m) ranged from .3 to 1.21%, 1.26 to 1.68%, and 10.40 to 14.51% respectively and were lower compared to the high fiber fractions from both barleys.

Introduction

The search for new high soluble dietary (SDF) fiber food sources that are easily and economically produced is a continual process. Various forms of milling have been employed throughout history to concentrate specific components in grains, such as protein and/or starch. Barley has been milled in the past to produce flour for baking purposes and ground to increase the digestibility of the whole grain to animals. In this examination of milling barley, the goal was to identify a milling fraction high in total dietary fiber (TDF) and SDF. Recent attention has been focused on hypocholesterolemic foods, particularly oat bran. Research scientists in public institutions and the private food industry have been looking for additional sources of soluble dietary fiber that are in a natural form, easy to produce and have the ability to reduce plasma cholesterol. Whole barley is naturally high in soluble dietary fiber and β-glucans which have been shown to reduce plasma cholesterol. Data obtained in a pilot study in our laboratory using a 6-roller Buhler

mill to process a hull-less barley, indicated that soluble dietary fiber was concentrated in the shorts fraction. Due to the lack of size and sophistication of the small Buhler mill, it was necessary to utilize a pilot mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA 99164).

Materials and Methods

Materials

Two hull-less barley cultivars, AZ-76-19-7 (AZ) and its waxy starch mutant daughter line, AZ-76-19-7-WX (AZWX) developed by Dr. Tom Ramage (USDA, ARS, University of Arizona, Tucson, AZ.), were selected for milling. Pilot studies in these laboratories demonstrated the efficacy of these barleys in lowering plasma cholesterol in cholesterol-fed chicks. AZWX is a chemical mutation from the parent line resulting in the starch being made up of $\approx 100\%$ amylopectin. The major differences in chemical composition between the two barleys are increased mixed linked $(1\rightarrow 3),(1\rightarrow 4)-\beta$-D-glucans and a decrease in starch in AZWX compared to AZ.

Milling

Milling was performed on a MIAG multomat 8-roller dry mill at the USDA Western Wheat Quality Laboratory (Washington State University, Pullman, Wa 99164). The milling flow sheet

is shown in Figure 1. The barleys were tempered to 13% moisture for 12 h prior to milling. Feed rate was set at 900 $g*m^{-1}$ on a vibrating feeder. Rollers were adjusted by a trial and error method to obtain a flour yield of approximately 50%. Fractions were collected, weighed, and bagged individually except that 2nd break flour through 4th middlings were combined, sifted and will be referred to as flour (2b-4m). Six fractions that were collected included: 1st break flour, flour (2b-4m), 5th middlings, red dog, break shorts, and bran.

Analysis

All milling fractions were analyzed for Kjeldahl protein, dry matter, ether extract, starch (Åman and Hesselman, 1984), total β-glucans (McCleary, 1988), insoluble dietary fiber (IDF) and SDF (Prosky et al., 1984). Aqueous extracts were obtained from autoclaved samples of each milling fraction to determine viscosity and molecular weight distribution (MWD) of the soluble fiber. Extraction conditions were: 4 g of each barley fraction after autoclaving was extracted by suspending 5 g in 100 ml of water then homogenized to insure uniform mixing. Suspensions were then placed in a rotating incubator at 37°C and shaken for 2 h. Solutions were then placed into 250 ml centrifuge bottles and centrifuge at 10,000 rpm for 20 m. The supernate was decanted and analyzed. Viscosity was determined with a Haake falling ball viscometer at 30°C.

Soluble dietary fiber extract MWD was determined by high performance size exclusion chromatography (HPSEC). The MWDs were determined by aqueous elution on a Beckman 5000-PWHR size exclusion column which contained beads with 1000 Å pores. Separation was carried out at 0.8 ml*m⁻¹ from a Waters 501 pump, the column was heated to a constant temperature of 35°C with a Waters TCM, and detection was by a Waters R-401 refractive index at 2x and negative polarity. Standards used were dextran fractions of specific molecular weight ranges T2000, T100, T10 (Pharmacia, Uppsala, Sweden), and α-D-glucose.

Results and Discussion

Analysis of the six milling fractions are shown in Table 1. Yields were approximately 50% flour and 20% red dog, with the remainder being shorts and bran. The red dog fraction had the highest concentration of SDF, mixed linked $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucans, and extract viscosity in both barleys. A greater percentage of SDF was found in each milling fraction from AZWX compared to AZ barley, whereas IDF was lowest in 1st break, flour (2b-4m), and break shorts but was higher in the red dog and bran from AZ. Percentages of β-glucans were greatest in each fraction of AZWX with the exception of the 5th middlings fraction which was about the same in both barleys. Viscosity measurements tended to increase with the increase of β-glucans and were highest in AZWX fractions except 5th middlings.

Viscosity of AZWX red dog was 86% greater than that of AZ red dog whereas β-glucans were only increased by 40%. Molecular weight distribution of the soluble fiber between all six milling fractions and both barleys were similar. Of the six fractions, the red dog from both barleys were lowest in starch (Table 2) and the percentage of starch in the various fractions varied between the two barleys. The fraction with the lowest concentration of fiber and ash, but the highest concentration of starch was 1st break flour and flour (2b-4m) from each barley. Protein and ether extract were highest in the non-flour fractions of both barleys (Table 3).

The differences in the milling characteristics of the two barleys is illustrated by the variation in the starch composition of the various milling fractions. This variation was great enough to influence the concentration of other components in some instances. It could be seen from a visual appraisal that a greater amount of the endosperm remained attached to the AZWX bran compared to the AZ bran. The greater percentage of TDF in the AZ bran compared to the AZWX bran (31.25 vs. 18.57%) may be partially explained by the greater percentage of starch in the latter fraction which acted as diluent. However, using the same logic, both the IDF and the SDF would have been increased in the AZWX bran making the IDF more equal between the two brans but increasing the difference in SDF. A similar relationship can be seen between TDF and starch in the 5th middling fraction, whereas in the

red dog fractions from the two barleys there was a large difference in the percentage of starch and no difference in TDF. Had the starch in the two red dog fractions been similar, the AZWX red dog would have contained a greater percentage of TDF compared to the AZ red dog. Thus it can be reasoned that by improving the milling technique to reduce the starch content in the traditional high fiber milling fractions, i.e. bran, red dog and break shorts, significant increases in β-glucans and extract viscosity would also occur in these fractions.

The comparative differences in milling characteristics of these two barleys that differed primarily in starch type, illustrate the necessity for further milling experimentation. The commercial cereal industry's interest in cereal products that are high in soluble fiber components, especially β-glucans, justifies more intensive research in this area. It is recognized that results of this study were obtained from only two barleys that were raised in one environment. Other waxy and non waxy hull-less barleys raised under different environments and years must be compared to determine if the same trends in milling characteristics occur. Additionally, different milling techniques should be investigated with these barleys to improve flour yields and further concentrate the dietary fiber components.

FIGURE 1. MILLING FLOW SHEET FOR MIAG MULTOMAT 8-ROLLER DRY
MILL (WESTERN WHEAT QUALITY LABORATORY, WASHINGTON STATE
UNIVERSITY, PULLMAN, WA 99164) Sieve cloth openings in
microns.
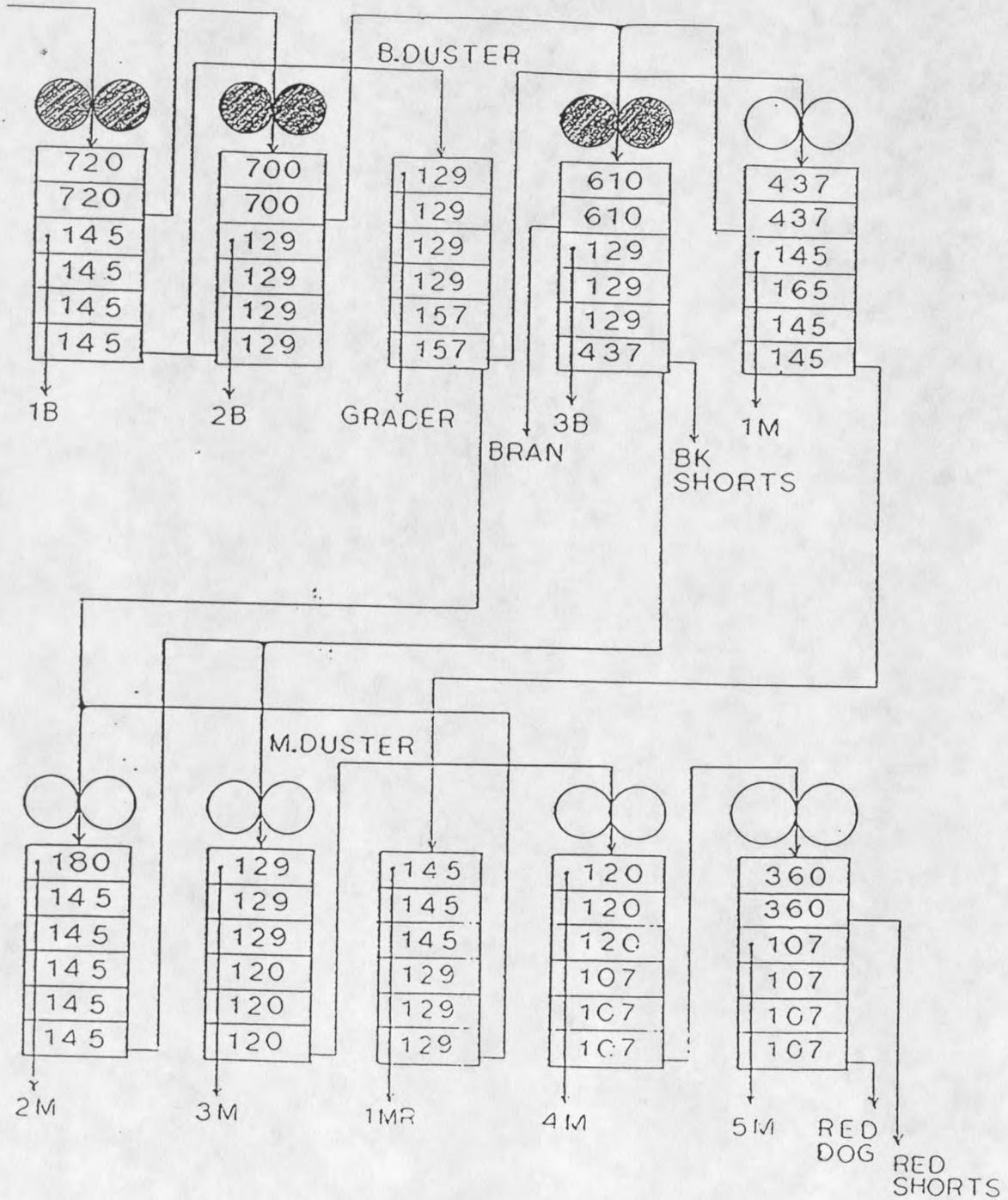
TABLE 1. DIETARY FIBER, β-GLUCAN CONCENTRATION AND EXTRACT VISCOSITY OF MILLING FRACTIONS FROM HULL-LESS AND WAXY HULL-LESS BARLEY, (DMB)

ITEM:[a]		SDF	IDF	TDF	β-GLUCAN	V I S C
BARLEY[b]	FRACTION[c]			%		cP
AZ	1B	1.46	2.38	3.84	2.42	1.60
	2B-4M	1.62	2.92	4.54	3.20	2.17
	5M	3.72	6.18	9.90	4.82	3.01
	Red dog	7.96	20.34	28.30	10.47	7.13
	Break short	6.83	8.99	15.83	6.85	4.15
	Bran	5.52	25.73	31.25	6.62	3.45
AZWX	1B	2.21	4.37	6.57	4.12	1.96
	2B-4M	4.32	4.32	8.64	4.01	2.26
	5M	4.38	5.35	9.73	4.94	2.78
	Red dog	13.64	14.01	27.65	14.64	13.24
	Break short	8.57	10.99	19.57	11.04	5.61
	Bran	6.55	12.01	18.57	10.69	6.08

[a]SDF = Soluble dietary fiber; IDF = Insoluble dietary fiber and VISC = Neutral extract viscosity.

[b]AZ = AZ-76-19-7 and AZWX = AZ-76-19-7-WX.

[c]B = Break flour and M = Middlings.

TABLE 2.   STARCH AND ASH CONCENTRATIONS OF MILLING FRACTIONS
           FROM HULL-LESS AND WAXY HULL-LESS BARLEY, (DMB)

Item:		Starch	Ash
BARLEY[a]	FRACTION[b]	———————%———————	
AZ	1B	75.09	1.26
	2B-4M	68.25	1.55
	5M	52.66	2.71
	Red dog	30.05	4.15
	Break short	58.36	2.08
	Bran	36.73	4.30
AZWX	1B	66.52	1.68
	2B-4M	70.44	1.54
	5M	56.39	2.06
	Red dog	42.30	2.30
	Break short	46.76	2.16
	Bran	47.32	2.25

[a]AZ = AZ-76-19-7 and AZWX = AZ-76-19-7-WX.

[b]B = Break flour and M = Middlings.

TABLE 3. PROTEIN AND ETHER EXTRACT CONCENTRATIONS OF MILLING FRACTIONS FROM HULL-LESS AND WAXY HULL-LESS BARLEY, (DMB)

Item:		Protein	Ether Extract
BARLEY[a]	FRACTION[b]	———————%———————	
AZ	1B	10.40	.34
	2B-4M	13.61	.77
	5M	20.41	3.91
	Red dog	19.56	4.37
	Break short	15.96	2.08
	Bran	16.65	2.15
AZWX	1B	12.32	1.01
	2B-4M	14.51	1.21
	5M	16.99	3.90
	Red dog	16.07	2.51
	Break short	16.09	3.13
	Bran	15.31	3.00

[a]AZ = AZ-76-19-7 and AZWX = AZ-76-19-7-WX.

[b]B = Break flour and M = Middlings.

Conclusion

Milling of waxy and non-waxy hull-less barleys in a wheat type dry roller mill produced high-fiber fractions that have potential as fiber sources for the food industry. These milling fractions will not require FDA clearance as they are natural products from edible plant materials.

Recent literature indicates that barley β-glucans are found in the endosperm cell walls (Woodward et al., 1983; Fincher and Stone, 1986; Wood, 1986; Åman and Graham, 1987). If the highest concentration of β-glucans is found in endosperm cell walls, the 1st break flour, which is only endosperm, should have had the highest concentration of β-glucans. However, the highest concentrations of SDF and β-glucans were found in the shorts and red dog fractions which originated primarily from the aleurone layers of the kernel. These data suggest that β-glucans in these barleys were more concentrated in the aleurone layers and not in the endosperm cell walls.

CHAPTER 4

METABOLIC RESPONSE OF CHOLESTEROL-FED WEANLING RATS FED DIFFERING AMOUNTS OF BARLEY SOLUBLE AND INSOLUBLE DIETARY FIBER

Summary

Thirty-five male weanling Holtzman rats were fed diets containing high fiber milling fractions of hull-less barleys with and without supplemental β-glucanase and a corn-starch casein diet containing alphacel. All diets were balanced for 20% total dietary fiber (TDF), 16% protein, and the first five limiting essential amino acids. Rats were provided unlimited access to feed and water. Barleys studied were AZ and its waxy starch mutant, AZWX. Barleys were milled in a MIAG 8-roller dry mill at the Western Wheat Quality Laboratory (Washington State University, Pullman, WA 99164). The milling fraction selected was red dog from both AZ and AZWX which contained 20.34% and 14.01% insoluble dietary fiber and 7.96% and 13.64% soluble dietary fiber, respectively. Rats fed red dog diets with and without β-glucanase had no significant differences (P > .10) in average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (FG). Rats fed the corn starch and casein diet were not significantly different (P > .10) in ADG from those fed red dog diets, but

were significantly higher (P < .05) in ADFI and FG. Rats fed
the control diet had significantly higher (P < .01) plasma
total cholesterol, triacylglycerol, and LDL-cholesterol. Dry
matter was significantly (P < .05) higher in feces from rats
fed the control diet, while fecal ether extract was
significantly lower (P < .05). These data demonstrate that
barley soluble dietary fiber had a marked effect on lipid
metabolism and fecal components compared to insoluble dietary
fiber. Based on these data, weanling rats are capable of
efficiently using nutritionally complete diets with 20% TDF
from barley for growth while experiencing marked changes in
lipid metabolism.

Introduction

Whole barley has been shown to reduce plasma total and
LDL-cholesterol in humans, rats, and chicks (Schneeman, 1986;
Ink and Hurt, 1987; Fadel et al., 1987; Newman et al., 1989).
Waxy hull-less cultivars have shown a greater efficacy in
lowering cholesterol than non-waxy types (Fadel et al.,1987),
but no investigations have been reported that examined the
efficacy of milling fractions of waxy or non-waxy hull-less
barley. Determination of a high dietary fiber milling
fraction from dry milling of waxy hull-less barley has not
been reported prior to this research (Chapter 3). A high
fiber fraction produced from oat milling to produce oat flour,
is defined as bran. Oat bran has a higher concentration of

soluble dietary fiber (SDF) compared to whole grain oat groats as well as a greater concentration of total dietary fiber (TDF). Rats fed oats and oat bran have also been shown to have lower plasma total and LDL-cholesterol and increased bile acid concentration in feces (Anderson and Chen, 1986). Increases in bile acid, neutral steroids, and other lipids found in feces decreases the efficiency of enterohepatic recirculation of bile acids, therefore causing a drain of the cholesterol pool (Story, 1986). Increases in SDF also increase fermentation in the caecum causing an increase in VFA production which may decrease hepatic cholesterol synthesis (Salyers et al., 1977a; Salyers et al., 1977b; Cummings, 1981; Chen et al., 1984).

The objective of this study was to determine the efficacy of a select milling fraction, red dog, from a non-waxy hull-less barley and its waxy starch daughter cultivar compared to a control diet containing an equal amount of TDF but void of SDF.

Materials and Methods

Five diets were formulated differing in quantity of SDF (Table 4). The fiber source used in four of the diets was the red dog milling fraction separated by an 8-roller wheat mill. Barleys milled were AZ, a hull-less barley, and AZWX, the daughter line to AZ which has a chemical mutation on chromosome 1 producing waxy starch. The red dog fraction was

chosen because it contained the highest concentration of SDF and β-glucans of the six different milling fractions (Chapter 3). All five diets were balanced to provide 16% crude protein, 19.3% TDF, and the requirement of the first five limiting essential amino acids. Two similar diets were prepared from each barley milling fraction with and without supplemental β-glucanase. A control diet was prepared from casein and corn starch with alphacel, an insoluble dietary fiber (IDF)(cellulose), as the fiber source. Dietary fiber was analyzed by the AOAC approved gravimetric method of Prosky et al.(1984).

Thirty-five male weanling Holtzman rats (initial weight = 72.33 g) were individually housed in suspended stainless steel wire mesh cages for a 24 d feeding period. Seven animals were assigned to each of five diets. They were allowed unlimited access to food and water. Body weight and feed intake were measured every 7 d and feed was replenished as needed during the course of the study. Average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (FG) were calculated for each rat for the 24 d period.

Thirty rats were randomly chosen for a 5 d metabolism study from d 18 to d 23 with equal numbers represented from each diet (n=6). Rats were housed in individual stainless steel metabolism cages fitted with screens and funnels to allow separate collection of feces and urine. Rats were allowed free access to food. Fresh water was provided daily

and waste feed was recorded. Total fecal collection for each day was combined and stored at -20° C until analyzed. Fecal dry matter was determined by lyophilization. Freeze-dried

TABLE 4. COMPOSITION OF DIETS FOR WEANLING RATS FED BARLEY RED DOG OR CORN STARCH - CASEIN - ALPHACEL[a], (DMB)

| Barley[b] | AZWX | | AZ | | Control |
Enzyme[c]	(-)	(+)	(-)	(+)	(-)
Item:			—%—		
Red dog	70.30	70.30	67.80	67.80	.00
Corn starch	13.21	13.16	17.62	17.57	50.80
Casein	5.40	5.40	2.90	2.90	19.38
Alphacel	.00	.00	.00	.00	19.30
Lard	5.00	5.00	5.00	5.00	5.00
AIN 76 min	3.50	3.50	3.50	3.50	3.50
AIN 76 vit	1.00	1.00	1.00	1.00	1.00
Choline	.20	.20	.20	.20	.20
Cholesterol	.50	.50	.50	.50	.50
Methionine	.40	.40	.48	.48	.07
Lysine	.24	.24	.45	.45	.00
Threonine	.00	.00	.15	.15	.00
Arginine	.00	.00	.15	.15	.00
β-Glucanase[d]	.00	.05	.00	.05	.00

[a]Diets were balanced for 16% crude protein and 19.3% TDF.

[b]AZWX = AZ-76-19-7-WX and AZ = AZ-76-19-7.

[c](-) = without supplemental β-glucanase and (+) = with supplemental β-glucanase.

feces were ground in a coffee mill for analysis. Ether extract was determined after acid hydrolysis with 2 N HCl. Apparent lipid digestibility was calculated by the difference of that ingested and that found in the feces. Rats were sedated with thiamylal sodium (Bio-Tal®) and blood samples drawn via heart puncture. Rats were sacrificed by cervical dislocation, livers removed, weighed, and immediately frozen

until analyzed for cholesterol. Plasma was separated from the whole blood in EDTA vacuum tubes and plasma lipids determined on a Kodak DT60 blood analyzer.

Data were analyzed by General Linear Model procedure and when effects were significant (P < .05) means were separated by least squares means PDIFF (SAS, 1987).

Results

AZWX red dog has a SDF:TDF ratio of .49 compared to .28 in AZ, its parent cultivar (Chapter 3). Their TDF values were very similar, 28.30% and 27.65% for AZ and AZWX, respectively. Due to this difference in the ratio of SDF to TDF between the red dogs of the two cultivars the diets made from each contained different amounts of SDF. The SDF:TDF ratio were .40, .26, and .01 for AZWX, AZ, and control diets, respectively (Table 5). Protein and ether extract levels were similar across diets, closely approaching the calculated levels for these nutrients.

Rats fed red dog diets with and without β-glucanase had no differences (P > .10) in ADG, ADFI, or FG (Table 6). No difference (P > .10) in ADG was detected between control and red dog fed rats. Control rats, however, had higher (P < .05) ADFI and consequently higher FG.

Rats fed the control diet had higher (P < .05) plasma cholesterol, LDL-cholesterol, liver weight and liver cholesterol than those fed barley red dog diets (Table 7).

TABLE 5.   FIBER COMPOSITION OF BARLEY RED DOG AND CORN STARCH
          - CASEIN - ALPHACEL DIETS[a], (DMB)

Barley[b]	AZWX		AZ		Control
Enzyme[c]	(-)	(+)	(-)	(+)	(-)
Item:[d]			%		
IDF	10.80	10.77	14.51	14.07	18.90
SDF	7.48	6.96	4.80	5.35	0.26
TDF	18.28	17.73	19.31	19.42	19.16

[a]Diets were balanced for 16% crude protein and 19.3% TDF.

[b]AZWX = AZ-76-19-7-WX and AZ = AZ-76-19-7.

[c](-) = without supplemental β-glucanase and (+) = with supplemental β-glucanase.

[d]IDF = Insoluble dietary fiber; SDF = Soluble dietary fiber; TDF = Total dietary fiber.

TABLE 6.   GROWTH PERFORMANCE OF WEANLING RATS FED DIFFERING
          LEVELS OF BARLEY SOLUBLE DIETARY FIBER

Barley[a]	AZWX		AZ		Control	CV%
Enzyme[b]	(-)	(+)	(-)	(+)	(-)	
Item:						
No rats	7	7	7	7	7	
ADG, g	8.66	8.35	8.73	8.48	8.52	8.67
ADFI, g	18.47[c]	17.70[c]	18.40[c]	18.30[c]	20.34[d]	8.31
F/G	2.14[c]	2.12[c]	2.11[c]	2.18[c]	2.39[d]	8.30

[a]AZWX = AZ-76-19-7-WX and AZ = AZ-76-19-7.

[b](-) = without supplemental β-glucanase and (+) = with supplemental β-glucanase.

[cd]Values in same row with different superscripts differ significantly (P<.05) due to diet.

HDL-cholesterol was lowest (P < .05) in rats fed the control diet and highest (P < .05) in those fed the AZ diets with the intermediate values for those fed AZWX regardless of enzyme supplementation.

TABLE 7. PLASMA AND LIVER CHOLESTEROL LEVELS OF WEANLING RATS FED DIFFERING LEVELS OF BARLEY SOLUBLE DIETARY FIBER

Barley[a]	AZWX		AZ		Control	CV%
Enzyme[b]	(-)	(+)	(-)	(+)	(-)	
Item:[c]						
No. rats	7	7	7	7	7	
Chol (mg/dl)	91.43[d]	91.43[d]	99.43[d]	90.57[d]	204.29[e]	34.22
TAG (mg/dl)	159.86	117.00	118.29	100.00	181.00	53.58
HDL-C (mg/dl)	56.00[d]	57.29[d]	63.86[e]	61.14[e]	46.00[d]	21.41
LDL-C (mg/dl)	3.46[d]	10.74[d]	11.91[d]	9.43[d]	122.09[e]	103.25
Liver Wt (g)	9.80[d]	9.71[d]	9.70[d]	10.03[d]	11.62[e]	12.64
Liver Chol (mg/g)	9.28[d]	10.01[d]	10.23[d]	9.71[d]	28.22[e]	43.18

[a]AZWX = AZ-76-19-7-WX and AZ = AZ-76-19-7.

[b](-) = without supplemental β-glucanase and (+) = with supplemental β-glucanase.

[c]Chol = Plasma Cholesterol; TAG = Triacylgycerol; HDL-C = High Density Lipoprotein - Cholesterol; and LDL-C = Low Density Lipoprotein - Cholesterol.

[de]Values in same row with different superscripts differ significantly (P<.05) due to diet.

Fecal wet weights were higher (P < .01) for rats fed AZ red dog with or without supplemental β-glucanase (Table 8). Fecal dry matter was not different between AZ red dog with or without enzyme, between AZ with enzyme and AZWX without, and between AZWX with or without enzyme. Feces from rats fed the control diet were higher (P < .01) in dry matter than those fed any barley diet. Feces from rats fed various diets differed (P < .01) in fecal ether extract (Table 8), with that from the control being the lowest, followed by AZ with enzyme, AZ without enzyme and AZWX with enzyme, and AZWX without enzyme.

TABLE 8. FECAL WEIGHT, FECAL DRY MATTER, FECAL ETHER EXTRACT, AND LIPID DIGESTIBILITY OF WEANLING RATS FED DIFFERING LEVELS OF BARLEY SOLUBLE DIETARY FIBER

Barley[a] Enzyme[b]	AZWX		AZ		Control	CV%
	(−)	(+)	(−)	(+)	(−)	
Item:						
No. rats	6	6	6	6	6	
Feces Wt (g)	41.28[d]	37.06[d]	57.97[e]	56.67[e]	34.88[d]	.62
Fecal DM%	48.52[ef]	52.37[f]	41.05[d]	42.86[de]	79.41[g]	.87
Fecal EE%	7.00[g]	5.99[f]	5.86[f]	4.92[e]	3.33[d]	.83
Apparent Fat Dig[c]	85.30[ef]	84.95[e]	83.61[d]	86.17[fg]	86.81[g]	.61

[a]AZWX = AZ-76-19-7-WX and AZ = AZ-76-19-7.

[b](−) = without supplemental β-glucanase and (+) = with supplemental β-glucanase.

[c]Apparent Fecal Lipid Digestibility. Five day total fecal collection.

[defg]Values in same row with different superscripts differ significantly (P<.05) due to diet.

Apparent lipid digestibility was highest and not different (P > .05) between the control diet and AZ with enzyme. AZ with enzyme and AZWX without enzyme were similar (P > .05), also AZWX with and without enzyme were not different (P > .05). AZ without enzyme had the lowest (P < .01) apparent lipid digestibility.

Discussion

The data showed no differences in rat ADG due to diet indicating lipid data were not biased by end weight differences of the rats. Plasma lipid data demonstrated that rats fed 4.8 - 7.5% SDF had similar reductions in plasma cholesterol, and LDL-cholesterol compared to control fed rats. The lack of differences in plasma cholesterol in the barley red dog diets may have been due to the high level of SDF and β-glucans in all diets and/or the possible presence of α-tocopherols in the red dog ether extract. Fecal dry matter suggested differences in physiologic response to IDF and SDF. Rats fed the control diet which contained almost 100% IDF had the highest dry fecal matter in comparison to those fed diets high in SDF. Fecal ether extract was lowest from rats fed the high IDF diet and highest in the feces from those fed high SDF diets which supports the hypothesis that SDF decreases the availability of dietary lipid for digestion and absorption. This hypothesis is further substantiated by the apparent lipid digestibility data. The control diet containing almost no SDF

had higher apparent lipid digestibility while diets with the highest SDF had the lowest apparent lipid digestibility.

Conclusion

Rats were able to utilize diets containing $\approx 20\%$ TDF for growth. A barley milling fraction from two cultivars that were high in SDF was highly capable of reducing plasma lipids, liver cholesterol, and apparent lipid digestibility in cholesterol-fed rats compared to a control diet high in IDF. This fraction has the potential for use in breads and other cereal products.

REFERENCES CITED

Aman, P. and H. Graham. 1987. Mixed-linked β-(1→3),(1→4)-D-glucans in the cell walls of barley and oats: Chemistry and nutrition. Scand. J. Gastroenterol. 22:42.

Aman, P. and K. Hesselman. 1984. Analysis of starch and other main constituents of cereal grains. Swedish J. Agric. Res. 14:135.

Aman, P. and C.W. Newman. 1986. Chemical composition of some different types of barley grains in Montana, USA. J. Cereal Sci. 4:133.

Anderson, J.W. and W.J.L. Chen. 1986. Cholesterol-lowering properties of oat products. In "Oat Chemistry and Technology," ed. F. Webster. Amer. Assoc. Cereal Chem., Minneapolis, MN.

Chen, W.J.L., J.W. Anderson, and D. Jennings. 1984. Propionate may mediate the hypocholesterolemic effect of certain soluble plant fibers in cholesterol-fed rats, Proc. Soc. Exper. Biol. Med. 175:215.

Chung, Tae Young. 1982. Isolation, description, associated traits and possible uses of three barley (Hordeum vulgare L.) starch mutants. Ph.D. Thesis. Montana State University.

Cummings, J.H. 1981. Short-chain fatty acids in the human colon. Gut 22:763.

Fadel, J., R.K. Newman, C.W. Newman, and A.E. Barnes. 1987. Hypocholesterolemic effects of beta-glucans in different barley diets fed to broiler chicks. Nutr. Rep. Int. 35:1049.

Fengler, A.I., F.X. Aherne, and A.R. Robblee. 1990. Influence of germination of cereals on viscosity of their aqueous extracts and nutritive value. Animal Feed Sci. and Technol. 28:243.

Fincher, G.B. and B.A. Stone. 1986. Cell walls and their components in cereal grain technology. Adv. Cereal Sci. Technol. 8:207.

Goering, K.J. and R.F. Eslick. 1976. Barley Starch. VI. A self liquefying waxy barley starch. Cereal Chem. 53:174.

Hockett, E.A.. 1981. Registration of hull-less and hull-less short-awned spring barley germplasm. Crop Sci. 21:146.

Ink, S.L. and H.D. Hurt. 1987. Nutritional implications of gums. Food Technol. 41:77.

Jenkins, D.J.A., D. Reynolds, A.R. Leeds, A.L. Waller, and J.H. Cummings. 1979. Hypocholesterolemic action of dietary fiber unrelated to fecal bulking effect. Am. J. Clin. Nutr. 32:2430.

Kay, R.M. and A.S. Truswell. 1977a. The effect of wheat fibre on plasma lipids and faecal steroid excretion in man. Brit. J. Nutr. 37:227.

Kay, R.M. and A.S. Truswell. 1977b. Effect of citrus pectin on blood lipids and fecal steroid excretion in man. Am. J. Clin. Nutr. 30:171.

Kirby, R.W., J.A. Anderson, B. Sieling, E.D. Rees, W.L. Chen, R.E. Miller, and R.M. Kay. 1981. Oat bran selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. Am. J. Clin. Nutr. 34:824.

Klopfenstein, C. 1988. The role of cereal β-glucans in nutrition and health. Cereal Foods World 33:865.

Lipid Research Clinics Program. 1984. J. Am. Med. Assoc. 251:351.

MacKey, J. 1981. Cereal production. pp. 5-23. In: Cereals a Renewable Resource: Theory and Practice, Y. Pomeranz and L. Munck, ed. Amer. Assoc. Cereal Chem., St. Paul.

McCleary, B.V., I. Shameer, and M. Glennie-Holmes. 1988. Measurement of (1→3),(1→4)-β-D-glucan. Methods Enzymol. 160:545.

Miettinen, T.A. and S. Tarpila. 1977. Effects of pectin on serum cholesterol, fecal bile acids, and biliary lipids in normolipidemic individuals. Clin. Chem. Acta 79:471.

National Heart Lung and Blood Institute. 1986. National cholesterol education program. Cholesterol Counts: Steps for lowering your patient's blood cholesterol. NIH, Washington, D.C.

Newman, R.K., C.W. Newman, and H. Graham. 1989. The hypocholesterolemic function of barley β-glucans. Cereal Foods World 34:883.

Poehlman, J.M. 1985. Adaptation and distribution. pp. 2-17. In: Barley, D.C. Rasmusson, ed. Agronomy Monograph. No. 26.

Pomare, E.W., W.J. Branch, and J.H. Cummings. 1985. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. J. Clin. Invest. 75:1448.

Prosky, L., N. Asp, I. Furda, J.W. DeVries, T.F. Schweizer, and B.F. Harland. 1984. Determination of total dietary fiber in foods, food products, and total diets: Interlaboratory study. J. Assoc. Off. Anal. Chem. 67:1044.

Reiser, S. 1979. Effects of dietary fiber on parameters of glucose tolerance in humans. In: Dietary Fiber: Chemistry and Nutrition, G.E. Inglett and S.I. Falkehag, ed. Academic Press, N.Y.

Salyers, A.A., J.R. Vercellotti, S.E.H. West, and T.D. Wilkins. 1977a. Fermentation of mucin and plant polysaccharides by strains of Bacteroides from the human colon. App. Enviro. Microbiol. 33:319.

Salyers, A.A., S.E.H. West, J.R. Vercellotti, and T.D. Wilkins. 1977b. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. App. Enviro. Microbiol. 33:529.

SAS. 1987. Statistical Analysis Systems Institute, Inc. Cary, North Carolina.

Schneeman, B.O. 1982. Pancreatic and digestive function. p. 73. In: Dietary Fiber in Health and Disease, G.V. Vahouny and D. Kritchevsky, ed. Plenum Press, N.Y.

Schneeman, B.O. 1986. Dietary Fiber: Physical and chemical properties, methods of analysis, and physiological effects. Food Technol. 40:104.

Schwartz, S.E., R.A. Levine, A. Singh, J.R. Schreidecker, and N.S. Track. 1982. Sustained pectin ingestion delays gastric emptying. Gastroenterology 83:812.