Suggested Short Title:

PARTICLE SIZE FRACTIONATION AND

EVALUATION OF FRACTIONS OF BSG

This thesis is dedicated to all the teachers who instilled in me the love of chemistry.

ABSTRACT

Dried brewers' spent grain (DBSG), not-ground, was subjected to a fractionation procedure based on particle size. In addition, the DBSG was milled before the fractionation. The DBSG was fractionated by mechanical sifting using different sizes of sieves (1000 μm, 500 μm, 250 μm, and <250 μm for the not-ground DBSG and 250 μm, 150 μm, and <150 μm for the ground DBSG).

The composition of each fraction (moisture, ash, protein, and dietary fiber soluble and insoluble) was determined. The following functional properties: water-holding capacity, cation exchange capacity, and *in vitro* bile salt binding capacity were determined in the fractions of DBSG and commercial fiber sources of wheat and oat bran. Protein content was negatively correlated with total dietary fiber content ($r = -0.99$, $P < 0.0001$). Ash content was positively correlated with total dietary fiber content ($r = 0.96$, $P < 0.001$). Water holding capacity was significantly different ($P < 0.05$) among different fractions. The cation exchange capacity was significantly lower ($P < 0.05$) in DBSG fractions when compared with commercial wheat and oat bran. In addition, bile salt binding capacities were higher in DBSG fractions when compared to commercial wheat and oat bran and correlated positively with the protein content ($r = 0.87$, $P < 0.005$ and $r = 0.84$, $P < 0.01$ for the taurocholate and cholate salts, respectively).

RESUME

Des fractions de drèche sèche (moulue et non-moulue) ont été obtenu per un procédé de méchanique utilisant des tamis. Des fractions de 1000 μm, 500 μm, 250 μm et <250 μm ont été préperé pour la drèche non-moulue, et des fractions de 250 μm, 150 μm et >150 μm pour la drèche moulue.

La composition de chaque fraction (taux d'humidité, minéraux, protéine et fibre diététique) a été déterminée. Les proprétés fonctionnelles telle que la capacité de rétention d'eau et de cation, et la complexation *in vitro* de sels bilières, ont été évaluées pour les fractions de drèche et des échantillons de commerciaux de blé et d'avoine. Une corrélation inverce a été observée entre le contenu en protéine et le contenu total en fibre diététique ($r = -0.99$, $P < 0.001$). Une correlation positive fut observée entre le contenu en minéraux et le taux de fibre diététique ($r = 0.96$, $P < 0.001$). Les différences de capacite de rétention d'eau entre les fractions se sont avérées significatives ($P < 0.05$). La capacité d'échange cationique des fractions de drèche a été moindre ($P < 0.05$) comparée au son de blé et d'avoine commerciaux. Les fractions de drèche ont démontré une capacité superiere de complexation de sels bilieres, comparées au son de blé et d'avoine. Cete capacite de complexation fur en corrélation avec le contenu en protéine ($r = 0.87$, $P < 0.005$ pour le taurocholate et $r = 0.84$, $P < 0.01$ pour le cholate).

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TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

BSG	Brewers' spent grain
DBSG	Dried brewers' spent grain
GDBSG	Ground dried brewers' spent grain
ADF	Acid detergent fiber
NDF	Neutral detergent fiber
TDF	Total dietary fiber
WHC	Water-holding capacity
CEC	Cation exchange capacity
r	Correlation coefficient
P	Probability (Statistical significance)
Prob.	Probability
Df	Degrees of freedom
Corr.	Correlation
Stnd. Error of Est.	Standard Error of the estimate

I. INTRODUCTION

More than 2300 years ago, Hippocrates, the father of medicine, recommended eating whole wheat bread "for its salutary effects upon the bowels" (Glicksman, 1982). However, it has been only over the last 25 years that food scientists, nutritionists, and clinicians have shown a concerted interest in dietary fiber because of its nutritional importance. Both epidemiological and experimental evidence suggests a relationship between the lack of fiber in the diet and chronic disorders and disease in Western countries (Burkitt and Trowell, 1975; Trowell et al., 1985). In addition, there are reports suggesting that diets rich in fiber reduce fat and protein digestibility and nutrient availability (Omaye et al., 1982; Thoma and Curtis, 1986).

Dietary fiber includes cellulose, hemicellulose, pectic substances, oligosaccharides, gums, lignin, and other minor components. The dietary fiber components are not digested by the normal enzymatic secretions, and not absorbed in the human upper digestive tract. However, some of the fiber macromolecules are digested by the microflora of the colon. These dietary fiber components occur and can be isolated from many plant sources, such as whole grain cereals, vegetable, and fruits.

The physicochemical properties of dietary fiber are considered to be related to the functional attributes of dietary fiber as functional ingredients in foods. These functional properties include fermentability by bacteria, water holding capacity, ion exchange capacity, and binding capacity of organic compounds.

The objectives of the study were :

i) To fractionate dried brewers' spent grain (DBSG) on the basis of particle size.

ii) To determine the composition and the functional attributes of the fibrous fractions.

iii) To determine, whether the different size particles provide distinct functional characteristics, when compared to commercial brans of wheat and oat.

iv) To investigate the relationships between composition of the ash, protein and dietary fiber contents in DBSG fractions and different functional properties.

II. LITERATURE REVIEW

II.A. COMPOSITION OF DIETARY FIBER

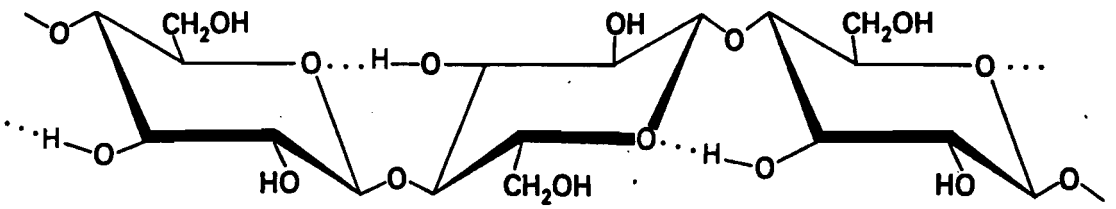
II.A.1. Polysaccharide components

This large group of carbohydrate compounds can be classified into its important individual components. This classification includes the main structural homoglycan cellulose and heteroglycans such as hemicelluloses, pectic substances, gums, mucilages, and other polysaccharides.

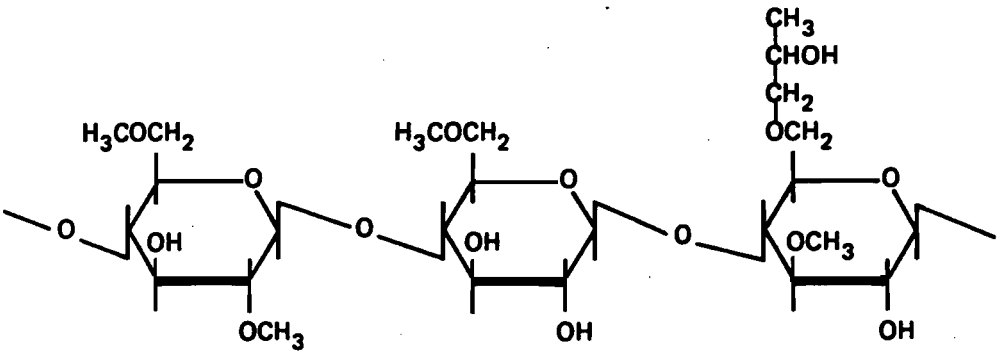
I.A.1.1. Cellulose

Cellulose is the most plentiful compound in the biosphere comprising more than half of all the organic carbon. Approximately 10^{10}Kg of cellulose is synthesized on earth each year (Stryer, 1988). Cellulose is a linear polymer consisting of β-1,4-linked β-D-glucose in the range of 300 to 15000 units (Dreher, 1987). Each glucose molecule is associated to the next by a rotation of 180° and the oxygen of the furanose is bound to the 3-OH group of the next by a hydrogen bond (Figure 1) (Stryer, 1988). Inter and intra hydrogen bonds can also be formed in different chains (Selvendran and Robertson, 1990). The configuration of the chains favours the formation of hydrogen bonds with water molecules between glucose units in the chain. The long straight chains are the result of the β-configuration.

Chains of cellulose can occur in crystalline and amorphous forms in the cell wall (Selvendran and Robertson, 1990). Cellulose is characterised by high mechanical strength and resistance towards chemicals, but can be partly degraded by colon microflora. The susceptibility of cellulosic materials to enzymatic hydrolysis can be



Cellulose
(β-1,4-linkages)



Hydroxypropylmethyl Cellulose

Figure 1. Structures of cellulose (top) and hydroxypropylmethyl cellulose (bottom).

increased by treatments which make the cellulose less crystalline. The crystalline regions are resistant to reagent and less susceptible to acid enzymatic hydrolysis.

Cellulose can be modified to produce derivatives with unique properties, these include microcrystalline cellulose and semisynthetic gums (Glicksman, 1986). These gums contain carboxymethylcellulose (CMC) which is a water soluble derivative of cellulose, hydroxypropylcellulose (Figure 1) which is soluble in water above 40^0C, and methylcellulose which is the methylether of cellulose (Dreher, 1987; Van Coillie, 1990).

II.A.1.2. Hemicelluloses

Hemicelluloses consist of mixed polymers of β-1,4 linked pyranoside sugar with side units consisting of galactose, arabinose, and uronic acid which can be methylated (Theander and Aman, 1979). The degree of polymerization is in the range of 50-200 residues and these substances are usually soluble in dilute alkali.

Hemicelluloses can be divided in the following three large categories; (i) xylans, (ii) mannans, and (iii) xyloglucans.

i) Xylans have a basic chain of β-1,4 linked D-xylose molecules with short side chains attached at C-6 of at least one half of the xylose residues. Single residues of glucuronic acid, and (4-O-methyl)α-D-glucuronic acid mainly attached by 1,2 linkages are bound to the main chain (Brett and Waldron, 1990). Lignin can form ester bounds with xylans (Selvendran and Robertson, 1990). Extended side chains containing L-arabinose and β-D-galactose subunits have additional substitution.

ii) There are several groups of mannans: simple mannans, glucomannans, and

galactoglucomannans (Theander and Aman, 1979). These polysaccharides consist of β-1,4 linked D-glucans of D-mannose and D-glucose. Units of α-D-galactose are bound by 1,6 linkages to these chains.

iii) The backbone of xyloglucans consists of β-D-glucose in which β-D-xylopyranose molecules are bound by 1,6 linkages (Theander and Aman, 1979; Olson et al., 1987).

II.A.1.3. Pectic substances

Pectic substances represent a complex group of colloidal polysaccharides in which α-D-galacturonic acid and rhamnogalacturans are the major subunits (Van Buren, 1991). Frequently, various monosaccharides occur along the backbone and side chains consisting of D-galactose, L-rhamnose, D-xylose, L-arabinose, L-fucose, D-apiose, and D-glucose (Theander and Aman, 1979). The basic unit in the main chain is D-galacturonic acid linked by α-1,4 bonds (from 200 to 1000 constituents) (Dreher 1987). The following three classes of pectins have been described (Olson et al., 1987).

i) D-Galacturonans (pectins or pectic acids) are the only acidic polysaccharides of these complexes (Figure 2). Galacturonans are often water insoluble because of the presence of calcium (Figure 2), and other divalent ions (Furda, 1979). Free acid groups can be neutralized with sodium, potassium, or ammonium ions. Some carboxyl groups can be esterified (Selvendran and Robertson, 1990) with methyl or acetyl groups.

Rhamnogalacturans represent a group of pectins with α-L-rhamnopyranose

units at intervals in the main galacturan chains with 1,2 linkages (Pilnik, 1990). Side chains of neutral saccharides partially acetylated and methylated can be found (Olson et al., 1987).

ii) Arabinans are linear polysaccharides of L-arabinose units connected by α-1,5 linkages; L-arabinose are joined by α-1,3 and /or α-1,2 bonds to the main chain (McCleary, 1990).

iii) Galactans consist of linear portions of D-galactose residues and can be divided in two groups: arabinogalactans which have α-L-arabinofuranose side chains attached to a galactose chain by 1,3 bonds and a more highly saturated galactan with modified chains of β-D-galactose residues attached by 1,3 and 1,6 bonds (Olson et al., 1987).

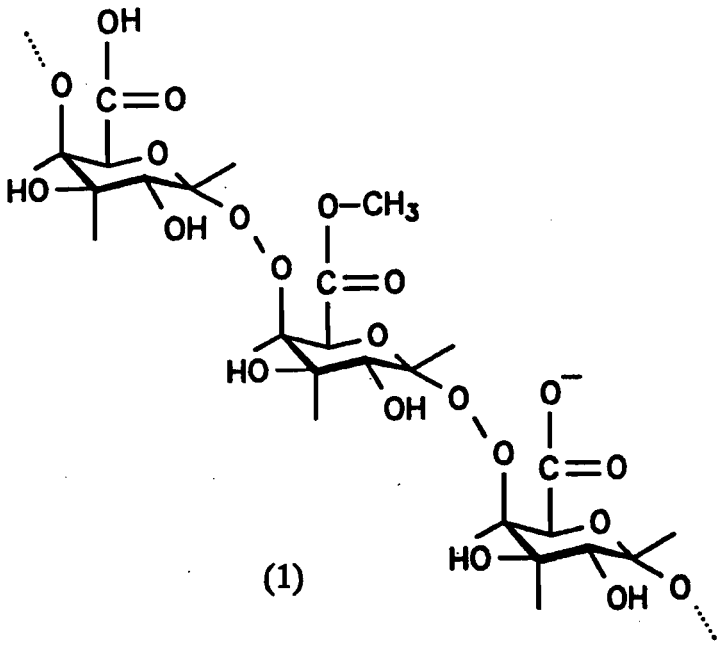
One of the most characteristic attributes of pectic substances is complex formation with high concentrations of Ca^{2+} (Figure 2) and Mg^{2+} (Furda, 1979).

II.A.1.4. Beta-D-glucans

β-D-Glucans are polymers of D-glucose which contain β-1,3 and 1,4 linkages in various ratios depending on the source (Dreher, 1987). Cereals like barley and oats are usually rich in β-D-glucans. These polymers are more soluble in water and less linear than cellulose.

II.A.1.5. Amyloids

Amyloids are structurally related to hemicellulose and consist of xyloglucans which react with iodine forming a blue complex like amylose. The structure is in the form of a main chain of β-1,4 glucose. Single units of D-xylose are joined to the main chain (Dreher, 1987).



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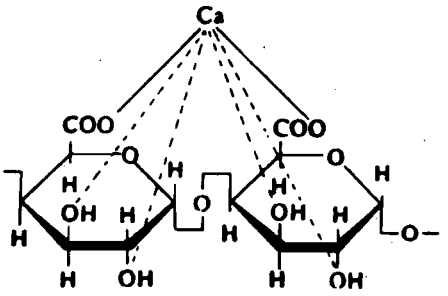

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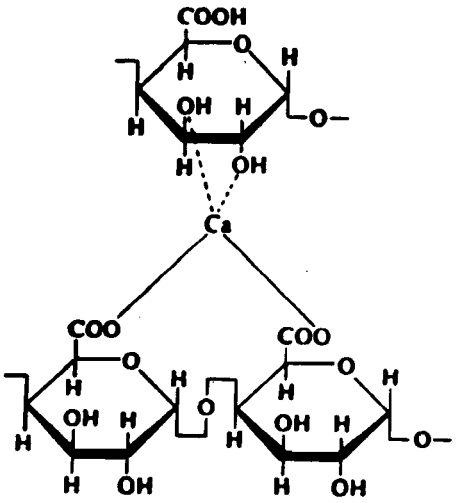

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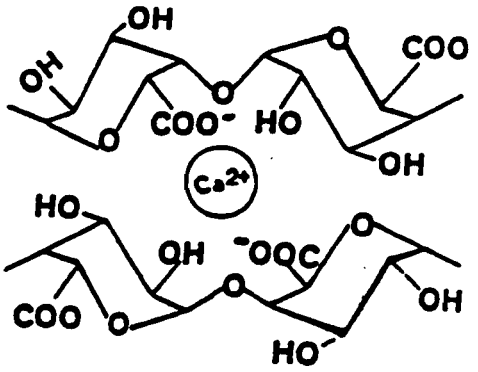

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Figure 2. Structure of pectic acids (1) and complexes, intramolecular (2) intermolecular (3, 4), between calcium (II) and pectic acids.

II.A.1.6. Carrageenans

Carrageenans are composed of galactose subunits alternately linked by 1,3- and 1,4- linkages and sulphated to various degrees. These sulphated esters of galactose and 3,6-anydrogalactose can contain potassium, sodium, calcium, and ammonium ions.

There are three major groups (Figure 3) of carrageenan hydrocolloids:

i) Kappa-carrageenan which consists of β-D-galactose-4-sulphate and 3,6 anhydro-α-D-galactose (Lahaye and Thibault, 1990).

ii) Lamda-carrageenan contains D-galactose-2-sulfate and D-galactose-2,6-disulfate derivatives (Glicksman, 1983a).

ii) Iota-carrageenan is similar to kappa, except that the 3,6 anhydrogalactose is sulphated at the second carbon molecule (Glicksman, 1983a).

II.A.1.7. Alginic acid

Alginic acid consists of α-1.4 linked α-L-glucuronic and β-D-mannuronic acid (Morris, 1990b; Dreher, 1987). The ratio of these acids in the hydrocolloid polymer is not constant.

II.A.1.8. Gums

Gums are hydrophobic polymeric substances which contain basically heteroglucans, and can give gelling and thickening effects when dissolved in water. They can be obtained from tree, plant exudates, seeds, microbial fermentations, seaweeds, and modified cellulose. The monosaccharic units consist of neutral sugars,

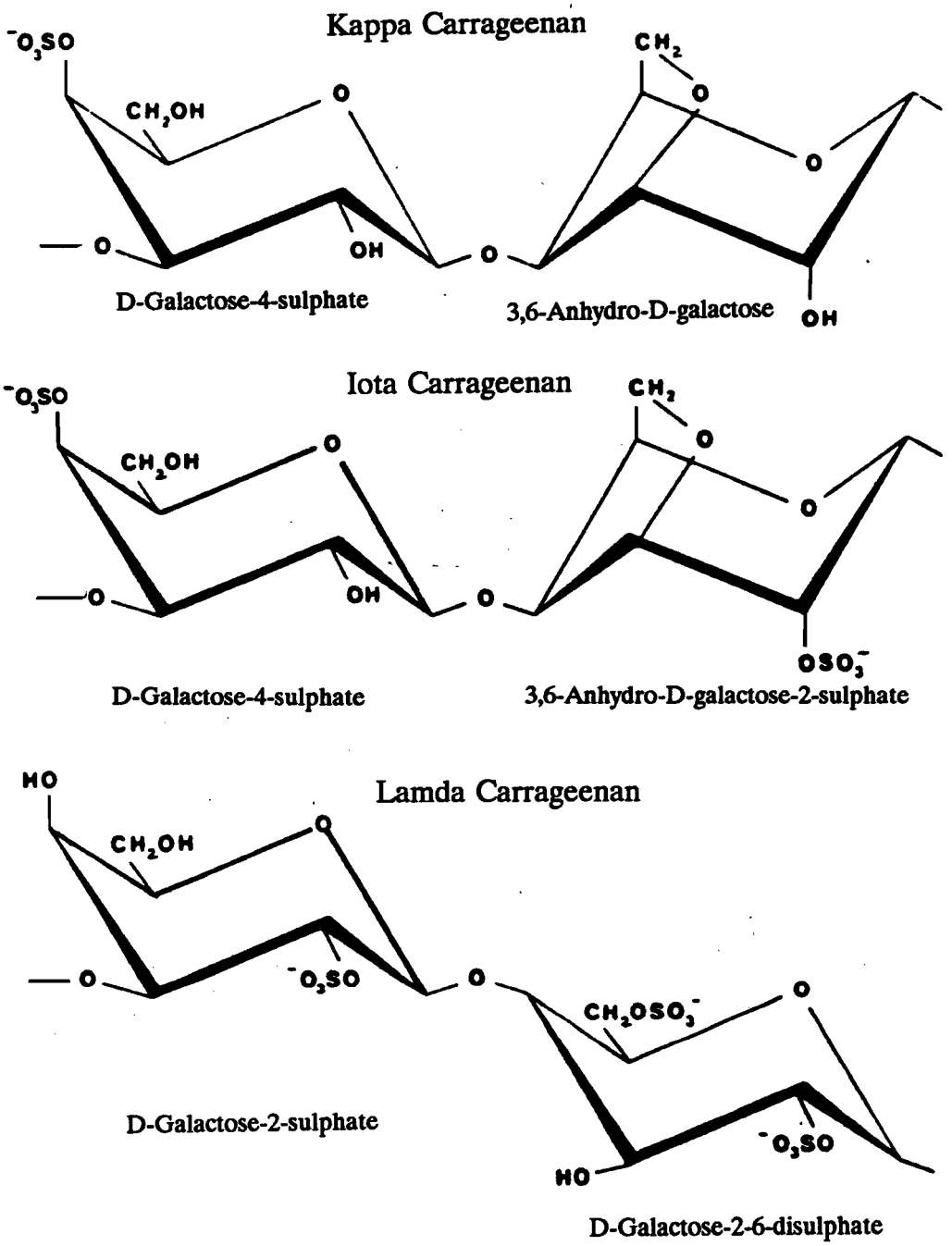


Figure 3. Structures of three major types of carrageenans (kappa, iota, and lamda).

uronic acids, and other acidic compounds (Glicksman, 1982). Three groups have been identified:

i. Mucilages and Seed Gums. Mucilages are usually a mixed group of neutral polysaccharides (Olson, 1987). The two main gums in this category are guar gum (a galactomannan) and locust bean gum (Wielinga, 1990).

ii. Plant exudates. Plant exudates are gums with complex structure as gum arabic, gum ghatti, gum karaya, gum traganath (Glicksman,1983b; Dreher, 1987; Stephen, 1990).

iii. Microbial Gums. These gums are produced by microbial fermentation of monosaccharide units (Dreher, 1987; Morris, 1990a).

II.A.2. Lignin and other Phenolic Components

II.A.2.1. Lignin

Lignin is an amorphous aromatic and hydrophobic polymer, which is formed by the enzymatic dehydrogenation and polymerization of phenylpropane units. The precursors of lignin (Figure 4) are coniferyl, sinapyl, and p-coumaryl alcohols (Theander and Aman, 1979). The monomeric units which are formed from these alcohols in the lignin complex are guaiacylpropane (3-methoxy-4-hydroxyphenylpropane), p-coumaryl (p-hydroxyphenylpropane), and syringylpropane (3,5-dimethyoxy-4-hydroxyphenylpropane) type units (Selvendran, 1984). Usually the bonds between the subunits are very stable. The substituents are linked together by carbon-carbon bonds which are extremely resistant to hydrolysis (Theander and

Aman, 1979). Chemical bonds and complexes among lignins, hemicelluloses, and cellulose have been observed (Selvendran and Robertson, 1990).

The rigidity and the resistance of the 3-dimensional structure of plant cells are associated with the properties of lignins. Further, lignin reduces the permeation of water across the cell walls and protects the cell against attacks by microorganisms and biochemical degradation.

Different fiber sources contain different amounts of lignin and various ratios of certain alcohols (Dreher, 1987).

II.A.2.2. Other phenolic compounds

Phenolic compounds include various polyhydroxyphenolic constituents, which are associated with fiber, such as hydrolysable tannins with sugar residues and condensed tannins, which are formed from flavonoid units (catechins, leucocyanidins) (Dreher, 1987). Moreover, some phenolic acids are esterified to carbohydrates in cell walls of cereals and grasses (Theander and Aman, 1979). These phenolic acids can be p-coumaric, ferulic, and diferulic acids (Figure 4).

II.A.2.3. Cuticular substances

Cuticular substances consist of waxes and other protective substances such as cutin, chitosan, and suberin (Dreher, 1987; Selverdran, 1984).

Cutin is a complicated plant polyester with hydroxy groups at C-8, C-9, and C-7, is linear and is a very hydrophobic polymer containing hydroxy and epoxy fatty acids (usually with C-16 and C-18) (Theander and Aman 1979). Suberin contains waxes, which are complex mixtures of esters of high molecular weight acids with long

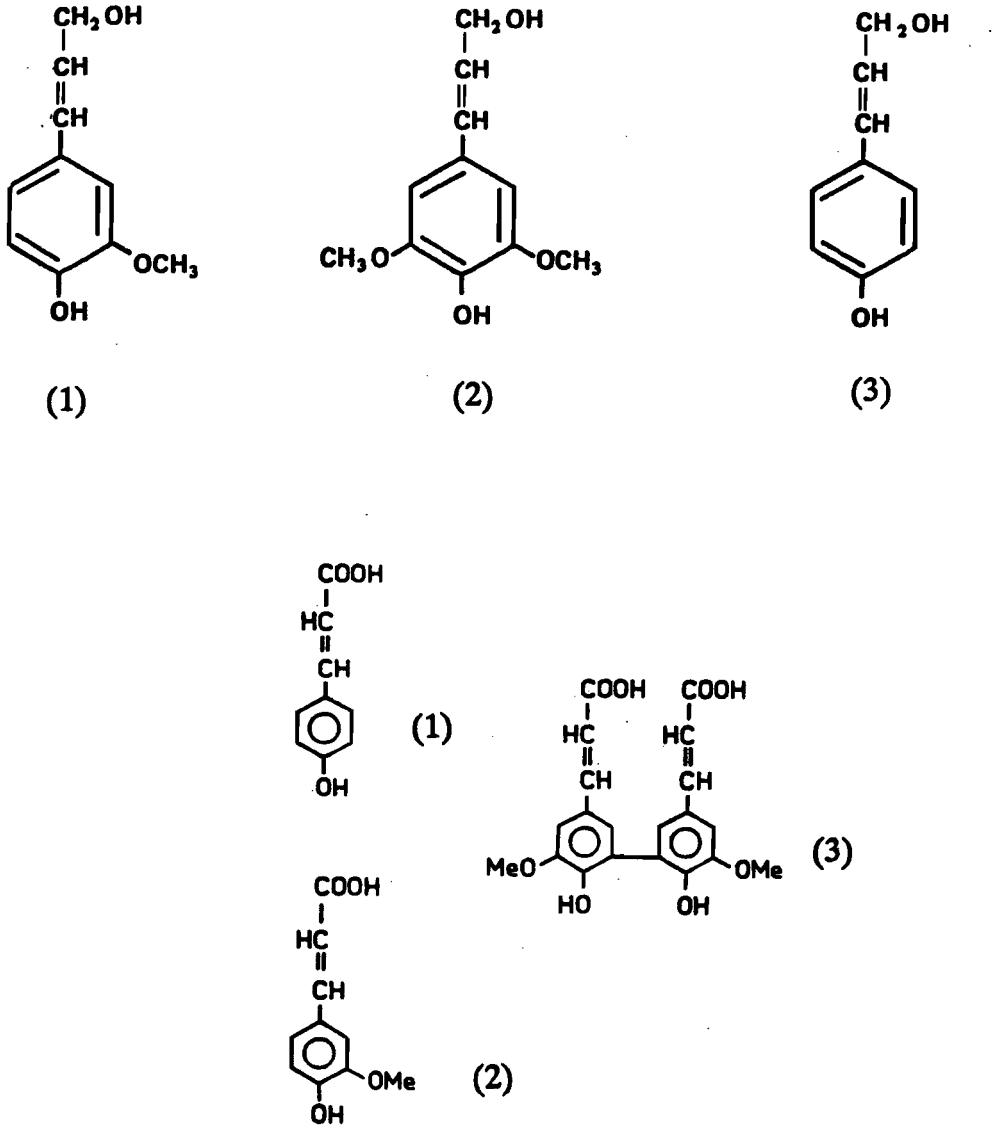


Figure 4. Precursors of lignin (top), (1) trans-coniferyl, (2) trans-sinapyl, and (3) trans-p-coumaryl alcohols; and phenolic compounds (bottom) found in cell walls, (1) p-coumaric, (2) ferulic, and (3) diferulic acids.

chain alcohols, aldehydes, ketones, and fatty acids with hydroxy groups. Suberin can be associated with other polysaccharides of fiber. Further, phenolic compounds such as p-coumaric acid and ferylic acid are present in very small concentrations in cutin and suberin.

II.A.3. Other Dietary Fiber Components

II.A.3.1. Proteoglycans and glycoproteins

Complexes of proteoglycans including pectic-hemicellulose-protein and pectic-hemicellulose-protein-polyphenol are also known to be present as components of fiber. The primary carbohydrates, which are involved in these complexes, are arabinoxylan, xylan, xyloglucan, and pectin arabinan residues.

Two types of proteins have been identified in proteoglycans and glycoproreins. One type may contain enzymes, such as peroxidase, which are immobilized by phenolic and glycosidic linkages, while the other type are connecting proteins in the cell wall.

Glycoproteins can be divided in two main categories; one that includes a protein in which hydroxyproline is abundant and the other which includes protein with low concentration of hydroxyproline (Selvendran and Robertson, 1990). The hydroxyproline rich glycoprotein named extensin and contains high concentrations of serine, arabinose, and galactose (Selvendran and O'Neil, 1982) its amino acid composition is similar to collagen (Dreher, 1987).

The glycoproteins can affect the solubility of some fiber components. These

components are related to hemicelluloses and pectins by formation of covalent bonds. Glycosidal bonds, between hydroxyproline and tetra-L-arabinose, are present in extensin (Theander and Aman, 1979).

II.A.3.2. Minerals

Metal cations and silica are associated with cell wall polysaccharides (Dreher, 1987) (Theander and Aman, 1979). Calcium, magnesium, iron, and zinc are associated with phytic acid. Calcium is also associated with pectin (Furda, 1979) and affects the rigidity of the cell wall components. Copper and zinc are associated with fiber polysaccharides. Other inorganic molecules such as calcium and magnesium carbonate, calcium oxalate occur in many fiber matrices.

II.A.3.3. Phytic acid

Phytic acid (myoinositol hexaphosphate) is a compound which is present in fiber components in the form of complexes with various metal ions (Theander and Aman, 1979; Maga, 1982; Dreher, 1987; Reddy et al., 1989).

II.A.3.4. Other minor components

Minor components including Maillard compounds, food additives, condensed tannins, silica, chitin and its deacetylated product chitosan are also included as dietary fiber components.

Some resistant starch is also included in the dietary fiber measurements. This is starch which is retained by fiber and escapes digestion in the small intestine, but is easily fermented by the large intestine microflora (Tomlin and Read, 1990). This starch is not hydrolysed, after gelatinization, by α-amylase and pullunase enzymes.

II.B. BREWERS' SPENT GRAIN (BSG)

Brewers' spent grain can be defined as the residue of malt and grain which remains in the mash-kettle after the liquefied and saccharified starch has been removed by filtration (Beldman et al. 1987).

The annual production of BSG by US breweries is over one billion pounds (Chaudhary and Weber, 1990a). Generally, BSG has been used as a source of feed for livestock. However, 400 pounds of cellulose can be obtained from every ton of dry spent grain; in addition industrial solvents, textiles, and plastics, after the conversion of spent grain to alkaline cellulose, can be produced from BSG.

Barley and its by-products can be used as a nutritionally important source of dietary fiber. It has been reported that BSG can be used as an ingredient in bread, as a food additive giving acceptable appearance and texture (Finley and Hamamoto, 1980; Dreese and Hoseney, 1982), and in cookies (Prentice and D'Appolonia, 1977; Prentice et al., 1978). It has been reported (Chandhary and Weber, 1990b) that by using bran flour from barley spent grain as a replacement ingredient in bread, an acceptable bread can be produced.

Brewers' spent grain can be dried and milled to produce a protein rich flour containing 35% protein and 35% total dietary fiber, and a flour containing 70% total dietary fiber (Weber and Chaudhary, 1987). The content of protein and dietary fiber in these flours is highly correlated with the particle size (Chaudhary and Weber, 1990). These flours contain the entire amount of the spent grain without generating other by-products.

Brewers' spent grain has been shown to have biochemical activity by inhibiting cholesterol biosynthesis (Weber and Chaudhary, 1987). Dry brewers' spent grain (DBSG) contains approximately 6.7 - 6.8 % fat rich in tocopherols and tocotrienols (90-100 ppm) (Qureshi et al., 1991a). These lipoids include d-a-tocotrienol and 1,3 dilinoleoyl-2-gama-linolenioylglycerol which inhibits the enzyme beta-hydroxy-beta-methyl glutaryl-CoA a reductase which catalyses the first step (rate-limiting enzyme) in the biosynthesis of cholesterol and other isoprenoids (Qureshi, et al. 1991a). Thus the amount of serum cholesterol and low density lipoprotein (LDL) cholesterol can be altered (Qureshi et al., 1991b).

The amount of phytic acid in brewers' spent grain has been found to be relatively low (Weber and Chaudhary, 1987). This reduction is due to hydrolysis of phytic acid by phytase during malting. However, brewers' spent grain is low in soluble dietary fiber, because of its prior procedure. In addition, BSG is too coarse for direct addition in foods and some type of modification is required in order to obtain a more suitable material in terms of functionality and acceptability.

II.C. METHODOLOGY FOR ANALYSIS OF DIETARY FIBER

There is ample evidence to show that total dietary fiber (TDF) has great physiological importance in human nutrition. However, nutritional studies indicate that TDF cannot always predict the actual physiological properties of dietary fiber. This increasing interest in TDF attributes accelerate the search for information on analytical methodology for quantitating TDF. However, the development of an

improved methodology has not been easy because dietary fiber exists as a complex mixture of different compounds.

A large number of analytical methods have been proposed for the dietary fiber analysis, but only a few methods have been recognized and accepted. The ideal method should give complete quantitation and fractionation of all individual fiber components, and be rapid and simple (Dreher, 1987).

II.C.1. Crude Fiber

The crude fiber method was developed in the nineteenth century and was for a longtime the only accepted method for the measurement of fiber (Dreher, 1987). The method is based on extraction of the sample with dilute acid and alkali and the subtraction of ash (AACC, 1983a; AOAC, Method 962.09, 1990). The technique cannot be applied in human nutrition because of the severity of the analytical conditions and the incomplete measure of dietary fiber components (Hall, 1989).

II.C.2. Acid Detergent Fiber (ADF)

ADF method is the first alternative method which could be used in foods using a detergent (cetyl-trimethylammonium bromide) in 1N sulfuric acid in order to solubilize non-fibrous components (Van Soest, 1963; AOAC, Method 973.18, 1990). The content of cellulose and lignin can be determined. By hydrolysing the residue further with 72% sulphuric acid the amount of lignin can be quantified.

II.C.3. Neutral Detergent Fiber (NDF)

This procedure involves treatment with the detergent sodium lauryl sulphate and EDTA (Van Soest and Wine, 1967). The remaining fiber residue from this method consists of cellulose, hemicellulose, and lignin. Modifications to correct for the presence of residual starch were done using enzymes such as alpha-amylase (Schaller, 1977; Robertson and Van Soest, 1981; Mogeau and Brassard, 1982a). However, the method can not determine soluble fiber and some amount of the dietary fiber is hydrolysed by enzymes. This procedure usually underestimates dietary fiber content.

II.C.4. Southgate Method

The technique of Southgate is capable of recovering the important fraction of soluble fiber. The procedure is based on chemical approaches and measures TDF and individual components. Treatments with methanol and diethyl ether, starch hydrolysis with enzymes and subsequent acid hydrolysis using dilute sulphuric acid are used in order to remove the non-cellulosic polysaccharide fraction. Strong sulfuric acid is used to hydrolyse the fiber. The sugar and sugar acids are determined colorimetrically (Southgate, 1969, 1981). The total content of dietary fiber by the Southgate method is usually 10% higher than values from other methods. However, the procedure is complicated and time consuming.

II.C.5. Englyst Method

In this approach according to Englyst and Cummings (1988) dimethyl sulfoxide (DMSO) is used to disperse the starch which is easily hydrolysed enzymatically by

pullulase and alpha-amylase. The monosaccharides are derivatised to alditol acetate esters and quantitified by gas liquid chromatography (GLC) (Englyst et al., 1982; Englyst and Cummings, 1982).The uronic acids and monosaccharides can be quantified colorimetrically (Englyst and Cummings, 1988). This method determines only non-starch polysaccharides. Other techniques for the evaluation of the individual monosaccharides utilize GLC (Kraus et al., 1990) and high-performance liquid chromatography (HPLC) (Linden and Lawhead, 1981; Slavin and Marlett, 1983; Neilson and Marlett, 1983).

II.C.6. Enzymatic Methods

The enzymatic-gravimetric method (Prosky et al., 1984; AOAC, Method 985.29, 1990) is the first method to be associated with the physiological definition of dietary fiber (Trowel et al., 1976) and the physiological significance of dietary fiber. The enzymes which degrade proteins and starch in the fiber sample, in this method, give results which partially correlate with the physiological action in the upper digestive track. Numerous reports have suggested the use of enzymes in the determination of dietary fiber (Hellenboorn et al., 1975; Asp et al., 1983). An interlaboratory study (Prosky et al., 1984) resulted in the first official acceptance in 1984 (Anonymous, 1985). Basically, the method was developed by Asp et al. (1983) and Schweizer & Wursh (1979). Prosky et al. (1984 and 1985) extended this method through a collaborative study which presented results with low coefficient of variation (CV). In this procedure, the sample is extracted and hydrolysed with alpha-amylase

and digested with protease and amyloglucosidase at different pH to eliminate protein and starch. The soluble fiber is precipitated with 78% ethanol (EtOH) and washed with ethanol and acetone. The residue is isolated, dried, and determined gravimetrically and the residual amount of protein and ash is determined in order to correct the measurements of fiber (Prosky et al., 1984).

This technique measures the amount of total dietary fiber (TDF); however, if the technique is modified by the addition of one additional filtration step (Furda, 1981), the two fractions of soluble (SDF) and insoluble (IDF) fiber can be separated (Prosky et al., 1988). The official AOAC method now is accepted in United States, Germany, Sweden, Denmark, and some other countries (Furda, 1989).

The method is relatively rapid, accurate, reproducible, and uncomplicated providing a good estimation of dietary fiber content. Resistant starch, that is not digested in the small intestine, is also evaluated in the determination of dietary fiber residue.

Nevertheless, there are some limitations and disadvantages. The filtration time with some types of fiber can be high (Prosky et al., 1984).The amount of polydextrose and synthetic hydroxypropyl cellulose, which are present in formulated food systems, cannot be measured because they are soluble in 80 % EtOH. It should also be noted that soluble oligosaccharides, such as raffinose and stachyose, cannot be recovered because they are soluble in 80% EtOH (Furda, 1989). In addition, there is a fraction of starch which is resistant to enzymatic hydrolysis and is counted in the amount of total dietary fiber.

Maillard products may create problems in the estimation of TDF (Prosky et al., 1984). Heat treatments of food can affect the content of dietary fiber components enhancing the amount of modified proteins and new formed polysaccharides in dietary fiber measurements.

II.C.7. Other Dietary Fiber Analytical Methods

A number of procedures have been described comparing various methods (Marlett and Navis, 1988), (Mongeau and Brassard, 1986) and introducing different techniques such as the separate determination of enzyme modified neutral detergent fiber and soluble dietary fiber (Mongeau and Brassard, 1990) and methods for fiber analysis using enzymes and gas liquid chromatography (Theander and Westerrlund, 1983). Some reports describe the use of near infrared reflectance spectroscopy (NIR) particularly for the quality assurance of total dietary fiber (Baker, 1985; Hall, 1989).

II.D. FUNCTIONAL PROPERTIES OF DIETARY FIBER

Dietary fiber in foods is derived from various food sources and tissues. Therefore the physicochemical properties of fiber are varied depending on the structure of the matrix, the nature of the tissue compounds, the measuring procedure, the chemical composition of the components including the functional groups (carboxyl, sulfate ester, hydroxy), the degree of substitution, branching, and the conformation of the components.

II.D.1. Particle Size

Particle size, which is related to surface area, is an important factor of fiber attributes. Particle size can affect the water holding capacity (Parrott and Thrall, 1978), the degree of degradation of components such as starch, proteins, and lipids, the extent of dietary fiber fermentation by colonic bacteria, the composition of fiber (Heller et al., 1977), and the bile salt binding capacities (Mongeau and Brassard, 1982b).

II.D.2. Water Holding Capacity (WHC)

The components of fiber have the ability to retain water in their complex structure. This property can be used to predict changes of the gastrointestinal tract (Stephen and Cummings, 1980; Eatwood et al., 1983), such as modification (induction) of stool weight, induction of the faecal rate of passage through the intestine (Stephen and Cummings, 1980). However, the WHC of fibers from different sources varies widely in terms of the amount of retained water by the colon. In addition, food functionality and quality can be altered by WHC (Labuza, 1985).

Water retention ability is also a physicochemical property of fiber that can be partially significant in the nutritional effect of dietary fiber in diets. Rich fiber diets increase stool yield (Eastwood et al., 1983), although, the relation between WHC of fibers and fiber function in diet is more complicated.

Fermentation of dietary fiber in the digestive track can influence the WHC, and these properties can affect the fermentation of fiber in colon (McBurney et al.,

1985). Fibers which hold large amounts of water can be degraded in the human gut by the microflora, altering the water holding ability. Further, partly degraded fibers have shown completely different water-holding properties than the original samples. Therefore, *in vitro* results of WHC are difficult to relate to the actual action in the gut and therefore to predict *in vivo* functional properties (Stephen and Cummings, 1980).

Results from WHC determination cannot predict the influence of dietary fiber in the diet. The faecal WHC of dietary fiber may be associated with the degree of degradation. For instance, vegetable fiber has higher WHC than cereal fiber, however, the bulking effect of vegetable fiber is lower than cereal bran (Robertson and Eastwood 1981b) because vegetable fiber is easily fermented. Cereal fiber cannot be easily fermented, therefore the water that is retained by fiber remains with little loss in the gut. The WHC, which is estimated in fiber, may differ from distribution of water in fiber in the gastrointestinal track (Robertson and Eastwood, 1981c). In addition, the WHC is associated with fiber fermentation in the gastrointestinal track providing in the more aqueous environment, better conditions for enzymatic and chemical activity to exist. The type of water, wether bound or readily available can affect the estimation of fiber properties. McBurney et al. (1985) studied the potential WHC (WHC of fermented residue) in relation to fermentation using dialysis membranes to obtain physiological conditions. In this report, it was suggested that the rate of passage and the degree of fermentability in fiber sources should be considered in WHC measurements.

The production of microbial mass after fermentation in gut can also influence the WHC of fiber in the large intestine (Eastwood et al., 1983 and McBurney et al., 1985). The most abundant component of human faeces is bacterial cells which can grow faster when fiber is provided (Stephen and Cummings, 1980). The bacteria can hold about 80% of water; therefore, bacterial population could significantly contribute to water retention in the gut. Stephen and Cummings (1979) reported an inverse relationship between retained water and faecal bulking effect that suggests an explanation for the complex relation of faecal weight and water held by fiber.

Water holding capacity is affected by the chemical composition (acidic polysaccharides), the structural conformation (Robertson and Eastwood, 1981b), and the configurational morphology of the fiber. Cereal fiber is less fermented than vegetable fiber in the caecum and colon because of its chemical composition and fiber structure (Robertson and Eastwood, 1981b). Therefore, the parameters of chemical composition and structure in cereals have greater ability to affect stool weight (Robertson and Eastwood, 1981b); nevertheless, cereal fiber tends to have lower WHC than vegetable fiber (McConell et al., 1974) which has higher ability to trap water. Furthermore, other factors such as particle size (Stephen and Cummings, 1979), sample preparation (Robertson and Eastwood, 1981c), temperature, pH electrolyte content (Labuza, 1985) and the osmolarity of the medium can also influence WHC.

Water can be held by fiber in three basic forms as follows:

i) water bound by hydrophillic polysaccharides, by hydrogen bonds, dipole

interaction, and ionic bonds (Eastwood et al., 1983); the characteristics of this type of water is the low chemical activity and vapour pressure.

ii) water retained in the fiber matrix (Robertson and Eastwood, 1981c) and held in capillaries by surface tension forces (Chen et al., 1984); the availability of this type of water depends on the pore size distribution and the surface tension forces in the fiber source (Eastwood et al., 1983).

iii) water trapped by the cell wall is available and has high chemical activity as in the case of wheat bran (Robertson and Eastwood, 1981a).

II.D.2.1. Methodology of WHC evaluation

Several methods are used for the determination of WHC. Some are applied to the prediction of faecal volume in the gut while others are used to evaluate the functionality of foods. These techniques are sensitive and can illustrate different measurements (Rasper & DeMan, 1980), due to the evaluation of different types of held water (Eastwood et al., 1983). Further, the obtained results are easily affected by external conditions and measuring methods which determine different mechanisms of retained water (Chen et al., 1984). Some common methods for measuring WHC are described below:

i) filtration: the sample is soaked in water filtered through glass wool (Robertson & Eastwood, 1981c) or filter paper (Eastwood et al., 1983) . After the filtration the sample is weighed, freeze-dried, and the WHC is calculated.

ii) centrifugation can be used as an external force to separate water. Several reports which use this technique apply various conditions such as time of

centrifugation, speed of the rotor, amount of water to estimate WHC (McConell et al., 1974; Heller and Halcker, 1977; Quinn and Paton, 1979; Eastwood et al., 1983).

iii) suction pressure: in this method (Stephen and Cummings, 1979; Robertson and Eastwood, 1981c) fiber samples are immersed in a solution of known osmotic potential initiated by an osmoticum eg. polyethylene glycol (PEG) with defined molecular weight (Robertson and Eastwood, 1981a). This osmotic potential is calculated from the known osmolarity of the solution. The procedure is applied to gels and physiological systems (McBurney et al., 1985) .

iv) the Baumann apparatus has been suggested by Chen et al. (1984) for determination of WHC in dry or semidry foods. This approach is based on liquid diffusion of water into fiber samples from a capillary system (Wallingford and Labuza, 1983; Labuza, 1985).

v) moisture sorption, this procedure is based upon equilibrium uptake of water vapour at a given external constant chemical potential of water (constant water activity). Usually the water activity is measured at $\alpha w=0.98$ (Wallingford and Labuza, 1983). The method involves a sample of certain initial moisture content that is exposed to a given relative humidity until equilibrium is achieved. (Labuza, 1985).

vi) Other methods that utilize one of the colligative physicochemical properties such as freezing point and NMR have been published (Labuza, 1985).

Centrifugation method gives elevated WHC values compared to Bauman and moisture sorption isotherm method because of prior swelling of water by surface forces (Chen et al., 1984). However, centrifugation is a precise and fast method that

provides an indication of water which is retained by fiber.

The values obtained during centrifugation procedure, depend upon the external pressure that formed during centrifugation the pore size distribution, the three-dimensional matrix of fiber, and the occurrence of minor components. The measuring technique, the experimental conditions, and pH which affects the swelling of polysaccharides alter the measurements. WHC measured by filtration is lower than WHC measured using centrifugation (Robertson and Eastwood, 1981c). Buffer solution, bile salt in different concentrations, and extended soaking have unimportant effect on WHC (Robertson and Eastwood, 1981c). When the structure (pore size) is open and the formation of protein is accumulated the water can be removed easier. Charged fiber molecules such as uronic acid enhance the water binding in fibers. By contrast, lignin give an inverse relationship with WHC (Stephen and Cummings, 1979).

II.D.3. Cation exchange Capacity (CEC)

Cation exchange capacity measures the ability of fiber to bind and absorb metal ions in its matrix (McBurney et al., 1986) and is defined as the number of ion equivalents in a specified amount of fibrous material. Uronic acids, pectins (Robertson et al., 1980; Rasper, 1979) and their carboxyl groups have been suggested to be associated with the binding of cations. Pectin guar gum gels and other dietary fiber sources form complexes by exchanging with divalent metals such as calcium (James et al, 1978; Furda, 1979), magnesium, iron, zinc and copper (Dintzis et al.,

1985; Platt and Clydesdale, 1987). Hemicelluloses bind various amounts of minerals using their glucuronic and galacturonic acids. Lignin can also affect significantly the cation binding because of the carboxyl, hydroxyl, and methoxy groups of the phenylpropane units (Van Soest et al.,1983; Platt and Clydesdale, 1987). Maillard and nitrogen compounds have also shown high CEC values (Rapser, 1979). Various functional groups, such as phenolic, ester, hydroxyl and amino group of non-digestible protein (McBurney, 1985) can affect cation exchange. Further, binding forces such as ionic, hydrogen, hydrophobic, and dispersion of fiber can affect the ion exchange attributes of fiber matrix (McBurney et al., 1983).

This mineral binding by dietary fiber is affected by the presence of phytate (Dintzis et al., 1985). CEC can also be affected by minor components of fiber such as free amino acids, phenolic compounds, aminosugars, and Maillard reaction products (McBurney et al., 1983) and is also affected strongly by pH, which influences the degree of ionization of the ionogenic groups and the pK value of the ion exchange fiber resins.

Dietary fiber can be characterized as either a mono- or polyfunctional cation exchanger. This cation exchanger can be either weak or strong. These various characterizations of dietary fiber can be shown using titration curves which indicate the neutralization of dietary fiber with acidic groups, by recording the changes of pH of the resulting solution when base is added (Rasper, 1979). The pH of the titration curve gives the number of ionogenic groups and their pK values. A strong cation exchanger gives a vertical transfiguration in the sigmoid titration curve (Dreher,

1987). McConnell et al., (1974) observed that cereal bran, oatmeal, and maize fibers act as weak polyfunctional ion exchangers. However, most fibers behave as weak monofunctional cation exchangers.

The property of fibers, such as wheat bran (Dintzis et al., 1985), to retain minerals in their matrix , suggests that mineral absorption in the gastrointestinal tract (Sandstead et al., 1978) may affect the electrolyte metabolism of the organism and reduce mineral bioavailability (Reinhold et al., 1976; Kelsay, 1978; James, 1980; Kelsay, 1982; Dreher, 1987) as well as the toxicity of some metals (Kay, 1982). This absorption of metals depends upon the pH, osmolarity, composition of the medium and can be related to microbial fermentation in the gut (McBurney et al., 1983; McBurney et al., 1986). When the pH is increased more negatively charged groups of fiber become available for cation exchange with cations. In addition to pH, microbial and enzymatic action in the gut may alter the cation exchange functional groups of fiber releasing new functional groups (McBurney et al., 1983), while physical characteristics of the fiber can also be modified. For instance, pectin and soluble dietary fiber have elevated CEC values (Van Soest et al., 1983), but is fermented by the microbial population of the human large intestine losing its ion binding abilities. Therefore, the actual *in vivo* situation in the gastrointestinal track is distinctive. Normand et al. (1987) reported that minerals bound by hemicelluloses can be released by enzymes found in the intestine such as trypsin, pepsin and hemicellulase.

II.D.3.1. Quantitation of cation exchange capacity

Two categories of methods are available for the measurement of CEC.

i) McConnell et al. (1974) reported the first technique to estimate the CEC in fiber using a titration procedure. In this method the carboxylic groups are converted to the H^+ (hydrogen cation) form by soaking the fiber in an excess of hydrochloric acid. The acid charged fiber is stripped by suspension in neutral sodium chloride solution and the amount of H^+ ions are slowly titrated with a standard solution (0.05N) of sodium hydroxide (Robertson et al., 1980). Titration curves can also be obtained by direct titration of fiber resins with sodium hydroxide (Rasper, 1979).

Several limitations to the titration procedure have been reported. Some amounts of dietary fiber can be removed during the acid conversion procedure. In addition, carboxylic groups can be released by the saponification of methoxylated functional groups (Rasper, 1979; Dreher, 1987), and therefore, the CEC can be overestimated.

ii) McBurney et al. (1983) suggested the use of mineral binding for the evaluation of CEC in fiber. Metal binding techniques can be applied as an alternative method in order to avoid the limitations of the titration method. Two metals, copper (Cu) and praseodymium (Pr), are commonly used to bind with the ion exchange groups of dietary fiber samples.

The metal binding procedure is based on the high capacity of copper ions (II) to be bound by dietary fiber functional groups (McBurney et al., 1983). The prepared

fibrous sample is incubated with cupric sulfate for a specified time at a predetermined temperature. The treated sample is then washed in order to remove unbound cupric ions. After the excess copper is removed, the fibrous sample is washed with hydrochloric acid. The washes are collected and the amount of copper in the washing is determinated colorimetrically (Williams and Morgan, 1954). The colorimetric method is based on the high and specific capacity of copper to form rapidly a blue water-soluble complex using as ligand, cuprizone (oxalic bis(cyclohexylidenehydrazide) or bis-cyclohexanone oxalyldihydrazone). This intense blue complex has a maximum absorption wavelength at 606 nm and pH optimum range at 8.2-9.0.

The metal binding method gives a good estimation of CEC; however, the CEC of dietary fiber, obtained by this method, can be different from that of the gastrointestinal track. The pH in the upper intestine is in the alkaline range (pH 7-8), and therefore more negatively charged carboxylic and other groups can be available for binding (McBurney et al., 1983; McBurney et al., 1986). The competition of Cu^{++} and H^{+} in the active fiber groups is increased when the pH is decreased and more H^{+} are bound to functional fiber groups. The binding of copper is done at low pH (pH 4.0-3.5) since at higher pH the cupric ions form the hydroxide which precipitates. Further, the removal of copper excess during the washing step is prevented when a gel is formed (Van Soest et al., 1983).

Allen et al., (1985) reported an alternative metal binding method for measuring CEC. This technique employs the tripositive rare element praseodymium

(Pr). The advantage of this method is that CEC can be measured at pH 7 which is closer to physiological pH of the small intestine. The technique gives higher CEC values (McBureny et al., 1986) because at the more alkaline pH, more negatively charged groups are available to bind with metal ions.

II.D.4. Bile Salts Binding

Organic molecules of bile salts, conjugated bile salts, and cholesterol can be absorbed by dietary fiber sources; This phenomenon can have a hypocholesteremic effect in serum (Vahouny, 1978; Jenkins et al., 1980; Kay, 1982) and is reported to be involved in certain etiologies of cancer (Reddy, 1975; Reddy et al., 1977; Spiller, 1978; Kay, 1982).

Eastwood & Hamilton, (1968) pointed out that primarily lignin and particularly methylated lignin bind bile acids. This binding ability is affected by the molecular weight, the presence of functional groups such as methoxyl and β-carbonyl groups on the lignin molecule and the degree of ionization (Eastwood and Hamilton, 1968; Kay, 1979). The bile binding capacity is reduced when phenolic hydroxyls of lignin are ionized.

In addition to lignin other components of dietary fiber have been shown to be related to bile salt binding, depending upon the chemistry of fiber. Saponins may absorb bile acids and cholesterol (Oakenfull and Fenwick, 1978). Other gel and acidic components of fiber, such as pectin and hemicellulose have been proposed to be involved in bile binding mechanisms (Furda, 1979; Story et al., 1982). High viscosity

soluble fiber can alter the diffusion of bile compounds in the bulk phase (Kay, 1982). Moreover, these acidic fibrous components can bind metals forming cationic bridges between trivalent cations such as Al^{3+} and Fe^{3+} and bile anions (Nagyvary and Bradbury, 1977; Furda, 1979). These polyuronic complexes can provide a means for bile acid absorption in the upper intestine. Dietary fiber sources, such as alfalfa (Kritchevsky and Story, 1974), guar gum (Vanhouny et al., 1980), resins like cholestyramine (Hagerman et al., 1973), and other non-nutritive components of diets (Eastwood and Hamilton, 1968; Kritchevsky and Story, 1974; Mongeau and Brassard, 1982), have given positive association with bile salt binding. However, other dietary fiber sources, such as cellulose, act neutral (Kritchevsky and Story, 1974; Story and Kritchevsky, 1976).

The binding capacity of dietary fiber components and particularly lignin, is also affected by the physicochemical nature of the bile compounds. Dihydroxy bile salts such as deoxycholate and lithocholate are retained with higher ability than trihydroxy salts, such as chenodeoxycholate and cholate (Story and Kritchevsky, 1976; Kern et al., 1978; Story et al., 1982). Polar conjugated bile acids such as taurocholate and glycocholate have lower binding capacity than the free bile acids. This reduction of binding when the polarity of bile salts is reduced may introduce hydrophobic interactions of bile salts with fiber.

Hagerman et al. (1973) indicated the importance of the bile salt structure on the binding ability of cholestyramine and the significance of ionic groups as well as the non-ionic attraction involved in the binding mechanism.

Reduction of pH can also increase the binding effect of bile acids (Eastwood and Hamilton, 1968; Kern et al., 1978; Kay et al., 1979; Floren and Nilsson, 1982). When the pH is decreased the acidic groups of the fiber become un-ionized suggesting the hydrophobic nature of the interaction.

In general, when the concentration of bile salt is increased the binding is increased (Eastwood and Hamilton, 1968; Kern et al., 1978) suggesting a linear relationship between dietary fiber and bile salt which is bound (Kritchevsky and Story, 1976; Eastwood et al., 1976). Other factors such as presoak of fibrous sample (Floren and Nilsson, 1982), water-holding capacity (Story et al., 1982) and particle size of dietary fiber (Burczak and Kellogg, 1979; Mongeau and Brassard, 1982) can also affect the binding procedure.

Studies using mixed micelles of bile salts with monoglycerides and fatty acids instead of bile salt solutions have been reported (Vanhouny et al., 1980). Binding of micelles on dietary fiber may be decreased when the amount of fatty acids, the degree of unsaturation and particularly the chain length is decreased (Eastwood and Mowbray, 1976). Bile conjugates may be within the micelle and prevented from being absorbed on to dietary fiber.

Story and Kritchevsky (1976) remarked that the binding mechanism of bile acids on a source of dietary fiber is complicated and a combination of binding mechanisms occurs (Story and Kritchevsky, 1978). Such mixed binding mechanisms with both hydrophobic and hydrophillic interactions have been reported by Floren and Nilsson (1982). Story and Kritchevsky (1976) suggested that the binding ability

depends upon the type and source of fiber which is used as a binding substance.

Bile salts bound to fiber was linearly related to the weight of wheat fiber (Floren and Nilsson, 1982). Kritchevsky and Story (1974) reported a linear relation between the fiber substance and the quantity of bile salt which was available for binding. Eastwood et al. (1976) also found a linear relation of bile binding and increasing amount of fiber substance (absorbent). The same uniform and monomolecular process was found by Kern et al. (1978). Further, some amount of bile salts was reversibly bound by fiber (Eastwood et al., 1976).

The hypothesis for binding of bile salts *in vitro* is that the reabsorption of these salts in ileum may be reduced when bile salts are bound by dietary fiber. Eastwood and Hamilton (1968) suggested that bile salts in the intestine can be bound to dietary fiber. Normally bile salts are reabsorbed in the ileum (Boyd et al., 1966) and returned to the liver using the enterohepatic circulation. The dietary fiber in foods retain these bile salts reducing the amount of the reabsorbed bile salts. This reduction of bile acids reabsorption can alter the cholesterol levels in plasma and tissue and lead to an increase in hepatic bile acid synthesis. In addition, increased bile acid excretion results in an increased synthesis of bile acids from cholesterol in the organism, while chenodeoxicholate synthesis is elevated because its inhibitor deoxycholate is absorbed by dietary fiber.

The pH in the intestinal tract is approximately pH 3.5 in the duodenum, increases to pH 6 in jejunum and pH 8 in the ileum where bile salts are reabsorbed. While the dietary fiber is passing through the duodenum, it may absorb bile salts at

low pH. The bile salts may be released, when the pH is increased in the ileum, and reabsorbed from the organism in the ileum eliminating the absorption of bile salts by dietary fiber.

The bile salts that are not absorbed in the ileum pass to the caecum where they are attacked, dehydroxylized and modified by colon bacteria to form a mixture of free and less water soluble bile acids (Eastwood and Mowbray, 1976). Bacterial flora can hydrolyse the amide linkage of conjugated bile salts, remove the 7α-hydroxyl group, and modify the remaining -OH groups. These metabolized sterols can be reabsorbed from the caecum and be carcinogenic (Mastromarino et al., 1976). The main bacterially degraded bile salt in the caecum and large intestine is deoxycholate. This salt was suggested to give mutagenic and carcinogenic potential (Mastromarino et al., 1976; Reddy et al., 1977; Macdonald et al., 1978). This acid can be retained by dietary fiber components. Dehydroxylation of primary bile acids by colonic bacteria may be decreased by fiber sources (Story and Kritchevsky, 1978). The metabolism of bile salts by caecal bacteria may increase when the bile salts are bound to fiber (Story and Kritchevsky, 1978).

II.D.4.1. Quantitation of bile salt binding

Eastwood and Hamilton (1968) suggested the first *in vitro* binding method using a fiber source from whisky maltings. The estimation of bile salts were done by the Pettenkofer reaction (Boyd et al., 1966). Kritchevsky and Story (1974) first used labelled bile salts for the binding measurements showing an *in vitro* procedure to evaluate the absorption. Floren and Nilsson (1982), using preparation of wheat bran,

mentioned the high precision in the calculation of *in vitro* bile using the decrease of radioactivity in the supernant. Other methods have been reported using a variety of conditions eg. different sample preparation procedures, amounts of fiber, concentration of bile salt, buffers, times of incubation, and detection methods for the bile salts (Kritchevsky and Story, 1974; Eastwood et al., 1976; Vanhouny et al., 1980; Mongeau and Brassard, 1982; Adiotomre et al., 1990).

II.D.5. Binding of other Organic Molecules by Dietary Fiber

Dietary fiber food sources also have the ability to bind organic molecules such as 2-amino anthracene which was extracted from fried beef and has mutagenic attributes (Moorman et al., 1983). Rubio et al. (1979) described the binding *in vitro* of N-nitrosodiethylamine (a carcinogenic substance) and therefore the reduction in bioavailability of this compound using lignin fiber preparations as absorbent. 1,2-Dimethylhydrazine (a carcinogenic compound) can also be bound by dietary fiber sources depending upon the pH of the medium (Smith-Barbaro et al., 1981).

Fiber preparations can also serve as a binding substrate for food colours such as erythrosine, ponceau SX, and brilliant blue FCF (Farber, 1981), thereby, exhibiting protective activity (Tsujita et al., 1979). This binding attribute of fiber is influenced by the pH of the medium and the concentration of the colour compound (Takeda and Kiriyama, 1979; Farber, 1981). Toxicological aspects of food colors have been suggested (Radomski, 1974; Grasso, 1983; Malaspina, 1987). One of the most important group of synthetic food colours are azo (-N=N-) hues. These colours can

be toxic because of their reductive degradation in the gastrointestinal track by bacteria (Rowland, 1981). Azo dyes are unstable to variations of pH. Food dyes are composed of isomers of the coloured compounds, by-products, and impurities that may be toxic. Colour compounds that are used as additives, such as brilliant blue, citrus red No2, yellow No5 (tartrazine), yellow No6 (sunset yellow), red No3 (erythrosine), have been reported to show toxic properties (Concon, 1988).

Steroid hormones, such as estone, estanodiol-17β, and testosterone, can be bound to natural and purified fiber sources (Shultz and Howie, 1986; Witten and Shultz, 1988). Therefore, dietary fiber diets and lignin may alter the value of hormones in the organism and affect the enterohepatic circulation of steroid hormones (Ross et al., 1990).

III. MATERIALS AND METHODS

III.A. ORIGIN OF FIBER SAMPLES

Commercial dried BSG was obtained from Molson Breweries of Canada Ltd, (Montreal, Quebec, Canada). Commercial dietary fiber sources (wheat bran and oat bran) were obtained from Ogilvie Mills Ltd (Montreal, PQ, Canada). Cholestyramine resin was purchased from Sigma Chemical Co (St Louis, MO) and was used as the control binder in bile salts binding experiments. The chemical reagents and solvents that were used were reagent grade (ACS approved) and were purchased from BDH Inc. (St-Laurent, PQ), unless otherwise indicated.

III.B. FRACTIONATION OF DRIED BREWERS' SPENT GRAIN (DBSG)

The dried brewers' spent grain (DBSG) was subjected to a dry fractionation procedure based on particle size grouping by mechanical sifting, using a set of different sieves. Particle size fractionation for the not-ground DBSG was processed using a set of three Canadian standard sieves (W.S. Tyler, St. Catharines, Ontario) with 1, 0.5, 0.25 mm size openings and in combination with a pan. The DBSG sample (25.0 g quantity) was placed in the upper sieve (1 mm), covered, placed on a Tyler Ro-Tap testing sieve shaker and agitated for 15 min. The fractionated DBSG was retained in each sieve and the pan was collected and weighed.

A portion of dry BSG was subjected to a grinding procedure. The DBSG was ground by a rotating impeller at high speed (10,000 rpm) in a Cyclotec 1093 sample mill (Tecator AB, Högönas, Sweden) fitted with a 1 mm screen. The ground DBSG

was subjected to the same fractionation technique as above, but using a set of sieves with smaller size openings (0.25, 0.15 mm) in combination with a pan. The fractionated samples obtained were sealed in plastic containers and stored at 20°C.

III.C. ANALYSIS OF DBSG

III.C.1. Moisture Content

Moisture content was determined using the vacuum oven method (AOAC, Method 925.09, 1990). A quantity (2 g) of sample was placed in metal pan and was dried in the apparatus at 80°C for 12 h under high vacuum. The covered dish was cooled in a desiccator with $CaCl_2$ as the desiccant.

III.C.2. Ash Content

The inorganic residue (ash) was determined according to the AOAC method (Method 923.03, 1990). A quantity (1 g) of dried sample was placed in a pre-weighed porcelain crucible that had been previously ignited. The samples were pre-ashed and ignited at 550°C for 5 h in a muffle furnace (Thermolyne Co., Dubuque, Iowa).

III.C.3. Crude Protein Content

The protein content was determined using a modification of the official micro-Kjeldahl method for total nitrogen determination (AOAC, Method 960.52, 1990). The analysis was performed using a Labconco Rapid III Kjeldahl system (Labconco Corporation, Kansas City, MO).

Appropriate quantities (0.2 - 0.5 g) of dried samples were transferred to Kjeldahl digestion tubes (250 ml). A Kjeltab catalyst tablet (5.0 g potassium sulphate

and 0.25 g mercuric oxide) (Profamo Analytical Services Inc., Dorval, PQ) was added to the sample in each tube. Concentrated sulphuric acid (8 ml) was added, the tube was agitated gently and then placed in the Labconco Rapid Digestion-4 apparatus pre-set at 410°C for 30 min.

After digestion, the tubes were removed, cooled for 10 min and 55 ml distilled water was added. The added water was carefully mixed and the tube was placed on the Kjeldahl distillation unit. A quantity (60 ml) of a solution of NaOH : $Na_2S_2O_3 \cdot 5H_2O$ in water (60%:5%) was automatically dispensed in the tube and the amount of liberated ammonia which was distilled after 5 min was collected in saturated boric acid (ACS certified reagent; Anachemia Canada Inc., Lachine, Montreal, PQ) solution (50 ml) and containing methyl red as indicator. The quantity of the collected ammonia was determined by titration using standard HCl (0.0900N).

The nitrogen content (%) was converted to protein content (%) using the multiplication factor of 6.25.

III.D. ENZYMATIC DETERMINATION OF DIETARY FIBER

Determination of insoluble, soluble, and total dietary fiber was performed using a combination of enzymes according to the method of Prosky et al. (1984) as modified by Prosky et al. (1988). The schematic diagram of this procedure is shown in Figure 5.

Duplicate (1.000 g) moisture free samples with fat concentration < 5% were weighed and placed in 250 ml conical flasks. A solution (50 ml) of phosphate buffer

(NaH_2PO_4-Na_2HPO_4, 0.08M, pH 6.0) was transferred to each flask and 100 μl α-amylase heat stable solution (Sigma Chemical Co., St. Louis, MO) was added in order to gelatinize alpha-linked polysaccharides. The flasks were covered with aluminium foil and incubated in a boiling water bath for 30 min after reaching the desired temperature of 95°C. The flasks were shaken gently every 5 min. The mixtures were cooled and the pH was adjusted to 7.5 by adding NaOH solution (0.275N, approximately 10 ml). Protease (5 mg; Sigma Chemical Co., St. Louis, MO) was added to the digestion mixture in order to digest protein. The flasks were covered with aluminium foil and placed in an agitating water bath (60°C, 30 min). The containers were cooled and a solution of hydrochloric acid (0.325N, approximately 10 ml) was added to obtain a pH of 4.5. Amyloglucosidase (300 μl solution from Aspergillus niger; Sigma Chemical Co., St. Louis, MO) was added to the flasks and the mixtures were incubated (60°C), as before, in order to hydrolyse enzymatically the starch content in the fiber sample.

Pre-ignited (525°C) Pyrex (gooch) crucibles (50 ml) with coarse fritted disc (Corning 32940, Fisher Scientific Limited, Montreal, PQ), with maximum pore size 40 - 60 μm containing acid washed Celite (0.7 g; Sigma Chemical Co., St. Louis, MO), were wetted and the Celite layer was redistributed with distilled water. The crucible was dried at 130°C for 2 h, weighed, and attached to a vacuum filtration apparatus. The solution of enzyme mixture was filtered into the crucible and the residue was washed with 2×10 ml portions of water and both filtrate and washings were reserved for the determination of soluble dietary fiber. The residue which contained the insoluble dietary fiber, was washed with 2×10 ml portions of 95% ethanol and 2x10

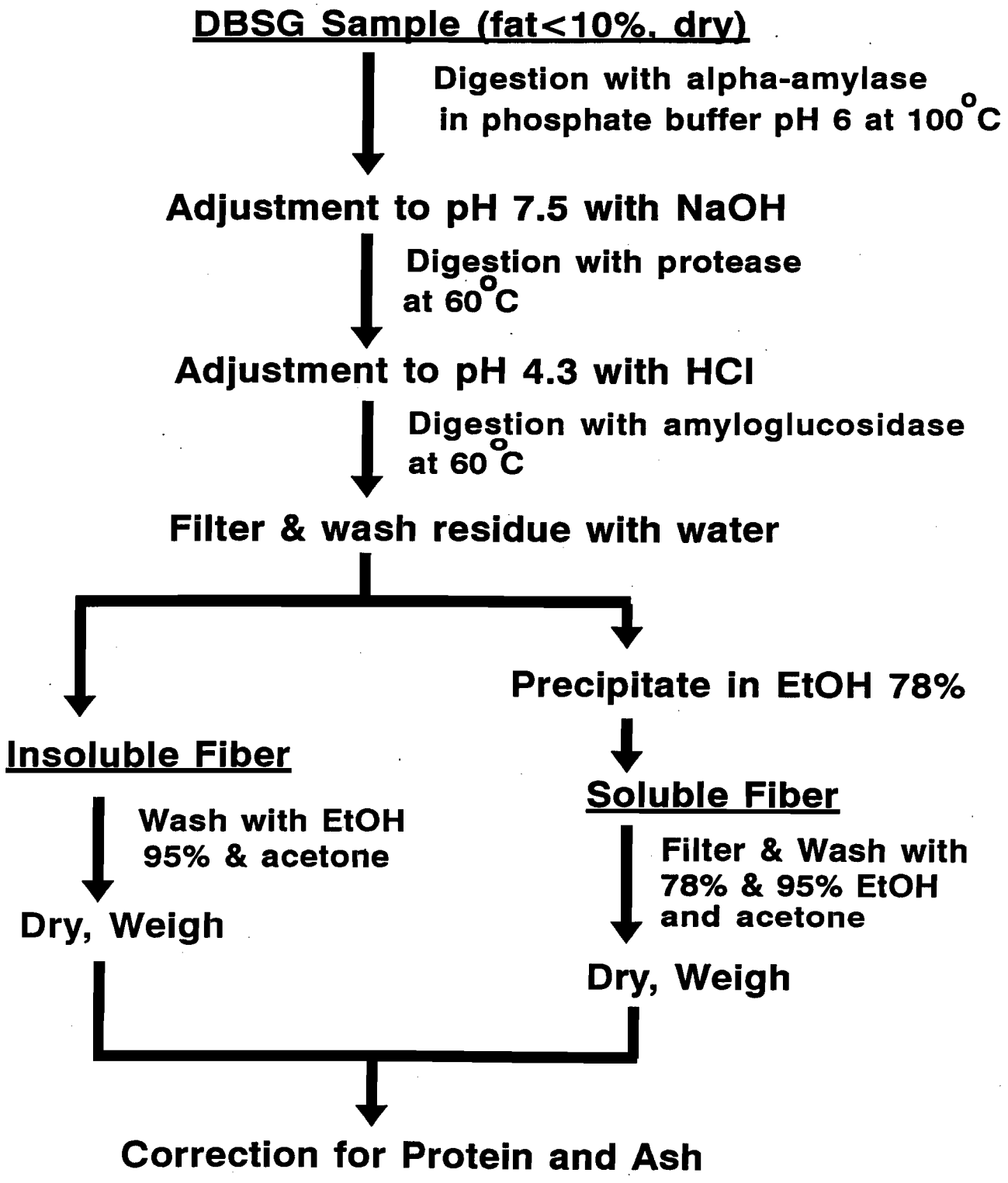


Figure 5. Schematic diagram of the enzymatic determination of soluble and insoluble dietary fiber.

ml portions of acetone.

The weight of filtrate and water washings that contained the soluble dietary fiber was adjusted to 100 g with distilled water. Ethanol (95%, 400 ml) which was previously heated to 60°C, was added to this solution and the mixture was cooled and held at room temperature for 90 min in order to precipitate the soluble dietary fiber. The precipitate was recovered by vacuum filtration using a coarse crucible containing Celite redistributed with ethanol (78%). The residue of soluble dietary fiber was washed with 3x20 ml portions of ethanol (78% v/v), 2x10 ml portions of 95% ethanol, and finally with 2x10 ml portions of acetone.

The duplicate crucibles containing the insoluble and soluble dietary fiber residues were dried for 12 h at 80°C in a vacuum oven. The crucibles were cooled and the weights of the residues were measured gravimetrically. One of the duplicate residues was analyzed for protein using the micro-Kjeldahl technique (section II.C.3.), and the other was used for the determination of ash by ignition for 5 h at 525°C in a muffle furnace. The protein and ash contents were subtracted from the weight of the residue in order to correct for these components. Blanks were run throughout the procedure to measure the contribution of reagents and enzymes to dietary fiber measurements.

III.E. WATER-HOLDING CAPACITY

Water-holding capacity (WHC) was measured as water hydration capacity (AACC, Method 88-04, 1983) by using the external centrifugal force method

(Beckman centrifuge, Model J2-21). Samples of DBSG fractions, wheat, and oat bran were dried (at 80°C) overnight in vacuum oven.

The determination of water holding capacity was done in two steps. First the weight of sample and the volume of water that would be used in final calculation of WHC, were pre-estimated. The determination of approximate WHC was performed by weighing a quantity (5 g) of the sample into a pre-weighed centrifuge tube (50 ml). An appropriate volume of distilled water was placed in this tube. The mixture was stirred with a glass rod until the tube contents were completely soaked. The tubes were centrifuged at low speed, 2000xg (5050 rpm) for 10 min. The supernatant was discarded and the tubes were inverted to drain for 15 min before weighing. The approximate WHC (ml/g) was estimated from the equation:

$$\text{Approx.WHC} = \frac{[(\text{weight of tube + sediment}) - (\text{weight of tube + 5.0})]}{5} \quad (1)$$

The weight of sample that was subjected to the determination of WHC was estimated using the following equation:

$$\text{Weight of sample (g)} = 15/(\text{approx. WHC} + 1) \quad (2)$$

The estimated weight of sample was placed in each of four tubes. Volumes of water 0.5 - 1.5 ml more than and 0.5 - 1.5ml less than the volume of water which was calculated from the equation (15-weight of sample) (3), were added to the tubes. The contents were mixed for 2 min using a stirring rod and centrifuged using the same conditions as before. After the centrifugation, the two adjacent tubes, one with and

one without supernatant, were used to measure the WHC. The WHC was calculated from the average of these two volumes divided by the weight of sample and expressed as the volume of water that is held by one gram of sample under the conditions of the experiment.

III.F. CATION EXCHANGE CAPACITY

III.F.1. Sample Preparation

A quantity (3 g) of each sample was washed in a Pyrex crucible (50 ml) with a coarse fritted disc. The washing was done with 4x40 ml portions of 78% ethanol, 3x20 ml portions of 95% ethanol, and 3x30 ml portions of acetone. The remaining sample "powder" was dried (at 80°C) for 12 h in a vacuum oven.

III.F.2. Preparation of Reagents

i) Cuprizone solution. A Quantity (0.2 g) of Cuprizone (oxalic bis(cyclohexylidenehydrazide), (Aldrich Chemical Company Inc., Milwauke, WI), was dissolved in ethanol (20 ml) by heating. This solution was diluted to 40 ml with deionized distilled water, the cuprizone solution contained 0.5% (w/v) cuprizone in ethanol:water (1:1).

ii) 10% (w/v) Ammonium citrate, dibasic (Anachemia Canada Inc., Montreal, PQ) solution in deionized distilled water.

iii) 0.6N hydrochloric acid in 70% (v/v) propan-2-ol, (reagent grade), was prepared by addition of 251 ml deionized distilled water, 700 ml propan-2-ol, and 49

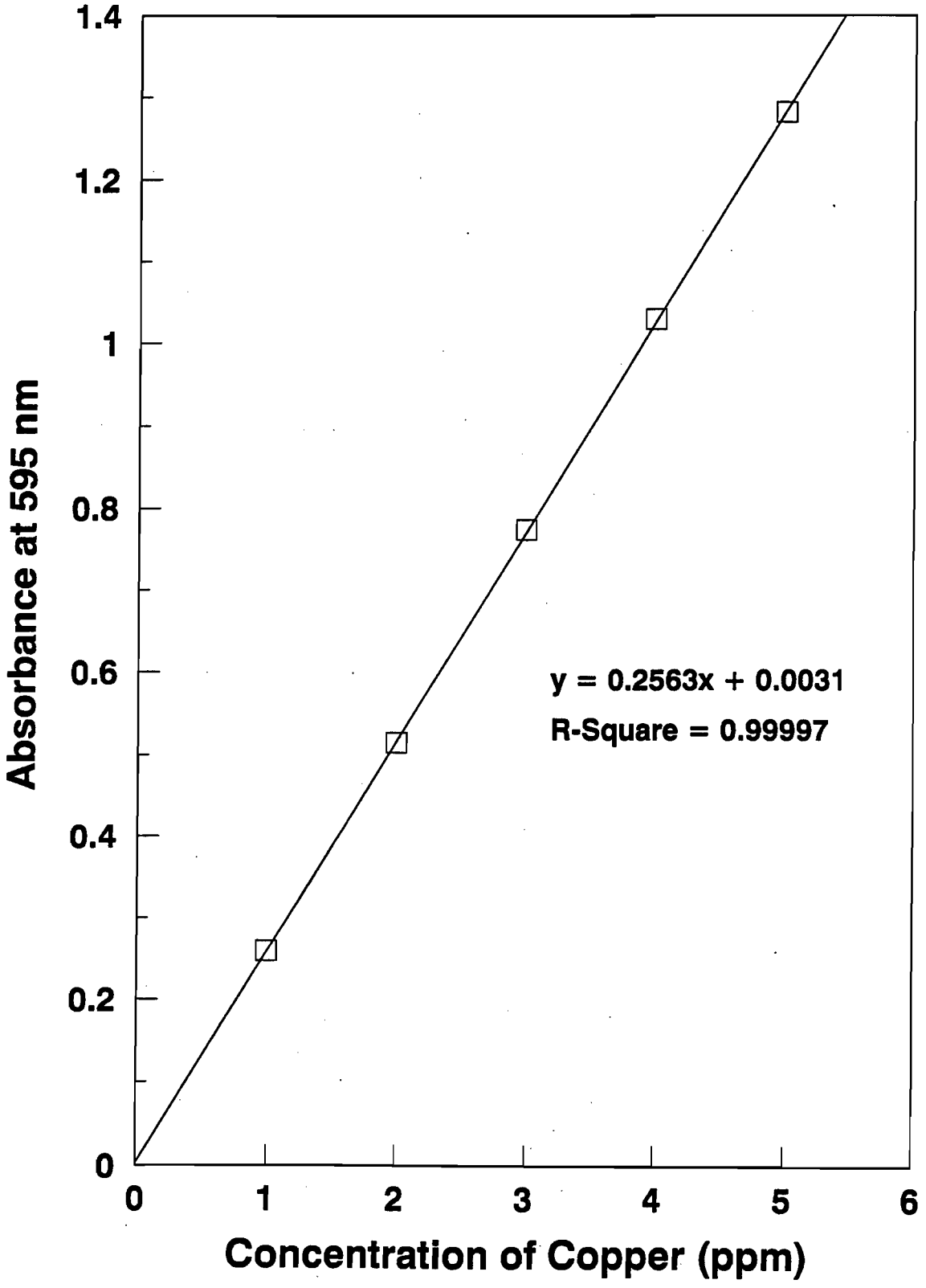
ml HCl (37%). The mixture was agitated and made up to 1 l by addition of 70 % (v/v) propan-2-ol.

III.F.3. Preparation of Standard Curve

A standard solution (100 μg/ml) of cupric ions was made by dissolving 0.3929 g of analytical reagent grade cupric sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 1 l deionized distilled water. A volume (10 ml) of this solution was diluted to 100 ml; this represented a stock solution (10 μg/ml). The pH of cupric solutions was adjusted to pH 3.5 in order to avoid formation of $Cu(OH)_2$ precipitate. A set of standard cupric sulphate solutions ranging from 0 to 5 ppm was prepared by diluting the appropriate volumes (0, 5, 10, 15, 20 and 25 ml) of stock solution (10 μg/ml) to 50 ml in volumetric flasks.

Ammonium citrate dibasic (1 ml, 10% solution), deionized distilled water (10-30 ml), isopropanol (2 ml, 70% in 0.6N HCl), and ammonia solution (4 drops, 25%) were added to the copper solution in the 50 ml volumetric flask. The pH was adjusted to the range 8.2 - 8.9 by use of dilute solutions of either NH_4OH or/and H_2SO_4. Cuprizone reagent solution (2 ml) was then added to the mixture in order to produce a coloured blue complex (Williams & Morgan, 1954). Deionized distilled water was added to bring the total volume of the flask to 50 ml. The final solution was thoroughly mixed, allowed to stand for 30 min and the absorbance was read within 1 h at 595 nm (LKB Biochrom Spectometer, Ultrospec II, Cambridge, England). A standard curve for the cupric ions, as shown in Figure 6, was prepared.

Figure 6. Standard curve for the estimation of the cupric ions concentration bound by dry BSG (λmax = 595 nm)



III.F.4. Determination of Cation Exchange Capacity

Cation exchange capacity measurements were performed according to the method of McBurney et al. (1983 , 1986) with slight modifications, as shown in Figure 7.

A quantity (0.500 g) of dried sample was placed in a Pyrex crucible (50 ml) containing a coarse fritted disc (gooch). The crucible was placed in a beaker (100 ml), and cupric sulphate solution (40 ml, 0.1M; pH<4) was added. The beaker was agitated and placed in a gravity convection incubator (2EG model, Precition Scientific Inc., Chigaco, Illinois) for 24 h at 38°C. After the incubation, the sample was washed with 5x40 ml portions of deionized distilled water under vacuum in order to remove unbound cupric ions. The crucible was then wiped and washed (by gravity filtration) with 4x20 ml portions of HCl (0.6M) in isopropanol (70%). The filtrate was collected in a 100 ml volumetric flask and isopropanol (70%) was added to adjust the volume to 100 ml.

An aliquot (2 ml) of the filtrate was placed in a 50 ml volumetric flask. Deionized distilled water (25 ml), ammonium citrate solution (dibasic, 1 ml, 10%), and ammonia solution (4 drops, 25%) were transferred to the volumetric flask. The solution was adjusted to the desired pH range (8.2-8.9) by the addition of either dilute NaOH and H_2SO_4 solutions. Cuprizone reagent solution (2 ml) was added, followed by deionized distilled water to bring the appropriate volume to 50 ml. The contents were thoroughly mixed and after 30 min the absorbance was measured at 595 nm. Blanks were prepared using the identical procedure except, that instead of

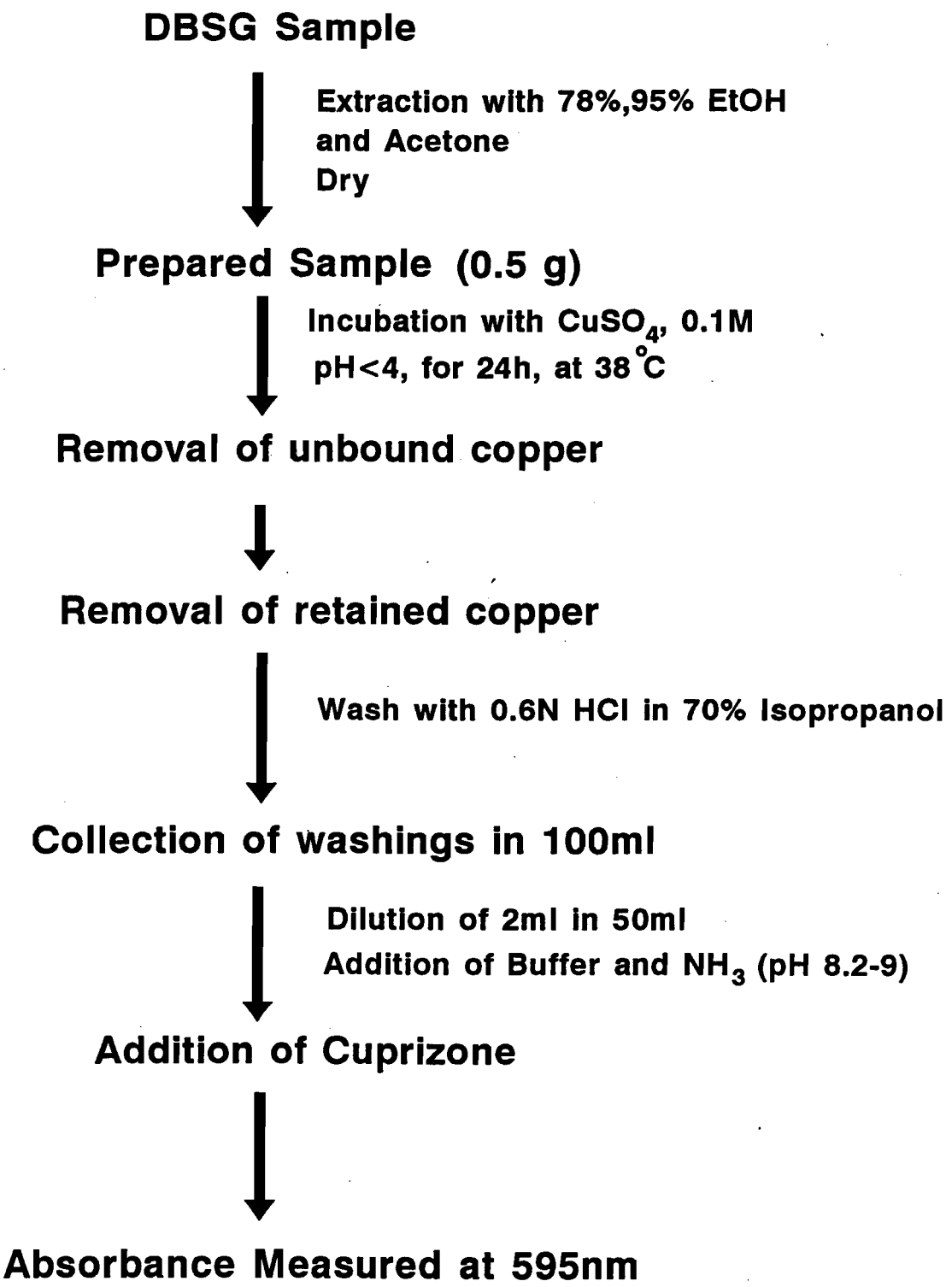


Figure 7. Schematic diagram of the determination of cation exchange capacity.

the cupric sulphate solution, sodium azide solution (0.1M) was added in order to prevent microbial degradation of the samples in the blank experiment.

III.G. BILE SALTS BINDING CAPACITY

Measurement of binding capacity of bile salts (*in vitro*) is based on the incubation of the sample with bile acid and measuring the amount of bile acid which is retained by the sample source (Eastwood and Hamilton, 1968). The binding capacity of bile salts was determined according to the methods of Kritchevsky and Story (1974) and Story (1982) with some modifications as illustrated in Figure 8.

The binding of taurocholic acid, 2[(3a, 7a, 12a-trihydroxy-24-oxo-5b-cholan-24-yl) amino]-ethanesulphonic acid in sodium salt form, a conjugated bile salt, and cholic acid, 3a, 7a, 12a, trihydroxy-5b-cholan-24-oic acid (sodium salt), an unconjugated bile salt, by fractions of DBSG, commercial fiber sources (wheat bran and oat bran), and cholestyramine (an anion-exchange resin) was measured. The samples were washed and vacuum dried as in section III.F.1 and stored in a desiccator.

III.G.1. Preparation of Solutions

Bile salt solutions at concentration 5.0 mM were prepared by dissolving 0.7172 g taurocholic or 0.5382 g cholic acid (sodium salt; Aldrich Chemical Company Inc., Milwakee, WI) in phosphate buffer (Na_2HPO_4-NaH_2PO_4; 250 ml, 0.1M, pH 8). The taurocholic salt solution was labelled by pipetting 62.5 μl radioactive [24-^{14}C]-taurocholic acid solution in MeOH:EtOH (1:3) (Du Pont Canada Inc., Mississauga,

Ontario) with concentration of 0.74 MBq/ml (0.02 mCi/ml) (0.39 μmol/ml), in the prepared bile salt solution. In the case of cholic salt solution, volume (12.5 μl) of radioactive [Carboxyl-^{14}C]-cholic acid solution in EtOH (Du Pont Canada Inc., Mississauga, Ontario), with concentration 3.7 MBq/ml (0.1 mCi/ml) (2.0 μmol/ml) was used. The final radioactivity in both solutions was 0.185 KBq (0.005 μCi/ml) and the specific activity was 37 KBq/mmol (1 μCi/mmol).

III.G.2. *In vitro* Determination

A quantity (100 mg) of each dry sample was placed in Röhre polyethylene sterile tubes (13 ml, Sarstedt, Numbrecht, Germany). Five millilitres of the individual bile salt solution (5 mM, 25 μmoles) in phosphate buffer (0.1M, pH 8) was added to the plastic tube. The tube was covered with a lined snap cap, sealed with paraffin paper and incubated (2 h, 38°C) on a reciprocal shaker-water bath (Precision Scientific Inc., Chicago, Illinois). The incubation mixture was shaken gently and continuously.

After the incubation, the plastic tube was centrifuged (IEC Centra-8R, International Equipment Company) at 5°C for 30 min at 2,000xg (3,300 rpm) to remove particulate material. The radioactivity of the supernatant was used as an indication of the quantity of unbound bile salt. 200 μl of the supernatant from each tube was transferred to a 7 ml polyethylene scintillation vial (Baxter Diagnostics Corporation, Point Claire, PQ) and 4 ml of scintillation fluid Ecolite(+), (ICN Biomedicals Canada Ltd., Mississauga, Ontario) was added; the contents in the vials

were mixed thoroughly and left to stand for 30 min. The radioactivity was analyzed for 10 min by liquid scintillation spectrometry in a LKB liquid scintillation counter (LKB-Wallac, 1209 Rackbeta, Turku, Finland) using external standardization and the appropriate window for ^{14}C. Control tubes, containing all the reagents without binding substance, were treated in the same manner.

The amount of bile salt that is bound to the sample, was determined by subtraction of the radioactivity that was recovered in the supernatant from the radioactivity that was initially added (control).

The effect of centrifugation speed on radioactivity measurement was examined. A quantity (2 ml) of the incubated mixture was centrifuged using different rotating speeds of 2,000xg and 14,000xg respectively (microcentrifuge Model 5415C, Eppendorf).

The determinations were performed on triplicate samples, and all isotope analysis of each incubation tube was done in duplicate.

III.H. STATISTICAL ANALYSIS

The data was analyzed statistically using the Statistical Analysis System (SAS) on McGill University's system. The regression curves in the appendix was performed using statgraphics software package (version 3.0) with the help of lotus freelance software.

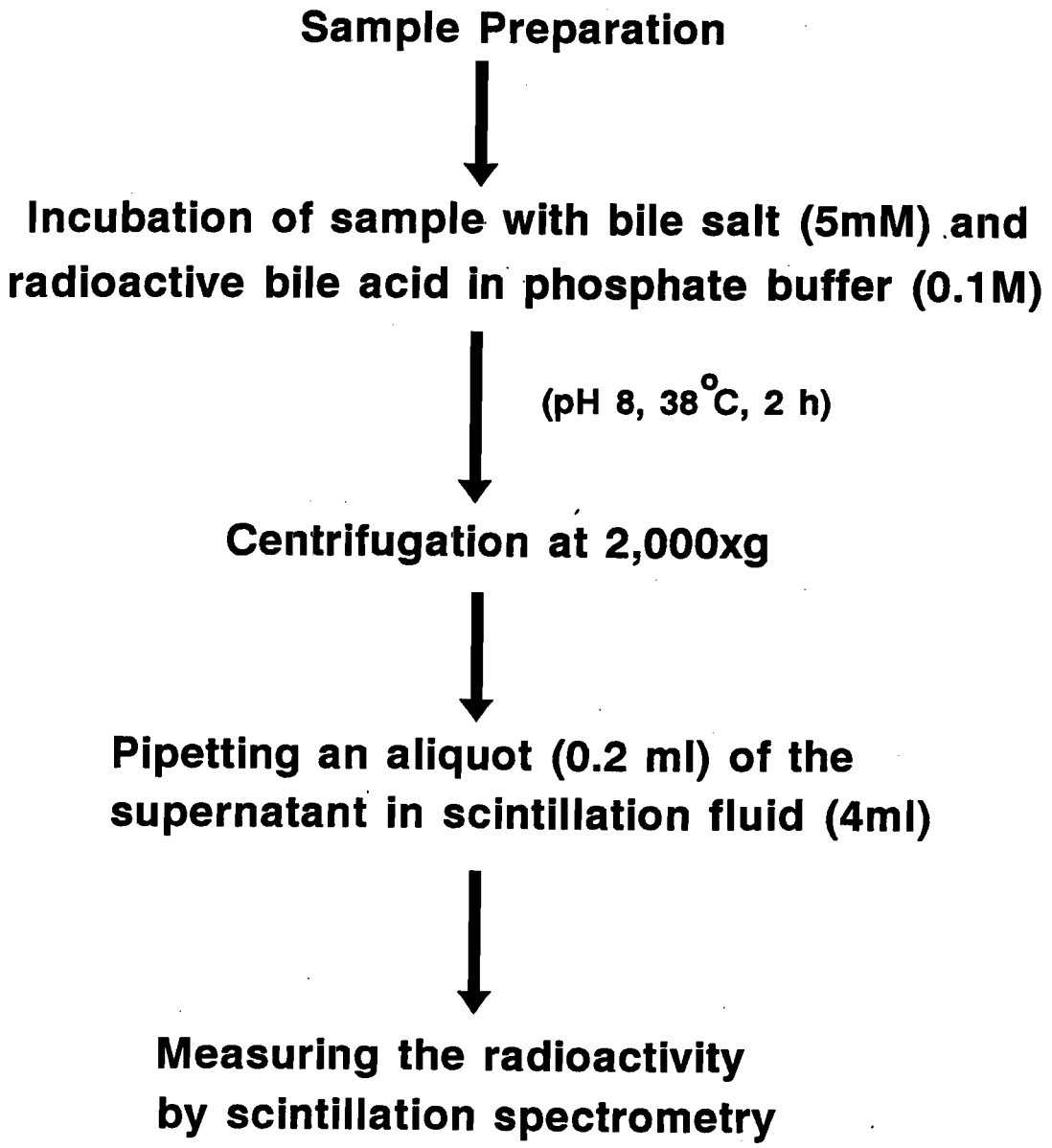


Figure 8. Schematic diagram of the *in vitro* determination of bile salt binding capacity.

IV. RESULTS AND DISCUSSION

IV.A. FRACTIONATION OF DBSG

IV.A.1. Preliminary Examination of Particle Size Distribution.

In order to determine the particle size distribution of dried brewers' spent grain (DBSG), a sample of not-ground DBSG (30 g) was subjected to a sieving procedure using a set of sieves ranging from 1400 to 250 μm attached with a pan. On the basis of this preliminary screening a set of four sieves (500, 250, 150, and 125 μm) were selected for use in the investigation of size distribution of milled (ground) DBSG. The sieve sizes were selected representatively to give sufficient quantities of fractionated DBSG in each screen.

IV.A.2. Particle Size Analysis

The relationship between the particle size and retained quantity of DBSG in each sieve using not-ground and ground DBSG samples are shown in Table 1. For the non-ground DBSG particles more than 90% (w/w) was retained in sieves with aperture of diameter greater than 250 μm. The not-ground DBSG gave fractions representing 36.3% (retained on 1000 μm screen), 30.3% (retained on 500 μm screen) and 24.2% (retained on 250 μm screen) of the total weight of the not-ground material. In the ground DBSG, only 30.9% (w/w) of the ground material was retained on the 250 μm screen. The two other fractions obtained from the ground DBSG represented 28.6% (w/w) (retained on 150 μm screen) and a significant amount 40.5% (w/w) (passed through 150 μm diameter screen) of the total weight of ground material.

Table 1a. Proportion of each fraction of not-ground dried brewers' spent grain retained on sieves.

Sieve size (μm)	>1000	500	250	<250
Mean[1] % (w/w)	36.3	30.3	24.2	9.2
SD[2]	2.25	0.25	1.77	0.52

[1] Results are means of triplicate determinations
[2] Standard deviation

Table 1b. Proportion of each fraction of ground dried brewers' spent grain retained on sieves.

Sieve size (μm)	>250	150	<150
Mean[1] % (w/w)	30.9	28.6	40.5
SD[2]	0.96	0.12	1.17

[1] Results are means of triplicate determinations
[2] Standard deviation

These results suggest that the particle size distribution of the not-ground DBSG was quite variable ranging from < 1000 μm to > 250 μm. As expected, the grinding procedure resulted in a significant change in the particle size distribution of the dried BSG.

Heller et al. (1977) ground fiber samples and determined the composition of the different size fractions. Parrott and Thrall (1978) investigated differences between particle size and physical properties of various fiber types. The authors determined the particle size distribution using sieves with screen aperture of 2.000, 841, 500, 208, and <208 μm. Chaudhary and Weber (1990a) reported a milling and sieving procedure to isolate two flour fractions from brewers dried grain with different protein and dietary fiber composition. In the same report milled sample was fractionated using a set of sieves (420, 297, 210, 149, 105, and <105 μm screen aperture) and the fractions were analyzed for protein and total dietary fiber.

The size reduction of the cell wall structure by milling can affect the functional properties of fiber source such as water-holding capacity and bile salt binding (McConnell et al., 1974; Mongeau and Brassard, 1982). The pore and radius volume can also be affected during the mechanical reduction of particles influencing the attributes of the dietary fiber. Excessive grinding may hydrolyse some polysaccharides. (Farber, 1982).

When the sample size is reduced, the surface area is increased providing more active sites for chemical and physical actions. However, when particle size becomes very small, the fiber bulk is more compact and the density increased (Parrott and

Thrall, 1978) reducing the activity of enzymes, the nutritional effectiveness of the food source and therefore the digestion. Larger particles can affect strongly the function of the bowels. Furthermore, the size distribution can affect the physical and chemical attributes of the fiber.

The remaining portion in each sieve can be affected by the shape of particles. The cereal fibers after grinding provide particles with rod shapes that can pass through certain aperture sieves (Rasper, 1979). Parrott and Thrall (1978) detected that the experimental data from sieving gave larger particles size results, while the microscopic analysis showed smaller values.

IV.B. MOISTURE AND ASH CONTENT

The results from the ash and moisture contents are shown in Tables 2 and 3. The different fractions of not-ground DBSG showed ash contents ranging from 3.07% (for the fraction with particle smaller than 250 μm) to 3.88% (500 μm fraction). The ground DBSG fraction showed a similar range of ash contents (3.00% to 3.83%). However, there is some heterogeneity in the ash contents for both the not-ground and ground DBSG as is shown in Figure 9. In the case of not-ground DBSG the 500 μm fraction contained the highest ash content (3.88%). For particle size less than 500μm, there was a reduction in ash content as particle size reduced for both not-ground and ground DBSG.

For the milled portions, the relationship between particle size and ash content can be associated with the milling process. Fibrous material (which may be high in

Table 2. Moisture and ash content of not-ground dried brewers' spent grain.

Screen Fractions	Moisture[1] ± SD[2]	Ash[1] ± SD[2]
(μm)	(%)	(%)
>1000	7.07 ± 0.08	3.29 ± 0.03 (b)[3]
500	6.88 ± 0.08	3.88 ± 0.05 (a)
250	6.79 ± 0.21	3.24 ± 0.07 (b)
<250	6.90 ± 0.17	3.07 ± 0.03 (c)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.01 level.

Table 3. Moisture and ash content of ground dried brewers spent grain.

Sieve Size	Moisture[1] ± SD[2]	Ash ± SD[2]
(μm)	(%)	(%)
>250	ND[3]	3.83 ± 0.02 (a)[4]
150	ND[3]	3.66 ± 0.15 (a)
<150	ND[3]	3.00 ± 0.10 (b)
GDBSG[5]	ND[3]	3.58 ± 0.15 (a)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Not determined

[4] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.01 level.

[5] Ground dried brewers' spent grain

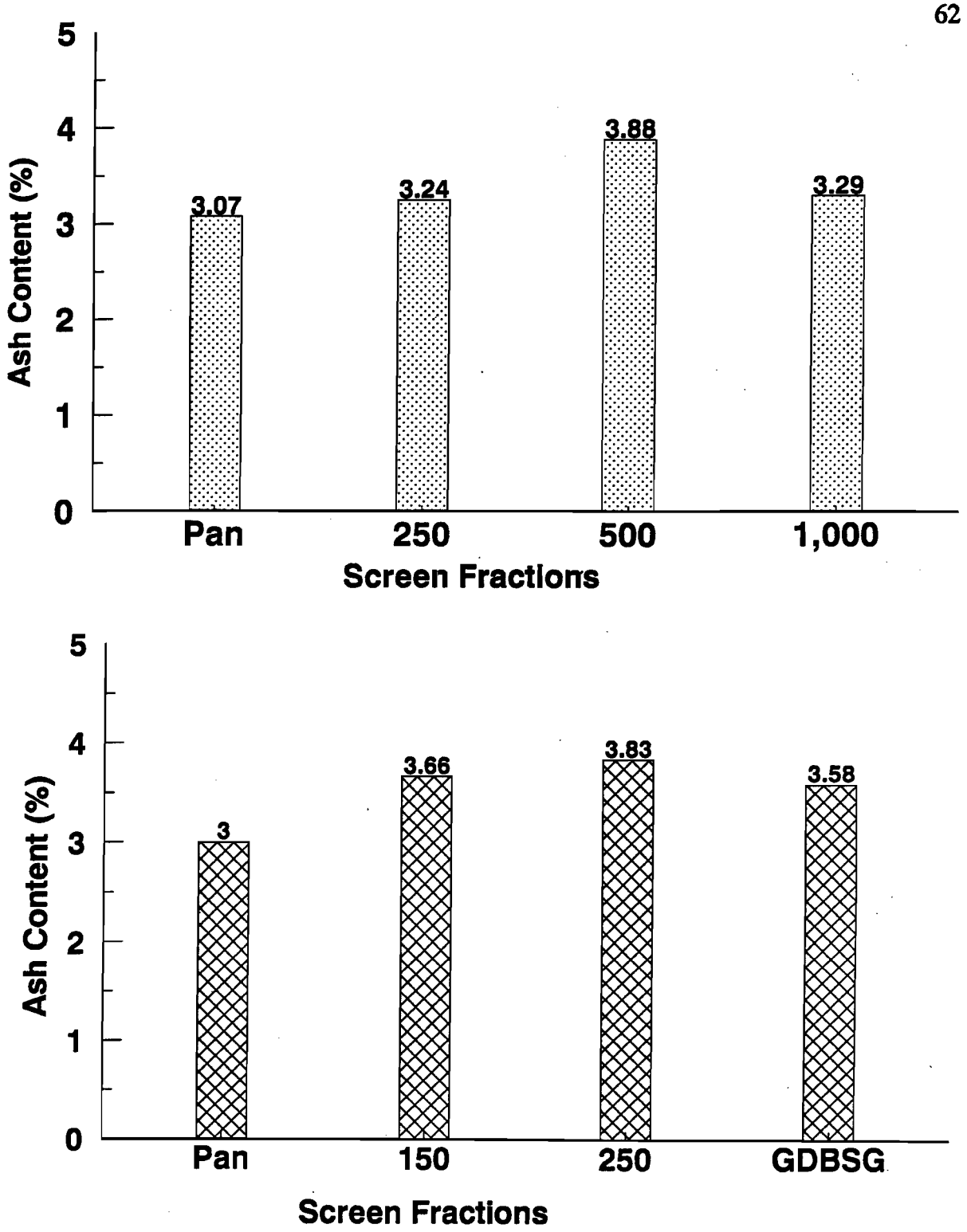


Figure 9. Ash content of fractions of not-ground dry BSG (top) and ground dry BSG (bottom).

mineral content) tends to be more rigid than non-fiber compounds, and the size reduction during grinding in these "fiber" components is more difficult than in non-fiber material. Hence, the larger particles eg fractions of >250 μm and 150 μm, contained more minerals (3.83 % and 3.66 %). The finer fraction (<150) gave an ash content of 3.00 %.

The ash content of DBSG fractions was correlated positively and significantly ($r = 0.96$, $P < 0.001$) with the total dietary fiber content (Tables S1 and S3; Figure S2) and was negatively correlated ($r = - 0.86$, $P < 0.01$) with the protein content (Tables S1 and S2; Figure S1). Further, negative correlations (Tables S1, S4, and S5; Figures S3 and S4) were observed between ash content and bile salts binding ($r = -0.83$, $P < 0.05$ and $r = -0.71$, $P < 0.05$ for the taurocholate and cholate respectively).

IV.C. PROTEIN CONTENT

The results of protein content (Tables 4 and 5) indicate that in both not-ground and ground DBSG, particle size is related to the total protein content of the fractionated DBSG. For the not-ground DBSG, the highest content of protein (41.83%, dry mater basis) was found in the fraction with the smallest particle size (<250 μm). The retained fraction on the 250 μm sieve gave an intermediate content of 38.81% protein while the lowest content of 22.22% protein was obtained from the 500 μm sieve fraction. However for the 1000 μm fraction, a higher content of protein (25.67%) was determined than in the 500 μm fraction. The results from Figure 10 suggest a relationship between protein content and particle size indicating that there

is a lower protein content associated with larger particles. The higher content of protein in the >1000 μm particles, when compared with the 500 μm particles, may be due to the morphological characteristics and nature of the specific particles.

In the case of protein for the ground DBSG, protein content was also related to particle size distribution. The lowest content of protein (22.47%) was found in the >250 μm fraction (the largest particle size fraction), while the 150 μm particle size and <150 μm particle size fractions showed significantly higher protein contents of 28.59% and 37.86%, respectively. These results indicate an inversely proportional relationship between particle size of DBSG and protein content (Figure 10). The protein percentage of non-fractionated ground DBSG presented an intermediate value of 29.1% protein, which is not significantly different from the protein content of the 150 μm fraction (28.59%).

Chaudhary and Weber (1990a) reported that a grinding and sieving technique similar to that used in this study, resulted in an increase in the protein content of ground brewers' dried grain when the particle decreased (the protein content of samples that were retained on the screen fractions of 420, 297, 210, 105, and <105 μm were 9.0, 14.0, 24.7, 41.5, and 45.9%, respectively). They also concluded that a combination of grinding and sieving techniques similar to that used in this study gave high-protein fractions and fiber-rich fractions. Prentice and D' Appolonia (1977) using milling and particle size fractionation of milled BSG also found higher concentrations of nitrogen in the smaller fractions.

The results obtained suggest that a relatively high protein content (37.86%)

Table 4. Compositions of screen fractions of not-ground DBSG on dry basis.

Fractions	Protein ± SD[1]	IDF[2] ± SD[1]	SDF[3] ± SD[1]	TDF[4] ± SD[1]
(μm)	(%)	(%)	(%)	(%)
>1000	25.67±0.80 (a)[6]	ND[5]	ND[5]	ND[5]
500	22.22±0.66 (b)	63.11±1.00 (a)	0.46±0.25 (b)	63.57±0.81 (a)
250	38.81±0.40 (c)	41.98±1.43 (b)	1.18±0.20 (a)	43.17±1.43 (b)
<250	41.83±0.19 (d)	35.44±0.96 (c)	1.53±0.18 (a)	36.97±0.80 (c)

[1] Standard deviation of means from triplicate determinations

[2] Insoluble dietary fiber

[3] Soluble dietary fiber

[4] Total dietary fiber

[5] Not determined

[6] Means were compared using the Duncan's multiple range test. Mean values with the same letter within the same column are not significantly different at 0.01 level.

Table 5. Composition of screen fractions of ground DBSG on dry basis.

Fractions	Protein ± SD[1]	IDF[2] ± SD[1]	SDF[3] ± SD[1]	TDF[4] ± SD[1]
(μm)	(%)	(%)	(%)	(%)
>250	22.47±0.31 (c)[6]	58.36±2.20 (a)	1.41±0.30 (a)	59.78±1.93 (a)
150	28.59±0.17 (b)	52.14±0.37 (b)	1.05±0.23 (a)	53.19±0.35 (b)
<150	37.86±0.35 (a)	41.43±1.84 (c)	1.08±0.14 (a)	42.51±1.75 (c)
GDBSG[5]	29.06±0.11 (b)	50.98±1.35 (b)	1.05±0.14 (a)	52.03±1.48 (b)

[1] Standard deviation of means from triplicate determinations

[2] Insoluble dietary fiber

[3] Soluble dietary fiber

[4] Total dietary fiber

[5] Ground dried brewers' spent grain

[6] Means were compared using the Duncan's multiple range test. Mean values with the same letter within the same column are not significantly different at 0.01 level.

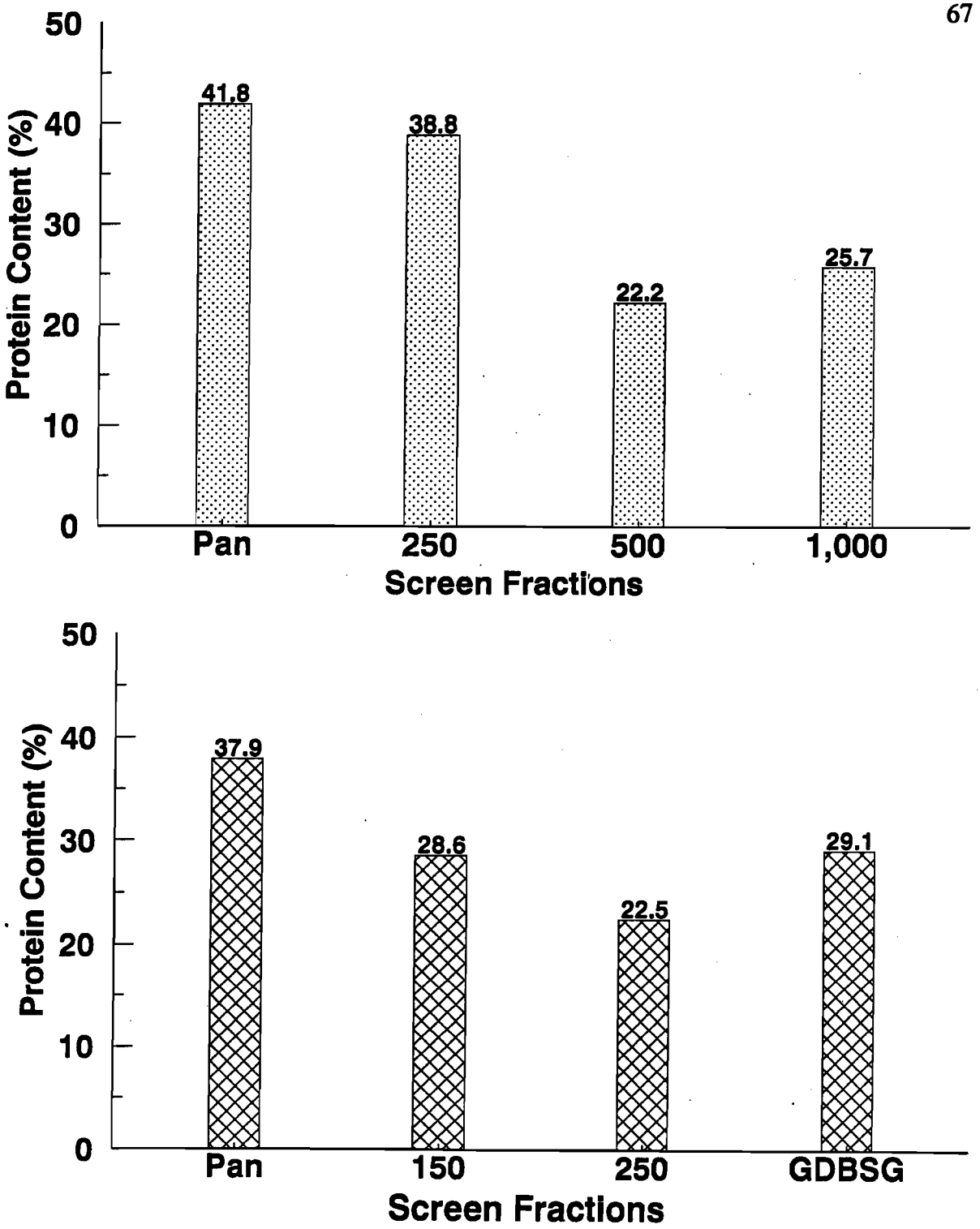


Figure 10. Protein content of different screen fractions of not-ground dry BSG (top) and ground dry BSG (bottom).

fraction representing 40.5 % of the ground DBSG was obtained by the grinding and sieving procedure. This suitable fraction could find used as a protein-rich food ingredient.

IV.D. DIETARY FIBER CONTENT

The results of analysis of soluble, insoluble, and total dietary fiber content in the different particle size fractions are shown in Tables 4 and 5 and are presented in graphic form in Figure 11. The total dietary fiber (TDF) content (dry basis) for the screen size of 500 μm (63.57% TDF) represented the highest value in the case of the not-ground DBSG. When the particle size was decreased, the content of TDF was also decreased. For example TDF contents for the 250 μm and <250 μm (pan) screen fractions were 43.17% and 36.97% TDF, respectively.

Similar results were observed in the case of ground DBSG (Figure 11). The largest particle size fraction (250 μm) contained the higher TDF content (59.78%). The 150 μm fraction contained 53.19% TDF and the smallest size fraction contained the lowest concentration of TDF (42.51%). An average percentage of TDF (52.03%) which was not significantly different from the TDF of the 150 μm fraction was determined in the milled DBSG (Table 5). All the other TDF percentages in samples of DBSG were significantly different according to Duncan's multiple variance test.

The contents of soluble dietary fiber (SDF) in all fractions were relatively small; SDF contents ranged from 0.46% to 1.53%. This could be the result of prior removal of soluble fiber components eg. β-glucans, pectins and other soluble dietary

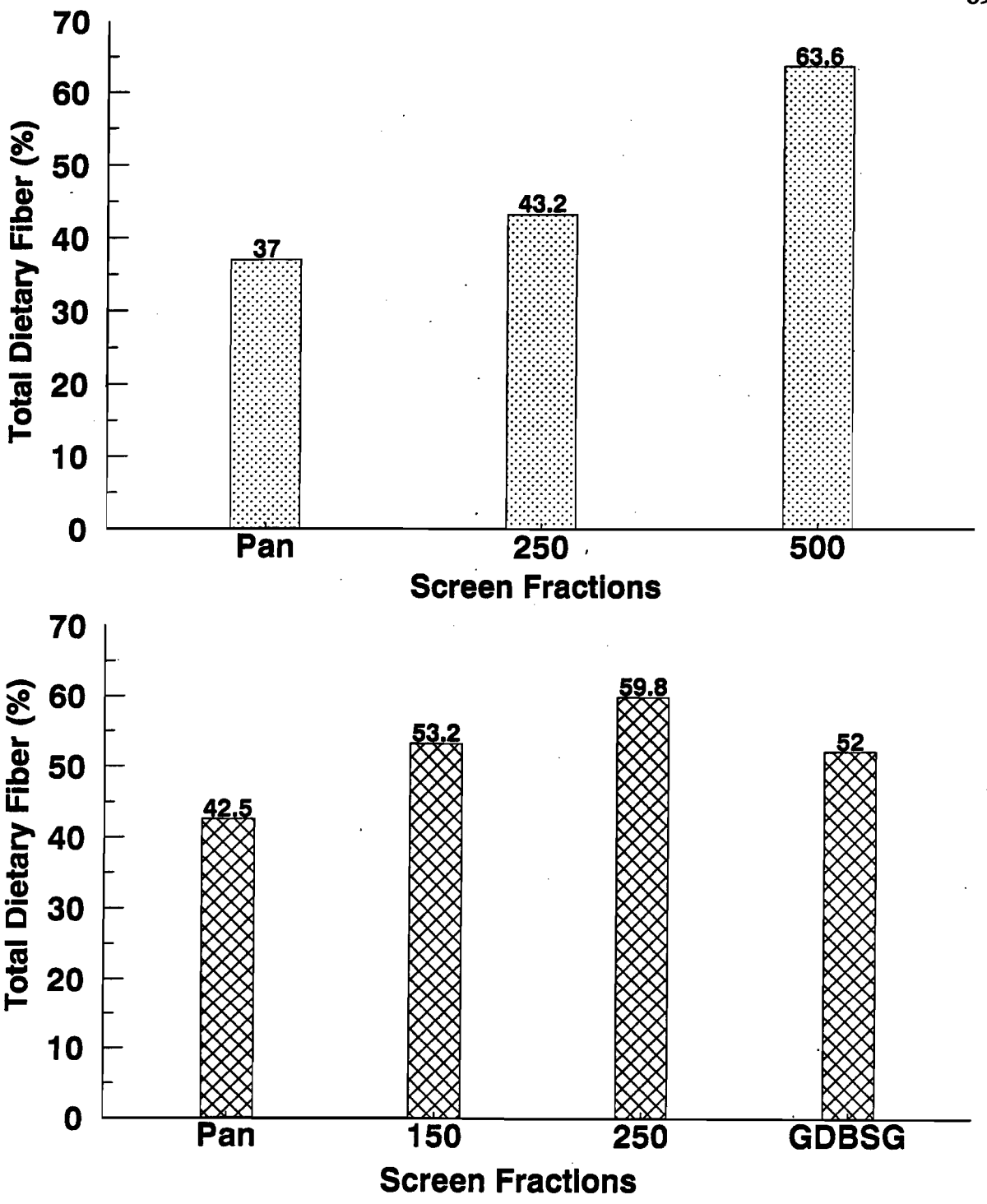


Figure 11. Total dietary fiber content of different screen fractions of not-ground dry BSG (top) and ground dry BSG (bottom).

fiber components during the extraction procedures in the brewing process. In addition, all the soluble dietary fiber contents for the ground fractions were not significantly different.

Chaudhary and Weber (1990a) showed a comparable relationship between the particle size of milled brewers' dried grain and total dietary fiber. The fractions with the larger particles gave higher concentrations of dietary fiber (85.0, 74.5, 57.2, 46.0, 38.0, and 29.3% TDF for the 420, 297, 210, 149, 105, and <105 μm fractions, respectively).

The fractions with high protein concentration contained low amounts of dietary fiber and and vice versa. Statistical analysis revealed that these two components are highly negatively correlated ($r = -0.99$, $P < 0.00005$) (Tables S1 and S6; Figure S5).

IV.E. WATER-HOLDING CAPACITY

Tables 6 and 7 show the water holding capacity of the fractions obtained from not-ground and ground fractions of DBSG. The results in relation to particle size are shown in graphic form in Figure 12. The values of the not-ground DBSG fractions vary among the different size fractions. These results indicate that the highest WHC value of 3.57 ml/g was found in the particles with sizes between 500 and 250 μm (250 μm fraction), while the largest particle size fraction (>1000 μm) had the lowest WHC of 1.88 ml/g. The other two fractions of particle size 500 μm and <250 μm gave intermediate WHC values of 2.25 ml/g and 2.91 ml/g, respectively.

The results indicate that there was a consistent relationship between WHC and

particle size with the ground DBSG fractions. As the particle size decreased from 250 μm to 150 μm to pan<150 μm fractions, the amount of water that was retained also decreased from 3.26 ml/g to 3.03 ml/g to 2.49 ml/g, respectively. The WHC results of not-ground and ground DBSG were statistically significantly different between different fractions.

The WHC of the commercial wheat bran (3.30 ml/g) was slightly higher and significantly different from the WHC of not-ground 1000, 500, and <250 μm DBSG fractions. However, the particle size fraction from not-ground DBSG that was retained on 250 μm sieve gave a higher but not statistically different WHC (3.57 ml/g) than wheat bran. A comparison of the ground DBSG fractions with wheat bran indicates that the concentration of water held by wheat bran is higher that the WHC of the two finer DBSG fractions. It should be remarked that wheat bran is a cereal source of dietary fiber with more elevated capacity to retain water within its matrix; in this type of bran, the high water-holding capacity is due to excessive quantities of trapped water within the bran structure (Robertson and Eastwood, 1981a).

Oat bran was another commercial source of fiber that gave the lowest and statistically different ($P < 0.05$) water holding capacity (1.38 ml/g) when compared to the fractions obtained from DBSG samples and wheat bran.

These results suggest that water held by DBSG fiber rely on the size of particles (Figure 12). The results do not show a significant correlation between WHC and fiber or protein content in the sum of DBSG samples (Table S1, S1). The capacity to hold water is a complex process and may involve the factors of

Table 6. Water-holding capacity of not-ground brewers' spent grain, wheat and oat bran.

Fiber Source	WHC[1] ± SD[2]
(μm)	(ml water/g dry sample)
>1000	1.88 ± 0.09 (e)[3]
500	2.26 ± 0.11 (d)
250	3.57 ± 0.12 (a)
<250	2.91 ± 0.14 (c)
Wheat Bran	3.30 ± 0.11 (b)
Oat Bran	1.38 ± 0.17 (f)

[1] Water-holding capacity

[2] Standard deviation of means from triplicate determinations

[3] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

Table 7. Water-holding capacity of ground brewers' spent grain, wheat and oat bran.

Fiber Source	WHC[1] ± SD[2]
(μm)	(ml water/g dry sample)
>250	3.26 ± 0.11 (a)[3]
150	3.03 ± 0.03 (b)
<150	2.49 ± 0.07 (c)
Wheat Bran	3.30 ± 0.11 (a)
Oat Bran	1.38 ± 0.17 (d)

[1] Water-holding capacity

[2] Standard deviation of means from triplicate determinations

[3] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

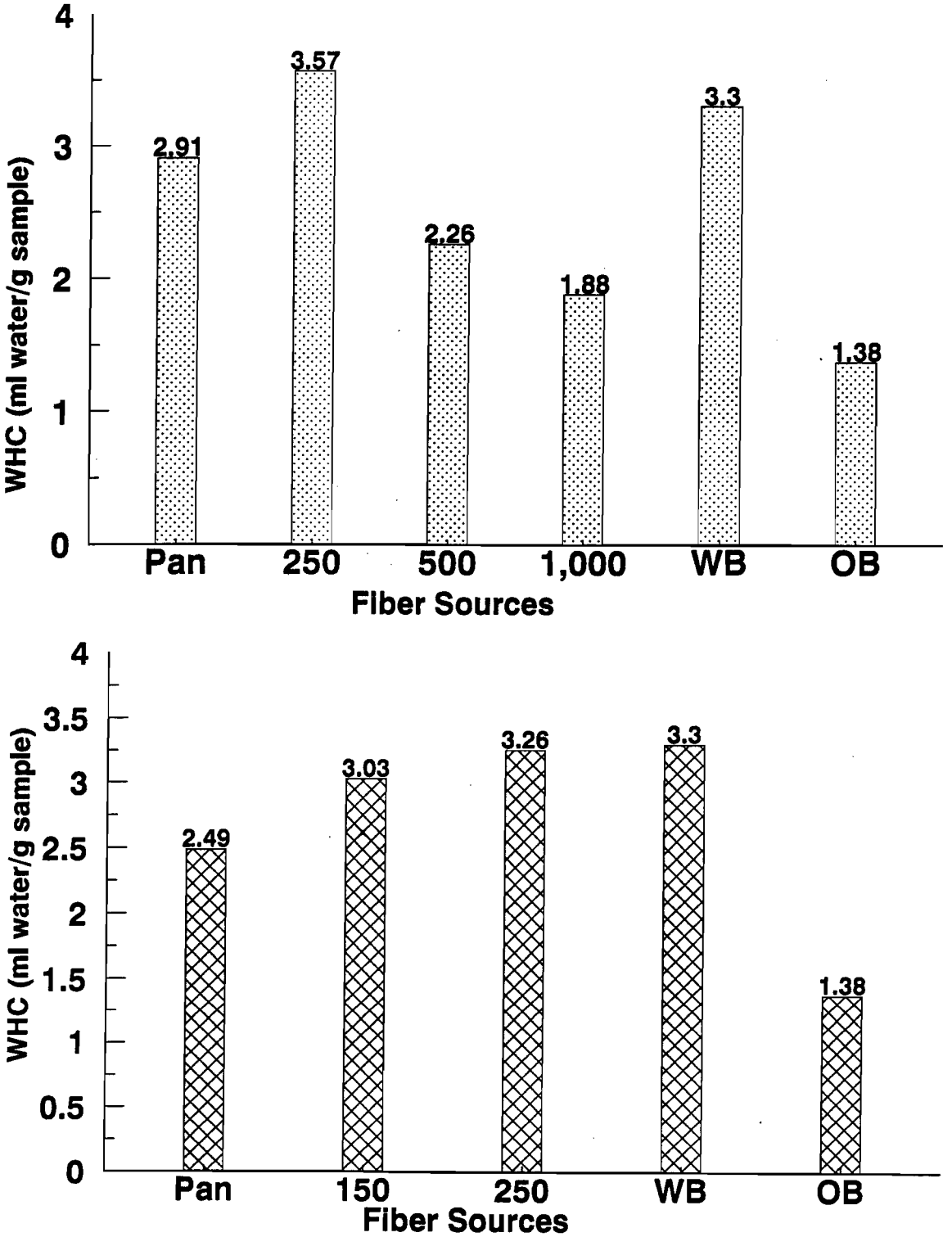


Figure 12. WHC of different screen fractions of not-ground dry BSG (top) and ground BSG (bottom) compared to commercial dietary fiber sources of wheat bran (WB) and oat bran (OB).

composition, surface area available for binding, and structural characteristics. In relatively large particles the surface area is relatively small and water is absorbed to a lesser extent. When the surface area is increased, the water can more easily be absorbed by a fiber. Although in very small particles the pore size is drastically eliminated and the three-dimensional fiber matrix that retains water, is destroyed. Furthermore, in smaller size particles the amount of fiber that is responsible for the water-holding effect is reduced and therefore the WHC is low.

McConnell et al., (1974) found a correlation between acid detergent fiber and WHC ($r=0.76$, $a=1.021$, $b=0.30$). However, Robertson and Eastwood (1981b) suggested that WHC is more related to structure than to chemical composition.

Kirwain et al. (1974) suggested that small particles of wheat bran had less ability to hold water; a similar phenomenon can be observed in Figure 14. In addition, Mongeau and Brassard (1982) demonstrated that water-holding capacity decreased as the mean of particle size of neutral detergent fiber from unprocessed brans was decreased; with a positive correlation ($r = 0.85$) between WHC and the mean of particle size in wheat products. Stephen and Cummings (1979) presented an elevation of the water-holding capacity as the particle size was reduced using two fractions with the same chemical composition. However, this appears to be related to the method of WHC determination that is used (Stephen and Cummings, 1979).

The standard AACC method which was used, can be compared to results of Robertson and Eastwood (1981a) who reported a value of 3.7 ± 1.7 g water/g fiber, for the wheat bran WHC using different experimental conditions (higher

centrifugation speed and time of soak 24 h). McConnell et al. (1974) reported wheat bran WHC of 3.0 g water/g acetone dried powder (soaked for 24h and centrifuged at 14000 x g for 1 h). Chen et al. (1984) used a similar centrifugation technique to measure the WHC and observed WHC of 2.63 ± 0.12 g H_2O/g solid for wheat bran and 1.42 ±0.09 g H_2O/g solids for oat bran.

Different methods evaluate water that is held by different mechanisms. The centrifugation method evaluates water that is bound by fiber, under certain types of compression using relatively low centrifugal force (2,000 x g) to remove free water. In our measurements, it is believed that water is bound by interacting with molecules of fibrous DBSG involving the use of dipole-dipole forces and hydrogen bonds. In addition, water can be held in capillary structures of fiber, and part of trapped water can be measured.

Water holding capacity can also be affected by the method of sample preparation such as drying (Robertson and Eastwood, 1981a). When the drying conditions are intense, the fiber structure can be damaged and the WHC can be decreased for the same fiber source (Robertson and Eastwood, 1981b). Therefore, mild drying conditions (vacuum oven) were used in order to remove the moisture content in our fiber samples. McConnell et al. (1974) reported an effect of grinding on WHC. They showed a decreased ability of milled fiber to hold water (from 5.0 to 3.1 g water/g dry powder) because of structure destruction. The same effect of grinding in the reduction of WHC was reported by Mongeau and Brassard (1982). Moreover, these structural modifications may affect the faecal bulking action of fiber (Robertson and Eastwood, 1981c).

IV.F. CATION EXCHANGE CAPACITY

IV.F.1. Quantification of Copper (Cu^{++})

The colorimetric method for copper determination is based on the reaction of cupric ions with a sensitive organic reagent oxalic bis (cyclohexylidene hydrazide) in slightly alkaline solution forming a blue colour complex (Nilsson, 1950). The molar absorbance index (17,120 at 606 nm) (Welcher and Boschmann, 1979) of the complex is relatively high compared to other chromogenic reagents that are used in the determination of copper. The coloured solution is clear, stable with constant absorbance at certain pH. This colorimetric method provides a fast and accurate estimation of copper (II). The sensitivity of the method is approximately 0.03 ppm (Peterson and Bollier, 1955). The optimum pH of the reaction is in the range between 7.0 - 9.0, the colour develops within 5 minutes, and is stable for one hour. The absorbance of the formed complex, in relation to copper concentration, is linear over the range of the standards (0-5 ppm of copper) that was used (Figure 6).

In this technique, none of the normal ions that can be detected in plant and other biological materials interfere with the quantitation of copper (Williams and Morgan, 1954; Peterson and Bollier, 1955). At least eight moles of cuprizone reagent are required to produce maximum colour development with one mole of copper. The stability of the formed blue compound depends upon the time, the temperature, the concentration of phosphates, and the concentration of ammoniun cations (Somers and Garraway, 1957).

IV.F.2. Cation Exchange Capacity of Fiber Sources

Tables 8 and 9, and Figure 13 show the cation exchange capacity of the fractions of DBSG, and the commercial dietary fiber sources of wheat bran and oat bran. The DBSG fractions showed a statistically significant lower cation exchange capacity when compared to commercial sources of wheat (1052.6 meq Cu^{++}/kg sample) and oat (526.6 meq Cu^{++}/kg sample) bran. The adverse effect of dietary fiber on mineral bioavailability by brans (Dintzis et al., 1985) and other fibre sources (Platt and Clydesdale, 1987) may be different in the case of fibre from brewers' spent grain. McBurney et al. (1986) measured the CEC of wheat bran using the elements praseodymium (III) and copper (II) and found values of 223.0 meq of H^+/kg neutral detergent fiber (NDF) using copper and 147.1 and 415.0 meq of H^+/kg (NDF) using praseodymium (at pH 3.5 and 7.0, respectively).

In the case of not-ground DBSG fractions the largest particle size fraction (>1000 μm) showed the highest CEC (352.2 meq Cu^{++}/kg), while the smallest size fraction (<250 μm) gave the lowest CEC value (296.3 meq Cu^{++}/kg). These resultst suggest lower CEC for smaller particle size fractions.

In the case of the ground DBSG the particles of size between >150 μm <250 μm gave the highest CEC value (304.9 meq Cu^{++}/kg). The lowest CEC (266.0 meq Cu^{++}/kg) was found in the smallest DBSG particles (<150 μm) and the 250 μm fraction gave a value of 280.9 meq Cu^{++}/kg. The CEC of the ground, but not fractionated DBSG, provided an intermediate value of 270.7 meq Cu^{++}/kg. In general, the not-ground DBSG values have relatively higher CEC values than the

Table 8. Cation exchange capacity values of not-ground brewers' spent grain, wheat and oat bran.

Fiber Sources	Absorbance[1,6] ± SD[2]	CEC[3,1,4] ± SD[2]
(μm)	(595 nm)	(meq Cu^{++}/kg sample)
>1000	0.577 ± 0.023	352.2 ± 13.86 (c)[7]
500	0.530 ± 0.008	323.6 ± 4.81 (d)
250	0.558 ± 0.012	340.5 ± 7.41 (c)
<250	0.486 ± 0.014	296.3 ± 8.29 (e)
Wheat Bran	0.860[5] ± 0.002	1052.6 ± 1.93 (a)
Oat Bran	0.861 ± 0.005	526.6 ± 3.13 (b)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Cation exchange capacity

[4] The cation exchange capacity was calculated from the equation CEC[3] = (C x 10000)/ 63.546 (meq/kg sample), where C is the concentration of cupric cations (ppm) in the diluted solution of the filtrate

[5] The initial volume of filtrate of cupric solution was diluted to 2 x volume

[6] The absorbance values is given after the subtraction of the blanks

[7] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

Table 9. Cation exchange capacity values of ground brewers' spent grain, wheat and oat bran.

Fiber Sources	Absorbance[1,6] ± SD[2]	CEC[3,1,4] ± SD[2]
(μm)	(595 nm)	(meq Cu^{++}/kg sample)
>250	0.461 ± 0.016	280.9 ± 10.03 (d)[8]
150	0.500 ± 0.004	304.9 ± 2.31 (c)
<150	0.436 ± 0.002	266.0 ± 0.97 (e)
GDBSG[7]	0.444 ± 0.012	270.7 ± 7.61 (e)
Wheat Bran	0.860[5] ± 0.002	1052.6 ± 1.93 (a)
Oat Bran	0.861 ± 0.005	526.6 ± 3.13 (b)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Cation exchange capacity

[4] The cation exchange capacity was calculated from the equation CEC[3] = (C x 10000)/ 63.546 (meq/kg sample), where C is the concentration of cupric cations (ppm) in the diluted solution of the filtrate.

[5] The initial volume of filtrate of cupric solution was diluted to 2 x volume.

[6] The absorbance values is given after the subtraction of the blanks

[7] Ground dried brewers' spent grain

[8] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

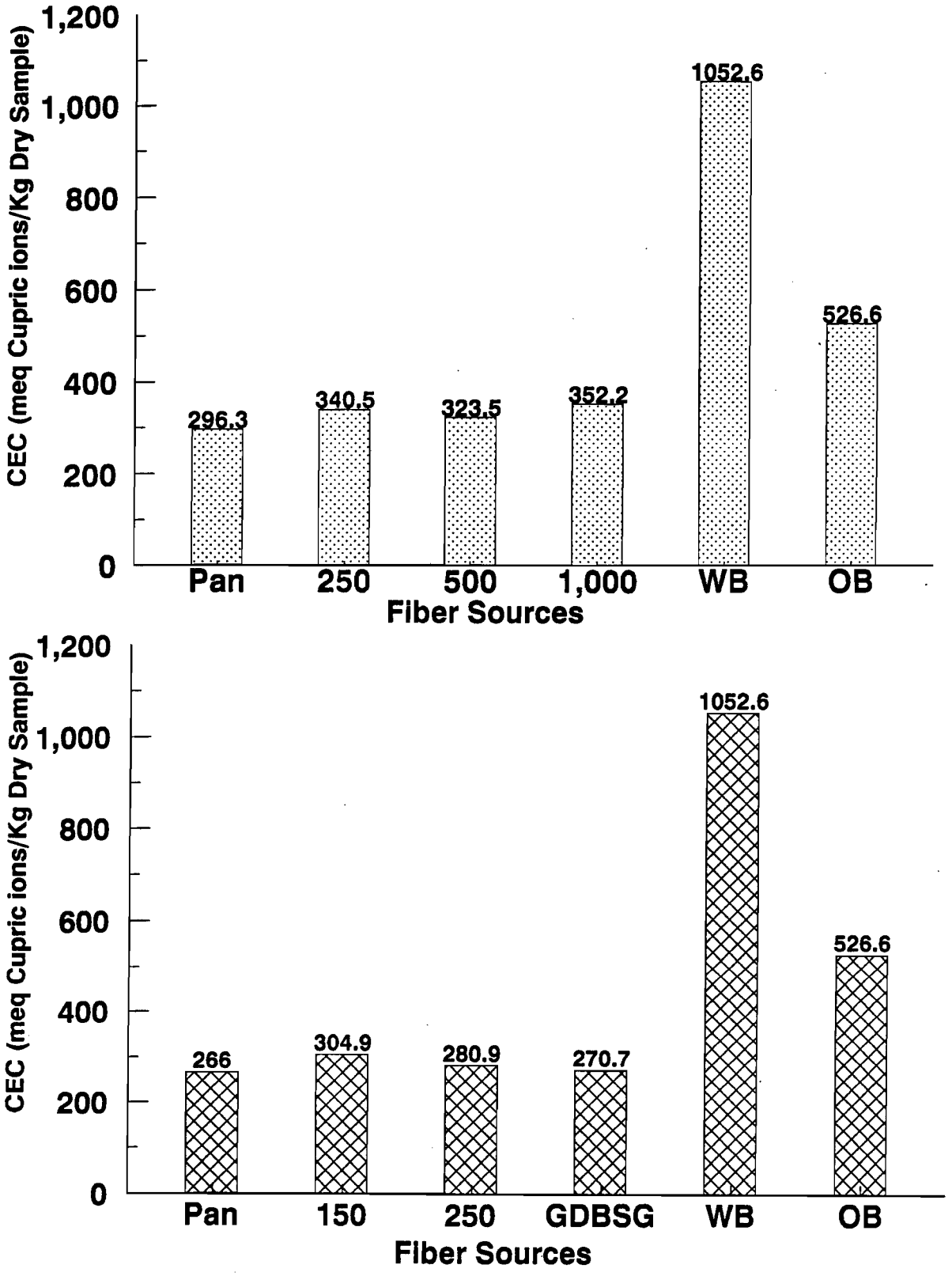


Figure 13. CEC (mequiv. of Cu^{++}/Kg prepared sample) different screen fractions of not-ground dry BSG (top) and ground BSG (bottom) compared to commercial dietary fiber sources of wheat bran (WB) and oat bran (OB).

ground fractions. This phenomenon maybe due to the grinding procedure which may alter the fibrous structure of DBSG and may modify the functional groups that are located in the surface area where the binding occurs.

McBurney et al. (1983) found a cation exchange capacity of 290 mmol H_2/kg cell wall for neutral detergent fiber from dried brewers' grains (1.0 M cupric sulphate, 60 min incubation). Another report (McBurney et al., 1985) gave a CEC value of 450.3 mmol H_2/kg neutral detergent fiber from dried brewers' grain using the rare earth trivalent element, praseodymium (Pr) (pH 7, 0.10M, 39°C, for 24 h).

One of the reasons for the relatively low CEC in the DBSG fractions, when compared to commercial fiber samples, may be the effect of the brewing procedure, which results in the removal of phytate and water soluble fiber. Phytate (phytic acid) is a compound in dietary fiber that can bind amounts of minerals and can be responsible for mineral deficiency in dietary fiber foods (James, 1980). Phytate is removed during the malting process by the enzyme phytase (Weber and Chaudhary, 1987). The amount of phytate in DBSG (0.048 %) is very low compared to other commercial sources such as wheat bran (1.50 %), corn bran (0.50 %) and rice bran (6.90) (Chaudhary and Weber, 1990a). The variable CEC values, with the deviation of particle size, may also be due to different chemical composition (ratio of fiber and protein) in various portions.

The ability of DBSG to retain copper (II) may be related to the chemical compositions of the different fractions. However, the physical nature (eg. structural configuration, pore size) may also influence the CEC values. McConnell et al. (1974)

observed a significant correlation between WHC and CEC in acetone dried fruit and vegetables (r=0.64, a=0.65, b=0.07); however, no correlation found between WHC and CEC in DBSG samples (Table S1). It has been shown (McBurney et al., 1986) that there is a positive correlation between CEC (using Cu^{2+}) and the content of nitrogen in different NDF samples (r=0.699, P<0.05), although no association was observed in DBSG fractions (Table S1). It has been reported (Kay, 1982) that the CEC of dietary fiber can be important in the absorption of bile salts with the formation of cationic bridges. However, a correlation of taurocholate and cholate with CEC in DBSG fractions showed that the correlation coefficient was not significant (Tables S1, S12, and S13; Figures S11 and S12).

The formulation of fiber foods can be helped by estimating *in vitro* the CEC (Rasper, 1979). In the case of BSG, the determination of CEC is important in the evaluation of the chemical and nutritional properties as a dietary fiber source. This evaluation can be used as an indicator in the investigation of ionized groups in the fiber matrix. Cation exchange capacity of fiber sources could be associated with trace element balance (James et al., 1978; Dreher, 1987). Dintzis et al. (1985) reported that significant changes of mineral concentrations occur when wheat bran and corn bran passed through the human intestinal tract. The concentration of metals such as Cu, Fe, Zn, and particularly Ca in ruminants was increased when compared to initial materials, suggesting an interaction between dietary fiber components and minerals. In addition, lignin can strongly bind substantial amounts of iron and thus may inhibit iron absorption in the intestine (Platt and Clydesdale, 1987).

IV.G. BINDING OF BILE SALTS *IN VITRO*

Tables 10, and 12 show the results (presented graphically in Figure 14) of bile salts binding of the not-ground fractions from DBSG. The particles of not-ground DBSG that were retained in 250 μm sieve showed the highest bile salts binding than any other fraction (15.09% and 15.93% for the taurocholate and cholate, respectively). A high amount of taurocholate (14.70%) (not statistically significantly different from the 250 μm fraction) was bound in the <250 μm fraction. The fraction of >1000 μm gave a lower value of 13.45% which, however, is higher and statistically different from the absorption of taurocholate from wheat (10.92%) and oat bran (9.76%). The lowest binding of taurocholate (11.43%) was observed in the fraction of 500 μm where the ash content (3.88%) and total dietary fiber content (63.57%) were the highest, and the content of protein (22.22%) was the lowest.

Similar observations can be seen in the case of cholate binding. The highest values were found in the fractions of 250 μm and <250 μm (15.93% and 15.68% respectively). These values were significantly different from the two fractions of >1000 μm and 500 μm with larger size particles where the values were lower (13.31% and 12.76% respectively). All the sodium cholate binding values using not-ground DBSG were statistically significantly different from the values of wheat bran (10.84%) and oat bran (10.46%).

In the case of ground DBSG (Tables 11 and 13; Figure 14) the amount of sodium taurocholate which was bound, increased when the particle was decreased giving the highest values of 14.68% and 14.14% for the <150 μm and 150 μm

fractions, respectively. Similar results were observed in the case of sodium cholate binding with the highest values of 13.48% and 12.81% in the finer fractions of <150 μm and 150 μm, respectively. This could be explained by the fact that there is a larger surface area to bind taurocholate and cholate in the smaller particle size material. Nevertheless, the smallest particles contain lower quantities of fiber but higher quantities of protein than the larger particles. Therefore, functional groups of both protein, as well dietary fiber protein, may have some ability to bind bile salts.

The binding of one conjugated (taurocholate) and one unconjucated (cholate) bile salt by DBSG was satisfactory when compared to the other commercial cereal fiber sources. The quantities of bile salts bound by the DBSG fractions not-ground or ground were statistically significantly different and higher than those of the commercial fibers. Only in the fraction of 500 μm in not-ground DBSG the amount of bound taurocholate was not significantly different from the commercial fiber sources. Wheat bran showed bile salts binding of 10.92% and 10.84% for the taurocholate and cholate, respectively, while oat bran showed bile salts binding of 9.76% and 10.46% for the taurocholate and cholate, respectively. Story and Kritchevsky (1976) reported bran binding capacities of 10.2% and 1.4% for sodium cholate and taurocholate, respectively (50 mg biding substance, 50 μmol bile salt in 5 ml buffer, at pH 7, for 2 h, at 37°C), and 60.7% and 80.7% for sodium cholate and taurocholate, respectively, using cholestyramine as binder. Normand (1979) reported binding of 17.8% and 17.3% for cholate and taurocholate, respectively, using wheat bran as binder (32 mg binding substance, 40 μmol bile salt in 2 ml buffer, for 16 h,

Table 10. Percent of taurocholic salt bound on fractions of not-ground BSG, wheat bran, oat bran, and cholestyramine resin.

Resins	Counting Rate[1] ± SD[2]	Bound Salt[1] ± SD[2]
(μm)	(cpm)[3]	(%)
>1000	1841.9 ± 11.0	13.42 ± 0.53 (c)[4]
500	1883.8 ± 6.4	11.43 ± 0.30 (d)
250	1807.1 ± 11.4	15.09 ± 0.54 (b)
<250	1815.3 ± 16.6	14.70 ± 0.79 (b)
Wheat Bran	1894.5 ± 12.4	10.92 ± 0.59 (d)
Oat Bran	1919.0 ± 10.9	9.76 ± 0.52 (e)
Cholestyramine	79.5 ± 1.3	97.40 ± 0.06 (a)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Counts per minute

[4] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

Table 11. Percent of taurocholic salt bound on fractions of ground BSG, wheat bran, oat bran, and cholestyramine resin.

Resins	Counting Rate[1] ± SD[2]	Bound Salt[1] ± SD[2]
(μm)	(cpm)[3]	(%)
>250	1850.5 ± 16.2	13.01 ± 0.77 (c)[5]
150	1848.0 ± 11.0	13.14 ± 0.53 (c)
<150	1815.6 ± 10.9	14.68 ± 0.52 (b)
GDBSG[4]	1827.0 ± 8.3	14.14 ± 0.39 (b)
Wheat Bran	1894.5 ± 12.4	10.92 ± 0.59 (d)
Oat Bran	1919.0 ± 10.9	9.76 ± 0.52 (e)
Cholestyramine	79.5 ± 1.3	97.40 ± 0.06 (a)

[1] Results are means of triplicate determinations
[2] Standard deviation
[3] Counts per minute
[4] Ground dried brewers' spent grain
[5] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

Table 12. Percent of cholic salt bound on fractions of not-ground BSG, wheat bran, oat bran, and cholestyramine resin.

Resins	Counting Rate[1] ± SD[2]	Percent Bound[1] ± SD[2]
(μm)	(cpm)[3]	(%)
>1000	1815.7 ± 10.8	13.31 ± 0.52 (c)[4]
500	1827.0 ± 5.1	12.76 ± 0.25 (c)
250	1761.5 ± 17.0	15.93 ± 0.83 (b)
<250	1766.85 ± 13.8	15.68 ± 0.67 (b)
Wheat Bran	1866.8 ± 11.0	10.84 ± 0.53 (d)
Oat Bran	1874.6 ± 9.1	10.46 ± 0.44 (d)
Cholestyramine	109.1 ± 4.8	96.19 ± 0.22 (a)

[1] Results are means of triplicate determinations

[2] Standard deviation

[3] Counts per minute

[4] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 levels.

Table 13. Percent of cholic salt bound on fractions of ground BSG, wheat bran, oat bran, and cholestyramine resin.

Resins	Counting Rate[1] ± SD[2]	Bound Salt[1] ± SD[2]
(μm)	(cpm)[3]	(%)
>250	1845.1 ± 12.0	11.89 ± 0.58 (c)[5]
150	1826.0 ± 6.4	12.81 ± 0.31 (b)(c)
<150	1812.3 ± 17.2	13.48 ± 0.83 (b)
GDBSG[4]	1834.4 ± 13.1	12.41 ± 0.63 (c)
Wheat Bran	1866.8 ± 11.0	10.84 ± 0.53 (d)
Oat Bran	1874.6 ± 9.1	10.46 ± 0.44 (d)
Cholestyramine	109.1 ± 4.8	96.19 ± 0.22 (a)

[1] Results are means of triplicate determinations
[2] Standard deviation
[3] Counts per minute
[4] Ground dried brewers' spent grain
[5] Means were compared using the Duncan's multiple range test. Mean values with the same letter are not significantly different at 0.05 level.

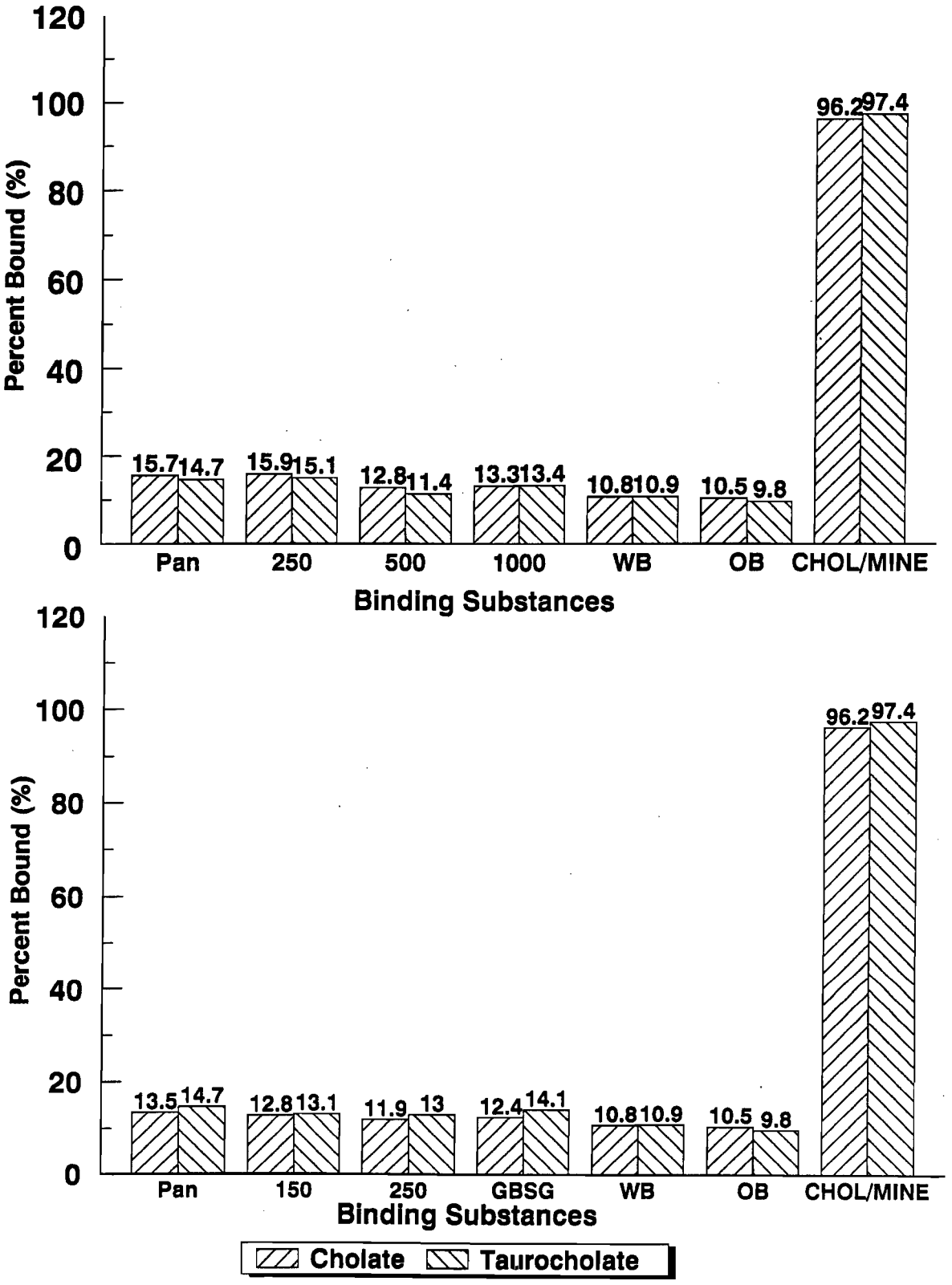


Figure 14. Absorption of bile salts of prepared screen fractions of not-ground dry BSG (top) and ground dry BSG (bottom) compared to commercial fiber sources of wheat bran (WB) and oat bran (OB).Cholestyramine was used as a control binding resin.

at 38°C).

Cholestyramine resin, a quaternary ammonium anion exchange resin with styrene-divilylbenzene copolymer matrix (Boyd et al., 1966), was used as control binding resin. The quantity (100 mg) of this anion exchange resin was able to bind large amounts of bile salts. The absorption of the ammonium group of cholestyramine on the sulphonic group of taurocholate was greater than cholate groups (97.40% and 96.19%, respectively). However, other binding forces of the more hydrophobic dihydroxy bile salts on the polystyrene matrix of the resin can occur (Hagerman et al., 1973). Kritchevsky and Story (1974) also found high binding capacity (81.5%) for the sodium taurocholate using cholestyramine as binder (40 mg binding substance, 100 μmol bile salt in 5 ml buffer, for 16 h, at 37°C).

The results indicate the involvement of composition and particle size in the binding process. The grinding and the active surface area may serve differently in the binding capacity of each DBSG fraction. The content of ash and total dietary fiber was found to be significantly negatively correlated (Tables S1, S4, and S9; Figures S3 and S8) with the binding of taurocholate in DBSG fractions ($r = -0.83$, $P < 0.05$ and $r = -0.91$, $P < 0.005$, respectively). The corresponding negative correlation was observed in the case of cholate binding ($r = -0.71$, $P < 0.05$ and $r = -0.80$, $P < 0.05$ for the ash and total dietary fiber content, respectively) (Tables S1, S5, and S10; Figures S4 and S9). A significant correlation was found between the concentration of protein in DBSG fractions and taurocholate binding ($r = 0.87$, $P < 0.005$) (Tables

S1, S7; Figure S6). In addition, the content of protein is significantly correlated ($r = 0.84$, $P < 0.01$) to the cholate binding in DBSG fractions (Tables S1, S8; Figure S7). The bound taurocholate was relatively poorly correlated to the bound cholate in DBSG fractions ($r = 0.66$, $P < 0.1$) (Tables S1, S14; Figure S13).

Burczak and Kellogg (1979) found a linear relationship between particle size of wood fiber and binding capacity. They reported a significant correlation (correlation coefficient 0.84) between the amount of bound cholate and the theoretical surface area of ground wood particles. Increase of bile salts binding occurs when the particle size was decreased because of the increase of the surface area. However, this phenomenon did not occur when oat fiber was incubated with trihydroxy bile acids of cholate and chenodeoxycholate.

Mongeau and Brassard (1982) reported a positive correlation ($r=0.86$) between taurocholate and the logarithm of mean of particle size in wheat products, this effect was not observed in our results.

Story and Kritcevsky (1976) reported the ability of alfalfa and lignin to bind bile salts. However, Mongeau and Brassard (1982) found no association between lignin content of fiber and its bile binding capacity. Wheat bran bound small quantity of bile salts while cellulose (Eastwood and Hamilton, 1968) was ineffective. Similarly, unimportant binding capacity for taurocholate and glycocholate was shown by cellulose and synthetic fibers, while alfalfa bound bile salts effectively (Kritchevsky and Story, 1974). Story et al. (1982) pointed out the significance of lignin, and pectins

in the absorption of bile acids and that the same amount of bile acid was reversibly absorbed to fiber components.

Variations in the absorption of bile salts by fiber can occur. For example, cholate may be adsorbed to un-ionized groups of lignin and other fiber components, while taurocholate is absorbed by other mechanisms (Eastwood and Hamilton, 1968). Sodium taurocholate may be absorbed by ionized groups of the fiber matrix acting synergistically with other binding forces using its strong sulphonic acid group of taurine. At pH 8 conditions, under which the experiment was performed, the functional groups of lignin and other active sites of the DBSG preparation were partly ionized. Hence, the hydrophobic interaction between fiber and hydrophobic bile molecule (cholate) is probably weak. Furthermore, the absorption of the polar taurocholate, that was ionized at pH 8, provided similar binding capacities with the less polar cholate. The 2 hour incubation time provided an effective binding time. This length of time is comparable to the time in which bile salts are exposed to fiber in the small intestine (2.5 h) (Eastwood and Hamilton, 1968). In the critical region of ileum, where the excreted bile salts are mainly reabsorbed and return to liver, the pH is approx. 8. Therefore, pH 8 of the incubation medium was chosen to measure the binding capacities of DBSG providing comparatively low binding values. The concentration of bile salts in the duodenum is 5 mM (Floren and Nilsson, 1982); therefore the same concentration was used in our incubation mixture.

Other researchers have also reported on the binding of sterols by fiber

compounds. For example, dietary fiber can absorb cholesterol providing an hypocholesteremic effect (Vanhouny et al., 1978). Guar gum has reduced total serum cholesterol (Jenkins et al., 1980).

V. SYNOPSIS

(1) Not-ground DBSG can be separated into the following particle size fractions: >1000 μm (36.3%), 500 μm (30.3%), 250 μm(24.2%), and <250 μm (9.2%). Ground DBSG can be separated into particle size fractions as follows: >250 μm (30.9%), 150 μm (28.6%), and <150 μm (40.5%).

(2) The ash content of ground brewers' spent grain varied in different particle size fractions. In the case of ground DBSG the mean of ash content was lower in smaller fractions. A significant positive correlation was observed between the contents of ash and total dietary fiber (TDF).

(3) Different particle size fractions of not-ground and ground DBSG varied significantly in protein concentration. The quantity of protein was higher in finer fractions than in fractions with larger particles in ground DBSG samples.

(4) The dietary fiber analysis suggests that: i) the particle size fractions varied significantly in insoluble and total dietary fiber content; ii) the soluble dietary fiber content in DBSG fractions was relatively small; and iii) there was a negative correlation between total dietary fiber and protein content in BSG samples (not-ground and ground) with different particle size.

(5) The evaluation of water-holding capacity (WHC) in DBSG indicates that: i) this property varied significantly among different particle size fractions of not-ground and ground DBSG; and ii) in the case of ground DBSG fractions, larger

particles gave higher WHC values.

(6) The determination of cation exchange capacity (CEC) revealed that: i) prepared DBSG fractions of different particle size showed different CEC; and ii) smaller particle size fractions showed significantly lower capacities to bind cupric (Cu^{2+}) cations when compared to commercial sources of wheat and oat bran.

(7) The results of *in vitro* bile salt binding suggest that: (i) significant amount of bile salts was bound by not-ground and ground ethanol and acetone washed DBSG fractions; (ii) the amount of bile salts, bound by DBSG fractions, was significantly more elevated than commercial sources of wheat and oat bran; (iii) there was a positive correlation between the bile salt binding capacity and amount of protein in samples from DBSG fractions.

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VII. APPENDIX

Table S1. Pearson correlation Coefficients with the Probabilities.

	X1	X2	X3
X1	1.00000	-0.86437	0.96236
	0.0	0.0056	0.0005
X2	-0.86437	1.00000	-0.98958
	0.0056	0.0	0.0
X3	0.96236	-0.98958	1.00000
	0.0005	0.0	0.0
X4	-0.05372	0.35003	-0.24819
	0.9089	0.4415	0.6354
X5	0.0017	-0.13188	0.07306
	0.9993	0.7556	0.8763
X6	-0.8289	0.87020	-0.90756
	0.0110	0.00495	0.0047
X7	-0.7113	0.84434	-0.80366
	0.0480	0.0084	0.0294

X1 Ash content variable
X2 Protein content variable
X3 Total dietary fiber content variable
X4 Water-holding capacity variable
X5 Cation exchange capacity
X6 Taurocholate bound variable
X7 Cholate bound variable

Table S1. Pearson Correlation Coefficients with the Probabilities.

	X4	X5	X6	X7
X1	-0.0537	0.00177	-0.82892	-0.71135
	0.9089	0.9993	0.0110	0.0479
X2	0.35003	-0.13188	0.87020	0.84434
	0.4415	0.7556	0.00495	0.0084
X3	-0.24819	0.07306	-0.90756	-0.80366
	0.6354	0.8763	0.0047	0.0294
X4	1.00000	-0.22695	0.41749	0.33732
	0.0	0.6246	0.3514	0.4594
X5	-0.22695	1.00000	-0.19000	0.36535
	0.6246	0.0	0.6522	0.3735
X6	0.41750	-0.19000	1.00000	0.66245
	0.3514	0.6522	0.0	0.07345
X7	0.33732	0.36535	0.66245	1.00000
	0.4594	0.3735	0.07345	0.0

X1 Ash content variable
X2 Protein content variable
X3 Total dietary fiber content variable
X4 Water-holding capacity variable
X5 Cation exchange capacity
X6 Taurocholate bound variable
X7 Cholate bound variable

Table S2. Regression analysis of ash content against protein content in DBSG samples using a multiplicative model ($y = ax^b$).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept*	2.40924	0.274878	8.76476	.00012
Slope	-0.346072	0.0806372	-4.29172	.00514

* NOTE: The Intercept is equal to Log a.

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.051766	1	.051766	18.41886	.00514
Error	.0168629	6	.0028105		
Total (Corr.)	.0686288	7			

Correlation Coefficient = -0.868498 R-squared = 75.43 percent
Stnd. Error of Est. = 0.053014

Figure S1: Regression of Ash Content (%) on Protein Content (%)

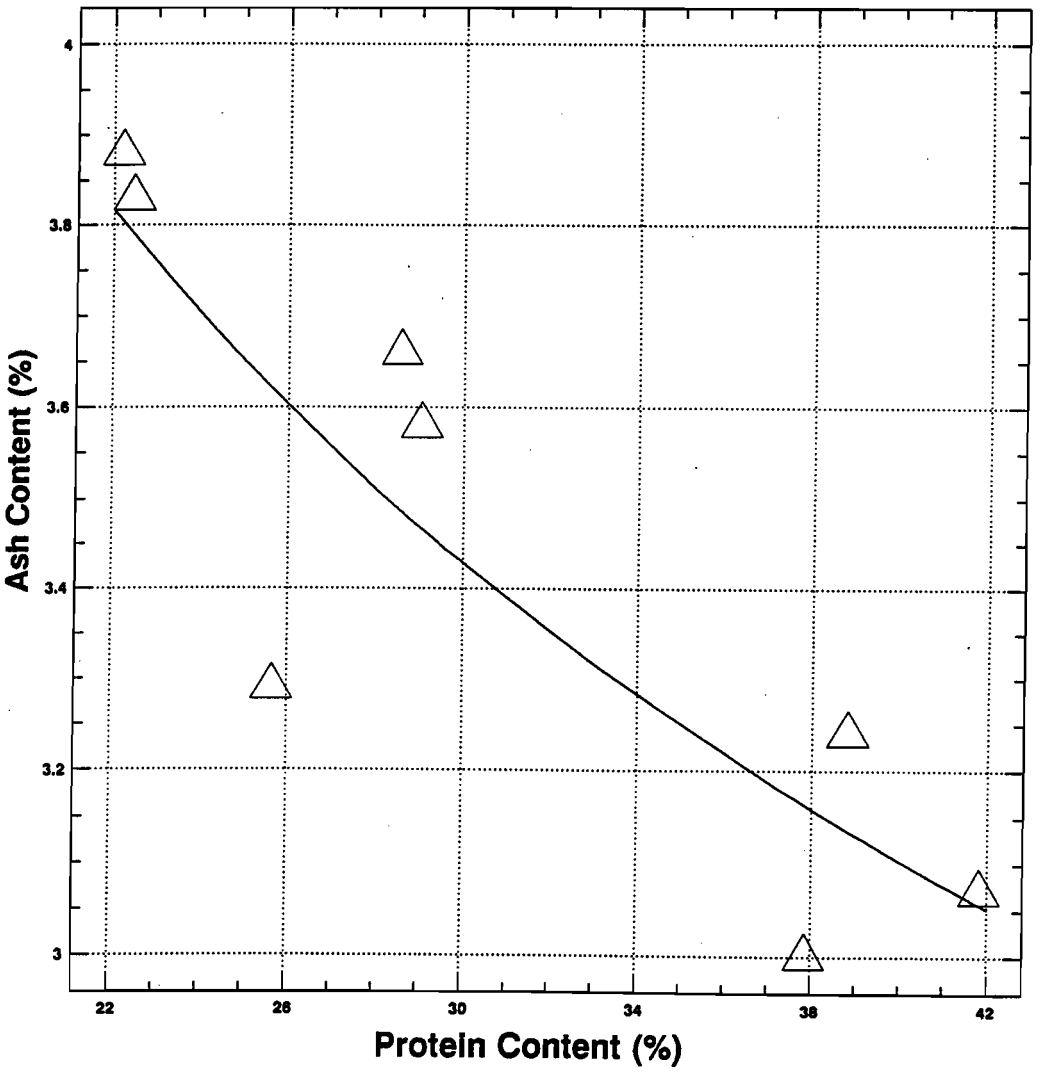

Table S3. Regression analysis of total dietary fiber content against ash content in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	-39.7274	11.4064	-3.48291	.01760
Slope	25.9403	3.27606	7.91815	.00052

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	524.57358	1	524.57358	62.6970	.00052
Error	41.833994	5	8.366799		
Total (Corr.)	566.40757	6			

Correlation Coefficient = 0.962362 R-squared = 92.61 percent
Stnd. Error of Est. = 2.89254

Figure S2: Regression of Total Dietary Fiber (%) on Ash (%)

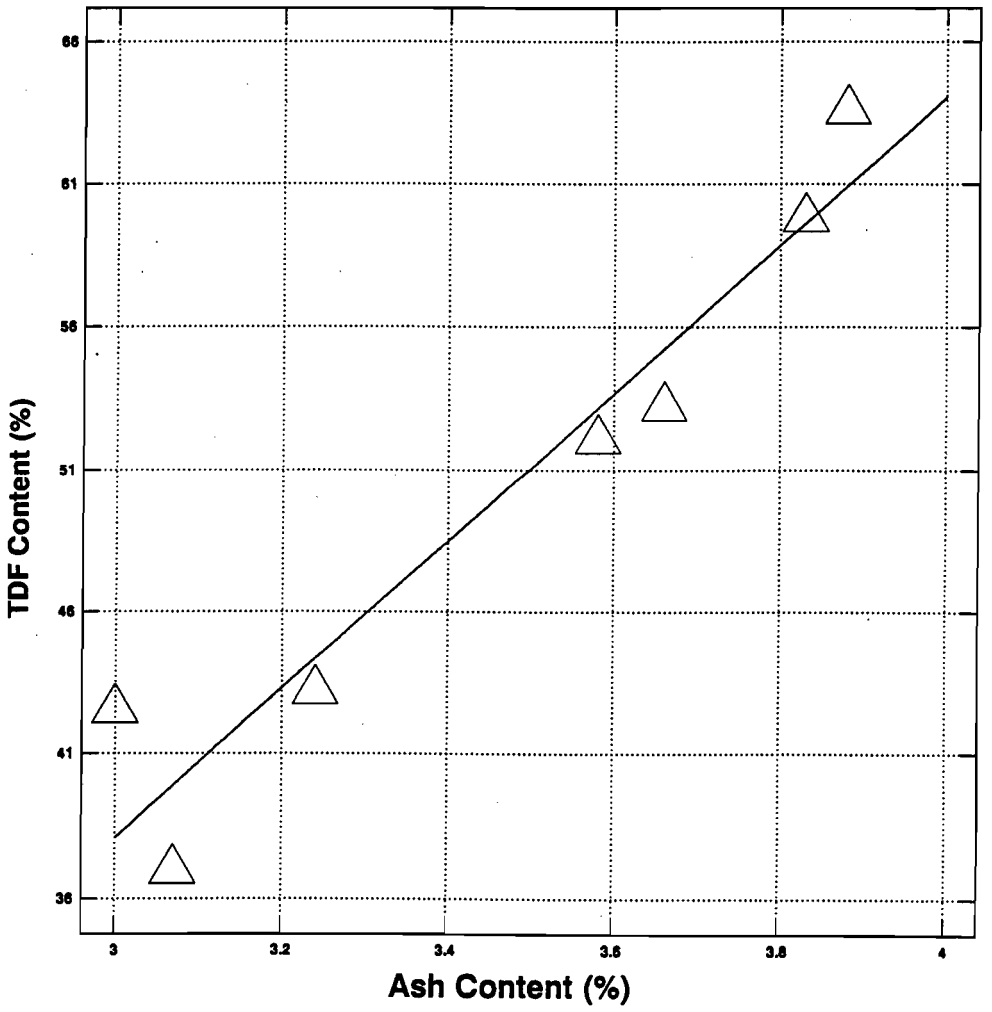

Table S4. Regression analysis of bound taurocholate against ash content in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	23.8061	2.79564	8.51544	.00014
Slope	-2.93426	0.808372	-3.62984	.01097

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	6.944623	1	6.944623	13.17572	.01097
Error	3.1624641	6	.5270774		
Total (Corr.)	10.107088	7			

Correlation Coefficient = -0.828918 R-squared = 68.71 percent
Stnd. Error of Est. = 0.726001

Figure S3: Regression of Bound Taurocholate on Ash Content

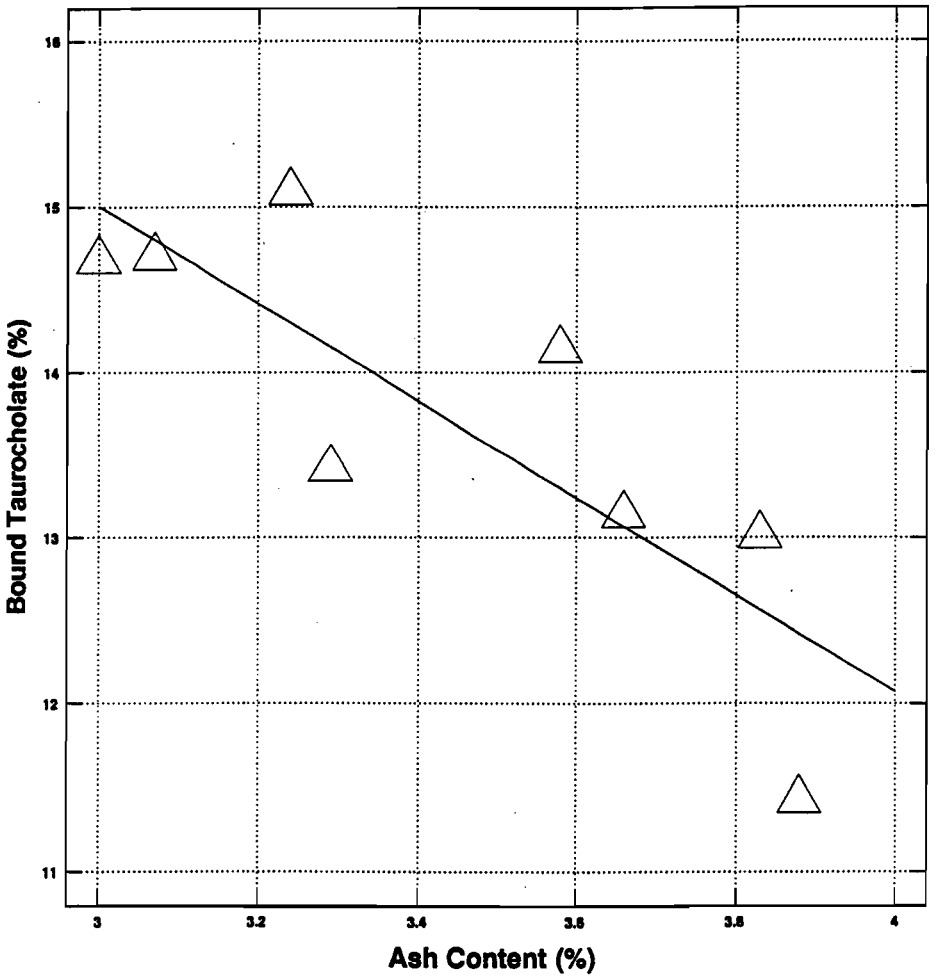

Table S5. Regression analysis of bound cholate against ash content in DBSG samples using a reciprocal model (1/y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	0.0172511	0.0214343	0.804838	.45162
Slope	0.0166597	6.19782E-3	2.68799	.03615

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.0002239	1	.0002239	7.225309	.03615
Error	.0001859	6	.0000310		
Total (Corr.)	.0004098	7			

Correlation Coefficient = 0.739138 R-squared = 54.63 percent
Stnd. Error of Est. = 5.56628E-3

Figure S4: Regression of Bound Cholate on Ash Content in BSG Fractions

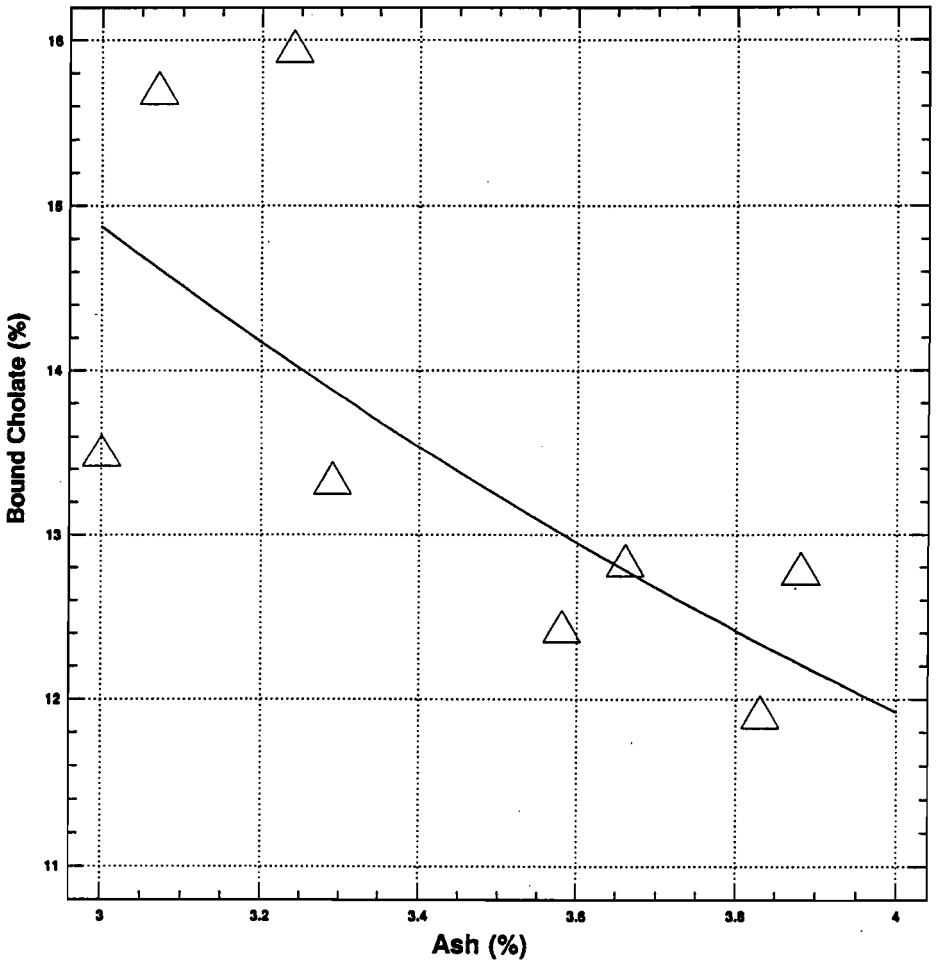

Table S6. Regression analysis of protein content against total dietary fiber content in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	72.3591	2.69828	26.8167	.00000
Slope	-0.813375	0.0529343	-15.3658	.00002

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	374.72362	1	374.72362	236.1068	.00002
Error	7.9354697	5	1.5870939		
Total (Corr.)	382.65909	6			

Correlation Coefficient = -0.989577 R-squared = 97.93 percent
Stnd. Error of Est. = 1.2598

Figure S5: Regression of Protein Content on Total Dietary Fiber Content in DBSG Fractions

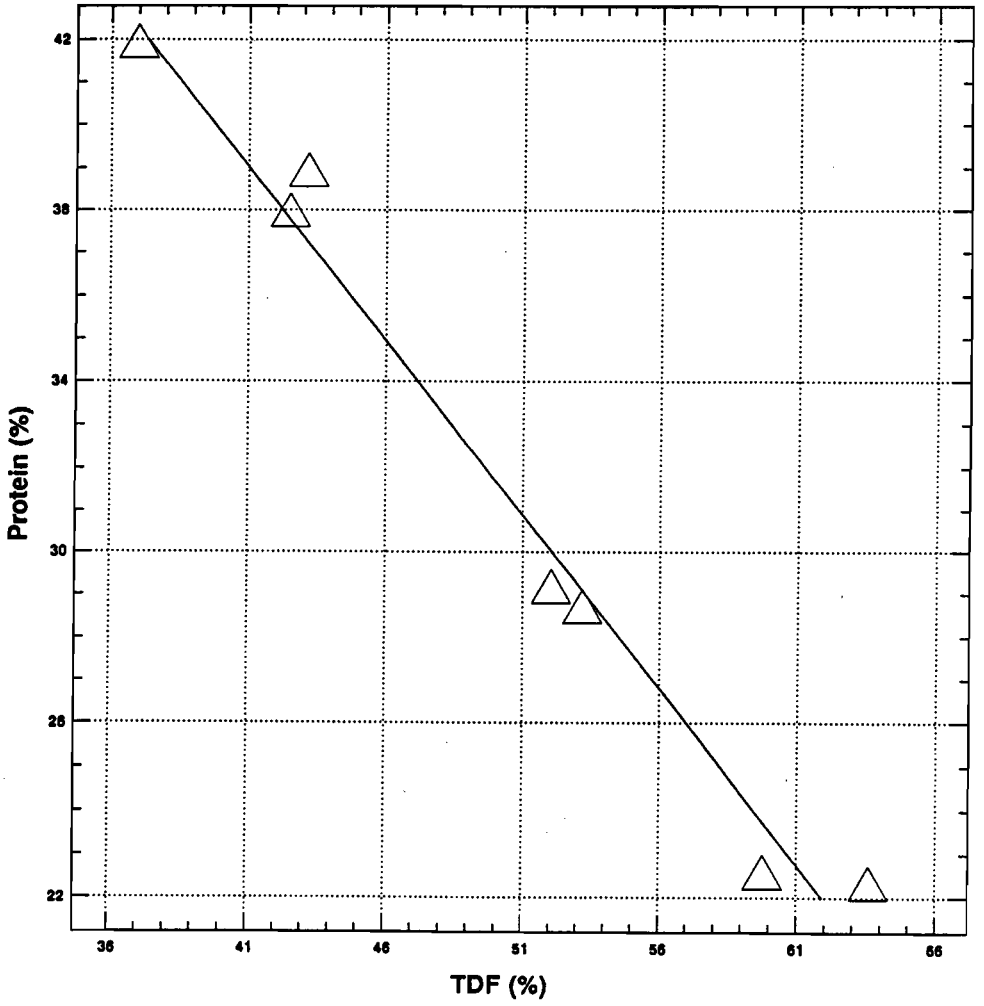

Table S7. Regression analysis of bound taurocholate against protein content in DBSG samples using a multiplicative model ($y = ax^b$).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept*	1.52884	0.247157	6.18572	.00082
Slope	0.319071	0.0725051	4.40067	.00457

* NOTE: The Intercept is equal to Log a.

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.044003	1	.044003	19.36585	.00457
Error	.0136332	6	.0022722		
Total (Corr.)	.0576364	7			

Correlation Coefficient = 0.873763 R-squared = 76.35 percent
Stnd. Error of Est. = 0.0476677

Figure S6: Regression of Bound Taurocholate on Protein Content in DBSG Samples

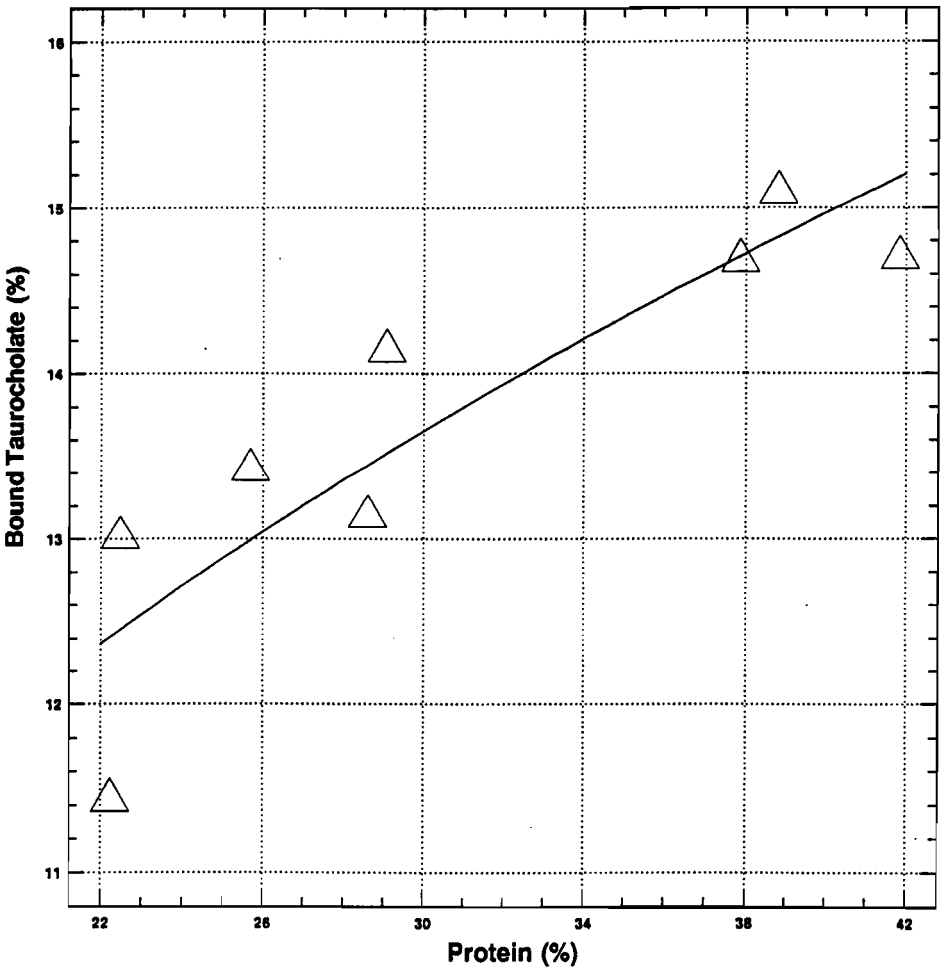

Table S8. Regression analysis of bound cholate against protein content in DBSG samples using a exponential model, y = exp(a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	2.23868	0.0948959	23.5909	.00000
Slope	0.0117303	2.99922E-3	3.91112	.00788

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.056815	1	.056815	15.29684	.00788
Error	.0222849	6	.0037142		
Total (Corr.)	.0790997	7			

Correlation Coefficient = 0.847507 R-squared = 71.83 percent
Stnd. Error of Est. = 0.0609438

Figure S7: Regression of Bound Cholate on Protein Content in DBSG Fractions

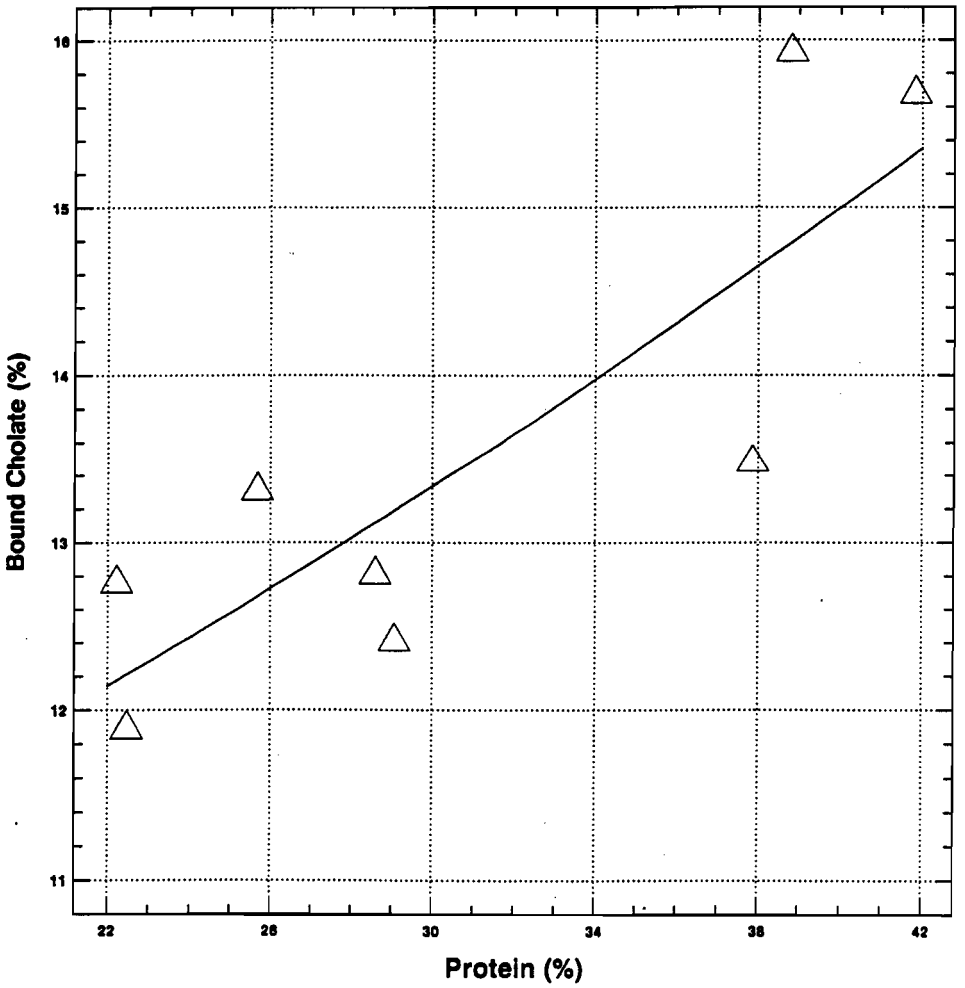

Table S9. Regression analysis of bound taurocholate against total dietary fiber content in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	19.797	1.27303	15.551	.00002
Slope	-0.12069	0.024974	-4.83262	.00475

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	8.250339	1	8.250339	23.35424	.00475
Error	1.7663468	5	.3532694		
Total (Corr.)	10.016686	6			

Correlation Coefficient = -0.907557 R-squared = 82.37 percent
Stnd. Error of Est. = 0.594365

Figure S8: Regression of Bound Taurocholate on TDF Content in DBSG Samples

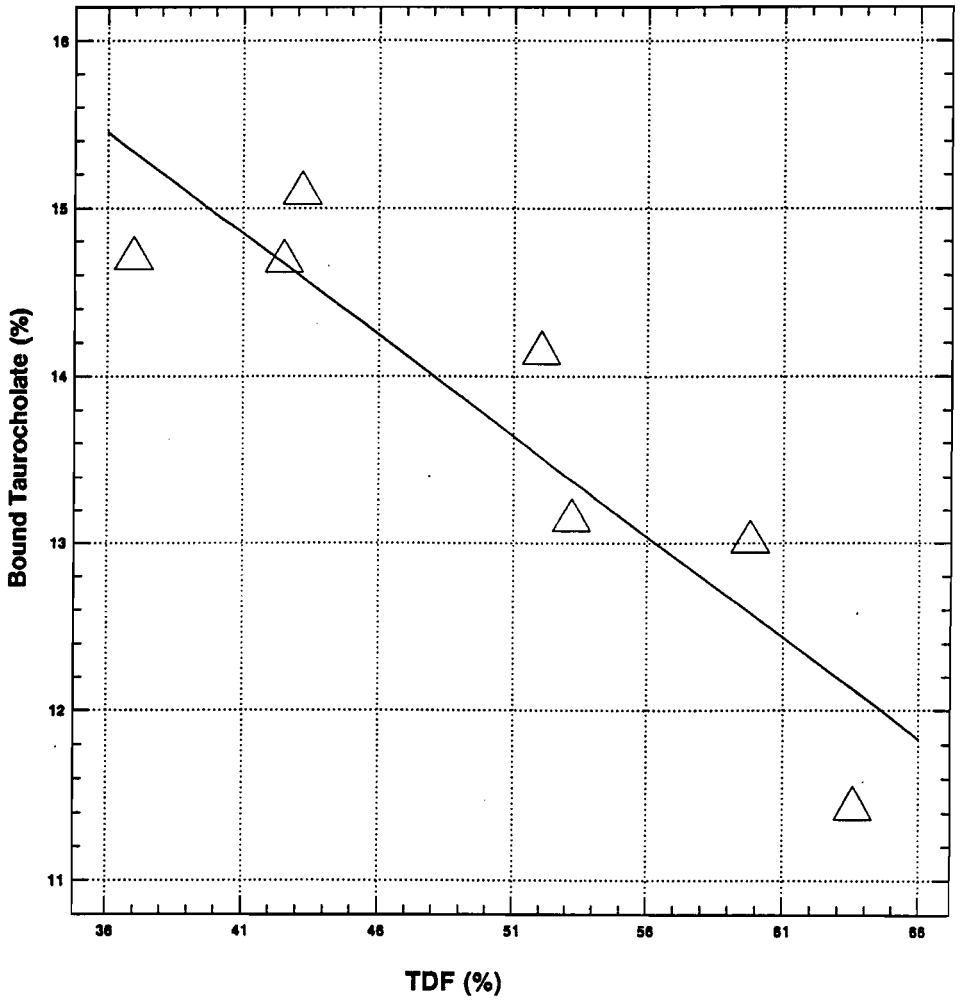

Table S10. Regression analysis of bound cholate against total dietary fiber content in DBSG samples using a multiplicative model ($y = ax^b$).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept*	4.47985	0.571032	7.84518	.00054
Slope	-0.481668	0.146293	-3.29248	.02165

* NOTE: The Intercept is equal to Log a.

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.054026	1	.054026	10.84042	.02165
Error	.0249190	5	.0049838		
Total (Corr.)	.0789455	6			

Correlation Coefficient = -0.827256 R-squared = 68.44 percent
Stnd. Error of Est. = 0.070596

Figure S9: Regression of Bound Cholate on TDF Content in DBSG samples

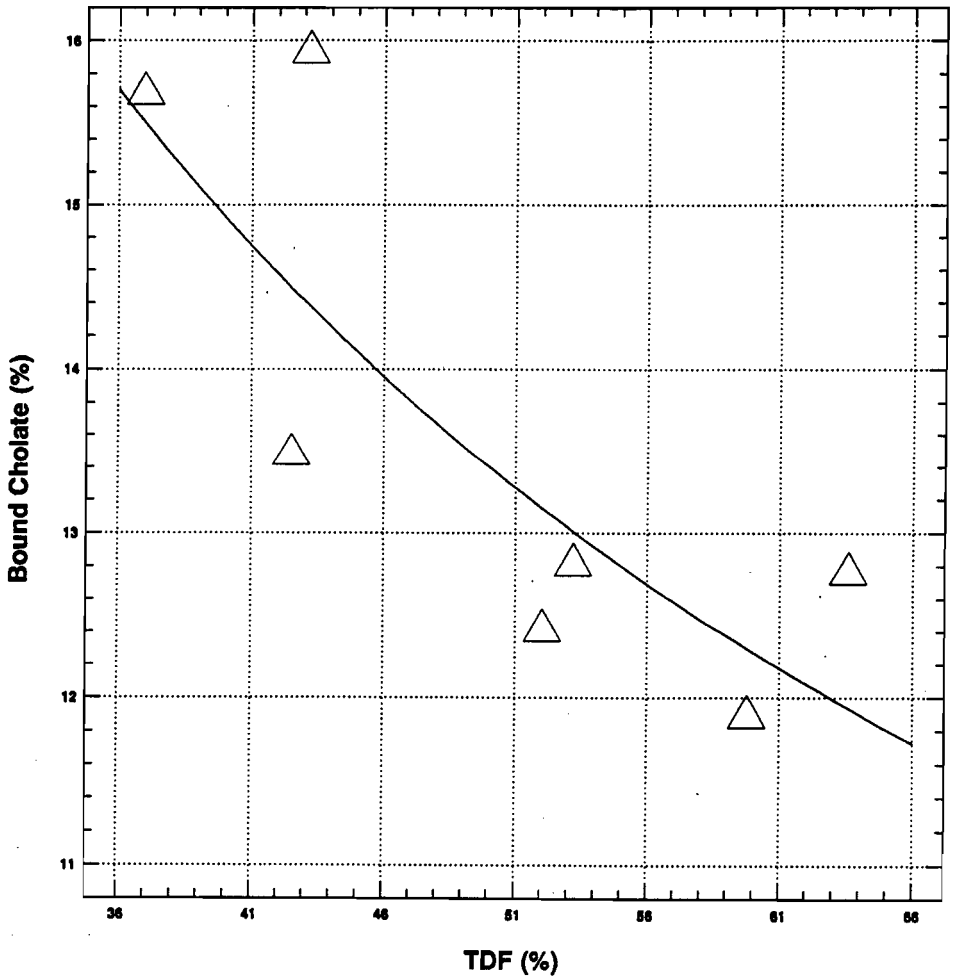

Table S11. Regression analysis of bound taurocholate against water-holding capacity in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	11.1267	2.49221	4.46459	.00661
Slope	0.906348	0.882203	1.02737	.35136

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	1.7233428	1	1.7233428	1.055486	.35136
Error	8.1637429	5	1.6327486		
Total (Corr.)	9.8870857	6			

Correlation Coefficient = 0.417495 R-squared = 17.43 percent
Stnd. Error of Est. = 1.27779

Figure S10: Regression of Bound Taurocholate on WHC in DBSG Fractions

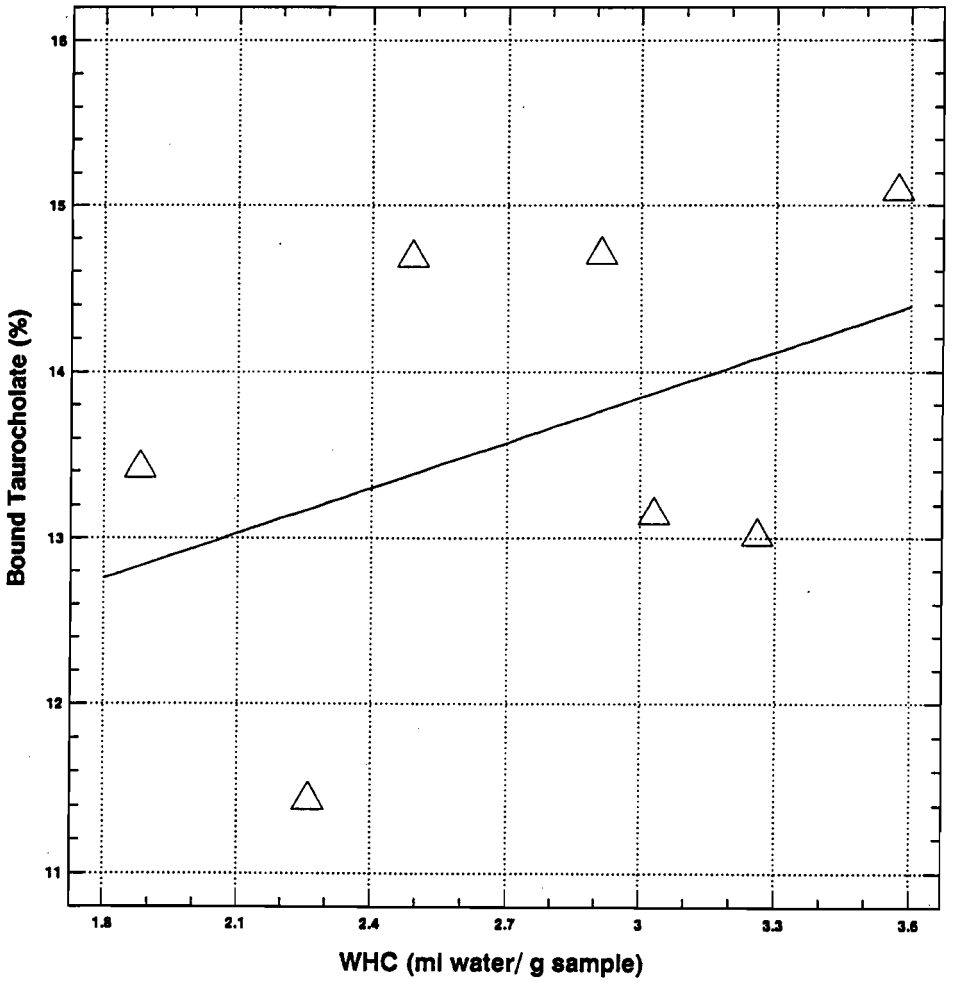

Table S12. Regression analysis of bound taurocholate against cation exchange capacity in DBSG samples using a multiplicative model ($y = ax^b$).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept*	3.67342	1.9784	1.85676	.11273
Slope	-0.18543	0.346219	-0.535587	.61150

* NOTE: The Intercept is equal to Log a.

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.0026298	1	.0026298	.286853	.61150
Error	.0550066	6	.0091678		
Total (Corr.)	.0576364	7			

Correlation Coefficient = -0.213606 R-squared = 4.56 percent
Stnd. Error of Est. = 0.0957485

Figure S11: Regression of Bound Taurocholate on CEC in DBSG Fractions

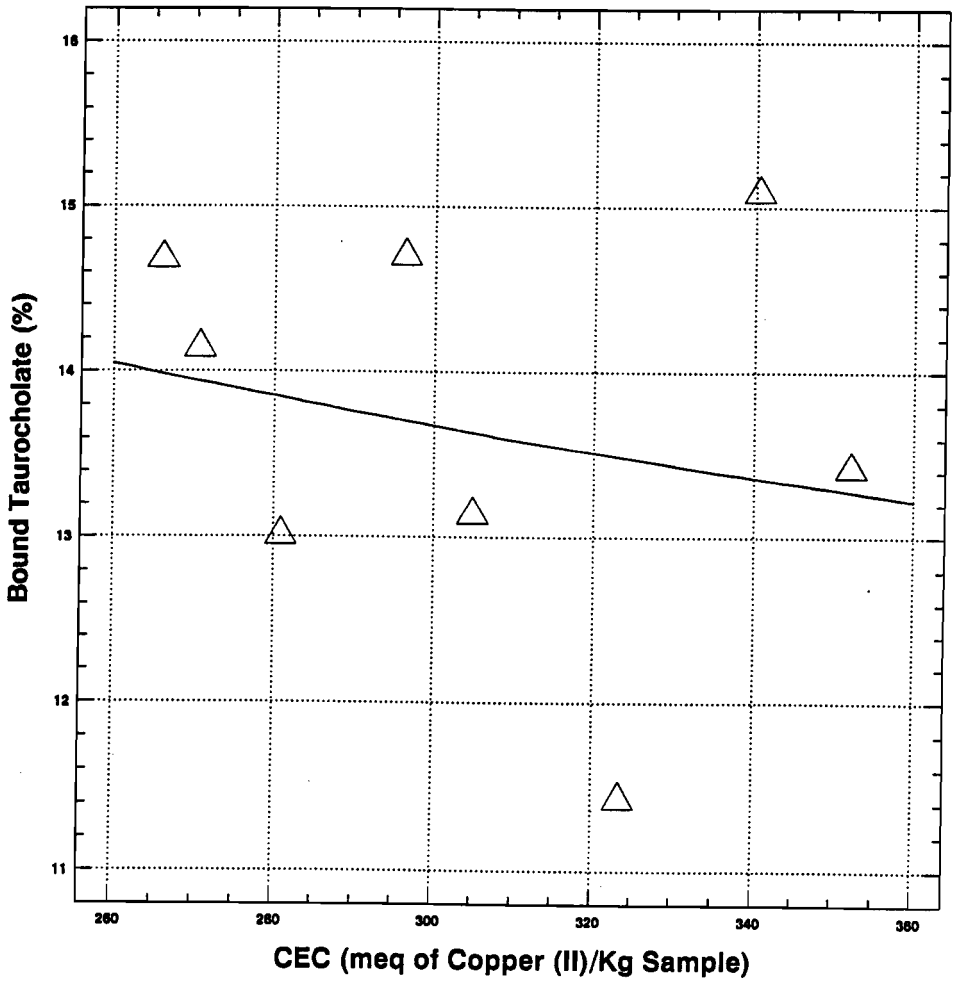

Table S13. Regression analysis of bound cholate against cation exchange capacity in DBSG samples using a reciprocal model (1/y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	0.10213	0.0276092	3.69912	.01010
Slope	-9.03716E-5	9.02716E-5	-1.00111	.35542

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	.0000586	1	.0000586	1.002218	.35542
Error	.0003511	6	.0000585		
Total (Corr.)	.0004098	7			

Correlation Coefficient = -0.378323 R-squared = 14.31 percent
Stnd. Error of Est. = 7.6498E-3

Figure S12: Regression of Bound Cholate on CEC in DBSG Samples

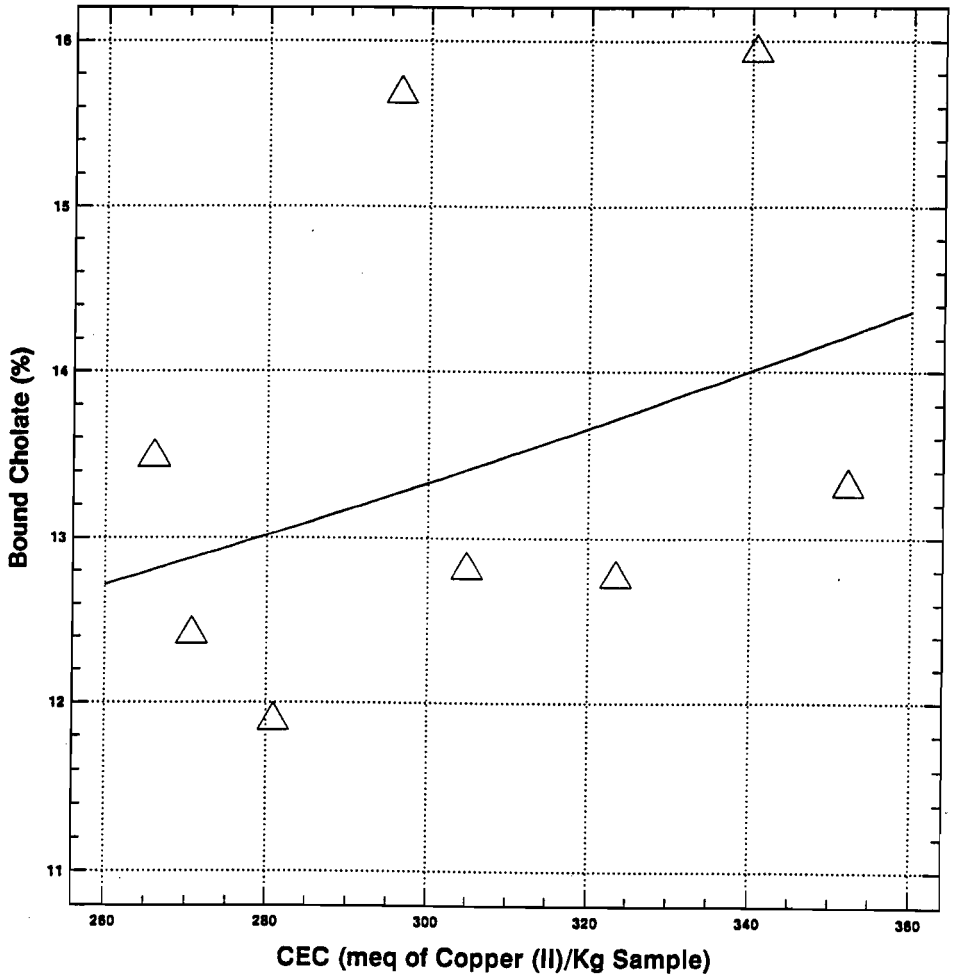

Table S14. Regression analysis of bound taurocholate against bound cholate in DBSG samples using a linear model (y = a+bx).

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	6.45889	3.36105	1.92169	.10302
Slope	0.535134	0.247043	2.16615	.07345

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	4.4354342	1	4.4354342	4.692213	.07345
Error	5.6716533	6	.9452756		
Total (Corr.)	10.107088	7			

Correlation Coefficient = 0.662453 R-squared = 43.88 percent
Stnd. Error of Est. = 0.972253

Figure S13: Regression of Bound Taurocholate on Bound Cholate in DBSG Fractions