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Binding of trace elements with various dietary fiber sources

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BINDING OF TRACE ELEMENTS WITH VARIOUS DIETARY FIBER SOURCES

by

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Charles W Weber — July 14 1989

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DATE

DEDICATED WITH LOVE TO MY BROTHER ALI

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ABSTRACT

Mineral binding capacity of rice bran, oat hull, soy bran, wheat bran, peanut, apple, tomato, and barley fiber were investigated. Defatted fibers were analyzed for ash, protein, acid detergent fiber, and lignin.

The fibers were washed with 1% HCl and acid washed samples studied for their mineral binding ability. The minerals tested were zinc, copper , and magnesium.

Analysis of acid washed samples showed that most of the original minerals had been stripped from the fiber. In most of the fibers studied, the order of binding was copper > zinc > magnesium. Among the eight fibers studied, oat hull and apple fiber had the lowest consistent binding capacity for the three minerals investigated.

This in vitro binding study has been run to duplicate intestinal condition of pH 6.8. Oat hull and apple fiber could be a good fiber source for application in the food industry regarding their low mineral binding capability.

CHAPTER 1

INTRODUCTION

The role of dietary fiber in nutrition and prevention of some disease is becoming important. Inclusion of fiber in the diet has been recommended, but how much and what type is important. The mineral binding ability of high fiber content in food is a concern and several workers have shown a reduced bioavailability of mineral with the consumption of wheat(Toma, 1986). Therefore, it is important to know the binding capacity of different fiber sources for minerals which are of concern to humans.

The purpose of this study was to investigate the binding capacity of rice bran, oat hull, soy bran, wheat bran, apple, peanut, tomato, and barley fiber for magnesium, copper, and zinc. In this study we have chosen pH 6.8 because it is the pH of the small intestine and is where most of the minerals are absorbed. This type of in vitro study could be a useful tool for further in vivo investigation.

CHAPTER 2

LITERATURE REVIEW

Fiber history and definition

Fiber is a complex mixture of plant material and there has been so much controversy over its definition and analysis. McCance and Lawrence (1929) proposed the concept of unavailable carbohydrate (including lignin), which enabled the pith or cell wall of plants to be cataloged in nutrition tables. In 1953 Hipsley (Trowell, 1979), a British colonial doctor in Fiji, introduced the term dietary fiber to cover what McCance and Lawrence had called the unavailable, that is undigested, carbohydrate of plants. At first there was little interest in dietary fiber. About six articles were published each year from 1953 to 1968. Then suddenly interest quickened (Trowell, 1979). The interest toward fiber started when Burkitt and Trowell (1975) hypothesized that populations subsisting on high residue diets exhibit fewer of the disease of Western civilization. They reached this conclusion by the observation of African life style. They had served a lifetime of medical service in Uganda and had observed the absence of colonic disease, diabetes and vascular disease both arterial and venous. They concluded this low occurrence was due to the diet and suggested that it was the fiber in the African diet that gave immunity from these disease.

The most widely used definition of dietary fiber is what

Trowell had given in 1976, as the sum of lignin and polysaccharides that are not digested by the endogenous secretions of the human digestive tract (Southgate, 1978).

COMPONENTS OF FIBER

Cellulose, hemicellulose, pectin, and lignin are the main components of fiber (Inglett, 1979). Cellulose, hemicellulose and some pectin associated with cell wall of plant and is considered structural polysaccharide. Lignin is the only component of fiber which is not a polysaccharide.

Cellulose is a linear polymer of glucose unit which are connected by beta 1-4 linkages. It is considered to be the main structural component of plant cell wall. There is no branching in a cellulose molecule. It contains about 300 glucose units, although values up to 10,000 units have been suggested. The beta 1-4 linkage strongly favors the formation of hydrogen bonding between sugar units in the chain and between adjacent chains (Inglett, 1979). The whole molecule folds into a flat, ribbon-like structure which X-ray diffraction studies have shown to have a helical conformation with a repeating unit every 10.3 A, each representing individual cellobiose units.

Cellulose is considered to be relatively insoluble and resistant toward chemicals, although it may be partly degraded by the colon microflora of man. The susceptibility of cellulosic materials to enzymatic hydrolysis may vary considerably and can be increased by treatments which make the

cellulose less crystalline, more swollen and/or less trapped by encrusting cell wall components such as lignin and silica (Inglett, 1979).

Hemicellulose is a heterogenous polysaccharide. The backbone is made of beta 1-4 linked pyranoside sugars (Selvendran, 1984) with a variety of branched sugars. The main chain consists of monomers such as xylose, mannose, galactose, and glucose; side chains consist of arabinose, galactose, and glucuronic acid (Schneeman, 1986).

Classification of hemicelluloses is hard; they are those cell wall polysaccharides preferentially solubilized by aqueous alkali after removal of water-soluble and pectic polysaccharides (Inglett, 1979). They exhibit a wide range of solubility with greater solubility being associated with a higher degree of branching. The individual classification is generally based upon the predominant monosaccharide residue(s), e.g., glucuronoxylans, galactoglucomannans and so on. Arabinoxylans are abundant in cereals (Theander and Aman 1979b). In hardwoods and softwoods 4-O-methyl glucuronic acid is a common residue.

Hemicellulose polysaccharides have generally a much lower degree of polymerization (50-200 residues) than has cellulose. Hemicellulose is frequently associated with cellulose by hydrogen binding.

Hemicellulose polysaccharides are important in human nutrition together with cellulose for their bulking effect.

The acidic types may have the capacity to bind cations. Hemicelluloses are digested by microbes to a greater extent than cellulose in the colon.

Pectin is a polymer of beta 1-4 D-galacturonic acid. It can have other carbohydrate moieties (rhamnose, arabinose, xylose, and fucose) linked to it. Partial methylation of the carboxyl groups on the galacturonic acid imparts important properties to pectin substances. Most of the pectins in plants are nonstructural substance. They are also found as part of cell wall and as intercellular cements. Association of pectin and pectin substances is not clearly understood, but they are considered highly soluble (schneeman, 1986). Pectins have gel forming and ion binding capacity because of free uronic acid groups. The cholesterol reduction effect is related to the methyl ester content of pectin.

Lignin is the only structural fiber which is not a polysaccharide. Lignin has a highly complex aromatic three dimensional structure which is builded up by phenylpropane units. Lignin is extremely inert, and in a majority of cases vigorous chemical procedures are required to free lignin from contaminating polysaccharide.

Food additives such as gums, gum arabic, alginate, carrageenan, guar gum, mucilage, modified polysaccharides (synthetic fibers, like carboxymethyl cellulose, modified starch, hydroxypropyl methylcellulose) are also included as food fiber (Selvendran, 1984).

METHODS OF ANALYSIS

Since dietary fiber includes a wide range of complex material, difficulties are encountered in its analysis. The analysis could be achieved by several methods. The methods are crude fiber, detergent methods, fractionation, enzymatic, total dietary fiber, soluble and insoluble fiber. Most of these methods may be used to isolate various fractions which are then measured gravimetrically. Methods also are available which measure specific carbohydrate.

Crude fiber analysis is the oldest and most commonly used method for analyzing plant fiber. It involves extraction with both acid and alkali, which measures the indigestible cell wall material. The method is highly empirical; in order to get a reproducible result we have to follow a precisely defined procedure. There are losses of fiber components which vary considerably from food to food. There are losses of hemicellulose and also solubilization of cellulose and lignin (Theander et al., 1979). It is evident that this method gives lower fiber values , because it does not include most of the cell wall polysaccharide which are not digested by man. This is not satisfactory for studies in human nutrition.

Van Soest proposed the two detergent methods of acid and neutral detergent fiber. These methods were originally developed to solve analytical problems which occurred in ruminant diets, particularly forage (Robertson et al., 1981).

Acid detergent fiber involves refluxing the sample for 60 minutes in 1N sulfuric acid containing cetyl trimethyl ammonium bromide (1%). This method resulted in residues containing most of the cellulose and lignin (Van Soest, 1963a, 1963b). The detergent solubilized partially some or all of the cellular components including starches,sugars, fat, nitrogen compounds, some minerals and hemicellulose. There exists a limitation when these methods are applied to human foods (Southgate, 1976). First is the size of sample. In a typical mixed diet the cellulose and lignin are of the order 1-2% of the dry matter, and a sample of 1 gram will give a final residue of only 10-20 mg. Precision is therefore difficult to achieve. Increasing the size of sample results in problems in foaming and filtration. The second limitation concerns the high levels of lipid found in mixed diet, since these intensify foaming and filtration problems (Southgate, 1976). In general, it is better to use a lipid extracted sample when measuring ADF in human foods. Decahyronaphthalene (decalin) is added as an antifoaming agent. The third objection involves precisely what the ADF measures. The plant cell wall contains a whole range of polysaccharides that are not hydrolyzed by human digestive secretions, while the dietary fiber does include all these polysaccharides. ADF, however, measures only a fraction of these.

Neutral detergent fiber was developed for the measurement of total dietary fiber. It involves refluxing the sample with

a neutral detergent solution which includes sodium lauryl sulfate, EDTA, sodium borate decahydrate, disodium hydrogen phosphate, and ethylene glycol (Van Soest and Wine, 1967). Difficulties involved with this method are the low concentrations of NDF found in food stuff, and the difficulty in achieving precision in measurements. The most common problem with the method is the difficulty in filtration. This usually is a problem of technique, but it can also be due to high concentration of protein , starch, mucilage and gums that occur in amounts which exceed the solubilizing capacity of the neutral detergent solution (Robertson et al., 1981). Sodium sulfite is included in this method because of its ability to aid in the solubilities of protein by attacking disulfide bridges and the linkages between the aromatic constituents. However, Hartly (1972) has shown the inclusion of sodium sulfite results in the loss of lignin subunits which outweighs the advantage which comes from the reduction in residual nitrogen. The proportion of starch and lipid, is much higher in most human foods than in the animal forages for which the method was developed. Therefore we have the problems with foaming and extraction. Also, in food rich in starch, starch remains insoluble in the hot detergent and is measured as NDF. Although the starch is soluble at 100° C, starch gel is formed as the crucible contents cool, making filtration impossible. The modification of the NDF method involves the use of alpha-amylase from Bacillus subtilis to solubilize the starch.

Methods available for lignin determination are 72% sulfuric acid which is called Klason lignin, and the use of potassium permanganate (Goering and Van soest, 1971). Lignin determination is run on acid detergent residue (Van Soest, 1963a and b). The 72% sulfuric acid is used to digest the cellulose. The residue is lignin plus mineral. By ashing the residue we can correct for the mineral present and obtain the lignin value. Potassium permanganate oxidizes the lignin phenylpropane unit, when added to ADF, and leaves a residue composed mainly of cellulose.

A flow chart of the Van Soest fractionation method can be found in Fig.1. For a complete breakdown analysis one should do first the NDF. This step separates the samples into NDF soluble, and insoluble fiber. Boiling the NDF fiber with acid detergent solubilizes the hemicellulose. Cellulose and lignin are left in the insoluble residue. Lignin determination can be made on this residue.

Southgate criticized the existing methods, because most involve determination based on differences. Also, the components are not analyzed for monosaccharide constituents. The fractionation method developed by Southgate (1969) measures the individual polysaccharides and lignin. The original protocol of Southgate (1969) is presented in Fig.2. The method was modified later on (Southgate, 1976), and the modified protocol is presented in Fig.3. The major difference in the two methods is the point of hot water extraction. The

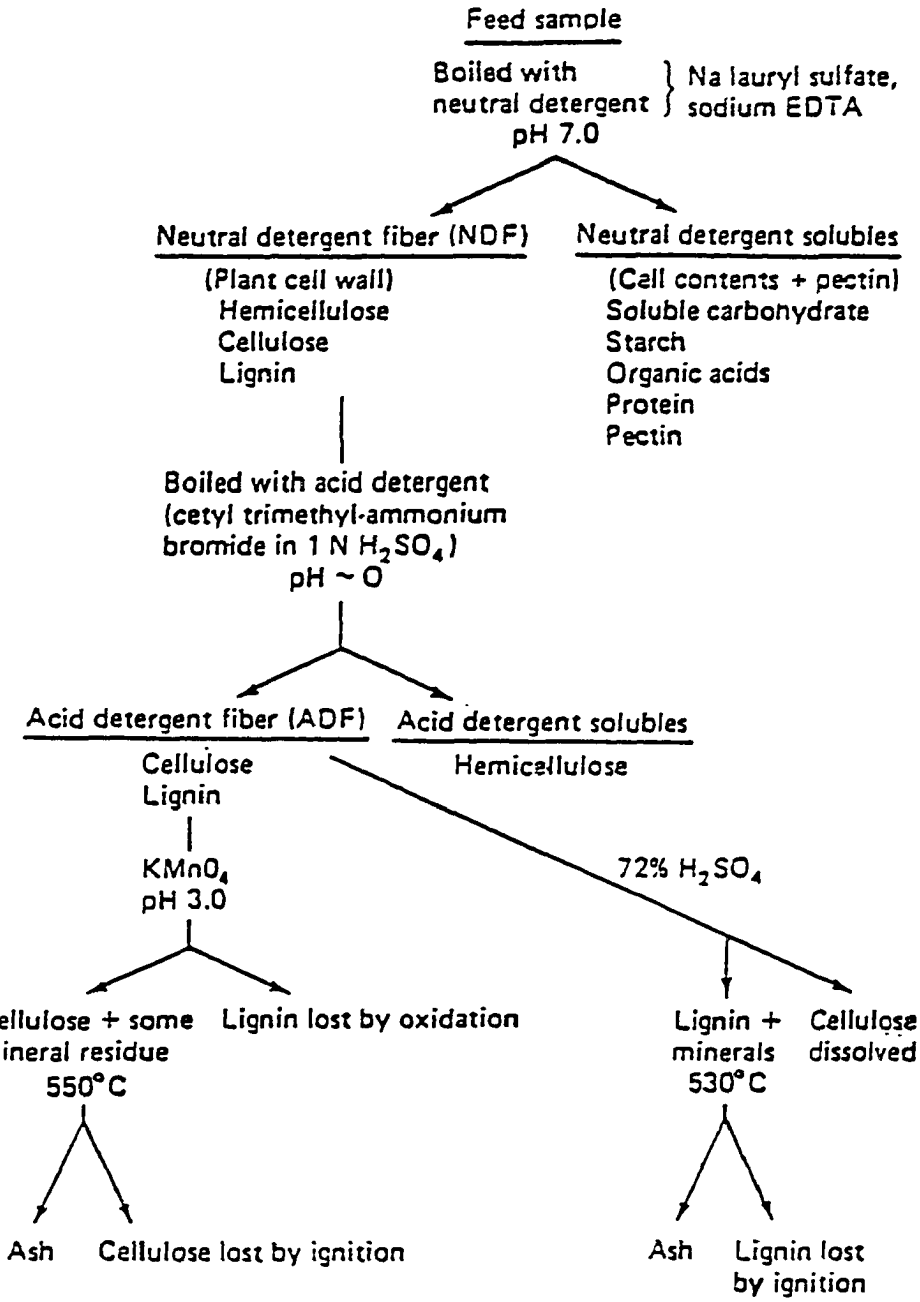

Fig. 1. The Van Soest fractionation method.

original method specified hot water extraction first, but in the modification starch was hydrolysed first. The water soluble polysaccharide was then extracted using hot water. The other steps are the same. The procedure as presented in Fig.3 consists of the following stages.

1. Treatment of the sample with 85% methanol to extract free sugars (monosaccharide, disaccharide, and higher oligosaccharides) together with some lipids, amino acids, pigments, etc. The remaining residue is then extracted with acetone or diethyl ether to remove lipids and pigments. The residue is then air dried and ground.

2. The ground sample is incubated with Takadiastase (amyloglucosidase) for enzymatic hydrolysis of starch. This method converts amylose and amylopectin to glucose.

3. The enzymatic hydrolysate is treated with four volumes of ethanol to precipitate any unchanged polysaccharides. The hexose (glucose) value in the supernatant is measured and is equivalent to water-insoluble starch as glucose.

4. The residue is treated with hot water. After centrifugation, 4 volumes of ethanol is added to supernatant. After centrifugation the precipitate is heated with 1 N sulfuric acid to hydrolyze the polysaccharide. The hexose, pentose, and uronic acid which is measured is equivalent to water-soluble non-cellulosic polysaccharides (which mostly derive from gum, mucilage, pectic substances and other soluble polysaccharides).

5. The residue of hot water extraction is subjected to hydrolysis by 1N sulfuric acid. After addition of ethanol and centrifugation, the supernatant is analyzed for hexose, pentose, and uronic acid which are primarily derived from hemicellulose (water- insoluble noncellulosic polysaccharide).
6. The residue from this dilute acid hydrolysis is dried and treated with 72% sulfuric acid which dissolves the cellulose. The hexose (glucose) measured in the supernatant is equivalent to cellulose.There are usually traces of other sugars in the supernatant, e.g., uronic acid and pentose which are derived from pectic substances. The residue insoluble in 72% sulfuric acid can be ashed . The loss in weight is equivalent to the lignin content.

The Southgate fractionation method produces more complete data on the individual polysaccharides, but it is time consuming and impractical for routine analysis of large numbers of samples.

The objection part of the Southgate method is the colorimetric determination of the sugars (pentoses, hexoses, and uronic acids). There are usually interferences by sugars in the determination, and there is lack of good correlation between sugars estimated by colorimetric methods and GLC technique for certain product (Selvendron et al., 1984). Now there are methods based on determination of sugars by GLC and HPLC (Schweizer and Wursch 1979, Collings and Yokoyama 1979). Anderson and Clydesdale (1980) performed fractionation of

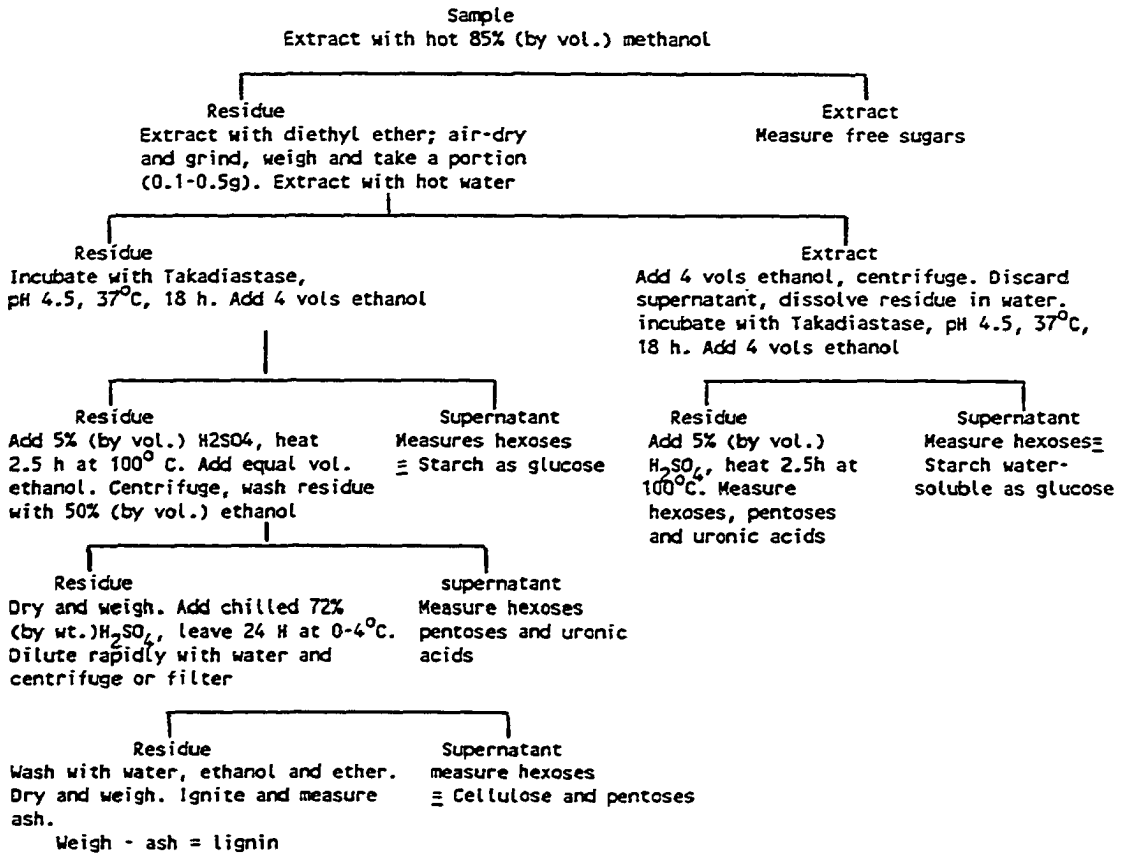

Fig. 2. Southgate (1969) fractionation method for measuring the components of the unavailable carbohydrate.

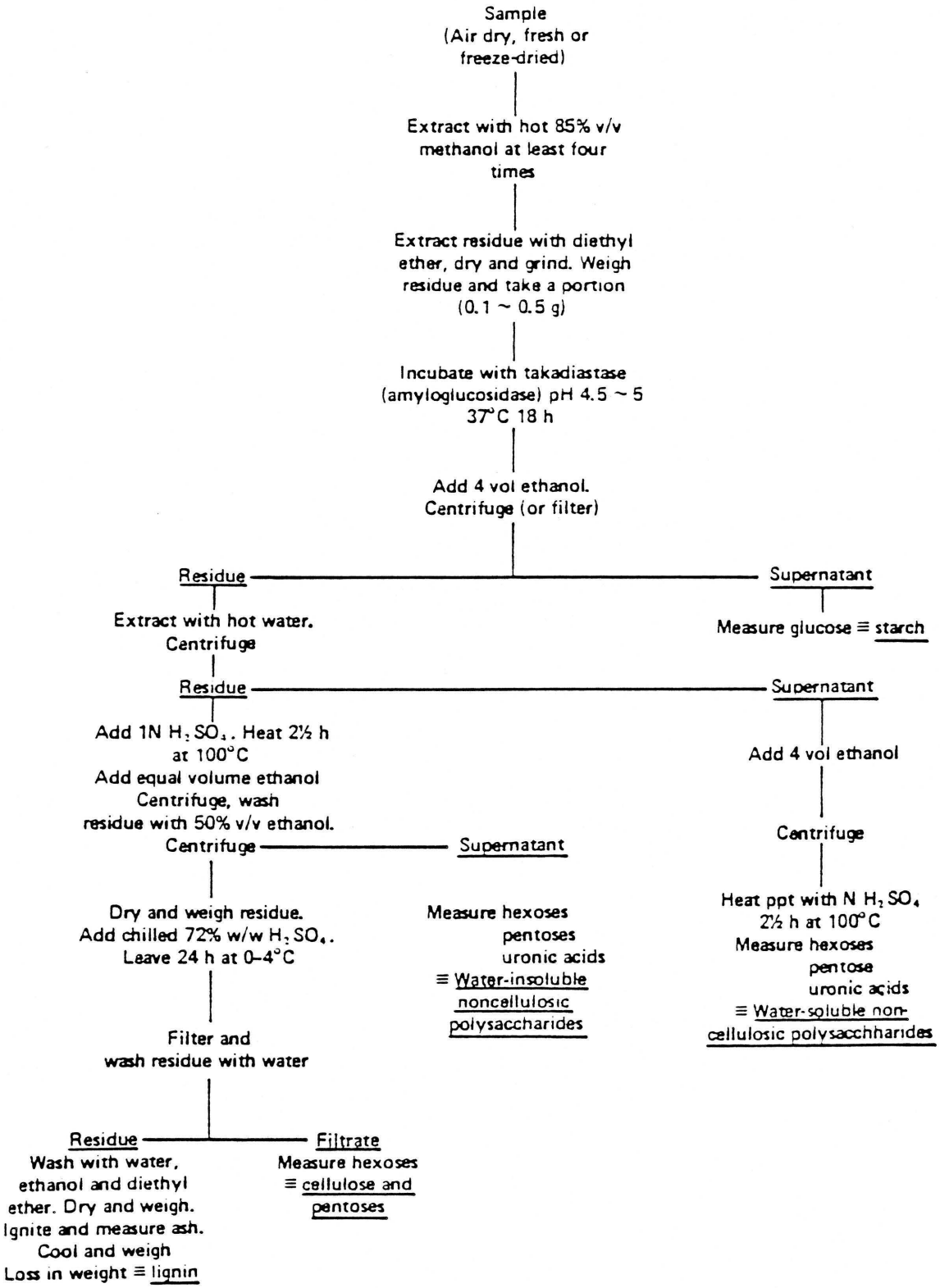

Fig. 3. Modified Southgate (1976) fractionation method.

wheat bran and analyzed the sugars by gas-liquid chromatograph.

Other methods used for determining plant fiber are enzymatic procedures. These methods were first employed by Williams and Olmsted (1935). Removal of starch and protein by pancreatin was followed by acid hydrolysis and by the identification and measurement of the sugar fractions. Hellendorn et al.(1975) used pepsin for hydrolysis of protein, pancreatin for subsequent starch hydrolysis, and then measured the fiber gravimetrically. Enzymatic procedures are designed to stimulate the in vivo enzymatic digestion for removal of the protein and starch, thereby isolating the polysaccharide and lignin fractions that represent fiber. In comparison to chemical methods, the technique produced higher values for fiber, but the relationship of these values to actual dietary fiber was still not completely understood (Baker et al., 1979). One of the problems with the enzymatic methods has been finding enzymes which are effective enough for complete digestion without removing the dietary fiber components (Theander et al., 1979a). Reproducibility of results by these methods is also difficult because of variation in enzyme purity. In addition, the cost of very pure enzymes becomes high for a large number of determinations (Baker et al., 1979). The Hellendoorn method has been expanded to include analysis of both water soluble and insoluble dietary fibers. Furda (1977) proposed a systematic methodology of

fractionation and isolation of water soluble polymers based on the usage of appropriate enzymes and inclusion of the soluble fiber fraction. Asp et al. (1983) and Asp (1977) evaluated some of the more widely used methods for the determination of dietary fiber and proposed enzymatic modification using pepsin, pancreatin, and termamyl, a heat-stable alpha-amylase. Theander and Aman (1979) introduced the use of the termamyl enzyme for combined gelatinization and starch degradation in dietary fiber analysis. At the 93rd AOAC Annual Meeting, Furda et al. (1979) reported that the use of mammalian as well as bacterial enzymes gave similar values for TDF determination when these enzymes were free from contamination of the fiber-splitting enzymes of cellulase, hemicellulase, and pectinase. In addition they sought to measure the soluble fractions of dietary fiber.

Asp et al. (1977) criticized most methods for measuring only the water insoluble components and ignoring the water soluble components, especially pectins and some types of hemicellulose. Asp et al. (1983) in their enzymatic procedure recovered soluble and insoluble fiber. They recovered the insoluble portion by filtration of hydrolysate. Alcohol was added to supernatant to precipitate the soluble fraction, which was then recovered by filtration. Theander et al. (1979a) preferred to recover the water-soluble polysaccharides (usually together with protein) via dialysis and freeze-drying rather than by precipitation from an aqueous solution by

ethanol. Their reason for this is that plant polysaccharides, such as arabinans and arabinogalactan, may remain in solution when ethanol is added. Prosky et al. (1984) in their total dietary fiber (TDF) method used a combination of enzymes of termamyl, protease, and amyloglucosidase plus a gravimetric procedure. After the gelatinization and enzymatic digestion steps, four volumes of 95% ethanol were added to precipitate the soluble dietary fiber. Total dietary fiber was recovered by filtration. Ash and protein determination were run on TDF to correct for these impurities.

The choice of analytical method for dietary fiber depends on the purpose and what one wishes to recover. For quality control a rapid procedure such as NDF may be suitable, provided that it is recognized that the values obtained are not total dietary fiber, and that the relation between NDF and the additional values obtained by the more time-consuming complete analysis is known. There is a time when specific, detailed and complete analysis of all components of fiber is needed, to understand, since the physiological effects of a given fiber are dependent on its particular chemical composition.

MINERAL BINDING

With the high interest toward fiber and recommendation toward increasing dietary fiber level, we need to consider one of the controversial issues of high fiber diet. One of the problem with high fiber foods is their mineral binding

ability. People at or near marginal intakes of minerals may be at some risk on elevated fiber intake. Although there are many studies that have shown high fiber diet adversely affects the mineral balance, the subject is still controversial. Which component of fiber is involved in the binding is also not completely understood. There are many factors that affect mineral binding by fiber. Many research papers discuss fiber influence on mineral balance, not always because of the polysaccharide, but perhaps because of the presence of phytate in the specific plant substances.

Reinhold et al. (1981) studied the iron binding by neutral detergent fiber (cellulose, hemicellulose, and lignin) from wheat and maize. They found that binding depends on iron concentration, pH, quantity of fiber, the presence of inhibitor, and quantity of inhibitors. Ascorbic acid, citrate, EDTA, and phytate among other substances markedly decreased binding of iron by NDF of wheat or maize and by wheat and maize brans. They found also that calcium and phosphate are strong inhibitors.

There are several in vitro methods used to measure mineral binding, but there is not any particular one which is agreed upon by all investigators. The methods which are used are either a centrifuge or an ion exchange column method. Most researchers have used the centrifuge method because of its simplicity, but the way that they determine binding is different .

Thompson and Weber (1981) studied the ion exchange capacity of wheat bran, soy bran, and oat hull for copper and zinc. They used the column procedure and either a neutral detergent fiber (NDF) or enzymatic treated fiber sources. They found all fiber residues bound more copper than zinc. And that the binding of both minerals was decreased as they were applied in combination. Probably there is competitive interaction for the binding site available.

pH of the medium is one of the factors which affect binding of minerals. Thompson and Weber (1979) looked at the binding of endogenous copper, zinc, and iron in six fiber sources. The binding was studied at three pH condition: (1) pH of 0.65 (2) pH of 6.8 (3) A sequential treatment of pH of 0.65, neutralization, then a pH of 6.8. They found after both pH 6.8 and sequential treatment most mineral remained bound in the residue, but after acidic treatment little mineral remained bound in the residue. They concluded that most of the mineral was released into the solution at pH 0.65, but was still bound after pH 6.8. If the endogenous mineral can be rebound by changing the pH from low (very acidic) to slightly acidic, there is a possibility that other minerals present in the diet could be bound to the fiber. Reilly (1979) studied the zinc, iron, and copper binding by dietary fiber at two different pH of 4.3 and 7.6. The extraction of the wholemeal bread and wheat bran was carried out by shaking a 1 gram sample with 50 ml 1M citrate buffer at different pH,

and analyzing the washed residue by atomic absorption, after dry ashing. He found that at pH 7.6 solubility, especially that of iron, was minimal. At pH 4.3 he found a higher solubility and more mineral being released when compared to the higher pH. Lyon (1984) studied the solubility of calcium, magnesium, zinc, and copper in cereal products containing different levels of phytate. He incubated samples with dilute HCl (pH=1) while shaking at room temperature and then centrifuged. Aliquots were analyzed for the minerals. He found a good extraction from all the cereals he studied in case of Zn, Ca, and Mg. Cu was not extracted as well as Zn and Mg. The effects of neutralizing the acid extract with bicarbonate was studied. They found the addition of sodium bicarbonate to make the pH 7.0 resulted in various degree of precipitation of different minerals. Precipitation was greatest for zinc in high-phytate cereals. Extracted copper was not precipitated. The addition of citrate and EDTA prevented precipitation.

Reinhold et al. (1975) were one of the pioneering research groups to look at interaction between fiber and mineral. They studied the binding of Ca, Zn, and Fe in breadstuff. They used dephytinized wholemeal bread and bran. They concluded in their study that the fiber largely determines the availability of bivalent metals of bread for absorption by the intestine. Other researchers have concluded that the phytate was the binding component, a view which is controversial in nature.

Ismail-Beigi et al. (1977) also examined binding of zinc

in vitro to wheat wholemeal bread (tanok), dephytinized tanok, wheat bran, wheat bran component, and cellulose at pH 5.0 to 7.50. They found that zinc binding was pH dependent, and it reached a maximum at pH 6.5 to 7.0. They found also the removal of phytate from tanok did not reduce its binding capability. Of the fractions they had separated from wheat bran, lignin and two of the hemicellulose fractions had high binding capability for zinc. Binding of zinc to various celluloses and dextrans was also demonstrated. Clydesdale and Camire (1983) reported degree of binding of minerals to soy flour did not correlate with the presence of phytic acid. However, Persson et al. (1987) in their mineral binding study showed that the incubation of fiber fractions with phytase reduced the complexing ability, indicating the active ligand to be phytate.

Rendelman (1982) studied the binding of zinc by bran and its components. He found high affinity of bran for zinc. Various gastrointestinal components were also investigated for their possible role in binding in the human small intestine. Sugar, saliva, amino acids, albumins and hydrogen carbonate ion at concentration expected in the intestine had little effect on the concentration of zinc. However, he found phosphate ion and mucin did cause significant zinc losses. He concluded this loss might be high enough to influence bioavailabilty of zinc.

Lee and Garcia-Lopez (1985) studied the iron, zinc,

copper, and magnesium binding by cooked pinto bean neutral and acid detergent fiber. They found the iron binding was increased by increasing pH. Both NDF and ADF bound Fe, Cu, Zn, but not Mg. Both NDF and ADF had a greater affinity for copper than for either iron or zinc. They also observed increase in binding with higher mineral concentration.

Nair et al. (1987) studied the binding of Cu, Cd, and Zn ions to some soluble and gelforming types of dietary fiber (guar gum, low and high methoxylated pectin and sterculia gum) potentiometrically. Binding to low methoxylated pectin was more pronounced than to high methoxylated pectin. Thus, the formation of complexes seemed to be due to the proportion of free carboxyl groups. The order of complex formation to low methoxylated pectin was Cu>Zn>>Cd. The binding to guar gum was negligible.

Since we normally process or cook our foods in some way or other, Camire and Clydesdale (1981) investigated the effect of pH and heat treatment on binding of Ca, Mg, Zn, and Fe to wheat bran, cellulose, pectin, and lignin. Lignin and pectin (methoxy content 7.5%) were found to have high metal binding. They found toasting (dry heat) had no effect on binding by cellulose but had a significant effect on the binding of metals by lignin and wheat bran. Boiling (wet heat) had a significant effect on the binding of metals by cellulose, lignin, and wheat bran. Clydesdale and Camire (1983) also studied the effect of pH and heat on mineral binding by soy

flour. The soy flour was found to bind more Fe, Ca, and Mg at pH 6.8 than at pH 5.0. They found higher binding occurred at higher pH. Boiling caused a significant increase in binding of zinc and magnesium at both pH value. Toasting also caused a significant increase in Ca and Zn binding.

The in vitro studies show that although fiber may bind minerals, there are numbers of agents in foods and in the digestive tract which may affect the amount of binding. It is difficult to compare results of studies, due to differences in experimental conditions. Different fibers vary in their capability to bind minerals. It is difficult to separate the capability of the different fibers, because they occur together in most foods (Kelsay 1986).

CHAPTER 3

MATERIALS AND METHODS

FIBER SOURCES

Fibers used in this experiment were obtained from commercial suppliers as follows: Hard red spring wheat bran, American Association of Cereal Chemists; rice bran, Riviana Food Inc. (Houston,Texas); soy bran, Archer Daniels Midland Co.(Decatur.Ill.); oat hulls, Quaker Oat Company; apple fiber, peanut fiber, tomato fiber, and barley fiber, Canadian Harvest; amalgamated bleached sugar beet pulp, Amalgamated Sugar Co.; and cellulose, ICN Pharmaceutical,Inc. (Cleveland,Ohio).

FIBER TREATMENT

Wheat bran, rice bran, oat hulls, and soy bran were ground with a Hammer mill using screen size 024 mesh. The fibers were shaken through 2 sets of sieves, 60 mesh(opening .0097 inch), followed by 100 mesh (opening .0059 inch). The result was three sets of sieved fibers of coarse, medium, and fine texture. The fiber fractions were saved in a plastic bags. Coarse fiber is the fraction retained on 60 mesh screen, medium is the fraction which passes through 60 mesh but retained on 100 mesh, and fine is the fraction which passes through a 100 mesh screen. Acid detergent fiber and total nitrogen were run on all three sieved fraction , to check for any fractionation and/or separation which might have occured during sieving. The fraction which passed through the

60 mesh and was retained on 100 mesh was used throughout the experiment after defatting.

Barley fiber, tomato fiber, and peanut fiber were received as a fine particle (100 mesh) from the commercial supplier and were used as received. Apple fiber used in this experiment was between 20-100 mesh. Amalgamated bleached beet pulp was used as received.

METHODS

All fiber sources having greater than 2% fat were defatted for 6 to 8 hours using a mixture of chloroform and methanol (2:1) in a soxhlet extractor. Barley and beet pulp were the only ones not defatted.

Mineral content of each sample was determined using a dry ash method (see appendix A for detail). Samples were analyzed for protein (microKjeldhl), moisture, ash using AOAC standard methods (AOAC, 1970), acid detergent fiber (ADF) and lignin using the methods of Van Soest (1963b), but omitted the use of asbestos.

These defatted samples were acid washed with 1% HCl in the following way.

ACID WASHING OF SAMPLE TO BE USED FOR MINERAL BINDING STUDY

To acid wash defatted sample in this experiment the procedure was as follows:

Ratio of fiber to acid was 1 gram to about 6.7 ml, with the exception of beet pulp, which had a high water absorption; therefore, more acid was added in order to make a slurry. A

weighed 150 gram sample of corresponding fiber (rice bran, oat hull, soy bran, wheat bran) was placed in a one gallon acid washed plastic bottle and 1000 ml 1% HCl was added to each bottle. In case of barley, peanut, tomato, apple and cellulose, 50 gram samples were weighed into a bottle and 334 ml 1% HCl added to each.

To a 30 gram beet pulp sample was added 511 ml of 1% HCl to make a slurry.

The slurries were shaken overnight at room temperature. They were poured into an acid washed porcelain filter funnel using a nylon filter cloth. The pH of the filtrate was read using pH meter.

The following pH was determined for the various fiber samples. Rice bran, 3.4; oat hulls, 1.98; soy bran, 1.43; wheat bran, 2.06; apple fiber, 1.23; tomato fiber, 2.45; and peanut fiber, 1.75.

The residue was washed 2 times with either 500 ml or 300 ml of 1% HCl, and the residue stirred in the funnel with an acid washed stirring rod.

To remove the acid from the residue: they were washed several times with deionized water (pH=7). The residue was considered to be free from acid when the filtrate didn't test acid to litmus paper. After filtration, the samples were spread out on a plastic cloth to air dry, using a fan to help with the drying. Some clumping occurred during acid washing, so the samples were ground with a mortar and pestle. In order to get

a homogeneous sample, they were placed on a 60 mesh screen and shaken. Most particles went through; if not, they were ground again and sieved again. The sieved sample which was acid and H_2O washed was used throughout the experiment for mineral binding. Mineral content of an acid washed sample was determined via dry ashing (see appendix A for the detail). These acid washed samples were used in the binding study using the centrifuge method.

CENTRIFUGE METHOD FOR MINERAL BINDING

Five grams of acid washed sample were weighed into a 250 ml acid washed centrifuge bottle. Thirty-five ml (or appropriate amount) of 1000 ppm standard mineral from American Scientific Product was added to each bottle. In the case of beet pulp 60 ml of additional water with a pH of 6.8 was added to it in order to make a slurry. This was necessary because beet pulp has such a high water binding capacity.

The pH of each bottle was adjusted to 6.8 $\pm$ 0.10 using either sodium hydroxide or hydrochloric acid of different concentration (10N, 1N, 0.1N NaOH or 5%, 1% HCl). The samples were shaken for 3 hours,then centrifuged for 15 minutes at 5000 rpm. After the centrifugation the filtrate was collected in a 50 ml test tube for pH determination. Fifty ml of double deionized water (pH=6.8) was added to each bottle, swirled to mix, then centrifuged at 5000 rpm for 10-15 minutes; filtrates were decanted. The water washing was repeated for 2 more times to remove the unbound mineral. After the last washing, the

residue was quantitatively transferred into a weighing boat by using a plastic spatula. The sample was covered with plastic wrap, frozen and placed into a freeze dryer. After drying, the samples were mixed by either an acid washed stirring rod or a pestle and then were transferred into a vial for storage until needed for further analysis. This material was our fiber bound mineral. Two gram samples in duplicate were dry ashed for analysis of desired mineral. The obtained value was considered as our binding capacity.

REACID WASHING OF FIBER BOUND MINERAL

A weighed 2 gram sample of fiber bound mineral was placed into a 250 ml centrifuge bottle, 15 ml of 1% HCl was added and swirled to mix to make sure it was in suspension. In case of beet pulp with it's higher water absorption, 20 ml of extra acid was added and swirled to make into a suspension. The slurry was shaken overnight, centrifuged at 5000 rpm for 10-15 minutes and decanted. The washing was repeated 3 times with 25 ml 1% HCl. The final residue was transferred into a weighing boat using a plastic spatula, and freeze dried. The samples were analyzed for desired mineral by the dry ashing method (see appendix A).

REAGENT AND INSTRUMENTATION FOR MINERAL ANALYSIS

GLASSWARE

All glassware was soaked in 25% nitric acid overnight and rinsed thoroughly with distilled deionized water(DDW).

REAGENT

The reference atomic absorption standards used in this experiment, purchased from American Scientific Product, are listed as follows: 1000 ppm magnesium standard (magnesium chloride in dilute hydrochloric acid),1000 ppm zinc standard (zinc nitrate dissolved in dilute nitric acid), 1000 ppm copper standard (copper metal dissolved in dilute nitric acid), 1000 ppm calcium, and 1000 ppm iron (Fe).

Lanthanum has been show to eliminate the interferences from phosphate and aluminum in the determination of calcium and magnesium. A 5% lanthanum oxide (La_2O_3) dissolved in 25% HCl was made in the following way: weigh 58.65 gram La_2O_3 in a large beaker, add 250 ml conc HCl, transfer quantitively into a volumetric flask, bring volume to 1000 ml with DDW, cool while making the solution.

STOCK SOLUTION AND THE STANDARD

To make 10 ppm mineral stock solution: Add 1 ml of reference atomic absorption mineral to a 100 ml volumetric flask and bring to mark with 5% HCl.

Running standards are made daily from the above stock solution. To make the standard curve 4 to 5 dilutions are made. The standard ranges for each mineral to give a linear curve are as follows: for copper and calcium 0.1 to 4 ppm, magnesium and zinc 0.05 to 1 ppm and for iron 0.1 to 3 ppm. The standards are made in 15 ml graduated test tube. To make the standard, an appropriate amount of stock solution is

pipetted into a test tube and the volume brought up to 10 ml with 5% HCl.

Magnesium and calcium standards should have La_2O_3 added to them for a final concentration of 1% lanthanum. For example, to make 0.1 ppm standard with 1% La_2O_3: pipet 0.1 ml of 10 ppm stock solution into a test tube, then pipet 2 ml of 5% La_2O_3 solution, adjust the volume to 10 ml with distilled deionized water.

APPARATUS

Mineral determination was done using an Hitachi 180-70 polarized Zeeman, atomic absorption spectrophotometer.Basic components of the instrument are :

a) light source is an external source of radiation (to be absorbed), usually these are line sources, emitting the spectral lines of the element to be determined. b) monochromator: adjustable monochromator for selecting the desired wavelength, rejecting all others which are not wanted. The entrance or aperture of a monochromator is a long, narrow slit with a width which is generally adjustable. c) Burner: adjustment of burner head relative to the light path of the instrument is necessary to obtain a maximum sensitivity. d) Flame: the main requirements of a satisfactory flame are that it has the proper temperature and fuel/oxidant ratio to carry out the enumerated function of the flame (decompose the sample into its constituents). Air/acetylene is the preferred flame of the determination for about 35 elements by atomic

absorption (AA). d) nebulizer: liquid sample drawn through sample capillary by the pressure differential generated by the high velocity gas stream. Solution reduced to an extremely fine spray and thoroughly mixed with fuel and oxidant. e) Detector: convert the light energy to electric energy. f) Display: shows the reading after it has been processed by the instrument electronics.

Separate hollow cathode lamps were used to measure copper, zinc , magnesium, iron and calcium at 324.8 nm, 213.8 nm, 285.2 nm, 248.3 nm, and 422.7 nm respectively. The optimum analytical operating conditions for each mineral are as follows:

For zinc the lamp current was 10.0 mA, slit width was 1.3 nm, burner height was 7.5, oxidant (compressed air) was 1.60 kg/cm^2, and fuel(acetylene) was 0.20 kg/cm_2.

For copper the lamp current was 7.5 mA, slit width was 1.3 nm, burner height was 7.5, oxidant was 1.60 kg/cm^2, fuel was 0.30 kg/cm^2.

The condition used for magnesium was: lamp current 7.5 mA slit width was 2.6 nm, burner height was 7.5, oxidant was 1.6 kg/cm_2, and fuel was 0.20 kg/cm_2: for calcium lamp current was 7.5 mA, slit width 2.6 nm, burner height 12.5, oxidant 2.60 kg/cm^2, and fuel was 0.40 kg/cm^2: the condition for iron was: lamp current 10.0 mA, slit width 0.2 nm, burner height 7.5, oxidant flow 1.6 kg/cm^2, and fuel was 0.30 kg/cm^2.

OPERATION AND CALCULATION

All samples to be analyzed for minerals have been dry ashed (see appendix A), and the ash dissolved in 25 or 10 ml 5% HCl. Appropriate dilution was made from this original solution for the analysis. National Bureau of Standard (NBS) reference material was ashed and run with the fiber sample to check the methodology and precision of the instrument. Again all the dilutions were made in 15 ml test tube with 5% HCl. TO operate the AA, set it with optimum condition for each mineral, zero the machine with 5% HCl (matrix of the standard and the sample). Read standards before and after reading samples, or every 10 sample if there are large numbers of samples for analysis, aspirate DD water through burner between each reading. Each sample was read 3 times, then averaged. The corresponding standards were averaged for calibration curve. Each sample's absorption reading was corrected by a reagent blank; concentration in each tube was determined using the standard linear regression of $r > 0.99$. In a spectrophotometric calibration curve, y would represent the measured absorbance and x would be the concentration of the standard while r is the correlation coefficient which is calculated for a calibration curve to ascertain the degree of correlation between the measured instrumental variable and the sample concentration.

The concentration in each sample calculated as follow:

$$\frac{\text{ug mineral}}{\text{gram sample}} = \text{ppm} = \frac{(A)(B)(C)}{(D)}$$

where:

A= Concentration of sample from linear regression

B= Volume (in ml) ash dissolved in

C= Dilution factor

D= Sample weight in grams

Dilution factor = E/F

E= Final volume in the tube

F= Volume of the original sample pipetted for dilution

Example of dilution factor: if two ml of sample stock solution is taken and diluted to 10, the d.f. is 10/2 = 5.

CHAPTER 4

RESULT AND DISCUSSION

The fiber sources of rice bran, oat hull, soy bran, and wheat bran were ground and sieved into 3 set of fiber fractions with different degrees of porosity: coarse, medium, and fine and were analyzed for total nitrogen and acid detergent fiber. The coarse fraction is the fraction which stayed on 60 mesh screen after sieving , the medium fraction is what passed through the 60 mesh but stayed on 100 mesh. The fine one is everything which passed through the 100 mesh screen. All these fractions were analyzed to see if any fractionation might have occurred during the sieving.

Sampling is important in all types of analytical work and having a representative sample is a necessity. Table 1 shows the protein value in gm/100gm of the various sieved samples. The rice bran had 17.08, 18.32, and 18.62 for coarse, medium, and fine fractions respectively. One way analysis of variance showed there is significant difference between these sample at the 95% confidence level. Thompson (1980) has analyzed rice bran as received from the supplier and found a value of 21.4% for protein based on dry weight. This value is comparable with the value reported here. The coarse fraction had the lowest protein, while the fine had the highest. The same trend was shown between the oat hull, wheat bran, and the soy bran. Soy bran was the exception in that there was no significant difference between the coarse and medium fraction, but both

Table 1. Protein content of ground and sieved fiber sources.[1]

Fiber sources	Coarse[2]	Medium[3]	Fine[4]
	----------gm/100 gm----------		
Rice bran	17.08 ± 0.06[A]	18.32 ± 0.04[B]	18.62 ± 0.12[C]
Oat hull	2.11 ± 0.07[A]	2.76 ± 0.01[B]	6.23 ± 0.02[C]
Soy bran	9.60 ± 0.06[A]	8.64 ± 0.20[A]	10.66 ± 0.50[B]
Wheat bran	16.91 ± 0.00[A]	16.23 ± 0.16[B]	19.76 ± 0.04[C]

[1]Calculated on wet basis,expressed as % of protein(Nx6.25)
Mean ± standard deviation (n=2)

[2]Fiber retained on 60 mesh screen

[3]Fiber passed through 60 mesh and retained on 100 mesh

[4]Fiber passed through 100 mesh

[A]Different letters on the horizontal line indicates, the means are significantly different at $P<0.05$

fractions were significantly lower in protein content than the fine fraction which had a 10.66 value. Coarse oat hull had 2.11 gram protein per 100 gram of sample while the fine value was 6.23. Thompson's (1980) value for oat hull protein was 4.6. Wheat bran was the exception; the coarse fraction had a protein value slightly higher than the medium. But still both coarse and medium fractions were lower in protein than the fine. The general trend has been that the coarser fractions have comparably less protein than the fine ones. Inglett et al. (1979) presented some protein data on materials collected on 4 different sieves. They have shown that the value of percent nitrogen increases as the sample become finer. Their observation was in agreement with our finding that nitrogen values are sensitive to particle size. What is the reason that more coarse fibers result in a lower protein value? Fibers are probably partioning according to the degree of their hardness. Harder materials are more difficult to pass through screens during the grinding. During the grinding, the softer material goes through faster, while the harder material stay on the top sieve. Probably the protein part of bran is softer material and, therefore, grinds to finer consistency. This was true for all of our samples. We found higher protein concentration in the finer fractions. This partioning could be due to the degree of silica or insoluble ash content of the sample. If insoluble ash was run, the coarse fiber probably would have the highest amount of ash. It was noticed that oat hulls

during mineral analysis had the highest insoluble ash after the acid was added to the ash. There was a 66% difference between the protein content of coarse and fine fraction of oats, which had the highest difference among the fiber sources tested. But the main point demonstrated here is the importance of a good sampling system. Having a homogenous material is an important key to good experimentation. Table 2 has the acid detergent values for the same samples of rice, oat, soy, and wheat. The fractionation is noticeable in this table, too. Rice bran ADF percentage for coarse, medium, and fine are 15.56, 12.1, and 8.83 respectively. Thompson (1980) found 10.0% ADF for the rice bran as received from the supplier. Statistics showed there was a significant decrease at $p<0.05$ in ADF value as the sample became finer. The same trend was shown between oat hull and wheat bran. In oat hull, the coarse fraction was 44.95% , medium was 43.70% and the fine was 34.15% ADF. Thompson's (1980) value for oat hull ADF was 42.2%, which is comparable with our medium fraction. The values for wheat bran were 15.86, 14.90, and 11.30 respectively for coarse, medium and fine. Soy bran was an exception. The coarse portion had significantly lower ADF than the medium and fine portion. The medium of 46.0 and fine of 44.67 were not significantly different, while the coarse was at 39.48 ADF. The reason why the coarse soy bran had a lower ADF than the fine is not clear, but could be due to softer fiber. The opposite was apparent for the rice, oat, and wheat

Table 2. Acid detergent fiber of ground and sieved fiber sources.[1]

Fiber Sources	Coarse[2]	Medium[3]	Fine[4]
	gm/100 gm		
Rice bran	15.56 ± 0.16[A]	12.10 ± 0.15[B]	8.83 ± 0.12[C]
Oat hull	44.95 ± 0.76[A]	43.70 ± 0.04[A]	34.15 ± 0.49[B]
Soy bran	39.48 ± 0.68[A]	46.00 ± 0.56[B]	44.67 ± 0.34[B]
Wheat bran	15.86 ± 0.37[A]	14.90 ± 0.04[B]	11.30 ± 0.19[C]

[1]Calculated on wet basis and expressed as Mean ± SD

[2]Fiber retained on 60 mesh screen

[3]Fiber passed through 60 mesh and retained on 100 mesh

[4]Fiber passed through 100 mesh

[A]Different letter on horizontal line indicates, the means are significantly different at $P<0.05$

with the highest value for ADF being found in the coarse portion and lowest in the fine portion. Fibers are harder than protein, therefore harder to grind, and probably that is the reason why they stay behind in coarse fraction. Fiber decrease in concentration as fines increase, with the exception of soy bran. It is also possible that larger particle sizes have a lower surface to volume ratio and this must reduce the access of detergent or chemical to the interior of the particle. That is probably why the coarse fraction had higher ADF values. Heaton et al. (1988) showed that in vitro starch hydrolysis by pancreatic amylase was faster with decreasing particle size with all three cereals they studied. In this experiment we wanted to work with the fraction with the highest fiber and the lowest protein concentration, but at the same time we did not want a material that was either too coarse or too fine. Medium fractions were chosen for the experiment since it is a compromise for both sieve size and the protein and ADF content.

Rice, oat, soy, and wheat fiber with medium characteristic were chosen for the mineral binding study. These samples along with other fiber sources, which are used as received from the supplier, were first defatted. Proximate analysis, acid detergent fiber, and lignin were performed on these defatted sample. Results of a duplicate analysis with their standard deviation can be seen in Tables 3 and 4.

Tomato fiber had the highest amount of protein 22.22%. Next

Table 3. Proximate analysis values of various defatted fiber.[1]

Fiber sources	Protein[2] %	Moisture %	Ash %
Rice bran	19.74 ± 0.18[b]	8.46 ± 0.14	12.32 ± 0.16
Oat hull	3.51 ± 0.03[g]	6.94 ± 0.04	6.07 ± 0.01
Soy bran	8.58 ± 0.02[e]	7.68 ± 0.01	4.32 ± 0.13
wheat bran	16.88 ± 0.93[c]	7.81 ± 0.03	6.80 ± 0.02
Apple fiber	4.65 ± 0.25[f]	5.96 ± 0.02	11.11 ± 0.06
Peanut fiber	18.96 ± 0.06[b]	7.25 ± 0.23	2.72 ± 0.07
Tomato fiber	22.22 ± 0.42[a]	5.96 ± 0.26	4.93 ± 0.08
Barley	11.80 ± 0.19[d]	4.94 ± 0.20	6.97 ± 0.14
Beet pulp	8.60 ± 0.08[e]	6.82 ± 1.15	4.66 ± 0.04

[1]Values on wet weight basis , presented as Mean ± SD
[2]Protein (nitrogen x 6.25)
[b]Different letters within column are significantly different at $p<0.05$

were rice bran and peanut fiber with values of 19.74 and 18.96% respectively. If one compares the value of protein in Table 3 for defatted rice bran, oat hull, soy bran, and wheat bran to those in Table 1, there is a small increase in protein when the sample is defatted (Table 3), which is reasonable. Rice and oats values obtained in this experiment were comparable with data reported by Thompson and Weber (1979), who had used the same type of fiber samples. Soy protein value was 8.58% which was lower in comparison to the value obtained by Thompson and Weber (1979), but the soy bran sample was not from the same source. James and Theander (1981) obtained a value of 16.1% for the wheat bran protein, while ours was 16.88%. Our analysis of Canadian Harvest's samples of apple, peanut, tomato, barley, and beet pulp fibers were close to those reported by the company. Our apple value of 4.65% was comparable with their reported value of 5%. However, James and Theander (1981) reported a value of 1.4% protein for apple pulp. Canadian Harvest reported a value for peanut fiber of 13-16%, while our value of 18.96%, was much higher than theirs. This higher value could be the result of defatting the sample. They have reported 12-15% fat for the peanut fiber. Correction for this amount of fat makes their protein value fall in the range of 14.8-18.82%. The 18.96 protein value for peanut was very close to their upper range. Tomato fiber had the highest protein value, 22.2%, which was in agreement with the 22.0% Canadian Harvest has reported. Our values for barley

and beet pulp, 11.8% and 8.60% was higher than the 8.0% and 7.0-7.5% of the Canadian Harvest. Moisture value were in agreement with the supplier value. Ash content reported by Canadian Harvest was in most cases higher than our analysis. Within our sample, rice bran and apple had the highest amount of ash , 12.32 and 11.11% respectively. Reported value for apple ash was 18.0%. Our values were based on defatted sample, and if we include the %fat, this makes our ash value even smaller. We can not explain why this discrepancy exists for our value versus the supplier value. However, James and Theander (1981) have reported 1.0% ash for apple pulp. This substantial difference could be due to different sample characteristics, and different samples or parts of apples being analyzed. Peanut fiber with a 2.72% ash value is the fiber with the lowest ash content. Ash reported by the company for the same sample was 3-11%.

Acid detergent fiber and lignin content of defatted sample appear in Table 4. ADF values ranged from 13 to 57% without including the cellulose. Analyses were based on duplicate results with the exception of six analyses for peanut, four for barley and three for cellulose. Many difficulties were involved in the analysis of these fiber samples. There were especially problems with filtration of the ADF sample and the lignin digest. Problems were probably due to fine particle size which makes the filtration difficult. Tomato and peanut gave the most problems in filtration of the lignin digest.

Table 4. Acid detergent fiber and lignin content of various defatted fiber sources.[1]

Fiber sources	ADF[2] %	Lignin %
Rice bran	13.06 ± 0.09[e]	4.70 ± 0.08[cd]
Oat hull	41.50 ± 0.05[c]	6.57 ± 0.03[c]
Soy bran	45.82 ± 0.42[b]	0.54 ± 0.05[d]
Wheat bran	15.14 ± 0.23[e]	3.93 ± 0.07[cd]
Apple fiber	57.23 ± 0.58[a]	15.03 ± 0.33[b]
Peanut fiber	55.74 ± 1.99[a]	25.55 ± 3.55[a]
Tomato fiber	46.56 ± 0.11[b]	23.58 ± 0.18[a]
Barley fiber	24.76 ± 0.24[d]	4.38 ± 0.11[cd]
Beet pulp	25.23 ± 0.91[d]	0.78 ± 0.10[d]
Cellulose	64.78 ± 2.59	8.50 ± 4.19

[1]Values on wet weight basis, presented as Mean ± SD (n=2 except 6 for peanut, 4 for barley, and 3 for cellulose

[2]Acid detergent fiber

[e]Different letters within column are significantly different at $p<0.05$

Filtration after addition of 72% sulfuric acid was very slow, making it necessary to leave the samples overnight, in order to accomplish the filtration the next day. It was necessary to analyze peanut fiber six times for periods of 2 days before achieving the complete filtration and washing desired. Addition of boiling water was beneficial in improving the filtration rate. This filtration problem could have been due to the gum or starch present in the sample which clogged the crucible. Boiling water was used to dissolve these materials and improve the filtration. Apple and peanut had the highest amount of ADF (this high value may be the result of high starch and gum), 57.23 and 55.74 respectively. Canadian Harvest reported that their peanut product had 15-35% crude fiber, 50-60% dietary fiber and 2-3% soluble fiber. Crude fiber should be lower than ADF which it was. Depending on which dietary fiber determination was used, dietary fiber value should be higher than the ADF. Because the ADF is mostly the insoluble portion of the fiber while the dietary fiber includes both the soluble and insoluble fractions. Reeves (1985) reported 73.9% ADF for peanut hulls.

The lignin contents ranged from 0.54 to 25.6%. Peanut and tomato had the highest lignin, while soy bran and beet pulp had the lowest lignin concentration. Ross et al. (1985) have reported dietary fiber and lignins content of various fresh and canned fruits and vegetables. Lignin ranged from 0.06-0.87 for apple, and 2.2-4.1 for tomato. However, since our samples

are from different sources, they were not comparable with those data.

ACID WASHING

Defatted fiber sources were acid washed with 1% HCl (pH about 0.75). The pH of the filtrate was measured and all the filtrates had higher pH after washing than the starting acid. It is known that fiber has the ability to reduce the acidity of the medium, because it acts as an ion exchange medium. Protein reduces the acidity of the medium by accepting hydrogen ion from it. Rice bran had the highest ability to reduce the hydrogen ion concentration, resulting in the filtrate having the highest pH of 3.4. The initial pH for a 1% solution of HCl was raised by rice bran. This reduction of acidity was consistent with protein content. Rice had the second highest protein content and also had the highest acidity reduction. Oat hull and apple fiber had the lowest amount of protein, and their filtrates had a lower pH of 0.98 and 1.23, respectively. Some additional components in our samples might have the ability to absorbed hydrogen ions, and/or cause a pH change.

Acid washing has stripped minerals from our fibers. The percent reductions of minerals have been demonstrated in Table 5. The percentage of extraction calculated as (acid washed value-endogenous value)/endogenous value x 100. Endogenous and acid washed value of minerals will be discussed under the binding study of each mineral. Lyon (1984) has run in vitro

Table 5. Percentage of Zn, Mg, and Cu extracted from different fiber sources with 1% HCl.

Types of Fiber	Zn	Mg	Cu
Rice bran	100.0	100.0	84.2
Oat hull	100.0	99.0	4.4
Soy bran	98.6	99.5	85.1
wheat bran	98.0	99.7	61.3
Apple fiber	100.0	98.8	-
Peanut fiber	94.6	99.2	85.5
Tomato fiber	93.2	99.4	31.7
Barley fiber	96.6	97.5	34.1
Beet pulp	-	97.4	100.0

studies for several cereal products in order to examine the likely release and fate of the Ca, Mg, Cu, and Zn present in cereals by simulating a passage through the stomach and intestine. He found Ca, Mg, Cu, and Zn were released by extraction with HCl (pH=1) for 0.5 hours. Zn, Ca, and Mg were more or less completely extracted except in the case of Muesli where Zn (46%) and Ca (7%) were poorly extracted. Our study agrees with Lyon for amounts of mineral released by acids. In all the cereals Lyon studied, Cu was not extracted well and had values ranging from 37% to almost 100% with a mean extraction of 63%. We had the lowest percentage of extraction, 4.4% for Cu from oat hull, while beet pulp had an extraction value of 100%. Oat hull and other fiber sources had a low extraction rate which was probably due to low initial Cu concentration (oat hull=1.5 ug/gm). In most cases, Cu was extracted to a level no lower than 1 ug/gm for fiber. This make the percentage of extraction low for copper when the initial concentration was low. We have a mean copper extraction of 61% for all the fiber study. Zinc and magnesium extracted well.

To investigate if any other changes might have occurred during the acid washing, protein and ash content of acid washed samples were analyzed. The mean protein value for all acid washed samples was 12.47, but, the defatted sample had a higher mean for protein, 12.77%. Overall acid washing resulted in lower protein content. Protein and ash content of

acid washed samples are presented in Table 6. Table 7 shows the percent change in protein and ash upon acid washing. Percent change was calculated as (value of acid washed sample-values of defatted)/value of defatted X 100. In most of the fibers we studied, a decrease in protein values was observed. Oat hull had the highest reduction in protein at 39%. Barley, apple, soy, tomato, and beet pulp fiber also had a reduction in protein content after being acid washed. This reduction in protein could be due to removal of nitrogenous (protein) compounds. Probably, we can assume that this loss was due to removal of water soluble protein and that the samples with highest protein losses are the ones with highest soluble protein. However, some of the samples such as rice bran, wheat bran and peanut fiber had increases in protein values after being acid washed; rice bran had 21.9% increase in protein value. This increase could be due to concentrating the protein in the sample. There are many other soluble compounds which could have been removed from the sample, e.g., free sugar, soluble ash, and soluble fiber. The extraction of these materials in the case of rice bran probably was higher than the nitrogenous material (protein). We have run ash analysis, and rice bran showed an 88.8% reduction in ash. Rice, wheat, and peanut were the samples which had an increase in protein concentration after acid washing. These three were among the samples which had the highest decrease in ash content of 88.8, 94.9, and 83.3% respectively. Percent of ash reduction of

Table 6. Protein and ash content of various defatted and acid washed samples.[1]

Fiber Sources	Protein[2] %	Ash %
Rice bran	24.06 ± 0.09	1.38 ± 0.10
Oat hull	2.14 ± 0.06	5.06 ± 0.04
Soy bran	7.58 ± 0.36	0.42 ± 0.05
Wheat bran	17.44 ± 0.13	0.35 ± 0.03
Apple fiber	3.97 ± 0.04	10.93 ± 0.07
Peanut fiber	19.62 ± 0.02	0.42 ± 0.06
Tomato fiber	20.82 ± 0.45	1.51 ± 0.00
Barley fiber	8.54 ± 0.12	4.84 ± 0.05
Beet pulp	8.07 ± 0.03	0.86 ± 0.06

[1]Result presented as Mean ± SD (n=2)
[2]calculated on wet basis, expressed as (Nx6.25)

Table 7. Percent changes in protein and ash content of various fiber sources after acid washing.

Fiber sources	% change in	
	Protein	Ash
Rice bran	+21.9	-88.8
Oat hull	-39.0	-16.6
Soy bran	-11.7	-90.3
Wheat bran	+ 3.3	-94.9
Apple fiber	-14.6	- 1.6
Peanut fiber	+ 3.5	-83.3
Tomato fiber	- 6.3	-67.5
Barley fiber	-27.6	-30.6
Beet pulp	- 6.2	-81.5

- means reduction in value by acid washing
+ means increse in value

samples is presented in Table 7. Soy bran had a high ash reduction of 90.3%. After ashing, it was observed the soy ash dissolved completely in acid. Soy primarily had soluble ash with negligible insoluble ash. Apple fiber and oat hull showed the lowest reductions in ash value, 1.6 and 16.6%. It was observed after ashing and the addition of acid that these two samples had the highest amount of residue, which was an insoluble ash (most likely silica). Therefore the lower reduction in ash content of apple and oats could be due to their high insoluble ash. The mineral analysis showed that most of the minerals were extracted from the sample. Mineral extraction by acid washing the sample had been our primary goal. We could then run the mineral binding study using these samples. However, acid washing probably removed many other water soluble components. Phytic acid was one that could have been extracted by acid washing, because the method of determination of phytic acid involves its extraction with HCl (Harland and Oberleas 1977).

ZINC BINDING CAPACITY

The potential of different fibers to bind zinc was investigated. The results of the binding study using wheat at 1, 3, 12, and 17 hours of incubation are presented in Table 8. Binding values ranged from 5.8 to 6.3 mg of Zn for the four times studied. At 1 hour of incubation, wheat bran binding for Cu was 5.8 mg. The 1 and 12 hour incubation times were not significantly different. At 3 hours wheat bran bound 5.9 mg

Table 8. Time study of acid washed wheat bran binding capacity for zinc.[1]

Time of Incubation	Fiber (gram)	Mineral added (mg)	Binding Capacity ug/gm
1 hr	5	35	5792 ± 214[a]
3 hrs	5	35	5914 ± 163[ab]
12 hrs	5	35	5679 ± 9[a]
17 hrs	5	35	6254 ± 56[b]

[1]Values presented as Mean ± SD (n=2)

[a]Different letter means they are significantly different at $P<0.05$

which was not significantly different from the 1 and 12 hour amounts. Because of the variability existing, 3 and 17 hours were not significantly different but 1 and 17 were different. Since we did not find any significant difference between 3 hours incubation time and the longer time periods, and because physiologically food does not remain normally in the stomach longer than 3 hours, the 3 hours incubation period was selected as the time period to be used in binding study. Using this time period, we were confident that an equilibrium would be reached between mineral and fiber.

To determine the ratio between fibers and minerals to be used, rice, oat, soy, and wheat were studied for their binding capacity at two different concentrations, while keeping a constant pH of 6.8. All the studies were run using 3 hours of incubation. Results are presented in Table 9. One gram of fiber was incubated with 5 ml of 1000 ug/gm stock zinc solution (5 mg). This same experiment was repeated two times; one study was run in quadruplicate. There was no significant difference at the 1:5 ratio among fiber. A higher ratio of 1:7 fiber to mineral was then studied. In this case 5 grams of fiber was incubated with 35 mg zinc. The higher concentration of mineral used for binding resulted in significant increase of binding capacity. Rice bound 7.8 mg at a 1:7 ratio. At 1:5 ratio the binding was 5.8 mg. In the case of rice the average binding capacity was slightly higher than the amount of mineral added to it. The error (SD) term helps partially to

Table 9. Zinc binding capacity of various acid washed fiber sources at 2 different mineral concentrations.[1]

Type of Fiber	Fiber (gram)	Mineral added to make the slurry (mg)	Binding capacity (ug/gm)
Rice	1	5	5846 ± 669[a]
Rice	1	5	5243 ± 936[a]
Rice	5	35	7820 ± 285[b]
Oat	1	5	673 ± 99[a]
Oat	1	5	925 ± 181[a]
Oat	5	35	1861 ± 155[b]
Soy	1	5	4613 ± 482[a]
Soy	1	5	5219 ± 1127[ab]
Soy	5	35	6713 ± 221[b]
Wheat	1	5	4841 ± 303[a]
Wheat	1	5	4535 ± 384[a]
Wheat	5	35	6713 ± 285[b]

[1]Value presented as Mean ± SD (n=2 except for the first determination of each fiber set n=4)

[a]Different letters in each sets mean they are significantly different at $p<0.05$

explain the higher binding. Rice, oat, soy, and wheat showed higher binding when the mineral concentration was raised. These results are reasonable, because increasing the mineral concentration up to a point will eventually saturate all the available binding sites. Many researchers have shown that an increase in mineral concentration resulted in higher binding (Rockway 1985, Lee and Garcia-Lopez 1985, and Rendleman 1982). Because of the higher binding at the 1:7 ratio, this ratio was chosen to be used in further studies. In the four fibers studied, rice bound higher amounts of Zn than the wheat, oat, and soy fibers, while oat bound the lowest amount. This was consistent for both concentrations of zinc.

Rice, oat, soy, wheat, apple, peanut, tomato, barley were investigated for their binding ability at a mineral concentration ratio of 1:7. Values related to zinc content and binding capacity of various fiber sources are presented in Table 10. An NBS liver standard which was run to verify the precision of the method for mineral analysis agreed with the reported certified value. NBS reported a Zn value of 130 $\pm$ 13 ug/gm for the liver. We obtained an average value of 151 ug/gm, and with the variation existing in analysis, there was no difference between these values. Endogenous zinc content ranged from 10.8 to 155.7 ug per gram of dry fiber for oat hulls and wheat bran respectively. The zinc contents of acid washed fibers are presented in Table 10. We can see that acid washing stripped most of the zinc from the fibers. Table 5

Table 10. Zinc content and binding capacity for various fiber sources.[1]

Types of Fiber	Endogenous[2]	Acid washed[3]	Binding[4] capacity	Reacid[5] washed
	ug/gm			
Rice bran	110.7 ± 1.1	ND[6]	7820 ± 285	149.7
Wheat bran	155.7 ± 5.9	3.2 ± 1.46	6990 ± 9	212.6
Soy bran	61.7 ± 0.0	0.9 ± 0.18	6713 ± 221	77.5
Peanut fiber	53.4 ± 3.2	2.9 ± 0.39	6399 ± 390	140.4
Barley fiber	52.0 ± 3.4	1.8 ± 0.30	6105 ± 71	10.8
Tomato fiber	41.4 ± 1.3	2.8 ± 0.21	4108 ± 64	63.8
Apple fiber	16.8 ± 6.0	ND	2020 ± 163	87.0
Oat hull	10.8 ± 3.1	ND	1861 ± 155	19.7
NBS liver	151.0			

[1]ug zinc/gram dry sample
[2]Zinc content of original defatted sample
[3]Zinc content of acid washed sample
[4]Total zinc content of fiber bound zinc
[5]Zinc content of reacid washed fiber
[6]Not detected

presented the percentage of zinc extracted for various fibers under acidic pH. The level of Zn left in the residue after acid washing ranged from zero to 3.2 ug/gm. Rice, apple, and oat had released 100% (Table 5 and 10). When 3.2 ug/gm remained in acid washed wheat bran, the extraction was 98.6%. Tomato fiber with 2.8 ug/gm zinc left in residue after acid washing was 93.2% extracted, which was the lowest extraction rate. The Zn extraction ranged from 93.2 to 100% with a mean extraction of 98.1%. It can be observed that most of the zinc in fiber has the potential of being available under acidic condition. Probably there was a weak bond (electrostatic) between fiber and zinc, which makes it possible for zinc to be removed by a pH change. Lyon's study (1984) showed that more or less Zn was completely released by extraction with HCl(pH=1) in all the cereals he studied with the exception of muesli. If one considers the condition in the stomach, most of the zinc from the bran should be freed and available. Thompson and Weber (1979) in vitro studies found little of the mineral still bound to fiber at pH 0.65.

Eight fiber sources: rice, oat, soy, wheat, apples, peanuts, tomatoes, and barley were studied for their Zn binding capacity. Incubation of 5 gram of fiber with 35 mg zinc (35 ml of 1000 ppm Zn standard), see Materials and Methods, for 3 hours at pH 6.8, resulted in an increase of zinc content in the fiber residue. This zinc value was considered to be the fiber binding capacity (Table 10).

Binding capacities are also graphed in Fig 4. We see that rice bran bound the highest amount of zinc. Oat hulls and apple fiber had the lowest binding capacity. Five of the fibers were able to bind more than the three others; they were rice which bound 7.8 mg, then wheat, soy, peanuts, and barley. Tomato fiber bound 4.1 mg. Apple fiber and oat hull with the lowest binding capacity bound 2.0 and 1.8 mg per gram of fiber respectively. This raises the question as to why some the fibers bind differently than the others?

There are many factors which affect the binding capacity of fiber. These factors include the pH (Thompson and Weber, 1981; Camire and Clydesdale, 1981), concentration of a mineral, heat treatment (Clydesdale and Camire, 1983) and types of fibers. Probably the method used in binding is important, as well as the presence of other compound, e.g., protein, other minerals, and vitamins (Greger 1987). In this experiment we can see the effect of the different fiber types. Rice bran had bound almost four times more zinc than the oat and apple fiber. Why do they bind differently? What component of our fibers were involved in the binding? In order to be able to answer this question, fractionation of different fiber should be performed and binding should be determined on the various fractionated fibers. In Table 10 we observed that the binding was correlated with the endogenous zinc content. As endogenous zinc content decreased, the binding capacity also decreased. Whether this was a coincidence is not known.

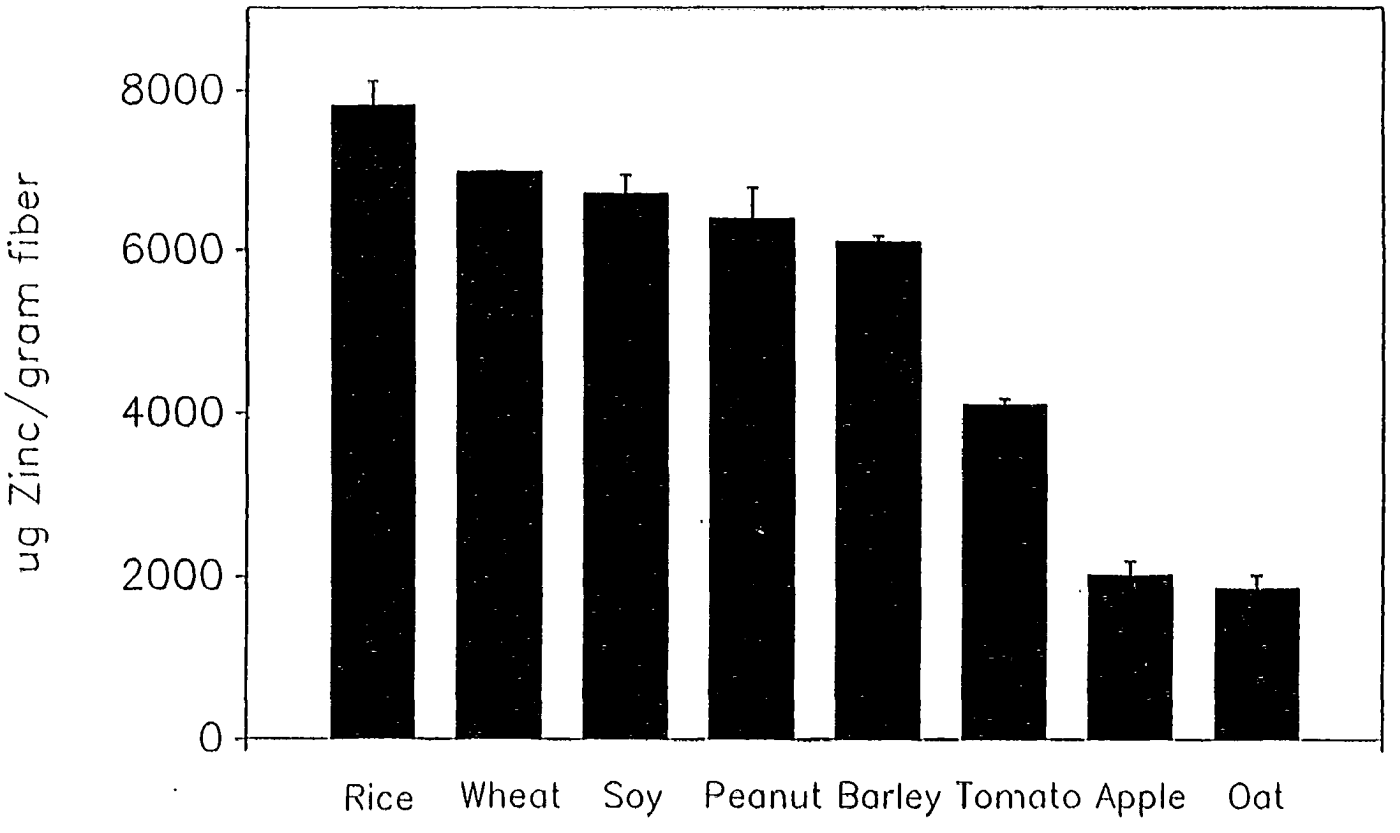

Fig 4. Fiber sources and Zinc binding capacity.

Statistical correlation was run to determine if there was a binding correlation between ADF, lignin, or protein values of the sample (Table 14). Pearson's correlation for zinc binding and protein concentration was high. The correlation was 0.65 with $p<0.005$. There are many other factors to be considered beside the protein binding effects. Comparing the individual binding values with protein concentration, we find a discrepancy in some cases. Therefore, protein levels can not be the only factor. Some of these variations are found in tomato with 21% protein level, but which only bound 4.1 ug/gm zinc. This was lower than the soy bran which had an 8% protein level, but bound 6.7 ug/gm zinc. However, we also see that rice bran with the highest protein (24%) bound the highest amount of zinc, and apple and oat with lowest protein levels, bound the lowest amount of zinc. Protein was found to be a significant factor when analyzing statistically but not the whole answer when comparing the individual binding and the protein data. The forms of protein, or reactive groups on the protein and/or amino acids, might be an additional factor in binding ability. Other types of binding compounds might also be a factor, but further work is needed to answer these questions. Pearson's correlation showed that Zn binding was negatively related to the ADF concentration. Correlation between lignin and zinc and its significance was very low.

The fibers which bound zinc were reacid washed again. This acid washing removed a large amount of the bound minerals

(Table 10), but the minerals were not removed to the low levels found after the first acid washing, or as low as the endogenous content. Zinc content in reacid washed fibers ranged from 10.8 to 212.6 ug/gm. It was possible that some type of strong complex formed which was not removable by acid washing.

MAGNESIUM BINDING CAPACITY

Values related to magnesium content and binding capacity are presented in Table 11. The endogenous magnesium content of fiber sources ranged from 566 to 13,736 ug per gram of fiber. Magnesium is one of the macrominerals, and as we see, most of fibers have high amounts of magnesium. Rice bran has an exceptionally high amount of magnesium, 13.7 mg. Wheat bran is the next highest with 8.0 mg. Apples, with lowest amount of magnesium, had 566 ug/gm. All these fibers after acid washing have lost a considerable amount of magnesium. The range of magnesium left in the acid washed sample was 1.6 to 49.6 ug/gm. Compared to the original concentration of magnesium which existed in the fibers, all the fibers have lost a large amount by acid washing. The percentage of extraction for magnesium ranged from 97.4 to 100. Rice bran had the highest amount of magnesium with 2.9 ug/gm remaining in the bran after acid washing . This resulted in an extraction rate of 100%. Barley had the highest amount of magnesium, 49.6 ug/gm, left after acid washing; the original magnesium level was 2010 ug/gm, and the extraction rate was

Table 11. Magnesium content and binding capacity of various fiber sources.[1]

Types of fiber	Endogenous[2]	Acid washed	Binding[3] capacity	Reacid washed
	ug/gm			
Peanut fiber	2324 ± 25	18.8 ± 8.7	4129 ± 15	29.9
Beet pulp	1530 ± 27	40.2 ± 6.7	3474 ± -	150.0
Soy bran	2554 ± 38	12.7 ± 2.7	3133 ± 300	11.0
Rice bran	13736 ± 159	2.9 ± 0.7	2918 ± 32	33.8
wheat bran	7995 ± 73	34.6 ± 26.6	2439 ± 58	36.7
Tomato fiber	3327 ± 38	19.1 ± 4.0	2091 ± 30	24.8
Barley fiber	2010 ± 18	49.6 ± 3.9	1427 ± 85	16.5
Apple fiber	566 ± 89	1.6 ± 0.1	1016 ± 163	6.8
Oat hull	820 ± 19	8.1 ± 4.5	525 ± 40	2.3
NBS liver	583			

[1]ug Magnesium/ gram fiber
[2]Magnesium content of original defatted fiber
[3]Total magnesium content of fiber bound

97.5%. Beet pulp had the lowest extraction rate, 97.4%. However, the Mg content of the acid washed sample ranged from as low as 1.6 to 49.6 ug/gm. Our poorest extraction rate was 97%, so magnesium extracted well. Lyon (1980) also showed that a large percentage of magnesium can be extracted from several cereals he studied. This shows that the magnesium is available from the bran under acidic condition. It also indicates that the binding was so weak that it makes the magnesium removable and therefore available.

Our NBS liver standard determination for magnesium was satisfactory. We obtained a value of 583 which is in agreement with the reported certified value of 604 $\pm$ 9. The magnesium binding study of various fibers demonstrated an interesting result which is not explainable. In five of the fibers we were not able to rebind as much magnesium as we originally removed, while four fiber samples bound more magnesium. The range of binding capacity for magnesium was 525 to 4129 ug/gm (Table 11, Fig.5). Peanut fiber had the highest ability for magnesium binding, 4.1 mg. The next highest binding fiber for magnesium was beet pulp. Rice, wheat, tomato, barley and oat were not able to bind magnesium at levels as high as endogenous materials. Rice and wheat had exceptionally low binding ability compared to the high amount of the endogenous magnesium they had. It is possible that by acid washing we altered the binding site. The binding capacity of rice bran was studied using the same fiber and mineral ratio of 1:7, but

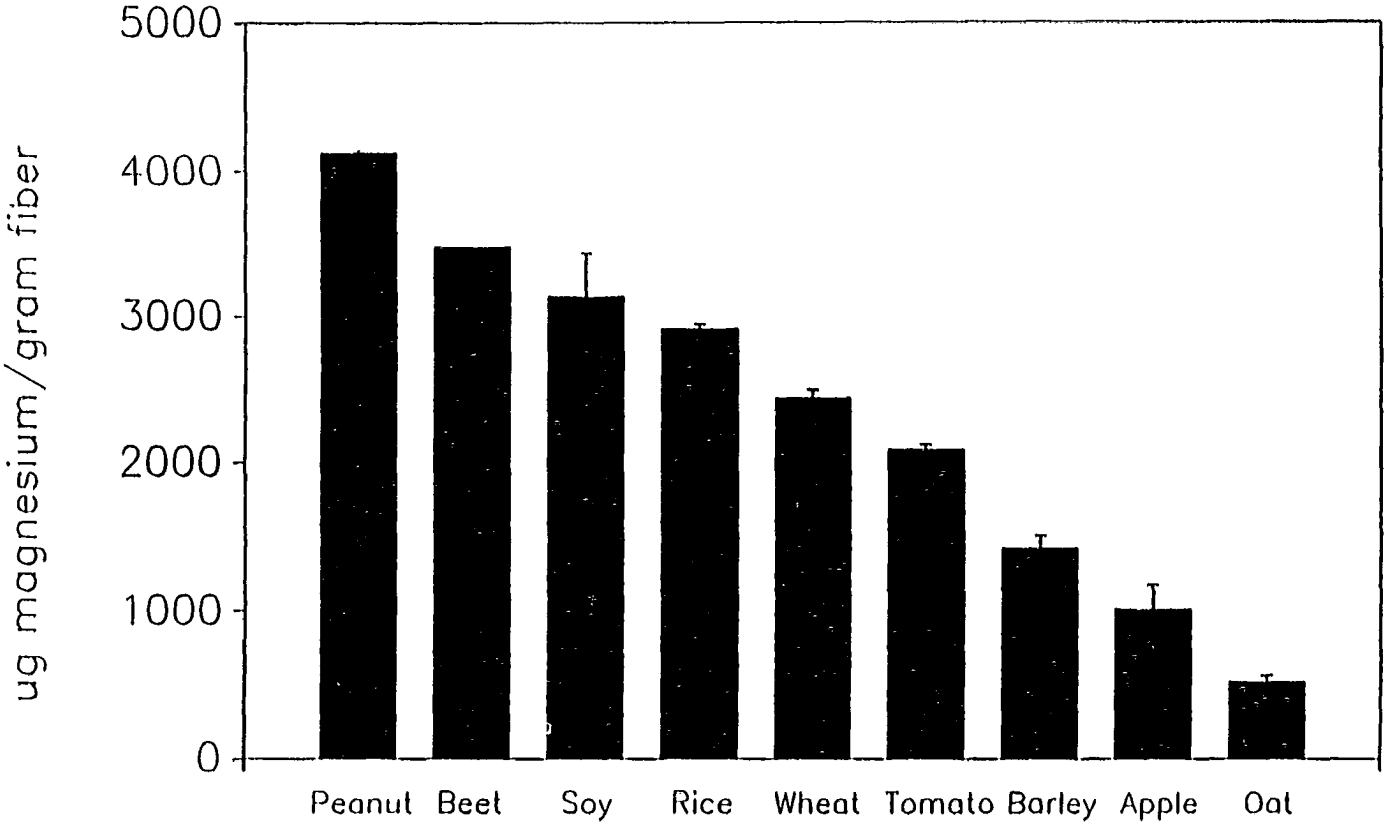

Fig 5. Fiber sources and magnesium binding capacity.

at different times of incubation. This was done to recheck if longer periods of time might have affected the total binding, since the binding at the usual time interval of 3 hours gave us a lower binding than the endogenous. The results of the study are presented in Table 12. Analysis of variance showed there was no significant difference for binding at different times. The rice, acid washed fiber, bound much less magnesium than endogenous content. Probably a higher mineral concentration was needed to saturate the binding site. As we see, magnesium binding capacities of several fibers are even lower than the endogenous content, which probably means the determinal effect of magnesium bioavailability is less likely to happen. Ward and Reichert (1986) reported canola cell wall had a higher binding affinity for Cu, Fe, Zn, and Mn and less affinity for magnesium. All the other fibers they studied had lower affinity for magnesium. They had studied the binding using an untreated sample. It seems that most fibers do not have much of a binding capacity for Mg, when compared to the endogenous level. Lee and Garcia-Lopez reported (1985) that NDF and ADF from cooked pinto beans did not bind magnesium.

Reacid washing removed the magnesium far below the endogenous content, and for the most part, magnesium was removed to the level of initial acid washed fiber. The only exceptions were in case of beet pulp and rice (Table 11). The range of Mg in the reacid washed fiber was as low as 2.3 ug/gm for oat hull and as high as 150 ug/gm for beet pulp. Not

Table 12. Time study of acid washed rice bran for magnesium binding.[1]

Time (hrs)	Fiber (gram)	Mg added to make slurry (mg)	Binding capacity ug/gm
3	5	35	3247 ± 152[a]
6	5	35	3054 ± 161[a]
9	5	35	3272 ± 239[a]

[1]Values presented as Mean ± SD

[a]Similar superscript means the means are not significantly different at $p<0.05$

being able to remove the bound magnesium in case of beet pulp could be a technique problem. Since beet pulp has a high water absorption, more acid and/or water probably should have been added to it. Also, when centrifuging the slurry in case of beet pulp, it was harder to obtain a compact pellet. This probably was due to the structure, and probably some of the unbound magnesium was left in the residue.

COPPER BINDING CAPACITY

Copper is one of the essential trace elements, and we have studied its binding capacity. The endogenous copper content of fiber ranged from below detection limit to 67.4 ug/gm (Table 13). Apple and cellulose copper content was so low that it could not be detected by our method. Peanut fiber had the highest copper content at 67.4 ug/gm. Wheat and rice bran were the next highest copper containing fibers. Barley, soy, tomato, beet pulp, and oat hull contained little copper. Acid washing extracted the copper to a very low detectable level. Beet pulp was extracted to a level at which no detectable copper remained in the residue; it was 100% extracted. Barley, soy, rice, tomato and oat hull were extracted to a level having about 1 ug/gm copper left. Rice and soy with higher endogenous copper had an extraction rate of 84% and 85%, respectively. Oat hull which already had a low level of endogenous Cu, 1.6 ug/gm, had an extraction rate of about 4%. Peanut fiber had the highest amount of copper left in residue after acid washing, but because it had a high endogenous

copper, the extraction was 85.5%. Lyon (1984) also did not observe a good copper extraction rate for the cereals, although he used a pH of 1. Reilly (1979) studied the extraction of wholemeal bread and wheat bran at pH 4.3 and 7.6 for zinc, iron and copper. He found that a small amount of mineral was released at pH 7.6. At pH 4.3 zinc was much more soluble than the copper and iron, with some 80% being dissolved compared with 30-40% of the iron and copper.

The copper value determined for NBS liver was 207 ug/gm. The certified value was 193 $\pm$ 10 ug/gm, and with the variability in analysis the two numbers are in agreement. Copper binding to various acid washed bran was studied. Many researchers have reported higher binding capacity at higher pH. At this pH more binding site (coo-) are probably available for binding. One of the problems involved in high pH study is the solubility behavior of the mineral, keeping the Cu in solution at high pH and avoiding hydroxide formation. This solubility problem at higher pHs was encountered in this work. At pH 6.8, at which other mineral binding was studied, we were observing a pronounced precipitate in case of copper. One researcher has suggested that the use of bicarbonate for adjustment of pH does solve the solubility problem, but this was not true in our work. The formation of hydroxide at different pHs with addition of NaOH was studied, and as a result, we decided to drop the pH for copper binding to 5.0. Therefore, 5 grams of acid washed fiber was incubated with 35

ml of 1000 ug/gm of Cu and pH adjusted to 5. After centrifugation and several washings, the residue was analyzed for copper which represented the binding capacity. The values for copper binding capacity appear in Table 13. The binding capacity data are graphed in Fig 6. Barley fiber had the highest binding capacity, 8.0 mg. Since all the fibers were studied at the same mineral and fiber ratio, the same pH and treated the same way, their binding capacities can be compared. The binding capacities of wheat, peanut, soy, and rice were not significantly different. Rice and tomato were not significantly different with binding values of 6.9 and 6.7 mg per gram of fiber, respectively. Beet pulp and apple binding were lower than the first six fibers at 5.5 and 5.1 mg/gm respectively. Analysis of variance showed that apple and beet binding values were not significantly different. Oat hull had the lowest binding capacity compared to all of the fibers studied. It bound 3.8 mg Cu per gram of fiber. Rockway (1985) studied the binding capacity of wheat bran and oat hull. She found that oat had a lower binding capacity than wheat, and had a maximum binding of 3.0 mg under the conditions she used. Cellulose has shown some binding capability, probably due to some site on it which makes the binding possible. Why some of the fibers bind more than others needs further study. Fibers clearly show selective binding. What components are really involved in the binding? Pearson's correlation showed the copper binding and protein were correlated with significance

Table 13. Copper content and binding capacity of various fiber sources.[1]

Types of fiber	Endogenous[2]	Acid washed	Binding[3] capacity	Reacid[4] washed
	ug/gm			
Barley fiber	2.9 ± 0.5	1.9 ± 0.3	7976 ± 232	47.5
Wheat bran	16.3 ± 0.6	6.3 ± 5.0	7134 ± 12	48.9
Peanut fiber	67.4 ± 3.7	9.8 ± 3.8	7116 ± 161	141.5
Soy bran	6.8 ± 1.3	1.0 ± 0.7	6915 ± 145	105.7
Rice bran	10.2 ± 0.2	1.6 ± 0.5	6855 ± 10	79.2
Tomato fiber	2.0 ± 0.2	1.4 ± 0.2	6680 ± 13	60.0
Beet pulp	5.2 ± 0.2	ND	5475 ± 43	702.2
Apple fiber	ND[5]	0.8 ± 0.5	5112 ± 387	73.9
Oat hull	1.6 ± 0.0	1.5 ± 1.3	3786 ± 213	12.4
Cellulose	ND	ND	1026 ± 4	5.0
NBS liver	207			

[1]ug copper/gram fiber. Values on dry weight basis
[2]Copper content of original defatted fiber
[3]Total copper content of fiber bound
[4]Copper content of reacid washed fiber
[5]ND=not detected

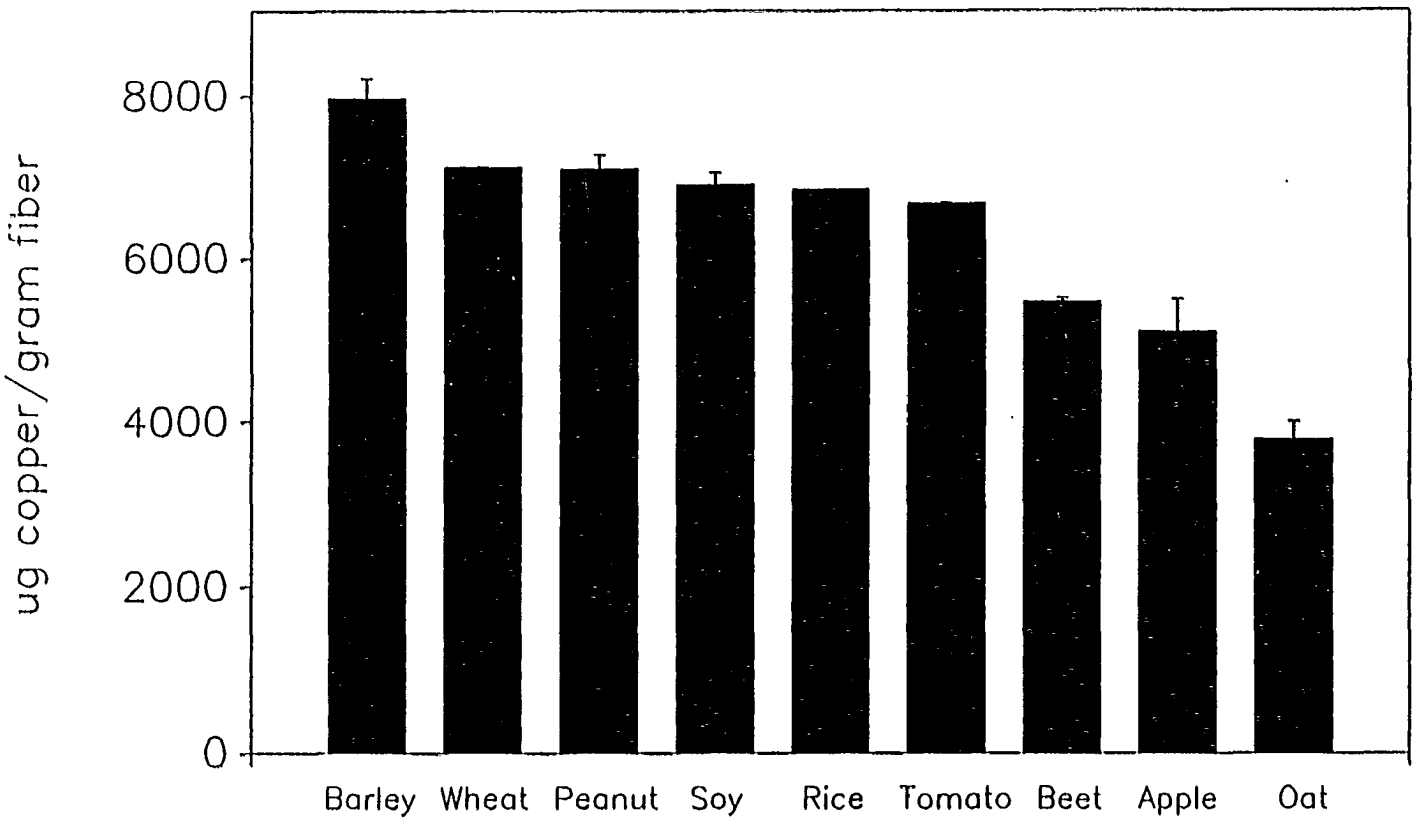

Fig 6. Fiber sources and copper binding capacity.

of $p<0.01$. The correlation for ADF and lignin were poor (Table 14).

The fiber bound copper were subjected to acid washing to determine how much of the copper could be removed, and/or the strength of the binding. The range of copper left in residue after acid washing was 5.0 to 702.2 ug/gm (Table 13). The large amount of Cu left in the residue after acid washing, especially in case of beet pulp, could be a problem in technique (see Mg binding), or formation of some complexes which can't be removed by acid washing.

Binding capacity of fibers for magnesium, zinc, and copper are presented in Fig.7. Most of the fiber had lower ability to bind magnesium compared to zinc and copper. The order of binding was Cu > Zn > Mg , with the exception of rice which bound more zinc than copper. Ward and Reichert (1986) have reported lower binding affinity of Mg compared to other minerals. Platt and Clydesdale (1987) have reported that lignin binds more Cu than Zn. We had higher Cu binding too, but our binding values were not correlated with the lignin content of the fibers. Further study needs to be done on fractionated fiber to determine the component involved in the binding. We have found that apple and oat had the lowest binding capacity for the minerals studied. Also, an additional calcium binding study was run using rice, oat, soy, and wheat. Oat was the fiber with the lowest binding ability. It would be informative if in vivo studies were run using these two

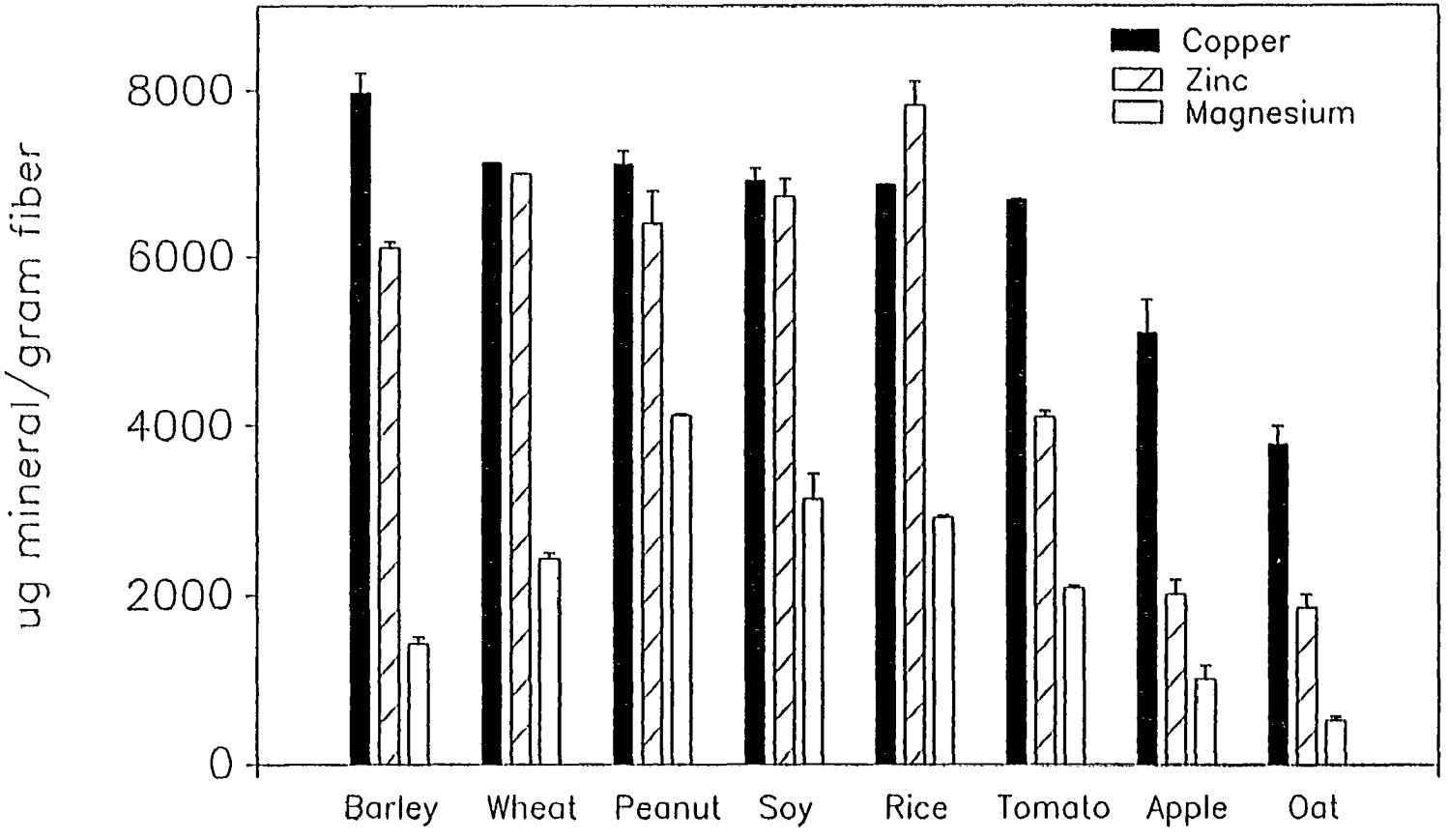

Fig 7. Fiber sources and Cu, Zn, and Mg binding capacity.

Table 14. Pearson's correlation coefficient for mineral binding capacity and fiber content.

	Mg	Cu	Zn
Protein	0.55 (0.02)[a]	0.59 (0.01)	0.65 (0.005)
ADF[b]	-0.07 (0.77)	-0.29 (0.24)	-0.58 (0.02)
Lignin	0.67 (0.79)	0.04 (0.87)	-0.30 (0.26)

[a]The first value is the correlation and the value in the parentheses is the significance of the correlation.

[b]ADF=Acid detergent fiber

types of fiber with low binding ability and using one of the fibers which had the highest binding capacity, to determine whether apple and oat have less effect on bioavability or absorption of mineral than the other fiber studied. Then we could confirm that the oat and apple fiber should be recommended fiber sources for inclusion in food products.

CHAPTER 5

SUMMARY

The importance of a good sampling system was seen in the first part of the experiment. It was demonstrated that different particle sizes of wheat bran, oat hull, soy bran, and rice bran had different compositional value. The general trend observed was that finer fractions have higher protein than coarse fractions. However, there is a decrease in ADF value as the fines increase, with the exception of soy bran. This difference could be due to degree of hardness of the fiber sample. The protein part of bran is probably softer and grinds to a finer consistency, while the fiber was harder than protein. Therefore, it was more difficult to grind and as a result it stays behind in the coarse portion while protein goes through.

Acid washing had considerable effect on the fiber sample. Most of the mineral was extracted from the fiber sources. Consequently, all the fibers had reduction in ash values. Protein value of most of the fibers studied was decreased with acid washing, which means they lost nitrogenous compound (protein content). Rice, wheat, and peanut were exceptions in having increased protein values, which was due to concentrating the protein. This was probably caused by the loss of the other soluble component.

Binding capacity of different fiber sources showed that they have different binding ability. Increasing the mineral

concentration added to the slurry caused a significant increase in the amount of the mineral bound. Rice had the highest zinc binding capacity, and it also had the highest protein content. Barley, wheat, peanut, soy, tomato, apple and oat bound more copper than zinc and magnesium. Binding capacity for magnesium was much lower than copper and zinc. This could be due to electronic configuration of Mg, Cu, and Zn. Zinc and copper are in the transition group of periodic table, and they need less energy to complete their outer shell. However, more energy is required for magnesium to complete its outer electronic shell, and probably that is why Mg binds less. In addition the hydration shell around Mg makes it less susceptible to interaction. According to all the fiber studies, apple and oat have lower binding than all the other fibers. This could be due to composition and their components. Further work is needed to determine the components which are involved in the binding. There are different methods that each investigator has used to measure the ability of fiber to bind mineral in vitro. Still, there is no common method which can be shared between the labs. One of the problems of binding at higher pH is the solubility problem of the mineral. In some of the methods, investigators measured the supernatant for mineral binding and considered the difference as the bound value. This overestimated the binding. In addition, measuring the residue overestimated the mineral binding since any precipitate which was formed might have been analyzed as the

bound value. In addition having a good control was very important.

APPENDIX A

DRY ASHING PROCEDURE

1. Place 250 ml acid washed vycor beakers in 100°c oven for a few hours.
2. Then place the beakers directly into a desiccator using crucible tongue.
3. Cool and weigh the beakers.
4. Weigh about 1 gram of sample (or appropriate amount) directly into the beaker.
5. Blanks are run throughout the experiment (this is a vycor beaker without any sample).
6. To dry the sample, place the beakers into 100°c oven for 2 hours.
7. After 2 hours, place them in desiccator, let it cool, and weigh.
8. Place the beaker in a muffle furnace, ash them overnight at 450°c.
9. Turn off the furnace, if possible let it cool down a little bit before opening the furnace door slightly, let the furnace temperature drop to at least 250°c.
10. Place the beakers directly into desiccator, let them cool, and weigh.
11. Add 5 ml concentrated HNO_3 to each beaker.
12. Place the beakers on a hot plate under the hood, and drive off the acid.

13. Ash again, as above, repeating 8,9, and 10.
14. Place the beakers on a tray, cover them with a paper towel to eliminate sample contamination from the dust.
15. Pipet 25 ml (or 10 ml) 5% HCl into each beaker.
16. Let them stand for a while.
17. Break up ash with acid washed glass rods, swirl it around well to dissolve the ash. Do the same with each beaker.
18. Transfer most of the liquid into a 30 ml acid washed polyethylene bottle, using a disposable pipet. Try not to transfer any of the insoluble ash which has settled.
19. The appropriate dilution can be made from the above stock solution for mineral analysis to be read by atomic absorption spectrophotometer.

    All dilution for samples and standard are made with 5% HCl. Appropriate blank dilution is made too.

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