EVALUATION OF SEVERAL SOURCES OF DIETARY FIBER FOR USE IN FOOD PRODUCTS

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CERTIFICATION OF THESIS WORK

We, the undersigned, certify that Ann-Marie John Cadden, B.S.H.Ec., M.Sc.
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ABSTRACT

Properties of several fibrous byproducts from sunflowers, psyllium, flax, mustard, wheat and field peas were evaluated as potential fiber supplements for use in food products. The effect of particle size was examined by subdividing the sunflower hulls into coarse and fine fractions that differed primarily in particle size, and the wheat bran into coarse (bran) and fine (shorts) fractions that differed in composition as well. Each fiber supplement was characterized by its color, pH, relative density, relative shape and distribution of particle size.

Fiber from cellulose (99.5% neutral detergent fiber [NDF]), pea hulls (45.5% NDF) and sunflower heads (36.2% NDF) contained a high percentage of cellulose, whereas fiber from the wheat bran fractions (51.7 and 36.4% NDF, respectively) contained a high percentage of hemicellulose. Fiber from flax hulls (30.6% NDF) and the sunflower hull fractions (97.0 and 98.0% NDF, respectively) contained high levels of lignin. Psyllium seeds (59.3% NDF) and mustard hulls (55.9% NDF) had high levels of both hemicellulose and cellulose. Sunflower heads contained 15-20% pectic substances. Psyllium seeds, flax hulls and mustard hulls contained 50, 21 and 12% mucilage, respectively. Approximately 30-50% of the mucilage was solubilized during the NDF analysis and 50-70% was measured as hemicellulose.

Sunflower heads, psyllium seeds, flax hulls and mustard hulls had the highest water absorption capacity, whereas cellulose had the lowest. The coarse fractions absorbed more water than the fine fractions. Sunflower heads and sunflower hulls, wheat bran and cellulose absorbed twice as much fat as psyllium seeds, mustard hulls and pea hulls.

Each fiber supplement was fed *ad libitum* to male weanling Sprague-Dawley rats for three weeks. Diets contained 10% NDF, 16% protein and 8% fat. The flax hull diet depressed growth whereas, the sunflower hull diet depressed appetite. Sunflower heads, psyllium seeds, flax hulls and mustard hulls were the most effective bulking agents in the gastrointestinal tract, and were less digestible than wheat bran, sunflower hulls, pea hulls and cellulose. Flax hulls had the greatest effect on increasing stool weight and volume, and on decreasing apparent digestibility. Sunflower hulls effectively increased stool volume, but not stool dry weight. Sunflower heads and flax hulls lowered serum triglyceride. Differences in serum cholesterol were not significant.

Fiber-supplemented breads were prepared using the sponge-dough procedure with each fiber supplement replacing 7.5% of the wheat flour. Cellulose bread resembled the wheat bread control. The quality of the pea hull and wheat bran breads was similar to the whole wheat bread, with the pea hull bread having a light crumb color. Flax hulls and sunflower hulls weakened the bread dough. Flax hull breads were small in volume and had a dark, red crumb. The coarse sunflower hulls imparted a gritty mouthfeel and a coarse crumb grain. The fine sunflower hulls also imparted a dark color to the crumb, although the fine particles interfered less with the bread structure than the coarse particles.

Fiber supplements were incorporated into bread and fed *ad libitum* to male weanling Sprague-Dawley rats for three weeks. Diets were formulated to contain 5% NDF, 16% protein and 8% fat. However, the bread diets were found to contain more lignin and less hemicellulose, cellulose and NDF than the raw ingredients. Rats fed the fiber breads had similar growth, feed

consumption, digestibility, serum cholesterol and serum triglycerides as rats fed whole wheat bread. The sunflower hull breads, especially bread containing the coarse fraction, were more effective in increasing stool volume than whole wheat bread. The cellulose bread was less effective. Stool volumes of rats fed flax hulls, wheat bran and pea hulls were similar to those of rats fed whole wheat bread.

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
df	degrees of freedom
HCL	hemicellulose : cellulose : lignin
LSD	least significant difference
MLPS	mean log particle size
ms	mean square
MWHC	mean water holding capacity
NDF	neutral detergent fiber
PER	protein efficiency ratio
RD	relative density determined by weighing sample contained in a known volume
RD_{mod}	relative density determined by using sedimentation cylinders to ensure even filling
SD	standard deviation
WHC	water hydration capacity

1. INTRODUCTION

Attention was directed towards the importance of dietary fiber when epidemiological observations were reported linking the lack of plant fiber with the prevalence of certain degenerative diseases in industrial societies. As a result, consumers are now demanding more fiber-enriched foods.

Certain crops grown in Western Canada have a large percentage of hulls or other plant material that is rich in fiber and may be suitable as sources for fiber supplementation in food products. Often this fiber-rich material is considered agricultural waste and discarded. The primary objective of this investigation was to identify sources of plant fiber suitable for further development as food products. Fiber supplements containing hulls from flax, mustard, sunflower and peas along with sunflower heads and wheat bran were prepared by simple laboratory methods. Microcrystalline cellulose was included as an example of a purified fiber source and psyllium seeds were added as an example of a mucilage-rich fiber source. Chemical composition, physical properties and physiological effects were considered for all fiber supplements. The next phase of the project was to determine the breadmaking characteristics of each fiber supplement and the effect of the breadmaking process on the fiber. Psyllium seeds, sunflower heads and mustard hulls were considered unacceptable in bread and deleted from the final investigations. Secondary objectives included examining the effect of particle size on chemical composition, physical characteristics, physiological effects and breadmaking characteristics. It was hoped that the results of this investigation would lead to the development of economically feasible methods of isolating dietary fiber from various plant sources and profitable utilization of the resulting fiber supplements.

2. LITERATURE REVIEW

2.1 DEFINITION OF DIETARY FIBER

Dietary fiber has been defined as the portion of plant material taken in our diet that is resistant to digestion by endogenous secretions of the human gastrointestinal tract (Trowell, 1972; Trowell *et al.*, 1976). Dietary fiber is therefore made up from the structural polysaccharides of the plant cell wall, other polysaccharides with related chemical structures and lignin, a noncarbohydrate (Southgate *et al.*, 1976). Major components of dietary fiber include hemicellulose, cellulose and lignin. Indigestible polysaccharides such as mucilages, pectins and gums are generally included in this definition (Trowell *et al.*, 1976) because of their close association with plant cell wall components (Table 2.1).

Table 2.1 Components of dietary fiber (Southgate, 1976)

Principal sources in the diet	Description	Classical nomenclature
Structural materials of the plant cell wall	Structural polysaccharides	Pectic substances Hemicellulose Cellulose
	Noncarbohydrate constituents	Lignin Minor components
Nonstructural materials either found naturally or used as food additives	Polysaccharides from a variety of sources	Pectic substances Gums Mucilages Algal polysaccharides Chemically modified polysaccharides

Certain important facts emerge from this general definition of dietary fiber as the plant polysaccharides and lignin resistant to hydrolysis by human digestive enzymes (Southgate *et al.*, 1976). First, dietary fiber is not a chemical entity, but a mixture of several different types of polysaccharides and lignin, combined for the most part in a complex physical structure, the plant cell wall (Southgate *et al.*, 1976). The composition and the physical properties of dietary fiber will vary with the foods present in the diet and within the same plant depending on its state of maturity and the conditions under which it was grown. Second, dietary fiber is a concept which relates to the mixture of indigestible dietary components that are present in the contents of the gastrointestinal tract at the end of the small intestine (Southgate *et al.*, 1976). In the large intestine, many of these polysaccharides are degraded and metabolized by the intestinal microflora. This degradation and the products produced may be an important part of the physiological role of dietary fiber. Therefore, dietary fiber should not be considered solely as the components of the diet that escape digestion and can be recovered in the feces.

2.2 ALTERNATIVE NOMENCLATURE FOR DIETARY FIBER

Some investigators are strongly opposed to the word "fiber" in "dietary fiber" since many components are not fibrous in nature and the term "fiber" is used in many other fields (Spiller and Shipley, 1977). Spiller *et al.* (1976) have suggested that the term "plantix" replace "fiber." "Plantix" was derived from the words "plant" and "matrix" because undigested "plant" materials form a "matrix" in the human gastrointestinal tract. "Dietary fiber," "purified plant fiber" and "plantix" all refer to

the sum of cellulose, hemicellulose, pectins, lignin, gums and mucilages (Table 2.2). The term "complantix" from "complex plantix" was used by Spiller *et al.* (1976) to describe the sum of "plantix" plus "associated indigestible plant cell wall factors" (Table 2.2).

2.3 Components of Dietary Fiber

Components of dietary fiber include cellulose, hemicellulose, pectin, gums, mucilages and lignin. The following discussion is limited to the structure of each component.

2.3.1 Cellulose

Cellulose is the major structural polysaccharide in the plant wall and the only truly fibrous component of dietary fiber (Cummings, 1976; Hodge and Osman, 1976; Southgate, 1976). Cellulose is found embedded in an amorphous gel composed largely of hemicellulose and pectic substances, together with a small amount of protein. In woody tissues, cellulose molecules are associated into partially crystalline microfibrils; however, the cellulose of vegetable pulp has little fibrous character. Microcrystalline cellulose differs from native cellulose in that the material in amorphous regions is hydrolyzed, leaving the crystalline areas intact in the form of tiny rodlike microcrystals.

Cellulose is a linear polymer of anhydro-D-glucopyranose units linked by β-(1→4) glucosidic bonds (Hodge and Osman, 1976). The degree of polymerization of native cellulose is believed to be larger than 10,000 or 15,000 since extraction procedures used to purify the cellulose are likely to hydrolyze the large molecules (Cummings, 1976; Southgate, 1976). Cellulose forms a crystalline structure in most plant cell walls (Cummings,

Table 2.2 Dietary fiber nomenclature[1]

Term	Reference	Substance
A. Nonnutritive natural fiber Nonnutritive plant fiber	Kritchevsky and Story, 1974 Spiller and Amen, 1975a	High fiber foodstuff (eg wheat bran) = Sum of B + E
B. Plant fiber Complantix Dietary fiber complex	Spiller and Amen, 1975a Spiller *et al.*, 1976 Trowell *et al.*, 1976	Components not digested before reaching the ileocecal valve; possibly digested by colonic microflora = Sum of C + D
C. Purified plant fiber Plantix Dietary fiber	Spiller and Amen, 1975a Spiller *et al.*, 1976 Trowell *et al.*, 1976	Sum of: cellulose + hemicellulose + lignin + pectin + gums + mucilages
D. Associated plant cell wall factors	Spiller *et al.*, 1976	Sum of: waxes + cutins + undigestible cell wall-bound proteins + undigestible cell wall-bound minerals + other cell wall-bound substances
E. Digestible components	Spiller *et al.*, 1976	Protein, fats and other lipids, carbohydrates, vitamins, minerals and water that are digested as such, or hydrolyzed by human digestive enzymes and then absorbed.

[1] Adapted from Spiller *et al.* (1976) and Spiller and Shipley (1977)

1976). The whole molecule folds into a flat, ribbon-like structure, which X-ray diffraction studies have shown to have a helical configuration. The structure is stabilized by extensive hydrogen bonding.

The linear, unbranched cellulose polymers pack together closely in a three-dimensional latticework and form microfibrils of cellulose (Cummings, 1976). These microfibrils form the basis of cellulose fibers, which are woven into the plant cell wall. Cellulose fibers are very strong, due, in part, to the length of the chains involved, and, in part, to the hydrogen-bonding capacity of the three hydroxyl groups.

2.3.2 Noncellulosic polysaccharides

Noncellulosic polysaccharides include all matrix polysaccharides from the cell wall together with all other indigestible polysaccharides other than cellulose (Southgate, 1976). This classification includes hemicellulose, pectic substances, gums and mucilage. According to Southgate (1976), the traditional division between hemicelluloses and pectic substances is an artifact of early extraction procedures and many structural features are shared by traditionally isolated hemicelluloses, pectic substances, gums and mucilages.

The term hemicellulose was used originally in 1891 by Schulze to describe the polysaccharides that could be extracted from the plant cell wall with dilute alkali (Cummings, 1976). The hemicelluloses seem to comprise a complex series of heteroglycans based on three types of homo-polymeric backbone chain, xylans, mannans and galactans and one type of mixed chains, the glucomannan (Southgate, 1976). These main backbones

carry side chains containing arabinose, galactose and 4-*O*-methylglucuronic acid. Less frequently, the side chains contain both xylose and arabinose. The hemicellulose molecule generally includes 50-200 sugar units and tends to be amorphous rather than crystalline (Cummings, 1976). Together with pectin, the hemicelluloses form the matrix of the plant cell wall.

Pectic substances are a group of complex polysaccharides that are found in the middle lamellae, primary cell walls and intercellular material of most plants (Southgate, 1976). Although pectic substances are characteristically soluble in hot water, those that make up an integral part of the wall are less readily soluble and appear to be present as calcium salts. The principal component is D-galacturonic acid. However, the sugars, D-galactose, L-arabinose, L-rhamnose and L-fucose are usually found while 2-*O*-methyl-D-xylose and 2-*O*-methylfucose are often present.

Plant gums are sticky exudates formed at the site of injury to plants, which dry to produce hard protective nodules (Cummings, 1976). Biochemically, the plant gums present a complex group of highly branched uronic acid-containing polymers, mainly of glucuronic and galacturonic acids, with neutral sugars such as xylose, arabinose and mannose. A high proportion of calcium and magnesium salts is formed and a significant number of the residues is acetylated.

Mucilages are associated with the endosperm or storage polysaccharides of plant seeds (Cummings, 1976). Their role is to retain water and so protect the seed against desiccation. Structurally, they resemble the hemicelluloses but are not classed biochemically with them because of their occurrence in a distinct part of the plant.

2.3.3 Lignin

Lignin adds strength and support to the cell wall by permeating the other constituents (Cummings, 1976). Lignin is very resistant to chemical degradation and appears to be more resistant to enzymatic digestion than any other naturally occurring polymer (Southgate, 1976).

Unlike other cell-wall structures, lignin is not a carbohydrate and it is a small polymer having a molecular weight of between 1000 and 4500 (Cummings, 1976). The basic units of the complex aromatic polymer, 4-hydroxyphenylpropane, 4-OH-3-methoxyphenylpropane and 3,5-dimethyl-4-OH-phenylpropane, are joined by carbon-to-carbon bonds. The structure of lignin is not known at this time, but a random type of polymer is expected (Southgate, 1976).

2.4 ANALYSIS OF FIBER

It was known from earliest times that some portion of plant materials was indigestible and had definite effects on bowel function, but no attempts were made to define this component until the early nineteenth century (Cummings, 1976).

2.4.1 Crude fiber

Einhof developed the original method for measuring indigestible matter in 1806 (Cummings, 1976; Van Soest and Robertson, 1977). However, his method involved maceration and hot water extraction. The values obtained correspond more to modern cell wall values by the neutral detergent fiber procedure than to present day crude fiber values.

Early chemists thought that the fiber that resisted retting and alkaline treatment was indigestible (Van Soest and Robertson, 1977).

Alkali extractions gradually replaced retting and acid extraction was introduced to further purify the noncarbohydrate components. Eventually, the crude fiber method was standardized to essentially its present day form (AOAC, 1975) and the term "Weende procedure" was applied to the method after a town in Germany where research in this field was been undertaken (Cummings, 1976).

Crude fiber represents the residue remaining after sequential extraction with solvent, dilute aqueous acid and dilute aqueous alkali (Spiller and Amen, 1975b). Fat is removed by the solvent (Figure 2.1). Acid extraction hydrolyzes carbohydrates and protein (Triebold and Aurand, 1963). Extraction with alkali causes saponification of the fatty acids not extracted by ether. Both treatments solubilize most of the mineral matter. The dry residue consisting mainly of fiber along with a small amount of mineral matter is weighed, ignited and reweighed. Crude fiber is represented by the weight difference between the dried and ignited sample.

Despite its established place in the history of animal nutrition, deficiencies in the crude fiber method have been recognized for over one hundred years (Cummings, 1976). Crude fiber has been criticized for both technical and nutritional reasons. First, crude fiber is a highly empirical analysis. Reproducibility is only satisfactory if the defined procedure is followed precisely. Second, crude fiber is not a chemically defined fraction. The composition of crude fiber varies from food to food, due, in part, to the partial solubilization of hemicellulose and lignin at acid and alkaline pH (Van Soest and Robertson, 1977). Cellulose is less soluble than hemicellulose and lignin in the acid and alkali used to prepare crude fiber. Lignin from monocotyledonous plants is more soluble in alkali than that of dicotyledonous plants, causing the loss of lignin from crude fiber to be

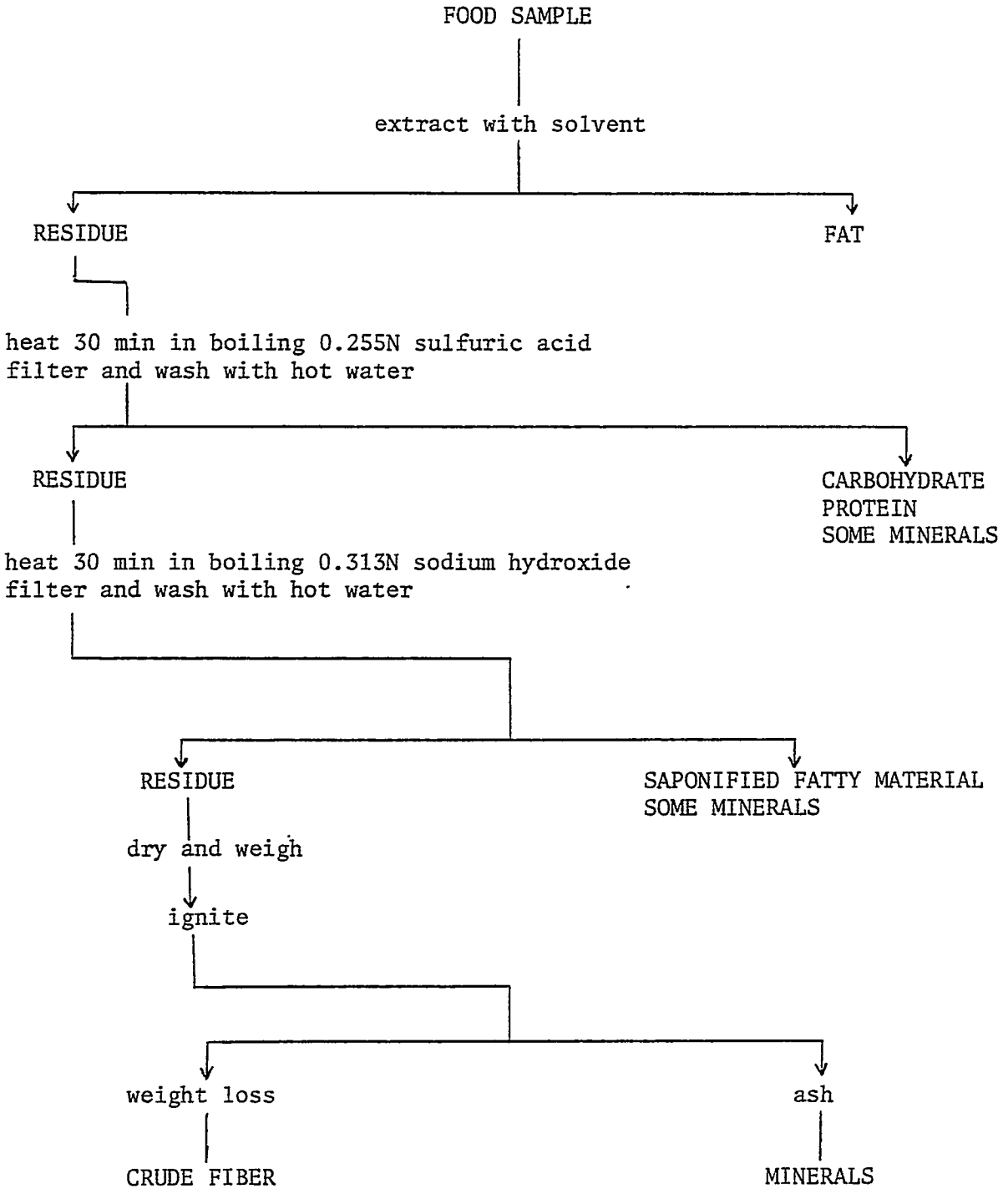


Figure 2.1 The crude fiber procedure

variable according to the plant species. More precise methods for measuring the components of the plant cell wall were developed, and the crude fiber method is now known to recover approximately 20% of the hemicellulose, 50-80% of the cellulose and 10-50% of the lignin (Spiller and Amen, 1975b). Pectins are totally lost in the crude fiber determination. The most severe limitation of the crude fiber method is that the procedure measures a variable proportion of plant cell wall constituents (Cummings, 1976). Since 80% of the hemicellulose is lost during analysis, the proportion of total cell wall constituents that are recovered depends to a large extent on the amount of hemicellulose in the cell wall. Cereals and other monocots that contain fibers high in hemicellulose and which are moderately high in lignin have a low recovery of cell wall constituents (Van Soest and Robertson, 1977). Legumes that contain high levels of lignin and low levels of hemicellulose are intermediate relative to crude fiber recovery. Highest recoveries are in the dicotyledonous non-legume plants where cell walls contain a high proportion of cellulose. The range of plant cell wall values relative to crude fiber values is from near equivalence to over five times greater.

2.4.2 Alternative methods of fiber analysis

Because of the many defects in the crude fiber procedure, alternative methods based on biochemical analysis, enzymatic digestion and chemical extraction with detergents have been developed to estimate dietary fiber (Schaller, 1978; Southgate *et al.*, 1976, 1978). According to Southgate *et al.* (1978), the ideal procedure must measure all components of dietary fiber, must provide separate values for the major components and should provide some information on the composition of the noncellulosic

polysaccharides present in the food or diet.

Any analytical procedure for the measurement of total dietary fiber must represent the compromise between a complete fractionation and measurement of all fiber constituents present, and a simplified system involving grouping of the different components in some arbitrary and often empirical way (Southgate, 1976).

Southgate (1969) measured the components of dietary fiber or "unavailable carbohydrates" by hydrolyzing the carbohydrates in each subgroup and measuring the resulting levels of hexoses, pentoses and uronic acids (Figure 2.2). Sugars were removed from the foodstuff with 85% aqueous methanol, and fat was extracted from the alcohol-insoluble residue with diethyl ether. Starch was measured as glucose after an enzyme hydrolysis. Water-soluble noncellulosic polysaccharides were extracted from the residue with hot water and precipitated with four volumes of ethanol. Both precipitate and residue were hydrolyzed separately with 1N sulfuric acid for 2.5 h at 100°C in a boiling water bath. Hydrolysate from the precipitate was analyzed for hexoses, pentoses and uronic acids. The hydrolysate from the residue was diluted with an equal volume of ethanol, filtered or centrifuged and the supernatant removed. The residue was washed with 50% aqueous ethanol. The combined supernatant and washings representing water-insoluble noncellulosic polysaccharides were analyzed for hexoses, pentoses and uronic acid. Cellulose was extracted from the residue with cold 72% sulfuric acid. Lignin was determined from the weight of the residue lost during ashing. The Southgate (1969) procedure provided a measure of total dietary fiber, provided separate measures of cellulose, lignin and noncellulosic polysaccharides and defined the composition of noncellulosic polysaccharides in terms of their component hexoses, pentoses and uronic acids (Southgate *et al.*, 1978).

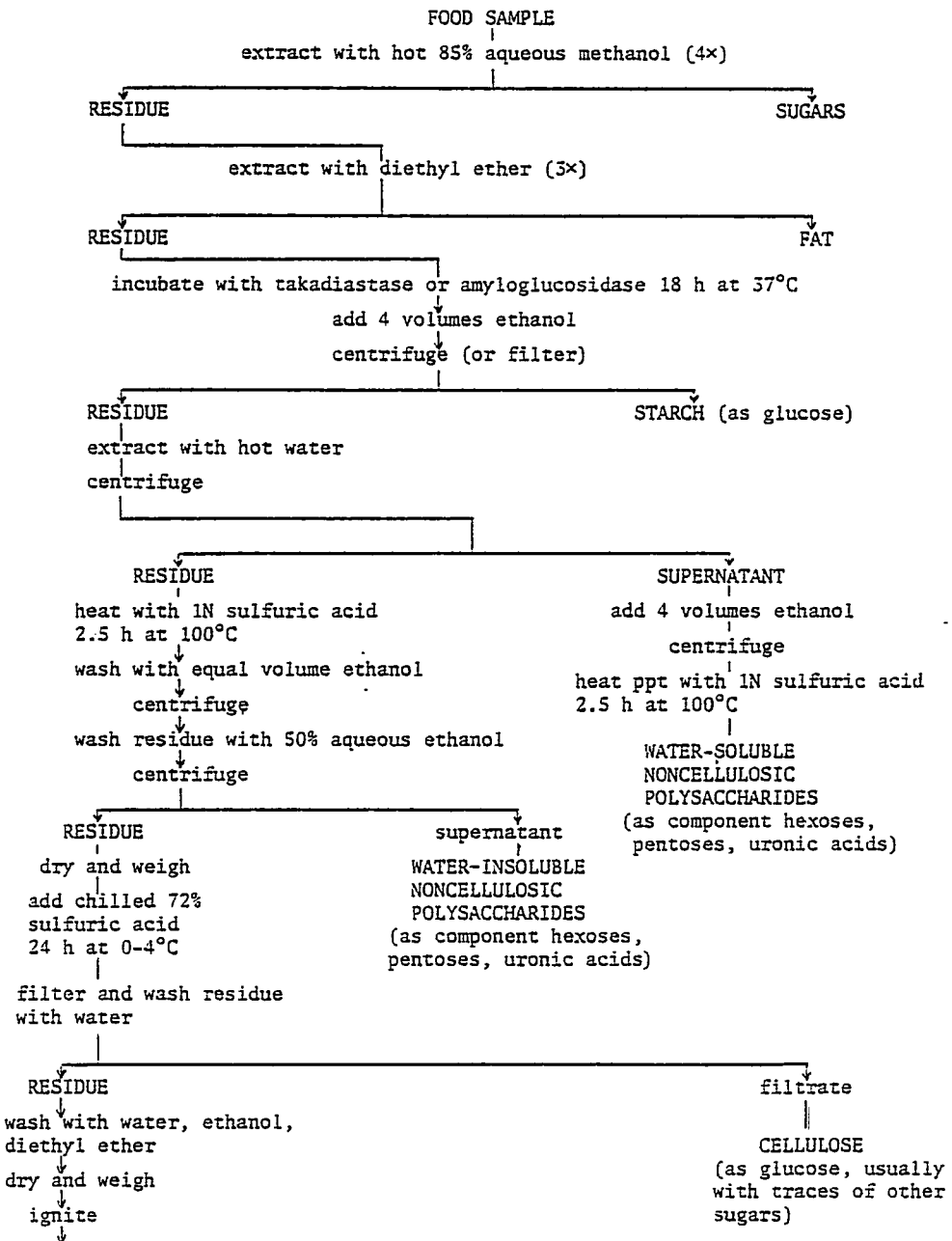


Figure 2.2 Measurement of dietary fiber or unavailable carbohydrate components by the Southgate (1969) method (adapted from Southgate, 1969, 1976; Southgate *et al.*, 1978)

Amounts of specific polysaccharides were not characterized.

The Southgate (1969) procedure for measuring carbohydrates was accurate and detailed, but time-consuming (Schaller, 1978). Simpler, more rapid methods have been developed to measure dietary fiber.

In the 1930s, McCance *et al.* (1936) measured total unavailable carbohydrates in a range of fruits, nuts and vegetables. By definition, their method was a measure of total dietary fiber since it included all unavailable carbohydrates plus lignin. The method involved measuring the starch and protein content of food residue that was insoluble in 80% aqueous ethanol (Figure 2.3). All components of dietary fiber were measured, but no information was given concerning fiber composition.

Enzymatic methods of measuring fiber have been developed which simulate digestive processes found in the human gastrointestinal tract. The origin of these methods was in the studies reported by Williams and Olmsted (1935) in which the indigestible residue in foods and feces was measured by an enzymatic procedure (Figure 2.4). Protein was removed by a prolonged incubation with pancreatin, followed by filtration of the residue and the subsequent removal of lipids by solvent extraction. The residue was treated with 72% sulfuric acid and left for 24 h at 0°C. The mixture was rapidly diluted and boiled under reflux for three hours. After neutralizing the hydrolysate, reducing sugars were measured before and after removal of fermentable sugars with yeast. Lignin was measured as the residue insoluble in acid. The non-fermentable sugars, as pentose, were considered to be a measure of the water-insoluble hemicellulose (pentosans) while the fermentable sugars, as glucose, provided a measure of cellulose. Their procedure was time-consuming and the final measurement procedures were relatively unspecific (Southgate *et al.* (1948). Later, Macy *et al.* (1973) modified the procedure to measure total

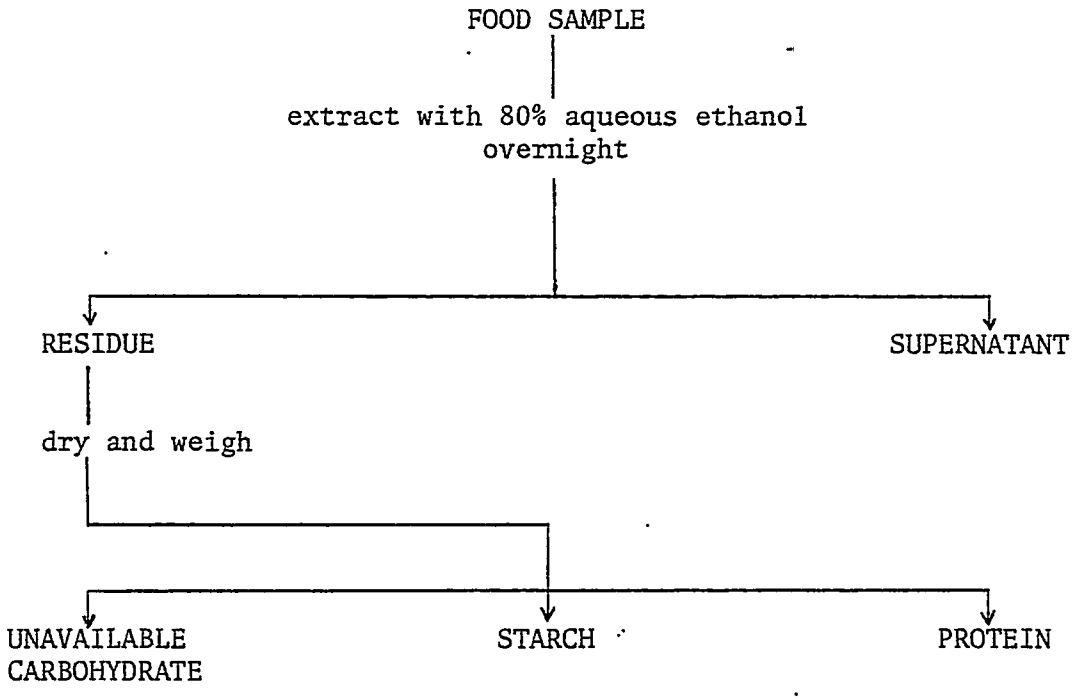


Figure 2.3 Measurement of unavailable carbohydrate by the McCance *et al.* (1936) procedure

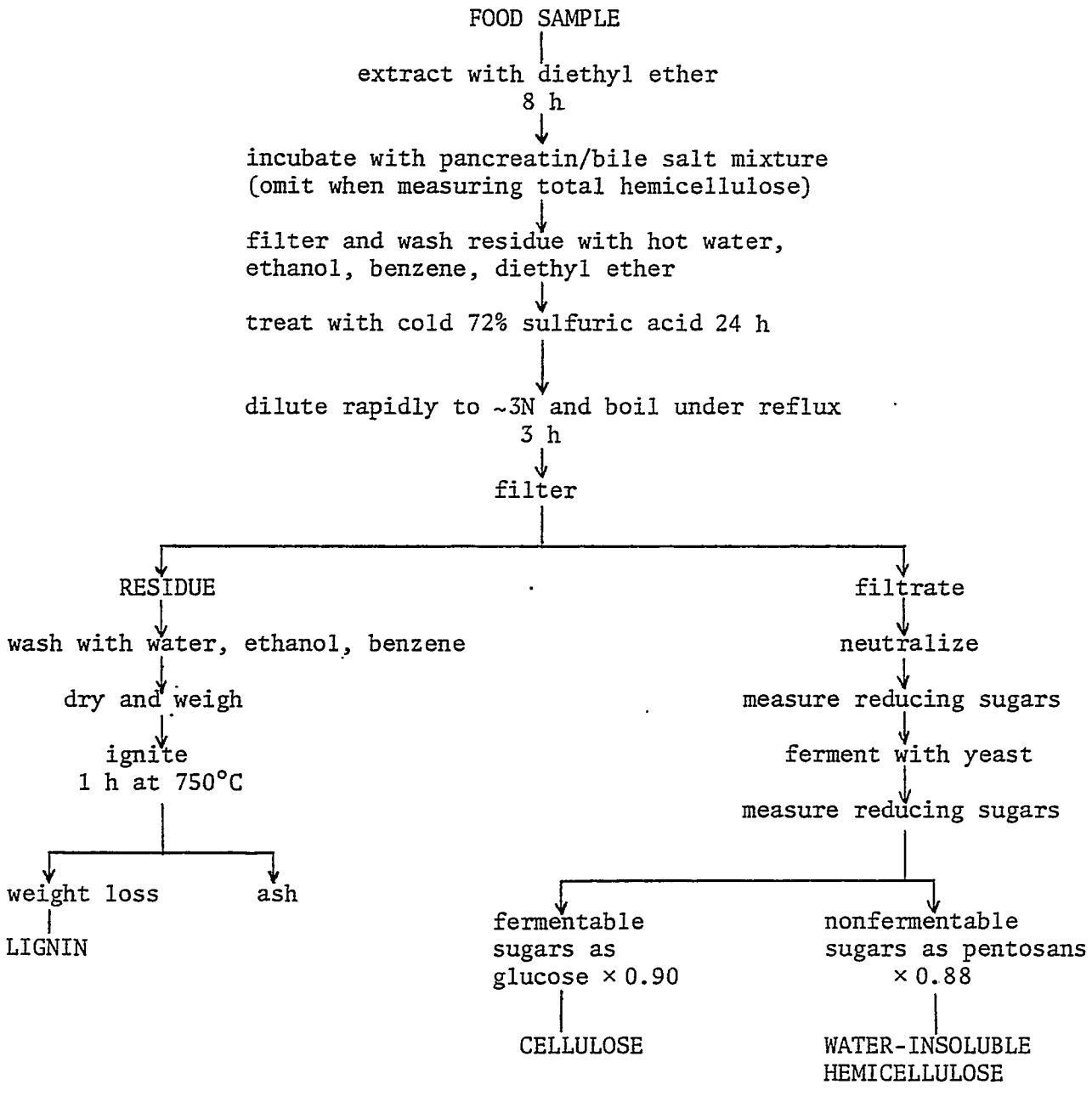


Figure 2.4 Measurement of indigestible residue by the Williams and Olmsted (1935) method as modified by Macy *et al.* (1943) (adapted from Southgate *et al.*, 1978)

hemicelluloses by omitting the pancreatin digestion step during which some hemicelluloses dissolved. Total hemicelluloses were then measured after total acid hydrolysis and removal of fermentable sugars.

More recently, Hellendorn *et al.* (1975) suggested a faster method (Figure 2.5) using a peptic digestion followed by pancreatin/bile salt digestion. After these enzymatic digestions, the residue was washed with water and dried. In its reported form, the method did not measure water-soluble components or readily hydrolyzable components that would be hydrolyzed with the protein in the 0.1M hydrochloric acid at 40°C.

Hellendorn *et al.* (1975) considered their method to be a more physiological approach to measuring fiber. However, Southgate (1977) believed this viewpoint needed to be established by *in vitro* and *in vivo* comparisons.

Saunders (1976) developed a simplified method of measuring *in vitro* dietary fiber using α-amylase from porcine pancreas and pronase, a fungal protease (Figure 2.6). Details of the method were published by Saunders and Hautala (1979). Water was used as a solvent and no buffers were required. As in the Hellendorn *et al.* (1975) procedure, the residue was washed and dried after enzymatic digestion. Undigested water-soluble polysaccharides were not measured by this procedure.

According to Schaller (1978), enzymatic methods such as the procedure used by Saunders (1976) are rarely as effective in removing digestible substances as actual intestinal digestion; and therefore, correction factors determined from animal feeding studies are needed to correlate the enzyme digestion results to the actual amount of dietary fiber. Also, enzyme preparations vary in activity from batch to batch so

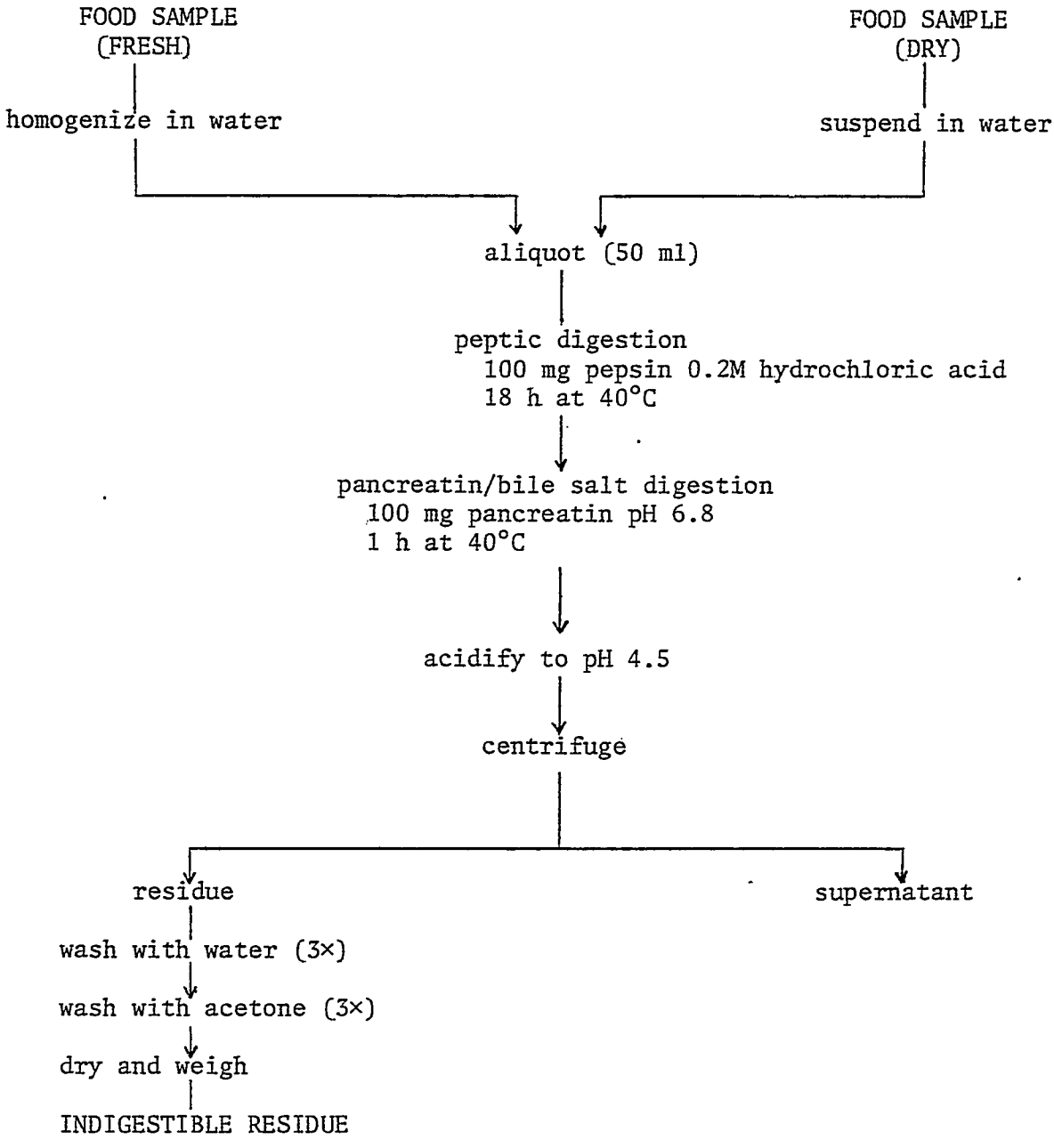


Figure 2.5 The Hellendorn *et al.* (1975) procedure for indigestible residue

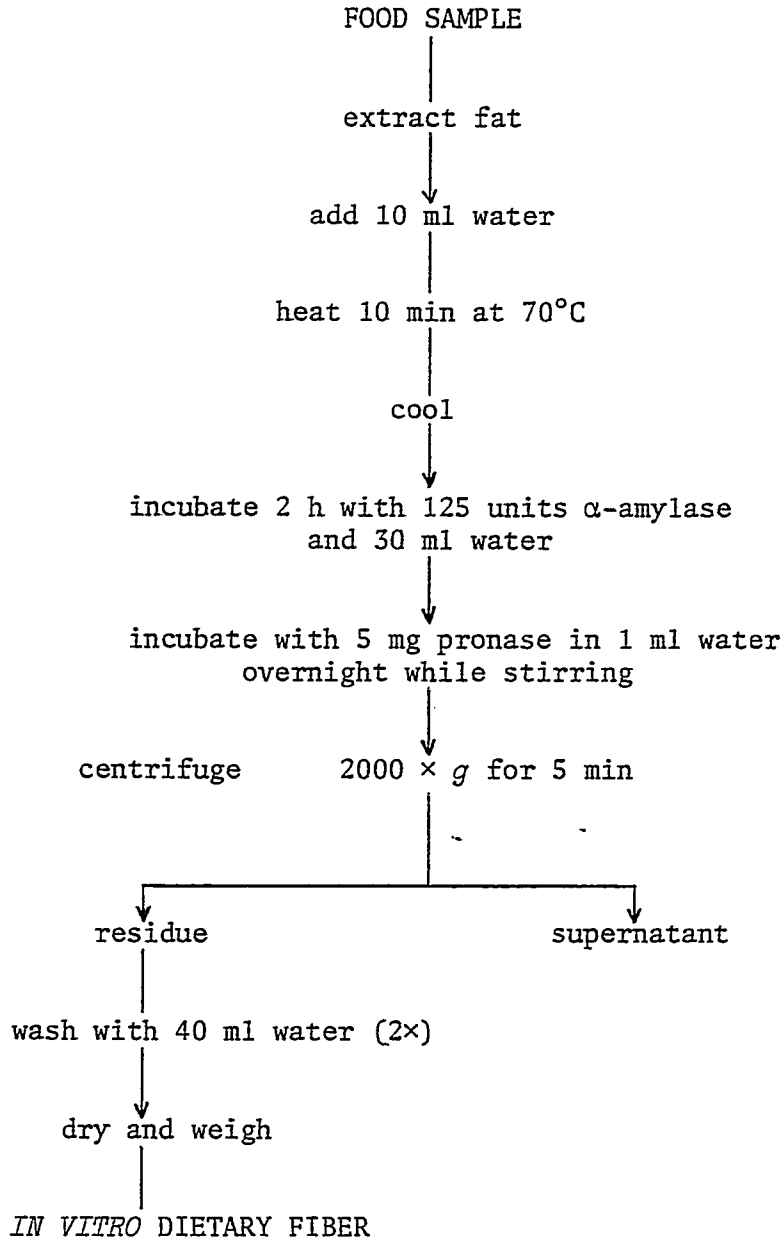


Figure 2.6 The Saunders (1976) procedure for *in vitro* dietary fiber

much care must be given to standardizing the procedure.

Chemical methods are similar to enzymatic methods in that protein and starch are extracted from residual insoluble fiber. The most commonly used chemical methods of isolating dietary fiber are the detergent fractionation procedures (Table 2.3) developed by Van Soest and his co-workers (Van Soest, 1963a, 1963b, 1966; Van Soest and Wine, 1967) and described by

Table 2.3 Basic scheme of fiber analysis using detergents (Spiller and Amen, 1975b)

Fraction	Reagent	Treatment	Yield
Cell contents		Calculate as 100—NDF	Lipids, sugars, organic acids, starch, soluble protein, nucleic acids, pectin
Neutral detergent fiber (NDF)	Sodium lauryl sulfate, EDTA-borate pH 7.0	Boil 1 h	Plant cell wall less pectins
Acid detergent fiber (ADF)	Cetyl trimethyl-ammonium bromide in 1N sulfuric acid	Boil 1 h	Lignocellulose + insoluble minerals
Lignin	72% sulfuric acid	3 h at 20°C	Crude lignin
Lignin	Potassium permanganate pH 3.0	1½ h at 20°C	Lignin as loss in weight by oxidation
Cellulose	None	Ash residue from lignin step	Loss in weight
Cutin	72% sulfuric acid	Treat cellulose 3 h at 20°	Residue is cutin
Hemicellulose	None	Calculate as NDF—ADF	

Goering and Van Soest (1970). Detergent fractionation procedures were originally developed to measure the fiber content of forages and were later successfully adapted to feedstuffs.

According to Goering and Van Soest (1970), the neutral detergent fiber (NDF) procedure separates the dry matter of feed into nutritionally available and soluble constituents from those that are insoluble or incompletely available and dependent on microbial fermentation. The procedure utilizes sodium lauryl sulphate as a detergent to solubilize lipids and proteins, ethylene diamine tetra-acetic acid (EDTA) as a chelating agent to remove minerals and heat to gelatinize starch at a neutral pH to prevent hydrolysis of hemicelluloses (Schaller, 1978). Pectin is converted to its soluble sodium salt and is essentially removed (Van Soest and Robertson, 1977). The NDF residue is composed of hemicellulose, cellulose and lignin with some accompanying cutin, minerals and protein. When food products of high starch content are analyzed, the residue also contains heat-resistant starch (Schaller, 1978). Residual starch in these samples is hydrolyzed by the addition of α-amylase. Silica is largely dissolved, in contrast to the ADF procedure where it is quantitatively recovered (Van Soest and Robertson, 1977). The analysis measures only the plant cell walls of fecal material since microbial cell walls are largely dissolved (Van Soest *et al.*, 1966).

Sodium sulphite was included in the original NDF procedure to reduce the large amount of cell wall protein associated with the NDF residue of forages (Van Soest and Wine, 1967). Later, Van Soest and Robertson (1977) suggested that sodium sulphite not be used for the analysis of foods since the sulphite might partially degrade the lignin fraction.

The acid detergent fiber (ADF) procedure provides a rapid method for lignocellulose determination in feedstuffs (Goering and Van Soest, 1970).

Cetyl trimethylammonium bromide (CTAB) in 1N sulfuric acid recovers the cellulose and lignin in the residue while removing more than 95% of both the hemicelluloses and nitrogen (Robertson, 1978). The residue represents essentially the crude lignin and cellulose fraction of plants but also includes the indigestible products of cooking, animal skin and hair, silica, pectins and tannins (Van Soest and Robertson, 1977). The difference between NDF and ADF is an estimate of hemicellulose; however, this difference does include some protein attached to cell walls (Goering and Van Soest, 1970).

There are two methods for determining the lignin content of foodstuffs by detergent fractionation. Both rely on the use of the ADF procedure as a preparatory step to remove protein and acid-soluble material that would interfere with the lignin determination (Goering and Van Soest, 1970).

The acid-detergent lignin procedure has been accepted by AOAC (1975) as a standard method for measuring crude or Klason lignin in feedstuffs. The ADF residue is treated with 72% sulfuric acid to dissolve the cellulose component. After washing, the resulting residue is dried, ashed to correct for the mineral content of the residue and reweighed to determine the crude lignin fraction. High lignin values can be a result of artifacts present in the lignin fraction. Cutin, the waxy coating on the surface of fruits and vegetables, is not chemically related to lignin but is insoluble in 72% sulfuric acid and contributes to the lignin fraction (Hartley, 1978). Similarly, heating a food can increase artifact lignin. Some of the products of the Maillard reaction are insoluble in 72% sulfuric acid, as are the products of the reaction between tannins and proteins. It can be argued, however, that artifact lignin is indigestible and therefore can justifiably be included as part of the fiber fraction (Van Soest and Robertson, 1976).

An alternative to the acid detergent lignin procedure is the permanganate lignin method (Van Soest and Wine, 1968). Lignin is oxidized with an excess of acetic acid-buffered potassium permanganate solution, containing trivalent iron and monovalent silver as catalysts (Goering and Van Soest, 1970). Deposited manganese and iron oxides are dissolved with an alcoholic solution of oxalic and hydrochloric acids, which leaves cellulose and insoluble minerals. Lignin is measured as the weight lost by these treatments. Cellulose is determined as the weight loss upon ashing. The ash residue is mainly silica.

Advantages of the permanganate method over the 72% sulfuric acid method include a shorter procedure for lignin *per se* while the residue is reserved for further analysis of cellulose and silica (Goering and Van Soest, 1970). The permanganate reagents are much less corrosive and require no standardization. The residue requires no filter aids, and lignin values are not subject to some interferences that affect the 72% sulfuric acid procedure. Values are less affected by heat-damage artifacts and are closer to true lignin.

Unfortunately, low values will result if the particle size is large and the sample is poorly penetrated by the permanganate reagents. Cellulosic carbohydrates can be lost from samples of low lignin content if the flow of permanganate solution through the crucibles is too fast.

Cutin is not measured by the permanganate lignin method (Goering and Van Soest, 1970). If a measure of cutin is required, acid detergent cutin can be determined by preparing permanganate cellulose and treating with 72% sulfuric acid and asbestos for three hours.

The buffered acid-detergent fiber procedure developed by Baker (1977)

is a compromise between the ADF and NDF procedures of Van Soest (Goering and Van Soest, 1970). Baker's (1977) method is based on the ADF procedure with the exception that a hydrochloric acid-potassium chloride buffer solution was used as a solvent for the detergent, CTAB. The buffer solution is a much less corrosive reagent than the 1N sulfuric acid used in the ADF procedure and is within the pH range of the human stomach digestive medium. Most of the starch and protein is removed from the sample while very little cellulose is lost. However, some hemicellulose is solubilized by this procedure.

2.4.3 Agreement among different measures of dietary fiber

Few detailed comparisons of different methods are available in the literature and more are needed to assess the relative merits of the different methods (Southgate, 1977).

According to Southgate (1977), values for total dietary fiber determined by the Southgate (1969) procedure are in agreement with values reported by McCance *et al.* (1936).

McConnell and Eastwood (1974) compared the ADF method of Van Soest (1963a, 1963b) and the Southgate (1969) method for unavailable carbohydrates. They reported that the two procedures gave similar values for cellulose and lignin in a range of foods.

Greenberg (1976) compared the detergent fractionation procedures with the Southgate (1969) method for unavailable carbohydrates. Cellulose and lignin values reported by Southgate (1978) show excellent agreement between the two methods. The hemicellulose values obtained by the Van Soest procedures are slightly lower than the noncellulosic polysaccharides as

would be expected because the latter includes water-soluble components.

Schaller (1978) reported that, in a collaborative study by the Food Fiber Committee of the American Association of Cereal Chemists, the dietary fiber contents of white wheat bran was 36% by the Van Soest enzyme-modified NDF analysis, 37% by the Southgate unavailable carbohydrate analysis and 35% by the Saunders *in vitro* enzymatic digestion method.

Rasper (1979) analyzed fiber-rich fractions of several cereals by detergent fractionation (Goering and Van Soest, 1970), buffered acid-detergent fiber (Baker, 1977), and *in vitro* fiber (Saunders, 1976). Southgate's (1969) method for unavailable carbohydrates was used to determine the composition of enzymatically undigested residue. As would be expected, buffered acid-detergent fiber values were intermediate between NDF and ADF values. Values determined by the NDF procedure were similar, although somewhat lower than values determined by the *in vitro* fiber method.

Saunders and Hautala (1979) measured and compared crude fiber, neutral detergent fiber, *in vitro* dietary fiber and *in vivo* dietary fiber (rats) in wheat milling fractions, wheat breads and wheat-based breakfast cereals. Although values for the same sample differed according to the method of analysis, all were closely correlated. The authors concluded that laboratory analysis for crude fiber, neutral detergent fiber or *in vitro* dietary fiber could accurately predict *in vivo* dietary fiber (rats) for wheat milling fractions when the laboratory result was adjusted by means of an appropriate regression equation.

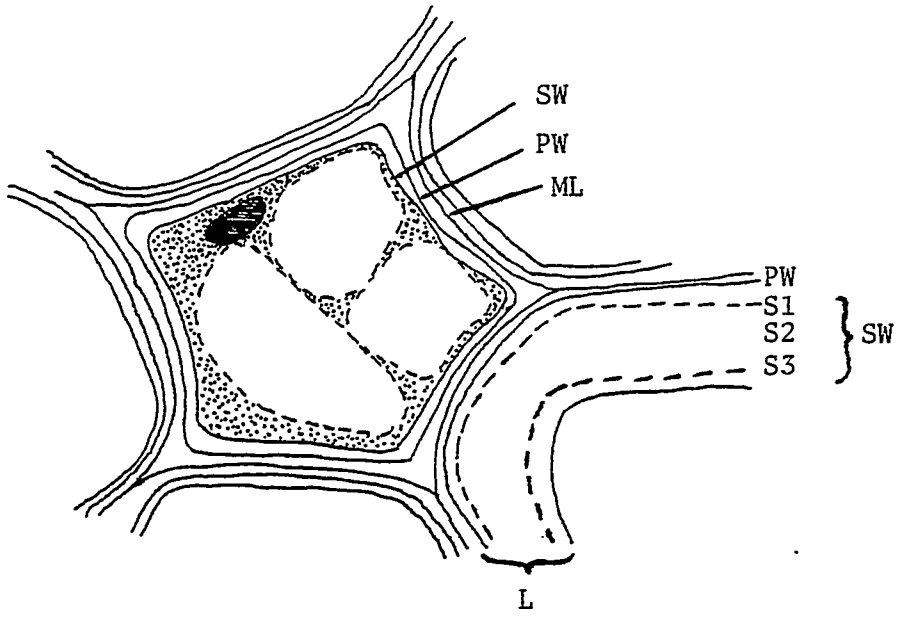
2.5 DISTRIBUTION OF DIETARY FIBER

2.5.1 Structure of the cell wall

Most dietary fiber is consumed in the form of plant cell walls and not as substances distributed randomly in the diet (Southgate, 1976). Some of the properties attributed to dietary fiber may be due, in part, to chemical and physical interrelationships among the various constituents of the plant cell wall.

Most detailed studies on the plant cell wall have been made on wood tissues and algae (Southgate, 1976). However, some structural features are common to many plants (Figure 2.7).

Expanding cells are surrounded by a thin primary wall which is deposited on the middle lamella (Southgate, 1976; Southgate *et al.*, 1976; Theander, 1977). The primary wall consists of a random network of cellulose fibrils deposited in a matrix of noncellulosic polysaccharides. The middle lamella or intercellular substance is rich in pectic substances. The cell wall thickens as cellulose fibrils are deposited in a regular parallel arrangement, surrounded by a noncellulosic polysaccharide matrix to form the secondary wall which is characteristically composed of a number of reasonably distinct layers. At the end of the thickening phase, formation of lignin becomes noticeable. Lignification usually starts in the middle lamella often at the junction of several cell boundaries and extends towards the cell lumen. Lignification is associated with an apparent increase in cell wall thickness which suggests that lignin is deposited within the original cellulosic structure of the wall and that lignification does not involve replacement of the noncellulosic matrix. When lignification is complete, the cell dies.



ML: Middle lamella which in the young plant is rich in pectic substances and where lignification starts as the cell matures

PW: Primary wall with a network of cellulose fibrils deposited in a matrix of non-cellulosic polysaccharides

SW: Secondary wall with several layers (S1, S2, S3) containing cellulose fibrils in a parallel arrangement in a matrix of non-cellulose polysaccharides

L: Lignified wall with lignin infiltrated around the other components of the wall and a dead cell

Figure 2.7 Idealized section of plant tissue (Southgate *et al.*, 1976)

The nature of interactions between the components of the cell wall has not yet been established (Southgate, 1976). However, it is now evident that many, but not necessarily all of the polymer fractions, are linked by both covalent and noncovalent bonds (Bailey *et al.*, 1978).

2.5.2 Dietary fiber content of foods

While the only measure of fiber in most food composition tables is crude fiber, levels of dietary fiber in common foods have been reported by Southgate *et al.* (1976), Shipley (1978), Southgate (1978), Jwuang and Zabik (1979) and Mongeau and Brassard (1979).

The major food sources of dietary fiber are cereals, vegetables, fruits and nuts (Southgate *et al.*, 1976).

Southgate *et al.* (1976) reported that the amount of dietary fiber in cereal foods depended on the type of cereal and the extraction rate of the cereal. As the extraction rate of wheat flour was increased from the level used in breadmaking (72%) to the rate used for whole wheat flour (100%), the level of fiber increased from 3-4% to 11-14% and the level of pentoses in the noncellulosic fraction increased five-fold (Southgate, 1978). Although the concentration of dietary fiber varied according to the type of cereal and the extraction rate, the composition of the dietary fiber was similar for all cereals studied by Southgate (1978); 70-80% noncellulosic polysaccharides, 15-25% cellulose and generally less than 10% lignin (Table 2.4).

Mongeau and Brassard (1979) found similar results to Southgate (1978) in their study of the NDF, hemicellulose, cellulose and lignin content of 44 kinds of bread. Although the NDF content of the breads was influenced by the degree of flour extraction as well as by the additions made to the bread, the composition of the NDF was similar for all breads: 65% insoluble

Table 2.4 Dietary fiber in selected foods

	Total dietary fiber (g/100 g)		Composition of the dietary fiber (%)			Composition of the noncellulosic fraction (%)		
	Fresh weight	Dry weight	Noncellulosic polysaccharides	Cellulose	Lignin	Hexoses	Pentoses	Uronic acids
Cereal products[1]								
White flour (72%)	—	3.45	80	19	1	80	11	9
Whole wheat flour (100%)	—	13.51	74	20	6	38	49	13
Wheat bran	—	48.00	74	18	7	19	69	12
Rice, long grain	—	2.74	78	22	tr[3]	82	9	8
Raw vegetables[1]								
Cabbage, white	2.66	27.4	66	23	11	7	42	50
Onions	1.30	18.1	74	26	tr	29	26	45
Peas, frozen	7.75	37.1	69	27	2	48	22	30
Peas, canned	6.28	34.1	61	39	tr	23	30	47
Carrots	2.90	28.4	60	40	tr	20	35	45
Potatoes	3.41	14.1	71	29	1	80	N.D.[4]	20
Fruits[1]								
Apple, flesh	1.42	9.16	66	33	1	20	35	44
Orange	1.90	13.7	71	14	15	19	37	43
Strawberries	2.12	19.1	46	16	38	22	33	45
Tomato, fresh	1.40	21.9	47	32	21	14	42	44
Nuts[2]								
Brazils	7.73	—	47	28	25	—	—	—
Peanuts	9.30	—	69	18	13	—	—	—

[1] Southgate (1978)

[2] Southgate *et al.* (1976)

[3] trace

[4] not determined

hemicellulose, 25% cellulose and the remainder mostly lignin.

The dietary fiber content of fresh vegetables and fruits is effectively diluted by the high water content at which most of these foods are consumed (Southgate, 1978). On a dry basis, vegetables and fruits are generally proportinately higher in cellulose and lower in hemicellulose than cereals (Table 2.4). Lignin values are low except when lignified seeds are eaten, as in the case of strawberries, or when skins are consumed, as in the case of fresh tomatoes (Table 2.4).

The dietary fiber content of nuts is usually higher than that of fruits and vegetables on a fresh weight basis (Table 2.4). The fiber composition of the nuts is similar to some fruits listed in Table 2.4.

The proportion of fiber constituents isolated from the more fibrous components of plant material (Table 2.5) follow the pattern of fiber composition in common foods (Table 2.4). Bran from cereals tends to be very high in hemicelluloses as compared to hulls from legumes and other dicotyledonous plants which are relatively high in cellulose (Table 2.5) (Van Soest and Robertson, 1976).

2.5.3 Factors influencing fiber composition

The composition and properties of plant fiber vary according to source, species of plant and physiological stage of growth (Van Soest, 1978). Fibers from related species within a plant family, such as cereal brans, are relatively similar as discussed in Section 2.5.2. Most vegetables and fruits are dicots and represent an immature stage of growth in contrast to the brans which are mature plant products. Generally, lignification and other factors promoting the development of a truly indigestible fraction increase with plant age.

Table 2.5 Typical fiber composition of selected plant materials

	Insoluble dietary fiber (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	% of insoluble dietary fiber		
					Hemicellulose	Cellulose	Lignin
Corn bran[1]	88.6	67.0	21.4	0.2	75.6	24.2	0.2
Rice bran[1]	21.8	11.0	6.9	3.9	50.5	31.6	17.9
Wheat bran, red[2]	44.5	29.1	12.0	3.4	65.4	27.0	7.6
Wheat bran, white[2]	39.5	27.9	9.9	1.7	70.6	25.1	4.3
AACC wheat bran[3]	40.2	28.3	8.7	3.2	70.4	21.6	8.0
Bagasse[4]	—	—	—	—	31	51	13
Beet pulp[1]	37.4	12.0	23.4	2.0	32.1	62.6	5.3
Pea hulls[1]	51.8	11.0	40.7	0.1	21.2	78.6	0.2
Peanut hulls[5]	86.2	12.2	39.4	34.6	14.2	45.7	40.1
Soybean hulls[2]	67.0	23.1	40.6	3.3	34.5	60.6	4.9
Sunflower hulls[4]	—	—	—	—	16	56	27
Citrus pulp[1]	24.8	0.0	23.4	1.4	0.0	94.4	5.6
Cellulose[1]	94.0	0.0	94.0	0.0	0.0	100.0	0.0

[1] Schaller (1978)

[2] Rasper (1979)

[3] AACC Standard Wheat Bran RO7-3691; Spiller *et al.* (1978)

[4] Van Soest and Robertson (1976)

[5] Childs and Abajian (1976)

Grinding fiber through a screen of specified size produces a spectrum of particle sizes (Van Soest and Robertson, 1976). The composition of the spectrum of fiber particles obtained on grinding is not constant, the more lignified pieces being more resistant to shearing. If coarser material is segregated in processing, the composition of the fiber may be altered.

The apparent fiber content of foods can be increased by products of the Maillard reaction produced through heating (Van Soest, 1978). The Maillard polymer is a brown, insoluble, indigestible substance with the physical properties of lignin. The polymer is quantitatively recovered in the residue from neutral detergent or acid detergent extraction and in crude lignin.

Heller *et al.* (1977) studied the effect of particle size and pH on the measurement of hemicellulose, cellulose and lignin in unprocessed and processed wheat bran, purified corn pericarp and peanut hulls. They reported that variation in sample particle size and that exposing the plant fiber to acid and alkali may change the fiber composition as measured by the Van Soest procedure. Grinding tended to reduce NDF values, but had no effect on ADF values. Consequently, hemicellulose values were dependent on particle size. The presence of acid or base and heat resulted in the solubilization of the hemicellulose fraction of plant fiber. Acid or base had little effect on other fiber components. Hemicellulose values for wheat samples ground through a 60 mesh screen of a Wiley Mill were 20% lower than for samples ground through a 20 mesh screen. Shaking red wheat bran for 24 h at 25°C solubilized 17% of the hemicellulose at pH 11.5 and 9% at pH 2.2. Refluxing for 60 min reduced the hemicellulose content by 62% at pH 11.5 and by 52% at pH 2.2. Similar losses were observed for purified corn pericarp while little change was found for peanut hulls.

2.6 PROPERTIES OF DIETARY FIBER

Properties of dietary fiber that are of biological significance include bulk density, fermentability, hydration capacity, cation exchange capacity, organic compound adsorption and gel filtration capacity.

2.6.1 Bulk density

Dietary fiber adds bulk to the contents of the gastrointestinal tract (Schaller, 1978) and promotes faster rate of passage (Van Soest, 1978).

Fibers from different sources have different effects on fecal bulk and transit time (Anderson and Chen, 1979). Fibers which are completely degraded in the colon may not increase fecal bulk. In general, fibers high in cellulose increase fecal bulk and decrease intestinal transit time, whereas soluble fibers such as pectin and guar gum increase transit time and have only a limited effect on fecal bulk.

Animal studies have shown that fine grinding of the fiber increases feed density and alters passage and character of gastrointestinal fermentations (Van Soest, 1978). Finer grinding increases the likelihood that fiber particles will flow with liquid, whereas coarser fiber will tend to mat and become occluded through filtration effects (Van Soest and Robertson, 1976). Finely ground fiber packs more densely and therefore has a reduced bulk effect. These effects become the mechanisms for differential passage of residues through the digestive tract.

2.6.2 Fermentability

Although fiber is generally considered indigestible, most animals including man have the ability to ferment fiber by virtue of their intestinal bacteria. Studies have shown that over 50% of dietary fiber in a western

diet can be broken down by bacterial enzymes into short chain fatty acids, water, carbon dioxide, hydrogen and methane (Cummings, 1973; Mendeloff, 1975). This process provides a significant energy source to both ruminants and animals with a large caecum that are able to absorb volatile fatty acids. Dietary fiber contributes less than 3% of the total energy of a mixed western diet and is probably unimportant as an energy source in human nutrition. However, the microflora of the human colon are able to digest hemicellulose and pectin to a significant extent as well as cellulose to a limited extent (Spiller and Amen, 1975b; Williams and Olmsted, 1936). Cellulose not digested by bacteria is probably intimately associated with the lignin polymer. Lignin remains relatively unchanged.

Southgate and Durnin (1970) studied the apparent digestibility of cellulose and pentosans in healthy human volunteers. Cellulose digestibility ranged from 0 to 84%. The variation in digestibility between subjects was attributed to variations in transit time. Subjects with the longest transit time tended to show the greatest apparent digestibility of cellulose due to longer incubation with microorganisms of the colonic microflora.

More recently, Gramstorff Fetzer *et al.* (1979) studied gastric disappearance of purified cellulose, hemicellulose and pectin as consumed by normal adolescent boys. Apparent disappearance of cellulose was 45-46% while hemicellulose disappearance was 76-90%. Pectin disappeared completely.

The way in which microorganisms degrade fibers depends on how well they can penetrate into the fibrils (Spiller and Amen, 1975b). Milling probably increases the susceptibility of the plant cell wall material to bacterial degradation by exposing more surfaces to bacterial action, which may reduce the capacity of the polysaccharides to bind water in the small

intestine (Anon., 1975). Lignin encrusts plant cell walls and reduces digestibility (Spiller and Amen, 1975b). It has been suggested that the amount of lignin in food regulates the digestibility of cellulose and hemicellulose. The wide variation in the proportion of fiber constituents digested probably depends on the relative amounts of each present, the physical structure of the fiber and on the variable bacterial flora and time spent in the gut (Cummings, 1973).

2.6.3 Hydration capacity

One of the most important physiochemical properties of dietary fiber is its ability to absorb water. This property contributes to alterations in intestinal transit time and to increases in fecal water associated with certain fibrous foods (Eastwood and Mitchell, 1976).

Most polysaccharides swell in the presence of water to form gels (Anderson and Chen, 1979). Pectins, gums, mucilages, storage polysaccharides and some of the structural hemicelluloses have high affinities for water and form gels in the small intestine. Cellulose has a moderate capacity, whereas lignin has a minimal capacity to absorb water (Spiller and Amen, 1975b).

Initially, water is adsorbed onto the fiber surface and later the interstitial spaces of the fibrous material are filled to saturation (Eastwood and Mitchell, 1976). Water subsequently added will be free water superfluous to the plant matrix. The capacity of plant fibers to hold water is determined by the chemical and structural properties of the macromolecules as well as by the electrolyte concentration and pH of the surrounding liquid. Grinding influences hydration capacity by increasing surface area and decreasing interior cell space (Van Soest, 1978).

Mongeau and Brassard (1980) reported a significant correlation between average particle size and water holding capacity of insoluble dietary fiber extracted from breakfast cereals and unprocessed wheat brans. Both dry bulk and water holding capacity decreased directly with mean particle size. Since dry bulk and water holding capacity were also closely correlated, the authors have suggested that grinding may have decreased the bulk which might be essential to hold interstitial water.

According to McConnell *et al.* (1974) the water absorptive capacity of any dietary plant is determined by its fiber content and the water holding capacity of the fiber. These workers estimated the water holding capacity of 26 fruits and vegetables using material that was insoluble in water at 40°C, dried with acetone and ground through a 1 mm pore sieve. The water holding capacity of the acetone dried powders appeared to be higher in vascular tissues such as lettuce, carrot, cucumber and celery, while lower in storage organs such as maize, oatmeal, potato, banana, wheat and wheat bran. Although acetone dried powder from wheat bran was a relatively poor binder of water, the large amount of dry matter present gave the original material excellent water absorption properties. Grinding in a rotary mill decreased water holding capacity of acetone dried powders from wheat bran, maize and winter cabbage. Cooking had a negligible effect.

Childs and Abajian (1976) studied the effect of incubation time (30, 60, 90, 120, 240 min), temperature (24, 29, 37, 45°C) and pH (4.6, 5.2, 6.8, 7.0, 7.4) on water holding capacity of ground peanut hulls. In general, water holding capacity increased with increases in both time and temperature of incubation. The pH had no significant effect at any of the times or temperatures employed.

Parrott and Thrall (1978) extracted fiber from almond skins, peanut hulls, rice bran (Protex 20 and Protex 40), coconut, soy bran and wheat bran (AACC Certified Bran and Nabisco All Bran) using the crude fiber procedure described in AOAC (1975) and compared the physical properties of these fibers with the commercial cellulose products Solka Floc (SW 40 and BW 100), Avicel 591 and Alphacel. Water holding capacity measured as a function of ionic strength (0.01, 0.1, 1.0M) adjusted with mono- and divalent cations (Na^{+}, Ca^{+2}) in deionized water and pH (2.69, 5.20, 7.33), proved to be highly individualized for each fiber source. Generally, increasing the ionic strength of sodium chloride and calcium chloride solutions from 0.1M to 1.0M resulted in decreased water holding capacity for most fibers studied. However, values for almond skins, Nabisco All Bran and Protex 40 increased with increasing ionic strength of a sodium chloride solution, while values for purified cellulose fibers less than 100μ in diameter evidenced a gradual increase in water holding capacity with increased calcium chloride concentration. The purified cellulose products showed maximum affinity for water in a neutral medium of pH 7.33. Water holding capacity of peanut hull fiber was highest in acid medium (pH 2.69) and declined with increasing pH values. In the weak acid medium (pH 5.20) water retention properties were maximized in the case of coconut residue and minimized in the case of rice bran. The pH had no significant effect on water holding capacity of fiber residues from wheat bran, soy bran or almond skins. When particle size alone was considered, the larger grade of Solka Floc (SW 40) had a correspondingly higher water retention at each pH tested than the smaller particle sized Solka Floc (BW 100). Water holding capacity of two different sized samples of rice bran were similar at pH 7.33. However, in the acid range, water

retention properties of the larger particle size (Protex 20) were consistently lower than the smaller grade (Protex 40).

2.6.4 Cation-exchange capacity

Acidic polysaccharides with uronic acid moieties, namely, pectins, alginates from seaweeds and to a limited extent, hemicelluloses, have the capacity to bind metals and act as cation exchangers (Spiller and Amen, 1975b). Cation-exchange capacity is constant for fiber from any particular plant source irrespective of the anatomical source (Eastwood and Mitchell, 1976). Species of plants have a characteristic cation-exchange capacity that is relatively independent of external nutritional factors in the soil.

The nutritional consequences of cation binding is uncertain at present (Anderson and Chen, 1979). However, short-term balance studies suggest that the initial weeks of a high fiber diet may enhance fecal excretion of calcium, iron, magnesium and zinc. Further studies are required to evaluate the long term effects of high fiber diets on mineral balance.

Most of the 26 fibers studied by McConnell *et al.* (1974) acted as monofunctional weak cation-exchange resins, whereas maize, oatmeal, bananas, cereal bran and new potatoes acted as very weak polyfunctional exchangers. Lettuce, cabbage, carrot, orange and turnip were in the range of commercially available weak cation exchange resins.

Work by Rasper (1979) confirmed that bran fiber was a weak cation exchanger with wheat bran having distinctly polyfunctional character. The polyfunctional characteristic was less pronounced in fibers isolated from corn bran, rice bran and barley hull. Cation-exchange capacity of fibers isolated from soybean hulls and peanut red skins was much higher than for fibers isolated from oat hulls and rice hulls due to the chemical nature of

the materials.

Mineral binding by dietary fiber appears to be pH dependent. Thompson and Weber (1979) studied the binding of endogenous copper, zinc and iron in wheat bran, corn bran, soy bran, oat hulls, rice bran and cellulose at pH 0.65, pH 6.8 and a sequential treatment of pH 0.65, neutralization, then pH 6.8. After both the pH 6.8 and sequential treatments, most of the minerals remained bound in the residues, while little remained bound after the acidic treatment. The authors reported that minerals were rebound when the pH was raised from very acidic to only slightly acidic.

2.6.5 Adsorption of organic compounds

Bile salts are adsorbed by the lignin component of dietary fiber (Eastwood, 1974). The bile acids excreted in the bile, taurine and glycine conjugates of cholic and chenodeoxycholic acid, are only weakly adsorbed (Eastwood, 1974; Eastwood and Mitchell, 1976). After chemical transformation by colonic bacteria, deconjugated bile salts are strongly bound and excreted in the feces. The adsorption is pH-dependent, being most strong at an acidic pH and weakest at an alkaline pH. Adsorption is possibly influenced by methoxylation of acidic hydroxyl moieties in the fiber.

Binding of bile acids and salts by fiber may cause increased excretion and, in turn, synthesis of bile acids from cholesterol (Story *et al.*, 1976). Also bile salts may be unable to interact in micelle formation, and, therefore, cholesterol adsorption may be inhibited. Both conditions would result in decreased adsorption and a loss of cholesterol from the body.

2.6.6 Gel filtration capacity

When plant fibers form a matrix in the gastrointestinal tract of nonruminants, a gel filtration system may develop which could exclude molecules or bacteria on the basis of size or ionic charge (Eastwood, 1973; Spiller and Amen, 1975b; Anderson and Chen, 1979). This would delay adsorption of carbohydrates and other nutrients in the small intestine while altering the availability of fiber, bile salts and nutrients for metabolism by bacteria. The physiological and nutritional consequences of these gel filtration systems have not been examined.

2.7 PHYSIOLOGICAL EFFECTS OF DIETARY FIBER

For years, dietary fiber was popularly and simplistically associated with the prevention of constipation. The attention of medical researchers, nutritionists, chemists and food technologists, as well as that of the lay press, was directed towards dietary fiber when epidemiological observations were reported that suggested dietary fiber played a role in preventing certain degenerative diseases common in most industrial societies and uncommon in primitive cultures. It is now known that dietary fiber has two major effects. First, it affects the functioning of the gastrointestinal tract by increasing stool weight and reducing intracolonic pressure. Second, it affects lipid metabolism by reducing the absorption and reabsorption of cholesterol and bile acids, respectively. The degree of biological effect varies with the fiber source.

2.7.2 Dietary fiber as a protective agent against disease

The incidence of certain diseases such as diverticulosis, ischemic

heart disease and diabetis mellitus (Table 2.6) is rare or nonexistent in countries that have been little affected by industrialization (Burkitt *et al.*, 1972; Burkitt, 1976). The main dietary changes that accompany industrialization include an increased consumption of animal fat and protein

Table 2.6 Western-risk diseases and conditions associated with low-fiber diets.[1]

Type	Disease or condition
Colonic	Constipation, appendicitis, diverticular disease, hemorrhoids, polyps and cancer of large bowel, irritable colon, ulcerative colitis
Metabolic	Obesity, diabetes mellitus, ischemic heart disease, varicose veins, venous thrombosis, pulmonary embolism, gallstones
Other	Dental caries, hiatus hernia

[1] Adapted from Bing (1976); Trowell (1978)

along with a marked decrease in the intake of cereal fiber. The "fiber hypothesis" considers dietary fiber to be a protective agent against colonic disease since plant fiber is the only component of the diet that reaches the large bowel in other than small proportions. Trowell (1978) has traced the development of this concept.

Epidemiological evidence collected principally from Africa and more recently from the Indian subcontinent, the Middle East and the Pacific suggests a fixed sequence of events (Trowell, 1976). The first stage is

the primeval diet of plant food eaters containing very large amounts of unprocessed starchy staples. The degenerative diseases of civilization are rare. The second stage, commencing westernization of diets, is accompanied by obesity and diabetes in privileged groups. During the third stage, moderate westernization of diets, constipation, hemorrhoids, varicose veins and appendicitis appear as common clinical conditions. In the final stage, advanced westernization of diets, ischemic heart disease, diverticular disease, hiatus hernia and cancer of the colon become common.

All epidemiological evidence suggest that these disorders result from contact with new environmental factors to which there has not been an evolutionary adaptation (Burkitt, 1976). One physiological characteristic that has been found to be closely associated with their prevalence is the size and consistency of stools. Large soft stools have invariably been found to be associated with low prevalences, whereas high prevalences are associated with small firm stools.

Many claims have been made regarding the benefits of dietary fiber. According to the Institute of Food Technologists' Expert Panel (IFT, 1979), dietary fiber is of definite value in relieving constipation problems, probable value in treating or preventing diverticular disease and possible value in reducing serum cholesterol and preventing other Western-risk diseases.

2.7.2 The effect of dietary fiber on gastrointestinal function

Eastwood and Kay (1979) have hypothesized that dietary fiber behaves as a sponge matrix with specific physiochemical properties dependent upon the structure and composition of the fiber components. The physiological

actions of dietary fiber, as it passes along the gastrointestinal tract, are dictated by the physiochemical properties of its components (Table 2.7) and may be quite different in different parts of the gastrointestinal tract.

Table 2.7 Physiological actions of fiber as it passes along the gastrointestinal tract (Eastwood and Kay, 1979)

Physiochemical properties	Type of fiber	Modifying
Gel formation	Pectin Mucilages	Gastric emptying Mouth to cecum transit Small intestinal absorption
Water holding capacity	Polysaccharides Lignins	Mouth to rectum transit Fecal weight Intraluminal pressure Fecal electrolytes
Matrix formation		Cecal bacterial metabolism
Bile acid adsorption	Lignin Pectin	Fecal steroids Cholesteral turnover
Cation exchange	Acidic polysaccharides	Fecal minerals
Antioxidant	Lignin	Free radical formation and action
Digestibility	Polysaccharides	Energy availability Chemical environment of colon Other physiochemical properties

Physiological actions of fiber in the small intestine are the result of gel formation, water holding capacity, cation exchange and bile acid adsorption (Eastwood and Kay, 1979). Because dietary fiber resists degradation by endogenous enzymes of the small intestine, structural changes are

relatively minor. However, the gel forming and water holding properties of dietary fiber may result in a reduced diffusion rate of soluble materials, such as glucose and cholesterol, towards the absorptive mucosal surface. Essential minerals as well as toxic materials may be irreversibly bound to dietary fiber as a result of its cation-exchange capacity and thus escape absorption in the small intestine. The addition of fiber to the diet is associated with reduced transit time. However, mouth to cecum transit time may be increased by gel-forming polysaccharides which result in reduced feedback inhibition of bile acid synthesis and increased size of bile acid pool.

Dietary fiber constituents, poorly digested by colonic microflora, form a matrix in the colon (Spiller and Sorensen, 1976). The matrix network changes the moisture and pH environment of the flora by diluting fecal components such as metabolites and bacteria, absorbing products of microbial metabolism in the colon and holding more or less moisture, cations and anions.

The right-hand side of the colon acts as a fermenter (Eastwood *et al.*, 1977). The sponge-like matrix contains bacterial enzymes from the colon, which are capable of selective degradations of fiber that can alter matrix structure and chemical environment of the colon (Eastwood and Kay, 1979).

Interactions may occur between bacteria, fiber and intestinal materials (Eastwood and Kay, 1979). Dietary fiber provides an adsorptive surface within the colon. Adsorption of bacteria and solutes to fiber may prevent or alter bacterial metabolism of the latter. Adsorption of other materials such as bile acids to fiber may prevent degradation by bacteria

and reabsorption from the colon. Fiber may thus alter the type and proportion of bile acids returning to the liver.

The role of the left-hand side of the colon is predominantly that of continence, the controlled evacuation of a formed stool (Eastwood and Kay, 1979). The laxative properties of dietary fiber are dependent upon the presence of a large, moist stool. The stool weight and consistency are determined by the water holding capacity of the sponge matrix and by the resistance of the matrix components to bacterial degradation. A relatively non-metabolized source of fiber that confines a large amount of water within a fiber or gel structure is most effective. Pectin, for example, has a high water holding capacity but does not promote stool bulk because it is degraded by colonic bacteria. The development of the stool depends on an ordered matrix structure provided by fiber that is kept lubricated by fat and bile acids. Methane, hydrogen and carbon dioxide, released during the bacterial degradation of fiber, may expand fecal bulk when facilitated by the presence of bile acids and fatty acids distributed through the fibrous matrix.

Generally, the physiological effects of dietary fiber on the gastrointestinal tract include reduced transit time, increased bulk of the stool and reduced intracolonic pressure.

Spiller and Amen (1975b) defined transit time as the time taken by an undigested particle to move from the stomach to the anus and be excreted in the feces. Brown bread passes from the stomach and through the intestines more rapidly than white bread (McCance *et al.*, 1953). The addition of wheat bran to the diet normalizes transit time. Slow transit times are accelerated while faster than average transit times are slowed down (Payler *et al.*, 1975). When transit time is reduced, nutrients move through the gastro-

intestinal tract faster and less time is allowed for digestion and absorption (Spiller and Amen, 1975b). Supplementation of diets with wheat bran results in increased fecal fat, nitrogen, energy and mineral excretion (Cummings, 1978). The mechanism of loss is through binding to fiber and through fermentation whereby nitrogen is incorporated into microbial matter (Van Soest and Robertson, 1976). The importance of nutrient loss is dependent on the nutritional adequacy of the diet being consumed.

Cummings *et al.* (1976) reported that adding wheat bran to metabolically controlled diets of six healthy volunteers resulted in increased fecal weight due mostly to increased water content, decreased mean transit time and increased excretion of fat, nitrogen, calcium and volatile fatty acids. Total bile excretion increased but the accompanying increase in fecal bulk resulted in a dilution effect with a net reduction in concentration.

Keim and Kies (1979) fed weanling mice semipurified casein-based rations altered by additions of hemicellulose, cellulose or lignin at levels of 5, 10 or 20% of the ration by weight. Diets contained similar amounts of protein and fat, but energy concentration varied. In general, as the level of fiber increased, feed consumption decreased as did weight gain, feed efficiency, protein efficiency, nitrogen balance, apparent digestibility of protein and fat, percent of fat in feces and percent carcass fat. Total fecal fat, fecal nitrogen and total fecal dry weight tended to increase with increased levels of fiber in the rations. Mice fed hemicellulose did not follow the same trends as mice fed the cellulose and lignin diets. The 10% hemicellulose group which had the best overall performance consumed more diet and thus gained more weight than did the 5 or 20% hemicellulose

group. This suggested a compensatory mechanism at the 10% hemicellulose level which was overburdened at the 20% level. Hemicellulose had a greater effect than cellulose and lignin in decreasing apparent digestibility of protein and fat. Liver abnormalities were noted in mice fed lignin but not in mice fed cellulose or hemicellulose.

Dintzis *et al.* (1979a) found compositional and morphological changes in brans of AACC wheat, dry milled corn and soybean hull after passage through the human alimentary tract. These materials, incorporated into bread to provide the major food fiber component of a controlled diet, were retrieved as identifiable particles from lyophilized feces of five healthy volunteers, analyzed for changes in major fiber components and examined by scanning electron microscopy. More than 90% of the corn bran was recovered and it seemed little affected by its journey. AACC wheat bran was recovered stripped of adhering endosperm and frequently of aleurone layer. The appearance of the wheat bran was changed greatly because remaining pericarp layers were folded or curled and approximately 15% cellulose and 60% apparent hemicellulose were lost from recovered material. Soybean hulls could be greatly disrupted by the human alimentary system with major losses of cellulose and apparent hemicellulose. Digestive effects on soy hulls differed greatly between individuals. In some cases cellulose and lignin were almost fully recovered, whereas apparent hemicellulose was about 50% recovered. Dintzis *et al.* (1979b) later reported that baking caused the bran particles of hard red spring wheat to curl and/or twist and that passage through the human gastrointestinal tract removed adhering endosperm and usually removed aleurone cell layers and degraded bran tissue.

Large numbers of epidemiological observations have shown that a log

function relationship (Figure 2.8) exists between fecal bulk and transit time (Burkitt *et al.*, 1974). Up to a certain fecal bulk of approximately 200 g feces per day, the bulkier the stool, the faster the transit time. Beyond this critical point, an increase in fecal mass has minimal effect on transit time. Many medical researchers believe a transit time of 2 to 3 days is desirable (Spiller *et al.*, 1977). Spiller and his co-workers (1977) have used the relationship between transit time and fecal weights to suggest that a recommended intake of dietary fiber would be that amount capable of inducing an average stool weight of no less than 140 to 150 g per day.

Bulky feces act as a physical buffer against intense muscle spasms which cause increased intracolonic pressure (Burkitt, 1976). The common pathogenic mechanism in such colonic diseases as diverticular disease, irritable bowel syndrome and ulcerative colitis is an intense spasm of the smooth muscle (Grimes, 1976). The strength of the spasm producing the increased pressure in the colonic lumen or wall and the length of time for which the colon has been affected probably determine the type of disease resulting.

Exaggerated muscular activity of the colon may lead to muscle thickening as well as to an increase in intracolonic pressure sufficient to force protrusions of the bowel lining mucosa through the overlying muscle wall (Figure 2.9).

2.7.3 The effect of dietary fiber on lipid metabolism

The adsorption of bile acids by dietary fiber has been previously discussed (Section 2.6.5). The hypocholesterolemic effect of certain plant fibers appears to be related to the enterohepatic circulation of bile.

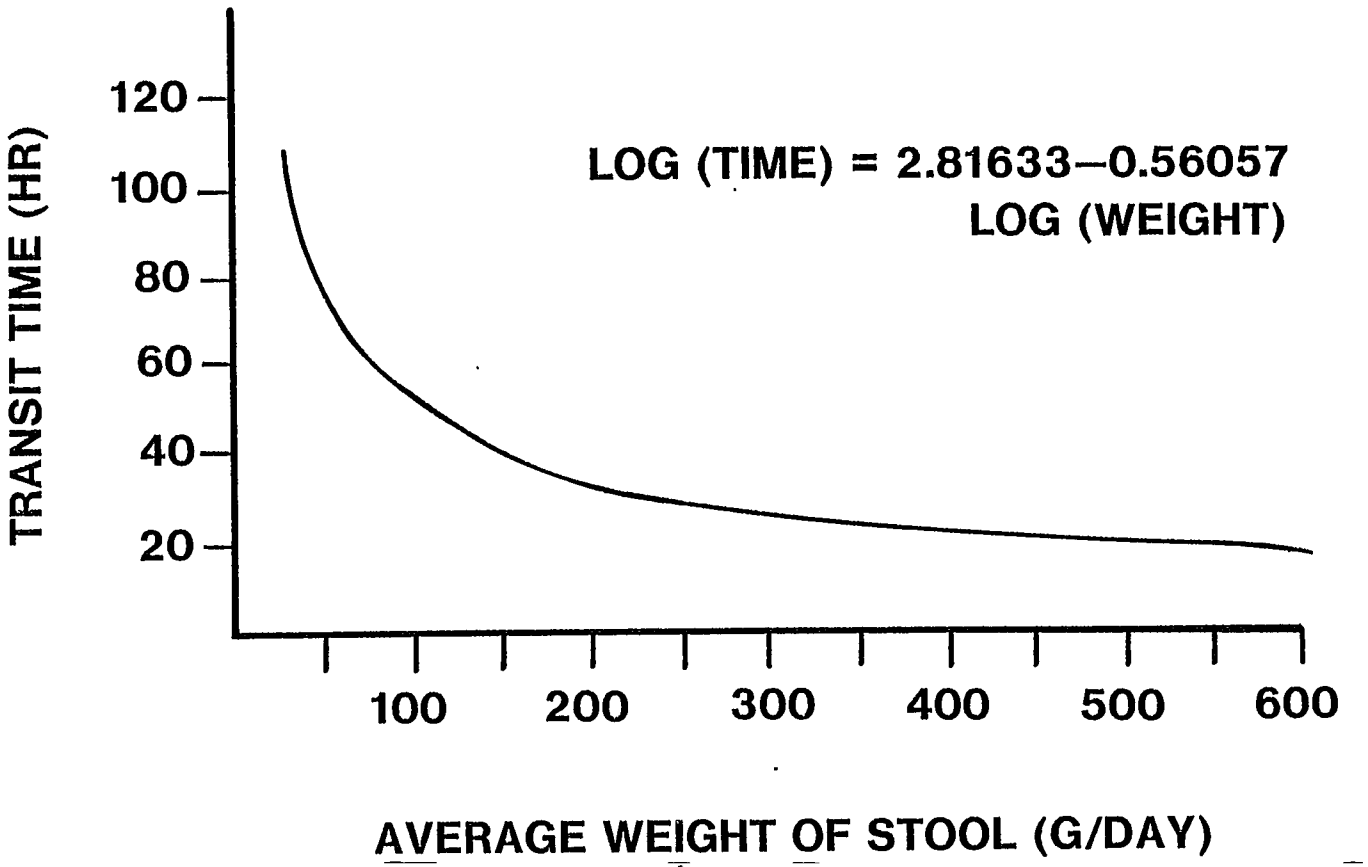


Figure 2.8 Relationship between intestinal transit time and stool weight (adapted from Burkitt *et al.*, 1974

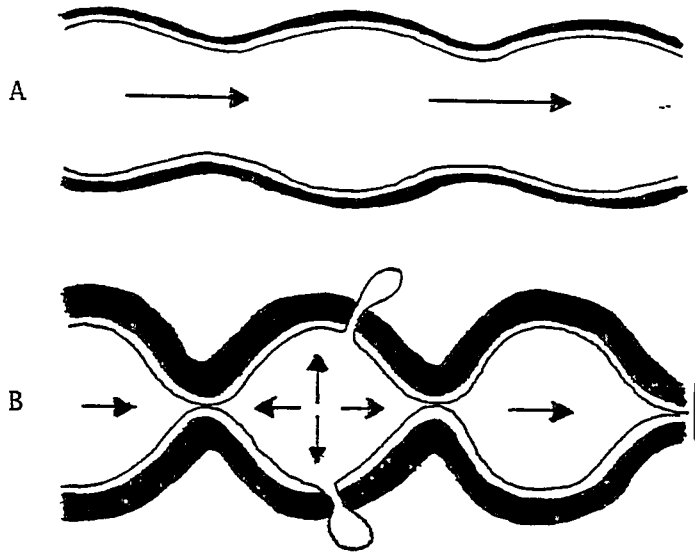


Figure 2.9 Diagrammatic representation of pathogenesis of diverticular disease of the colon. (A) The situation with soft bulky feces. (B) The situation with firm small feces (Burkitt, 1976)

Bile acids are synthesized from cholesterol in the liver, conjugated with taurine or glycine by an amide linkage and secreted into the duodenum where they aid in the absorption of lipids (Guyton, 1971; Hamilton, 1977; Lehninger, 1975). The primary site for bile acid reabsorption is the ileum where 80-90% of the conjugated bile acids are absorbed by an active transport mechanism. The small amount of conjugated bile acids which escape ileal absorption is degraded by bacteria and partially absorbed by passive non-ionic diffusion in the colon. After intestinal absorption the bile acids are transported to the liver via the portal vein and by means of this enterohepatic circulation gain access to the intestinal lumen again. The system is in a steady state. The rate of bile salt synthesis is controlled by feedback inhibition from the reabsorbed bile salts. Synthesis rate equals fecal loss of bile acids.

Ingesting certain types of high fiber diets causes an increased excretion of bile acids, sterols and fat (IFT, 1979). This implies that the fiber compounds "bind" bile acids thereby preventing absorption of cholesterol and fat as well as preventing reabsorption of bile acids. The body then draws upon its cholesterol stores to synthesize more bile acid, presumably resulting in a lowering of the blood (serum) cholesterol level.

Plant fibers influence the absorption and metabolism of triglycerides and fatty acids but these effects may not be apparent by measuring serum triglycerides after an overnight fast (Anderson and Chen, 1979). Modest reductions in fasting serum triglyceride values have been observed. A small triglyceride lowering effect was reported by Munoz *et. al.* (1979) in healthy men consuming soft white wheat bran, corn bran, soybean hulls or hard red spring wheat bran after consuming a low fiber basal diet. However, the

major effects of plant fibers on fasting triglyceride values appear to be in patients with fasting hypertriglyceridemia suggesting that dietary fiber may have a role in the treatment of patients with this condition (Anderson and Chen, 1979).

2.7.4 Physiological effects of dietary fiber from different sources

Different physiological effects can be produced by different sources of fiber varying in composition (Cummings, 1978). For example, gel-forming polysaccharides such as guar gum and pectin alter the pattern of glucose absorption and are hypocholesterolemic; fiber from cereal is not hypocholesterolemic, but exerts a pronounced effect on the colon. Insoluble plant fibers are relatively ineffective in lowering serum cholesterol while water soluble fibers are moderately effective (Anderson and Chen, 1979). Transit time is decreased by cellulose-containing fibers and increased by soluble fibers. Studies with bran and cellulose suggest that some responses may be dose-related.

Cummings *et al.* (1978) added approximately 20 g/day of concentrated dietary fiber from carrot, cabbage, apple, bran and guar gum to the controlled basal diet of 19 healthy volunteers. Fecal weight increased by 127% on bran, 69% on cabbage, 59% on carrot, 40% on apple and 20% on guar gum. These changes in fecal weight were correlated with an increased intake of pentose-containing polysaccharides from the fiber. On the basal diet there were pronounced individual differences in fecal weight, and from these the response of subjects to the fiber preparations could be predicted. Addition of fiber shortened mean transit time through the gut and significantly diluted an inert marker in the feces. According to Cummings *et al.*

(1978), diet-induced changes in colonic function may explain international differences in the prevalence of colonic disease, while personal variation in the response to dietary fiber may determine individual susceptibility to colonic disease within a community.

Stephen and Cummings (1980) continued the work of Cummings *et al.* (1978) and reported that 36% of the wheat bran fiber and 92% of the cabbage fiber were degraded in the gut, probably by colonic microflora. According to Selvendran *et al.* (1980), the mechanisms whereby wheat bran and cabbage fiber affect the human colon are different. Constituent fiber polysaccharides in wheat bran were not readily degraded by bacteria in the large intestine and therefore not fully metabolized due to the protective effect of lignin. Residual polysaccharides bind water and make a major contribution to the observed increase in fecal weight. Alternatively, cabbage fiber, rich in pectic substances but low in lignin, stimulated microbial growth. In this case, the increased fecal weight was due to increased bacterial mass.

Forsythe *et al.* (1978) fed adult male rats cellulose, wheat bran, wheat middlings, oat bran, oat flour, sugar beet pulp, soybean hulls and psyllium seeds for 28 days. All diets were formulated to contain similar amounts of nitrogen, fat and carbohydrate per kcal. Fibers extensively fermented by gut microflora as measured by increased volatile fatty acid excretion, caused an increase in fecal moisture and a decrease in intestinal transit time. Particle size of wheat bran and cellulose had no effect on fecal mass, moisture or dry matter. However, the cellulose with the larger particle size decreased intestinal transit time. No fiber source lowered serum cholesterol, but serum cholesterol was increased in rats fed 16 and

30 mesh wheat bran. The authors concluded that each fiber had a highly individualized effect on laxation but little effect on serum cholesterol in rats.

Ranhotra *et al.* (1977) substituted coarse, medium and fine wheat bran for the sucrose component of a hypercholesterolemic diet. Increasingly finer milling of bran caused a progressive decrease in diet, lipid and cholesterol intake of rats and in their body and liver weights. The serum lipid lowering effect along with the fecal cholesterol and bile acid losses were increased slightly with decreasing particle size.

Several studies have suggested that particle size is an important parameter in assessing the effect of cereal fiber on human colonic function (Brodribb and Groves, 1978; Heller *et al.*, 1980; Kirwan *et al.*, 1974). Generally, cereal fiber with the larger particle size was associated with larger fecal mass and fecal moisture and shorter mean transit times.

A rat-feeding experiment was conducted at Michigan State University to determine if cellulose in reduced-calorie high-fiber bread had a different effect on weight gain than did the same type of cellulose added to a dry ration (Colmey, 1978). Rats fed the reduced-calorie high-fiber bread gained less weight than rats fed regular enriched white bread. Weight gains and feed intakes tended to be slightly higher when cellulose was fed as a separate entity with regular bread rather than incorporated into high-fiber bread. This suggested a satiety effect for the high-fiber breads.

Mickelsen *et al.* (1979) used cellulose-containing bread in a weight reduction program for overweight college-age men. A similar group was fed regular white bread and results were compared. The reduced-calorie bread had greater satiety value than the regular bread. There was a greater

recovery of cellulose in the stool and greater fecal loss of energy. Absorption of fat and protein was not impaired.

Wyman *et al.* (1976) reported that cooked wheat bran had less effect on the intestine of healthy men fed a low fiber basal diet than did a comparable amount of raw bran. Raw bran (12 and 20 g/day) increased fecal dry weight. Only the higher dose decreased transit time and increased fecal volume. There was no significant effect on stools when up to 22 g cooked bran was ingested per day.

Physiological effects of dietary fiber in humans have been extensively reviewed by Kelsay (1978). Bulkier stools were reported when wheat bran, bagasse, cellulose or fruits were added to the diet. Bile acid excretion was increased by bagasse and Bengal gram. Serum cholesterol levels were lowered by pectin, rolled oats, guar gum and legumes, but not by wheat fiber, bagasse or cellulose. The serum cholesterol lowering effect was more pronounced when the cholesterol levels of the subjects had been increased by a high fat or high cholesterol diet.

According to Truswell (1977), serum cholesterol can be lowered by lignin, pectin, guar, psyllium seed gum, locust bean gum, carrageen, carboxymethyl-cellulose, rice bran, rolled oats and some legumes. Wheat bran, cellulose and bagasse do not have a serum cholesterol lowering effect.

The fecal bulking action of dietary fiber appears to be independent of its hypocholesterolemic effect (Jenkins *et al.*, 1979). Generally, efficient bulking agents such as wheat bran have no effect on serum cholesterol, whereas refined fiber preparations such as guar and pectin that lower serum cholesterol produce only small increases in daily stool output.

2.8 FIBER IN FOODS

Although consumers are now demanding high fiber foods, some caution should be exercised in choosing a suitable fiber supplement. Studies concerning supplementation of bread with different fibers are reviewed.

2.8.1 Market demand for high fiber foods

In recent years the attention of medical researchers, nutritionists, chemists and food technologists, as well as that of the lay press, has been directed towards dietary fiber. Although medical findings have not conclusively established a cause and effect relationship between dietary fiber and degenerative disease, interest has grown in increasing levels of fiber in the North American diet and many fiber-supplemented foods have appeared on the supermarket shelves (Spiller *et al.*, 1978). Manufacturers of bran-based breakfast cereals were among the first in the food industry to react to the impact of fiber in their advertising campaigns by emphasizing their product's crude fiber content along with the laxative effects of bran. Bakers soon followed the cereal manufacturers by providing consumers with fiber-enriched baked goods and emphasizing their product's reduced caloric content. Although fiber is generally added to breakfast cereals and breads, there is some interest in utilizing the functional properties of dietary fiber in other bakery products, meat systems, pastas, snack foods, diet breakfast drinks, salad dressings and toppings (Anon., 1977; McCormick, 1976).

After determining the neutral detergent fiber content of 44 kinds of bread, Mongeau and Brassard (1979) suggested that the replacement of white bread by breads of higher fiber content would be a practical way to

gradually increase the intake of dietary fiber.

Consumers are becoming increasingly receptive to high-fiber breads. The "back-to-nature" movement of the 1970s resulted in a small decline in the U.S. production of white bread and a dramatic increase in the production of variety breads (Mrdeza, 1978). Technology made white bread the basic staple for most bread eating peoples of the world and variety breads such as rye, whole wheat, bran and raisin have traditionally shared a smaller portion of the market. Today white bread on the table is no longer a sign of affluence and dark breads have become a contemporary status symbol. Fiber breads are relatively new to the variety bread market and have become associated, for the most part, with "naturalness." Their popularity was assured by reports in the lay literature concerning the importance of dietary fiber to good health.

2.8.2 Criteria for choosing a suitable fiber supplement

Factors to be considered in choosing a suitable fiber supplement include its cost, shelf life and availability as well as its effect on the color, flavor and texture of the food product (Dubois, 1978). Cereal brans, for example, should be added to foods where the natural whole grain flavor and the color and texture imparted will not be a problem (Colmey, 1978).

Often, high levels of dietary fiber are concentrated in plant materials that, until recently, have been regarded as animal feed or agricultural wastes. Before using these materials for human studies, consideration should be given to their suitability for food use, their physical form and the problems associated with hydrophyllic gums (Mullen, 1978). Also attention should be given to any legal restriction which may

impair use of the chosen fiber supplement.

Suitability for food use

Any fibrous material fed to humans should not contain dangerous levels of microorganisms, mycotoxins, pesticide residues or heavy metals (Mullen, 1978). Special care should be given to sanitation during processing and storage. There should be no evidence of insect and rodent contamination.

Physical form of food fiber

Some fiber-rich materials disintegrate into sharp, needle-like particles upon grinding (Mullen, 1978). Caution is urged in using materials such as bagasse due to the possibility of physical damage to the alimentary tract.

Caution should be exercised in feeding humans large quantities of oat hulls (Mullen, 1978). Oat hulls, unlike other cereal grains, contain a spiculum that is relatively hard and might cause irritation depending on the grinding and cooking procedure used.

Hydrophyllic gums

Concentrated hydrophyllic dietary fiber sources such as the plant gums and mucilages form viscous solutions upon hydration (Mullen, 1978). If large portions of these sources are consumed in a dry form, subsequent hydration and swelling in the gastrointestinal tract can lead to blockage.

Legal requirements

In Canada, use of fibrous components of plant materials must comply with the Food and Drugs Act and Regulations. Fiber supplements can be used

in any food for which a standard is not prescribed in the Food and Drugs Regulations providing these foods meet the general provisions of the Act dealing with matters of safety and deception (Sarwar, 1978). There are no special provisions regarding the presence of antinutritional factors in high-fiber raw materials.

Legally, fiber breads should be considered specialty breads and not replacements for white bread. White bread is considered to be a standard product and its ingredients are regulated by the Canadian Food and Drugs Regulations. According to Section B.13.021(i) of the Act, white bread is the food made by baking a yeast-leavened dough prepared with flour and water, and it may contain not more than 5% by total weight of whole wheat flour, entire wheat flour, graham flour, wheat meal, wheat starch, nonwheat flour, nonwheat meal or nonwheat starch, any of which may be wholly or partially dextrinized. According to Section B.13.029, specialty breads may contain ingredients listed in Section B.13.021(i) in an amount greater than 5% along with fruits, nuts, seeds and special flavoring ingredients not provided for in Section B.13.021(i).

Microcrystalline cellulose is considered to be a food additive under the Canadian Food and Drugs Act and Regulations. Its use as a dietary fiber source is restricted to that of a filler in calorie-reduced dietetic foods which are defined in Section B.24.006 as foods that provide not more than 50% of the calories normally present. In the United States, microcrystalline cellulose is considered a GRAS substance under the 1938 Federal Food, Drug and Cosmetic Act (Anon., 1978). Foods with one-third calorie reduction can bear reduced calorie claims.

There is no policy on dietary fiber claims in Canada at the present time due to the lack of standard methods for determining dietary fiber, and the lack of conclusive experimental data on the physiological

significance of dietary fiber and its various components (Sarwar, 1978).

Since there was evidence in the literature to establish the effectiveness of bran as a source of dietary fiber and many of the bran-based cereals had been promoted for years as a source of "bulk" in the diet, Food Directorate's Bureau of Nutritional Sciences have recommended to the Department of Consumer and Corporate Affairs that the manufacture of any food providing 18 g bran per reasonable daily intake (RDI) could make a claim to the effect that the food contains bran, a source of dietary fiber (Sarwar, 1978). The value of 18 g was selected because it is approximately the amount of bran found in a RDI (25 g) of the traditional bran cereals. Health Protection Branch would consider extending fiber claims when appropriate data were available.

2.8.3 Fiber in bread

Fiber can be added to foods to dilute calories, to act as preventative medicine against degenerative disease or for the functional properties of the particular fiber source. High-fiber ingredients differ in functional characteristics such as water and fat absorption, as well as in their nutrient concentrations, including protein and the component amino acids (Anon., 1977). By incorporating a blend of high-fiber ingredients, the end food product can be formulated to contain a higher nutritional level while also retaining or increasing the fiber content to or above that of whole grain.

According to Scala (1974, 1975) fabricated foods offer a unique opportunity for fiber additions since fiber's water binding and absorptive properties exert a significant effect on the organoleptic characteristics of the food product. The questions to be asked by food technologists include what kind of fiber, how much fiber, in what food systems and how

should it be balanced (Scala, 1975).

Much has been written about the physiological benefit of dietary fiber and its relationship to disease, but few publications discuss the technology of adding fiber to food (Spiller *et al.*, 1978). This may be due to the unavoidable lag between medical and nutritional findings and the application of this information to new or modified food ingredient systems, and subsequent publication of such work. Also knowledge in this area has been restricted through patent protection and/or industrial classified information, effectively restraining the flow into the literature for two to three years or longer.

Khan *et al.* (1976) replaced 0, 5, 7.5, 10, 12.5 and 15% of wheat flour in bread with coconut residue. The formula included 0.5% sodium stearoyl-2-lactylate and 2% nonfat dry milk. Water absorption increased with the addition of coconut residue. Acceptable bread was obtained with up to 7.5% of the coconut residue in the bread formulation.

Lorenz (1976) studied the feasibility of using triticale bran by replacing 0, 5, 10 and 15% of the wheat flour with coarse and fine triticale bran and rye bran. Addition of 10 and 15% triticale and rye bran increased mixing time and decreased proofing time. There was no decrease in loaf volume even at the 15% level of replacement. Breads baked with the fine bran had a more uniform grain and a smoother texture than those baked with the coarse bran.

Volpe and Lehman (1977) produced an acceptable high-fiber bread using the 70/30 sponge dough method by replacing 10% of the flour with a blend of 88.6% purified cellulose and 11.4% vital wheat gluten added to the dough side. The dough ingredients included 75 ppm potassium bromate

and 0.5% sodium stearoyl-2-lactylate.

Pomeranz *et al.* (1977) studied the effects of seven celluloses, four wheat brans and two oat hulls on functional properties of bread by replacing 0, 3, 5, 7, 10 and 15% of the flour. Water absorption was reduced by oat hulls, but increased by wheat bran and cellulose. The addition of up to 5% fiber materials decreased loaf volume to an extent expected from dilution of functional gluten proteins. At levels above 7%, fiber materials decreased loaf volume much more than expected from gluten dilution. The large decrease resulted from lowered gas retention rather than from unsatisfactory gas production. Oat hulls imparted an objectionable gritty texture to bread. Bread taste and mouthfeel were slightly modified by cellulose. Wheat bran modified taste and mouthfeel but the modification was not objectionable. Overall effects on color from added fiber materials were smallest for the celluloses and largest for wheat bran.

Subsequently, Pomeranz *et al.* (1976) studied the effects of AACC certified food grade wheat bran and two samples of brewer's spent grains on functional properties of bread by replacing 0, 3, 5, 7, 10 and 15% of the wheat flour. The bran-enriched bread was superior in loaf volume, crumb grain and crumb color to bread in which flour was replaced with spent grains. About 7% white wheat bran could be added without significantly modifying characteristics of an acceptable white bread.

D'Appolonia and Youngs (1978) studied the effects of oat bran and wheat bran on dough properties and bread. At equal levels of addition, loaf volume was lower for oat than for wheat bran. However, bread containing 10 or 20% oat bran was more acceptable to panelists than was bread containing the corresponding level of wheat bran.

Lorenz (1978) evaluated the baking properties of confectionary sunflower seeds. Sunflower hulls along with raw or roasted whole and dehulled meal from the variety Greystripe replaced 1, 3, 5, 10 and 15% of the wheat flour in pup loaves baked by the straight dough procedure. The addition of ground sunflower hulls resulted in breads with poor grain, texture and flavor. However, satisfactory loaves were produced with blends of wheat flour and up to 10% replacement of raw, whole or dehulled sunflower meals. Roasting the seeds prior to grinding produced a more open grain than was observed in breads baked with the non-heat-treated products. The dehulled, roasted meal caused a more dramatic decrease in bread volume with higher levels of substitution than was measured previously with products which were not roasted. The roasted, whole sunflower meal did not have this volume-depressing effect. Roasting improved the flavor of the sunflower breads. Panelists described the flavor of the breads containing roasted sunflower products as "nutty" and preferred the flavor of these loaves over that of the white bread control.

Satin *et al.* (1978) of Steinberg Foods Limited, Montreal evaluated several sources of natural vegetable fiber (microcrystalline cellulose, sugar beet pulp, hulls of yellow field peas, malt hulls, wheat bran and alfalfa) for their applicability in the preparation of a white bread. Baking studies, chemical analysis and physiological experiments were used to characterize the various sources. The conventional sponge and dough method was used and an acceptable product was made by adding 7.5% of 20-40 mesh pea hulls at the dough stage. The bread was later marketed in Ontario as "Le Pain Quotidien" or "Daily Bread", a commercial natural fiber white bread containing pea hulls.

The practical application of adding fiber to breads has been discussed by Dubois (1978). Major types of commercial high-fiber breads are listed in Table 2.8. Aside from the traditional whole wheat loaf, wheat bran, corn bran, soy bran and powdered cellulose are the major fiber sources being used in bread today. Of these, wheat bran and powdered cellulose are most common.

Table 2.8 Types of commercial high-fiber breads (Dubois, 1978)

Fiber source	Marketing approach	Relative fiber content
Multiple grain Multiple fiber source No cellulose	All natural	Slightly more than whole grain
High amounts of grain or legume fiber No cellulose	Relatively high crude fiber All natural	Slightly higher than above class
Whole grain and/or grain fiber and/or legume fiber plus	Calorie reduction High crude fiber Fiber-rich appearance	Fiber content several times that of whole wheat bread
Cellulose as major fiber source	Calorie reduction Highest crude fiber	Highest of this type bread, several times that of whole wheat bread

The addition of more than 7-10% of any of the fibrous materials produces major changes in processing techniques and quality characteristics of bread (Dubois, 1978). Gluten is diluted, which causes a weakening of the cell structure. Also, the fibrous materials tend to cut the gluten strands, thereby reducing gas retention. Some fiber materials have a high flavor content, thus altering the normal flavor of the product.

Adjustments must be made for the high level of fiber material and the high absorption requirement for these materials (Table 2.9).

Table 2.9 General formulation for commercial high-fiber breads[1]

Ingredient	Type	Amount (%)
Flour	High protein Spring wheat or Spring/Winter blend	100.0
Water		115.0-120.0
Vital wheat gluten		10.0
Yeast		3.0-5.0
Yeast food	Bromate type	0.5-0.75
Salt		2.5-3.0
Sugar	Sucrose, dextrose, high fructose, brown sugar, molasses	Total, 8-10
Fiber	Powder cellulose, wheat bran, soy bran, corn bran	Total, 15-25
Milk products	NFDM or milk replacer	3.0
Shortening		0
Sodium stearoyl-2-lactylate		0.5
Potassium bromate		50-75 ppm
Calcium propionate		0.25-0.50

[1] Adapted from Dubois (1978)

Although breads with added fiber are being produced successfully using the straight dough and pre-ferment procedures, most fiber-supplemented breads are produced by the sponge-dough method (Dubois, 1978). In most

breads 60-70% of the flour is used in the sponge and 40-30% in the dough. Compared to white bread, sponge fermentation time is generally reduced to 2.5-3.5 h and additional dough mixing time is required to fully develop the dough. Requirements for water, yeast, sugar, salt and oxidizing agents are increased by the addition of fiber to bread (Table 2.9). If more than 7-10% fiber is added to the formula, vital wheat gluten is needed to maintain a quality product. Dough conditioners which strengthen gluten have been found beneficial. Milk products are added primarily for nutritional benefits. Shortening is often removed from the formula so that the bread can be sold as a reduced calorie food. Normal to slightly high levels of calcium propionate are recommended since, due to their high moisture content, high-fiber breads are susceptible to mold growth.

3. MATERIALS AND METHODS

3.1 PREPARATION OF FIBER SUPPLEMENTS

Sources of dietary fiber used in this study included sunflower heads, psyllium seeds, flax hulls, mustard hulls, wheat bran, sunflower hulls, pea hulls and microcrystalline cellulose (Figure 3.1). As well as these dietary fiber sources, commercial straight grade and whole wheat flours, used to study the effect of fiber supplementation on breadmaking, have been included with the chemical and physical analysis of fiber supplements.

Fiber supplements were prepared from sunflower heads, flax hulls, mustard hulls, wheat bran and sunflower hulls using available laboratory equipment. As part of the preparation procedures, the proximate composition of each subfraction was determined by standard AACC (1962) and AOAC (1975) procedures (Section 3.2.1) and the concentration of dietary fiber was estimated as buffered acid-detergent fiber (Baker, 1977).

3.1.1 Straight grade wheat flour

Baker's flour (not enriched, not improved) from hard, red spring wheat was obtained from Saskatchewan Wheat Pool Flour Mill, Saskatoon, Saskatchewan.

3.1.2 Whole wheat flour

Commercial whole wheat flour from hard, red spring wheat was obtained from the Saskatchewan Wheat Pool, Saskatoon, Saskatchewan. This source contained 45 ppm potassium bromate plus Maturox (azodicarbonamide).

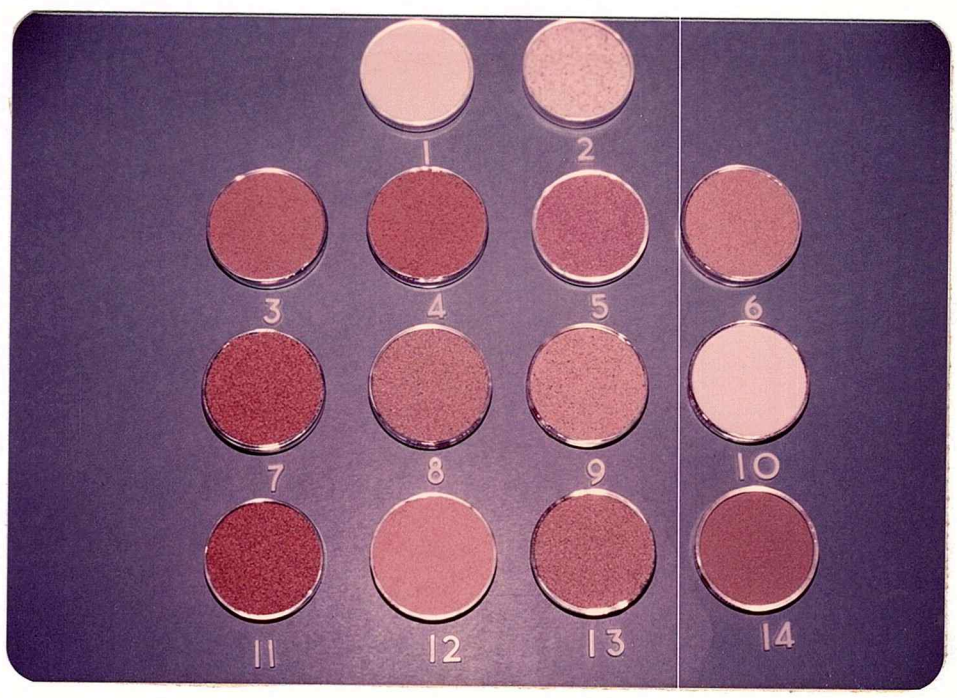


Figure 3.1 Fiber supplements

1. straight grade wheat flour
2. whole wheat flour
3. sunflower heads
4. psyllium seeds
5. flax hulls
6. mustard hulls
7. wheat bran (coarse)
8. sunflower hulls (coarse)
9. pea hulls
10. cellulose
11. wheat bran (coarse)
12. wheat bran (fine)
13. sunflower hulls (coarse)
14. sunflower hulls (fine)

3.1.3 Sunflower heads

Sunflowers are presently grown for their seeds as a source of vegetable oil, feed protein supplement and confectionary ingredient. After harvest of seeds, heads and stalks are left on the ground as agricultural wastes. This waste material is rich in pectic substances. Lin *et al.* (1975) reported that the total pectin content of the head, bracts, neck and stalk of the field-grown Peredovik sunflower were 19, 11, 7 and 5% respectively, but the stalk accounted for 45% of the total plant pectin. The level of pectin in four cultivars varied from 15 to 24% in the heads and 4 to 7% in the stalks, depending on the maturity stage at harvest, cultivar and weight of plant parts. Although pectin can be extracted from sunflowers (Kim *et al.*, 1978a, 1978b; Lin *et al.*, 1976, 1978; Sabir *et al.*, 1976), it was hoped that the head and stalk could be used directly as a fiber supplement.

The heads used in the present study contained about 15-20 cm of neck and stalk as well as the bracts to maximize the proportion of pectin-containing material. The first step was to separate the plant material components according to their tissue structure (Figure 3.2). Each fraction was ground to pass through a 60 mesh (250μm) US sieve and proximate analysis was carried out (Table 3.1). Small fractions which could not be ground with available equipment were discarded. Since high levels of buffered acid-detergent fiber were found in all sunflower head fractions, a composite sample, 1976 high cut, containing 60% heads and 40% neck and stems (Lee, 1978) was used in this investigation.

The large heads and stalks were first broken in a Wiley mill that had the screens removed, and then ground in a Jacobson hammermill, Model 160-D through a $\frac{1}{16}$ in screen once and three times through a $\frac{1}{32}$ in screen.

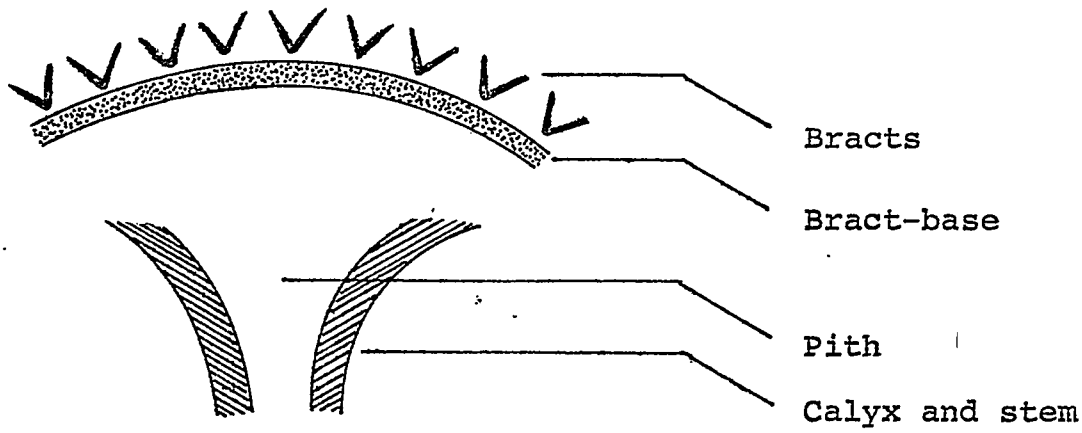


Figure 3.2 Fractionation of sunflower heads

Table 3.1 Analysis of sunflower head fractions

	Particle size (μm)	Yield (%)	Crude protein (%N × 6.25)	Ether extract (%)	Ash (%)	Crude fiber (%)	N-free extract (%)	Buffered acid-detergent fiber (%)
Bracts	<250	94	5.4	4.3	9.2	30.8	50.3	65.7
Bract-base	<250	98	9.3	4.5	18.0	14.2	54.0	60.3
Pith—coarse	>250	16	10.7	3.2	13.7	24.4	48.0	49.6
—fine	<250	84	9.1	1.9	17.1	15.2	56.7	72.8
Calyx and stem	<250	91	7.6	2.1	15.3	23.7	51.3	76.7
Composite—coarse	>250	35	6.1	2.4	13.8	27.1	50.6	59.1
—fine	<250	65	6.2	2.8	16.6	19.5	54.9	50.0

After each pass through the hammermill, the ground material was screened through a 60 mesh US sieve using a Kason Centri-Sifter and the coarse fraction was reground (Table 3.1).

Due to the splinter-like appearance of the coarse fraction only the fine fraction was considered suitable for use as a source of dietary fiber in foods. Consequently, in later investigations only the fine fraction was considered.

3.1.4 Psyllium seeds

Psyllium seeds were obtained commercially from Canadian Soya Industries Ltd, Vancouver, B.C. and whole seeds were ground through a 1 mm screen in a Cyclone Sample Mill.

3.1.4 Flax hulls

Flax seeds (Dufferin cultivar) were obtained commercially from Early Seed and Feed, Saskatoon. Hulls were separated from extracted linseed meal using the liquid cyclone fractionation process described by Sosulski and Zadernowski (1980).

Fractionation process

Flax seed was first flaked by roller milling (Figure 3.3). Linseed oil was extracted from the flaked seed using Skelly B (bp = 60-68°C) and a soxhlet apparatus for 10-16 h. Following extraction, solvent was removed by placing the defatted meal on mesh trays in a fume hood for several days. Defatted linseed meal was pinmilled, suspended in Skelly B and separated into hull and flour fractions using a liquid cyclone. Residual solvent was removed as previously described.

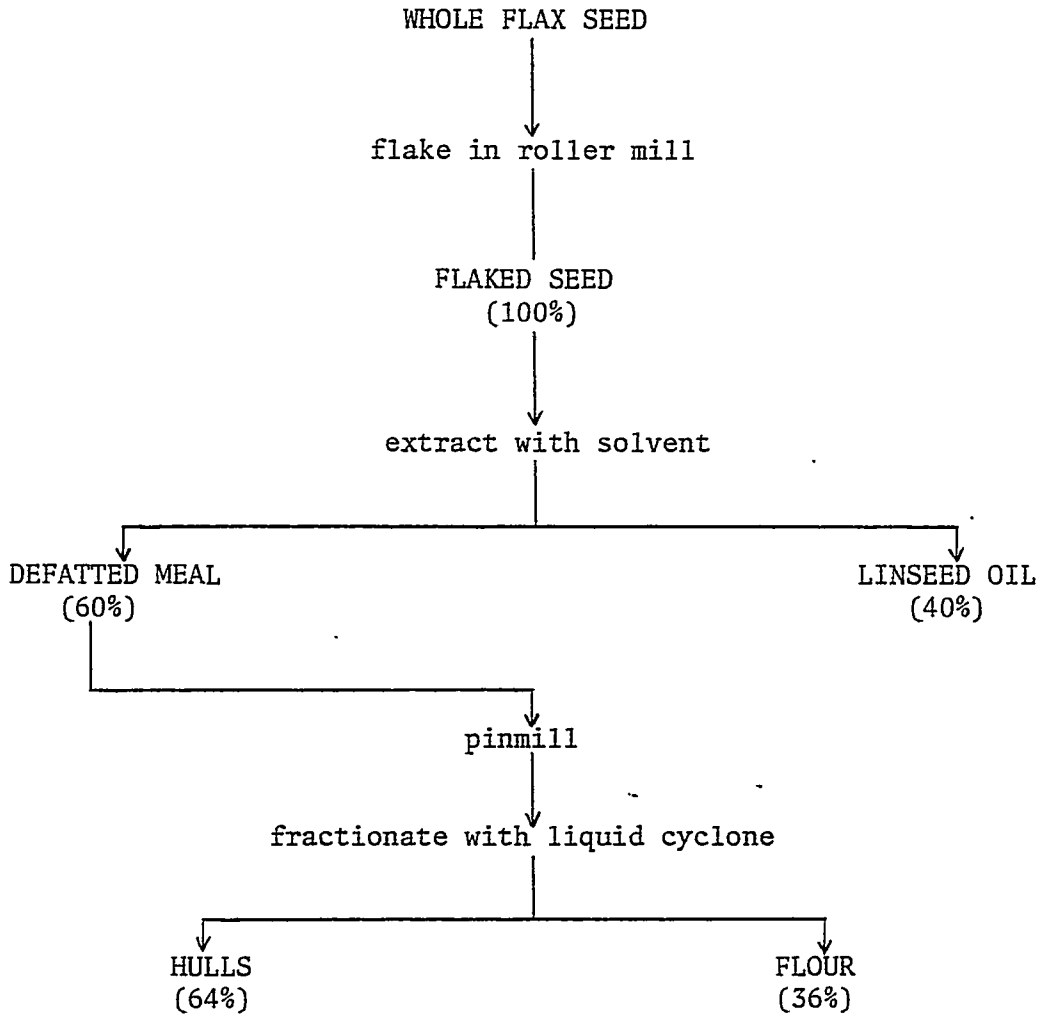


Figure 3.3 Separation of flax hulls and flour by fractionation process

Modified fractionation process

Since only hulls from flax seeds were of interest in this investigation, the Sosulski and Zadernowski (1980) procedure was slightly modified by removing fine flax meal, that contained a limited quantity of hull material from the flaked seed, prior to the extraction of linseed oil. The modified fractionation procedure was a faster method of preparing flax hulls since only the hull-rich fraction needed to be extracted.

Flax seed was flaked in a roller mill (Figure 3.4). Fine material which passed through a 20 mesh US sieve was removed. The remaining hull-rich fraction was partially defatted by immersing the sample in a large container with Skelly B and allowing it to soak 24 h. The sample was stirred occasionally. After 24 h the partially defatted meal, oil and solvent were filtered through a coarse fritted glass filter under vacuum. Solvent was removed from the partially defatted meal prior to pinmilling and separation into hull and flour fractions by the Sosulski and Zadernowski (1980) procedure.

Analysis of flaxseed fractions

Use of the modified fractionation process was justified since both fractionation procedures for separating flax hulls from linseed meal had similar calculated yields: approximately 40% defatted flax hulls, 20% defatted flax flour and 40% linseed oil. The theoretical yield of full fat hulls, determined by hand dehulling 25 flax seeds, was 53% by weight. If the level of linseed oil in full-fat flax hulls was also 40%, the theoretical yield of defatted flax hulls from flaked linseed meal would be 32%. Since the calculated yield of defatted flax hulls was higher, the flax hull fiber supplement used in this investigation contained some flax flour and

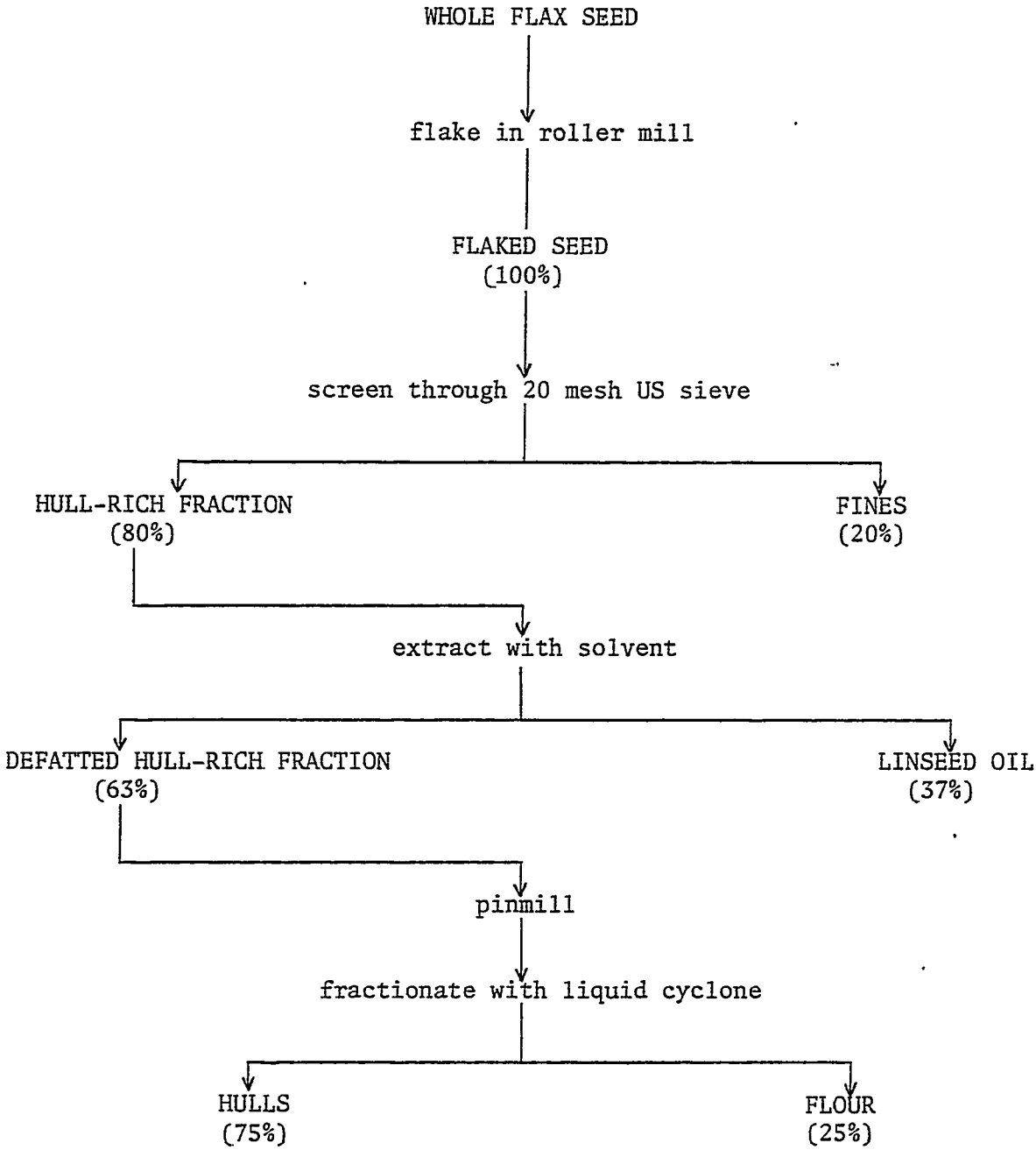


Figure 3.4 Separation of flax hulls and flour by a modified fractionation process

was not a pure hull fraction. Flax hulls prepared by the modified fractionation procedure contained a higher level of dietary fiber as measured by buffered acid-detergent fiber and lower level of protein (Table 3.2). This would suggest that the prepared hull fraction contained marginally less flour contamination than if hulls were prepared by the original Sosulski and Zadernowski (1980) procedure.

3.1.6 Mustard hulls

Yellow mustard seed (Sabre cultivar) was obtained locally from Saskatchewan Wheat Pool, Saskatoon, Saskatchewan and hulls were separated from fat-extracted meal by an aspiration process (Figure 3.5).

Mustard seed was first flaked by roller milling, then partially defatted by immersing the flaked seed in a large container with Skelly B for 24 h. After 24 h the partially defatted meal, oil and solvent were filtered through a coarse fritted glass filter under vacuum. Solvent was removed by placing the partially defatted meal on mesh trays in a fume hood for several days prior to the aspiration process. Fine material which passed through a 20 mesh US sieve was removed. The remaining hull-rich fraction was aspirated twice using a North Dakota Blower allowing 10-15% air inlet. The aspirated mustard hulls contained 5.0% ether extract (Table 3.3), which was considered to be too high for good storage. After the partially defatted hulls were ground in a KT grinder, residual oil was removed by solvent extraction using a soxhlet apparatus and Skelly B for 10-16 h.

3.1.7 Wheat bran

Commercial germ-free wheat bran from hard, red spring wheat was

Table 3.2 Analysis of flax seed fractions

	Crude protein (%N × 6.25)	Ether extract (%)	Ash (%)	Crude fiber (%)	N-free extract (%)	Buffered acid-detergent fiber (%)
Fractionation process						
Flaked meal	22.6	46.4	2.4	5.4	23.2	11.3
Defatted meal	37.8	6.2	4.2	9.4	42.4	20.9
Hulls	32.5	1.1	4.9	12.6	48.9	25.1
Flour	55.8	1.8	4.6	4.5	33.3	13.9
Modified fractionation process						
Flaked meal	22.6	46.4	2.4	5.4	23.2	11.3
Fines	25.1	56.0	2.7	2.7	13.5	5.6
Hull-rich fraction	21.9	43.4	2.4	6.3	26.0	13.0
Defatted hull-rich fraction	28.9	25.2	3.2	8.4	34.3	17.1
Hulls	20.3	2.0	4.1	16.4	57.2	30.4
Flour	60.0	3.5	4.8	3.5	28.2	10.3

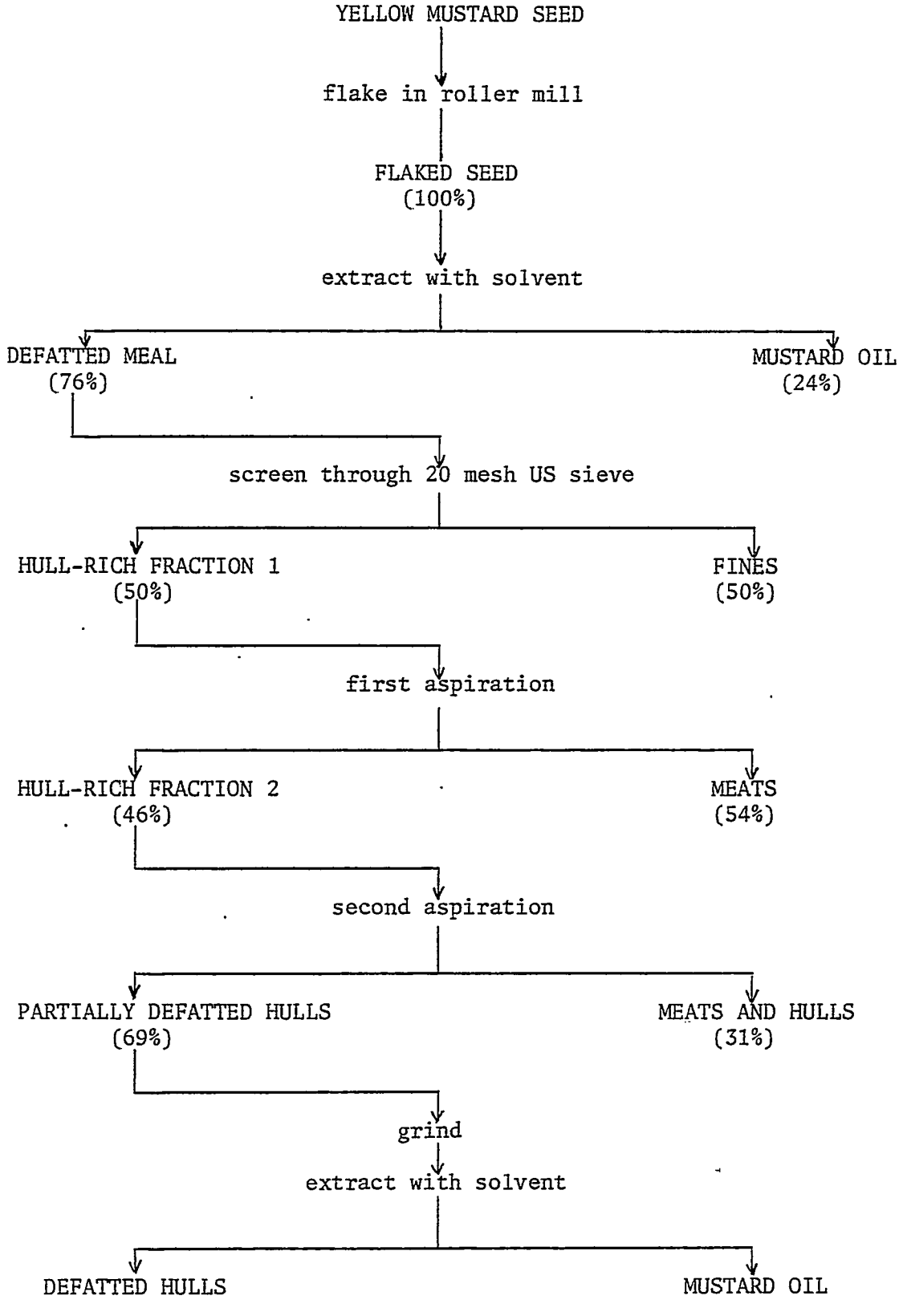


Figure 3.5 Preparation of yellow mustard hulls

Table 3.3 Analysis of yellow mustard meal and hulls

	Crude protein (%N × 6.25)	Ether extract (%)	Ash (%)	Crude fiber (%)	N-free extract (%)	Buffered acid-detergent fiber (%)
Flaked meal	32.5	29.0	4.4	6.8	27.3	13.0
Defatted meal	40.9	5.4	5.8	10.0	37.9	20.8
Hand selected hulls						
—full fat	18.4	8.2	5.1	23.7	44.7	54.9
Aspirated hulls						
—partially defatted	17.7	5.0	4.9	24.4	48.0	51.9
—defatted	17.5	0.1	4.9	27.1	50.4	62.0

obtained locally from Saskatchewan Wheat Pool, Farm Services Division, Saskatoon, Saskatchewan. The wheat bran was ground twice in a Jacobson hammermill Model 160-D through a $^{1}/_{32}$ in sieve and screened through a 60 mesh US sieve using a SWECO separator (Figure 3.6). Overs were reground twice and screened once again. Remaining overs were reground three times and screened once more. The ground material was screened through 40 and 60 mesh US sieves. The result was a fine, powdery product with a lower ash and crude fiber content, and a coarser granular product with a higher ash and crude fiber content compared to the whole wheat bran (Table 3.4).

3.1.8 Sunflower hulls

Commercial confectionary type sunflower seeds were obtained from Early Seed and Feed, Saskatoon, Saskatchewan and later mechanically dehulled. This source contained 50% hulls by weight.

One of the major problems in using sunflower hulls as a fiber source was to find a suitable method for reducing particle size. Sunflower hulls tended to form splinters when ground, which were not reduced by further grinding. Breads prepared in a preliminary investigation with pinmilled sunflower hulls were unacceptable due to their grey-green color and coarse mouthfeel. Therefore, it was necessary to remove color and splinters from the ground sunflower hull product. The dark pigments were removed by a flotation process and the splinters were removed by screening (Figure 3.7).

After pinmilling, the ground sunflower hulls were suspended in water (approximately 12.5% solids) to form a slurry. The ground material was allowed to settle until three distinct layers formed: a supernatant layer, a layer of dark pigmented material and finally, a layer of light colored material. The supernatant and dark fractions were decanted. The light

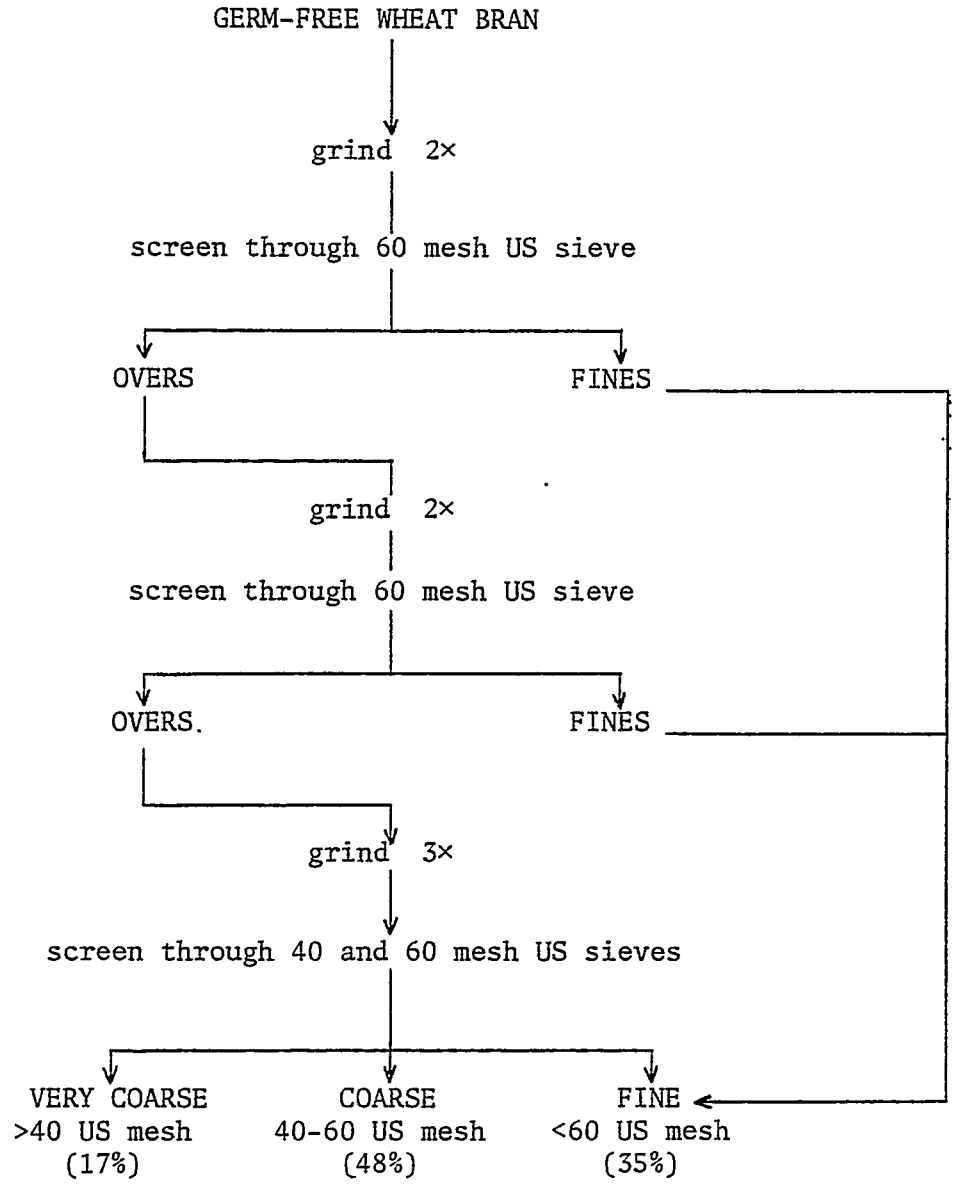


Figure 3.6 Fractionation of wheat bran

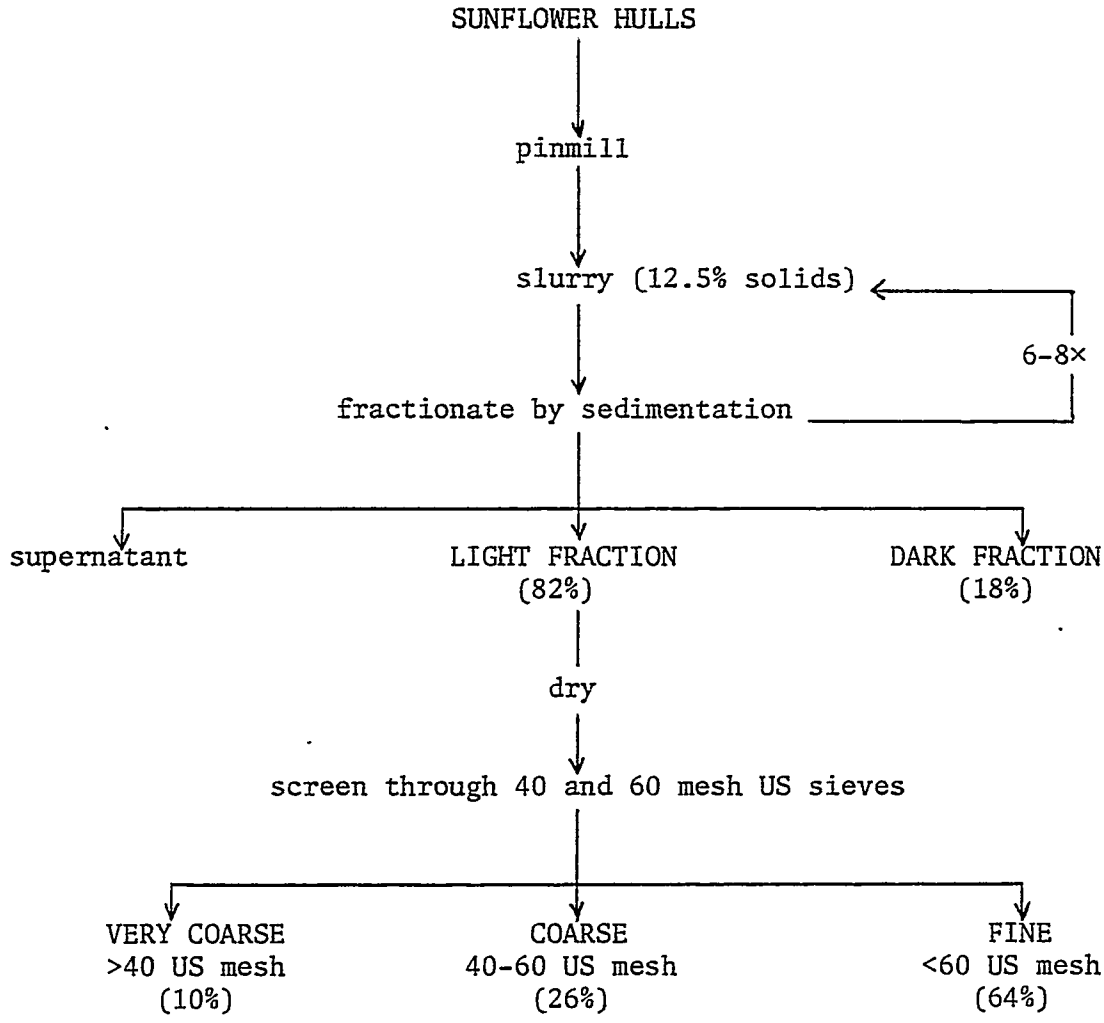


Figure 3.7 Fractionation of sunflower hulls

Table 3.4 Analysis of wheat bran

	Crude protein (%N × 6.25)	Ether extract (%)	Ash (%)	Crude fiber (%)	N-free extract (%)	Buffered acid-detergent fiber (%)
Whole bran	17.6	4.9	5.4	9.8	62.3	27.8
Bran fraction						
coarse	15.4	5.4	7.2	12.5	59.5	33.7
fine	18.7	4.2	4.8	8.7	63.6	24.2

fraction was resuspended and allowed to settle. A minimum of six extractions were required to remove the majority of the dark pigments. Each fraction was dried 48 h at 60°C in an air oven. The dry light fraction was screened through 40 and 60 mesh US sieves to remove unwanted splinters. The splinter-like material which did not pass through a 40 mesh US sieve was unsuited for use in human foods.

Compared to the intact sunflower hull and the prepared light fractions, the dark fraction was higher in protein and ash and lower in fiber content (Table 3.5). Although relatively high in fiber content, the dark pigment made the dark fraction unsuitable as a fiber supplement. The remaining "coarse" and "fine" fractions were compared in this investigation.

3.1.9 Pea hulls

The field pea hulls used in this investigation were supplied by Pro Star Mills Ltd, Saskatoon, Saskatchewan. This product, which has been hammermilled through 16 mesh US sieve (Pro Star Mills Ltd, 1978), was the same as used in "Le Pain Quotidien" or "Daily Bread", a bread marketed in central Canada by Steinberg Foods Limited, Montreal, Quebec.

Table 3.5 Analysis of sunflower hull fractions

	Crude protein (%N × 6.25)	Ether extract (%)	Ash (%)	Crude fiber (%)	N-free extract (%)	Buffered acid-detergent fiber (%)
Whole hull	3.1	1.2	2.2	57.4	36.1	91.7
Hull fraction						
Light—coarse	2.0	0.6	0.7	63.8	32.9	96.3
—fine	3.2	1.0	1.3	60.2	34.3	87.1
Dark	8.2	1.6	5.2	48.4	36.6	75.9

3.1.10 Cellulose

Avicel PH-101, a microcrystalline cellulose, a product of FMC Corporation was used in this investigation.

Avicel PH-101 is a purified depolymerized alpha cellulose derived from fibrous plants (FMC Corporation a, b). The product can be used as a direct replacement for high caloric ingredients, such as flour, fat and sugar, in low moisture food products.

3.2 CHEMICAL COMPOSITION OF FIBER SUPPLEMENTS

The chemical composition of each fiber supplement was compared with each other and with straight grade wheat flour and whole wheat flour.

3.2.1 Proximate analysis

Proximate analysis was carried out on each fiber supplement by standard AACC (1962) and AOAC (1975) methods. Moisture was determined by measuring weight loss of samples that had been dried in a 130°C air oven

for 1 h (AACC Method 44-15). Protein was determined by the micro-Kjeldahl procedure (AACC Method 46-13). The digestion step was modified to eliminate the use of mercury. Samples were digested with 5 ml concentrated sulfuric acid and 3 g of a catalyst mixture containing 3 parts copper sulfate to 100 parts potassium sulfate. Protein was calculated from nitrogen using the conversion factor 6.25. Fat was extracted from samples with anhydrous ethyl ether for 6 h using Goldfisch extractors (AACC Method 30-20). Samples were ashed for 2 h in a 600°C muffle furnace (AACC Method 08-03). Crude fiber was determined by AOAC Method 7.050 using California buchner funnels to filter acid-extracted and alkali-extracted residues. Details of the crude fiber procedure were previously described in Section 2.4.1. Nitrogen-free extract was determined by difference. Results were reported on a dry basis.

3.2.2 Detergent fractionation

The insoluble dietary fiber content and fiber composition of each fiber supplement was determined by detergent fractionation (Goering and Van Soest, 1970) according to AACC (1962) and AOAC (1975) procedures. Insoluble dietary fiber was measured as neutral detergent fiber (NDF) by AACC Method 32-20. Residual starch was removed by incubating the NDF residue with α-amylase from porcine pancreas 18 h at 37°C. Acid detergent fiber (ADF) was determined by AOAC Method 7.055. Hemicellulose was calculated as the difference between NDF and ADF. Crude lignin was measured by the acid detergent lignin procedure (AOAC Method 7.058, 1975). Cellulose was estimated by the amount of ADF residue dissolved in 72% sulfuric acid during the lignin determination procedure.

3.2.3 Mucilage extraction

The mucilage content of flax hulls, mustard hulls and psyllium seeds was determined by a modification of the Bolley and McCormack (1952) method and recovered by the Neville (1913) method (Appendix A). Separation of these fiber supplements into hull and mucilage fractions has been illustrated in Figure 3.8. Detergent fractionation described in Section 3.2.2 was carried out on the mucilage-free hull samples.

3.3 PHYSICAL CHARACTERISTICS OF FIBER SUPPLEMENTS

Physical characteristics of each fiber supplement were compared with each other and with straight grade wheat flour and whole wheat flour.

3.3.1 Color

Color of fiber supplements was measured by means of a Hunterlab D25D2M Digital Color and Color Difference Meter equipped with an M optical head. The instrument was standardized with a white tile (No. C2-5470; L = 94.7, a = -0.9, b = 0.5).

3.3.2 pH

The natural pH of the fiber sources was measured on 10% dispersions of the fiber supplement in distilled water.

3.3.3 Particle size and shape

Particle size was determined by a modification of the screen analysis method of Heller *et al.* (1977) (Appendix B). The aperture at which 50% of the sample passed, the mean log particle size (MLPS), was determined with probability graph paper by plotting the cumulative percent

Figure 3.8 Separation of flax hulls, mustard hulls and psyllium seeds into hull and mucilage fractions.

Row: 1. flax hulls 2. mustard hulls 3. psyllium seed.
Column: 1. hull and mucilage 2 mucilage-free hull
3. mucilage

undersize on the normal probability axis against the corresponding sieve aperture on the log axis (Lapple, 1968).

Particle shape and relative size were recorded by photomicrography using Kodak Panatomic-X film and Ilfobrom 3 single weight glossy paper.

3.3.4 Relative density

There were no standard methods available for measuring relative density. The method proposed by Parrott and Thrall (1978) was not reproducible. Their method for directly measuring density was dependent upon filling a graduated cylinder to the same level in exactly the same manner every time. Details of their method were not published and it was found that values varied with the diameter of the graduated cylinder, the method of filling and with the level chosen due to compaction of the sample material.

To overcome the problem of reproducibility the author developed two methods of measuring relative density (Appendix C). The first method (RD) was based on filling a small container of known volume. The second method (RD_{mod}) used sedimentation cylinders to ensure even filling. Data were evaluated independently and means for each test were compared with Duncan's multiple range test.

3.3.5 Capacity for holding water and fat

Childs and Abajian (1976) defined water holding capacity as a function of incubation time, temperature and pH. In their study, there was a significant interaction between time and temperature, but pH had no significant effect. The capacity of each fiber supplement to hold water and fat was tested at three incubation times (30, 60 and 120 min) and two

temperatures (24°C and 37°C) by a modification of the Childs and Abajian (1976) (Appendix D) method. Three replicates were performed for each fiber supplement under each time-temperature condition. Results were analyzed by analysis of variance and Duncan's multiple range test.

Results obtained by the modified Childs and Abajian (1976) procedure were compared with water hydration capacity determined by the Quinn and Paton (1979) method and fat absorption capacity determined by the Sosulski *et al.* (1976) modification of the centrifuge procedure of Sosulski (1962). These methods are discussed in detail in Appendix D.

3.4 PHYSIOLOGICAL EFFECTS OF HIGH-FIBER DIETS

The physiological effects of ingesting relatively high levels of each fiber supplement were studied in weanling rats. Of particular concern in this investigation were the effect on growth and feed consumption, the effect on gastrointestinal function and the effect on serum cholesterol and serum triglycerides.

3.4.1 Experimental design

The experimental design used was a completely randomized experiment with eight replications. Means were compared by Duncan's multiple range test.

3.4.2 Diet formulation

Sunflower heads, psyllium seeds, flax hulls, mustard hulls, wheat bran (coarse), sunflower hulls (coarse), pea hulls and cellulose were incorporated into isonitrogenous and isocaloric diets (Table 3.6) at levels to provide 10% NDF. All nutritional requirements of growing rats were

Table 3.6 Diet formulation (dry basis)

	Sunflower heads	Psyllium seeds	Flax hulls	Mustard hulls	Wheat bran	Pea hulls	Sunflower hulls	Cellulose
Casein	14.82	12.66	6.81	13.78	13.64	13.58	16.54	16.75
Corn oil	7.17	6.33	7.45	7.45	6.96	7.63	7.94	7.98
Cornstarch	41.05	50.28	47.39	53.90	52.86	49.61	58.01	58.02
Vitamin mixture[1]	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Mineral mixture[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sunflower heads	29.76	—	—	—	—	—	—	—
Psyllium seeds	—	23.53	—	—	—	—	—	—
Flax hulls	—	—	31.15	—	—	—	—	—
Mustard hulls	—	—	—	17.67	—	—	—	—
Wheat bran	—	—	—	—	19.34	—	—	—
Pea hulls	—	—	—	—	—	21.98	—	—
Sunflower hulls	—	—	—	—	—	—	10.31	—
Cellulose	—	—	—	—	—	—	—	10.05

[1] U.S. Biochemical Corporation. Vitamin supplement.

[2] U.S. Biochemical Corporation. Draper 4164.

supplied by the diets (Warner and Breur, 1972). Commercial vitamin and mineral supplements from United States Biochemical Corporation (Appendix E) were incorporated. Chromic oxide was added as an indicator of digestibility. Diets were formulated to contain 16% protein and 8% fat. Protein and fat not supplied by each fiber supplement were supplied by casein and corn oil, respectively.

3.4.3 Animal management

Eight male weanling Sprague-Dawley rats were randomly allotted to each treatment and individually housed in wire bottomed cages in an environmentally controlled room with 12 h light and 12 h dark. Feed and water were offered *ad libitum* during the three week test period. Rats were weighed at the end of the first week and twice weekly thereafter. At the end of the three week test period, feed jars were removed and the rats fasted overnight. The following day, rats were weighed and slaughtered by decapitation. Blood was collected from the neck region in 15 ml centrifuge tubes and cooled in ice until serum could be prepared. The liver and kidneys of each rat were removed, washed in saline solution and weighed.

3.4.4 Fecal collection procedure

Five day total collections were made at the end of the second and third weeks. Feed intake was determined for each time period. Feces were dried overnight at 60°C in a forced draft oven and weighed. Volume was measured in a 25 ml graduated cylinder. During the time period that fecal collections were not being made, feces uncontaminated by spilled feed and urine were collected, pooled within treatments and refrigerated for subsequent chemical analysis. After fecal trays had been cleared, fresh

fecal pellets from individual rats were collected over a 2 h period in capped glass vials. Moisture was determined by drying the samples overnight at 60°C in a forced draft oven and measuring weight loss.

3.4.5 Chemical analyses of diets and feces

The proximate composition of the diets was determined by standard AACC (1962) and AOAC (1975) procedures. Crude protein and ether extract content of feces were determined. Methods have been previously described in Section 3.2.1.

Dietary fiber content and composition of the fiber was determined by the detergent fractionation procedures discussed in Section 3.2.2. However, to eliminate filtration problems starch was removed from the NDF residue of rat diets by a heat resistant α-amylase from *Bacillus subtilis*, Sigma A6505, as described by Mongeau and Brassard (1979). Sodium sulfite was omitted to prevent the partial degradation of the lignin fraction (Van Soest and Robertson, 1977). To eliminate the severe filtration problems which resulted when determining the NDF content of rat feces, a centrifugation step was included between the refluxing and filtration stages (Appendix F).

Gross energy of the diets and feces was determined with a Parr Oxygen Adiabatic Bomb 1241 Calorimeter equipped with a Parr 1655 Digital Thermometer.

Diets and feces were analyzed for chromic sesquioxide according to the method of Bolin and Lockhart (1960).

3.4.6 Preparation and analyses of rat serum

Serum was prepared from the freshly collected blood by

centrifugation at 10,000 × *g* for 30 min at 4°C. Serum was promptly removed from the separated cells with a Pasteur pipette and stored tightly capped and frozen for further analysis.

Total serum cholesterol was measured by the enzymatic procedure of Röschlau *et al.* (1974).

Serum triglycerides were measured by a colorimetric procedure using the "Triglyceride C-37 Rapid Stat Kit" available from Pierce Chemical Company, Rockford, Illinois.

3.4.7 Response criteria

Growth response was measured by calculating weight gains, feed intakes, feed efficiency ratios and protein efficiency ratios and by recording fasted weights, liver weights and kidney weights at the time of slaughter. The laxative properties of the fiber supplements were compared by measuring dry weights, volumes and densities of feces voided during two five-day collection periods and determining fecal moisture on fresh feces voided between the two total collection periods. Chemical composition data were used to calculate digestibility coefficients for all nutrients. Calculations were based both on the use of chromic oxide as an indicator substance and on the use of total collection data collected over two five-day periods. Serum cholesterol and serum triglyceride levels were compared.

3.4.8 Statistical analyses

Statistical analyses were facilitated by the use of SPSS procedures (Nie *et al.*, 1975).

The completely randomized experiment was analyzed by a one-way analysis of variance comparing each fiber source. Means were compared using

Duncan's multiple range test.

Weight gains, fasted weights, organ weights and serum triglycerides were adjusted for differences in dietary intake by analysis of covariance.

Interrelationships between response criteria were studied by means of a correlation analysis.

3.5 BREADMAKING CHARACTERISTICS OF FIBER SUPPLEMENTS

Breadmaking characteristics of flax hulls, wheat bran, sunflower hulls and cellulose were compared using flour blends containing 7.5% fiber supplement and 92.5% straight grade wheat flour. The 7.5% replacement level was chosen because it was similar to the level of pea hulls used in "Le Pain Quotidien" (Satin *et al.*, 1978) and because it was known from preliminary investigations that this level would produce a reasonably acceptable loaf. Sunflower heads, psyllium seeds and mustard hulls were not included in these investigations.

3.5.1 Mixograph study

Dough strength of straight grade wheat flour, whole wheat flour and flour blends containing 7.5% fiber supplement were compared by a Mixograph study (AACC method 54-40, 1962). The water level used was based on optimum baking absorption (sight absorption).[1]

3.5.2 Breadmaking procedure

All breads were baked by a conventional sponge and dough procedure (Appendix G). The basic bread formula included 100 g flour blend (14%

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[1] Sight absorption is the amount of water required to form dough of a predetermined consistency.

moisture basis), 0.5% sodium-stearoyl-2-lactylate (SSL),[1] 4 g non-fat dry milk,[2] 3 g shortening, 0.75 g yeast (dry basis), 5 g sucrose, 1.75 g salt, 0.1 g ammonium phosphate monobasic, 75 ppm potassium bromate, 0.3 g malt and water to optimum absorption. The fiber supplement was added at the dough stage. The sponge, containing 70 g straight grade flour, SSL, yeast, ammonium phosphate monobasic, malt and water was mixed until smooth (45 sec in a GRL mixer) and allowed to ferment 3.5 h at 30°C and 80% RH. Remaining ingredients, including the fiber supplement, were added to the sponge and mixed in a GRL mixer to optimum dough development as determined by the mixograph study. Optimum mixing time was the time required for the mixograph curve to peak minus the time required to mix the sponge. The dough was punched and panned on sheeting rolls according to AACC method 10-10 (1962). Loaves were baked 20 min at 220°C in an experimental rotary oven.

Weights and volumes of baked breads were determined 1 h after the loaf was taken from the oven. Loaf volumes were determined by rapeseed displacement. Specific volumes were calculated from weight and volume data.

Crumb compressibility was measured with a Food Technology Corporation Texture Test System. Three slices of bread measuring 4 × 4 × 1.5 cm were cut from each loaf and tested with the standard shear-compression cell operated by the 300 lb ring at 100 psi. A range of 1000 was used on the chart recorder. Peak areas were measured with a planimeter.

Color of bread crusts and 1.5 cm slices were measured with the Hunterlab D25D2M Digital Color and Color Difference Meter equipped with an

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[1] EMPLEX supplied by the Patco Products Division of the C. J. Patterson Company, Kansas City, Missouri.

[2] Alpha Dry Skim Milk, Spray Process, High Temperature, Bakers Brand supplied by Alpha Milk Company, Red Deer, Alberta.

M optical head. A white tile (No. C2-5470; L = 94.7, a = -0.9, b = 0.5) was used to standardize the instrument. Total color difference (ΔE)[1] was calculated to facilitate comparisons between fiber-supplemented breads and breads containing no fiber supplement.

Loaf volume, specific volume, crust compressibility, crust color and crumb color were analyzed by analysis of variance and Duncan's multiple range test.

3.5.3 Quantitative descriptive analysis

Sensory properties of fiber-supplemented breads were compared by quantitative descriptive analysis (Stone *et al.*, 1974). The anchor points of the scale used were "very fine" to "very coarse" for measuring visual appearance of the crumb grain, "very smooth" to "very gritty" for measuring mouthfeel of the crumb, "very bland" to "very strong" for measuring flavor intensity of the crumb and "very soft" to "very hard" for measuring crust firmness (Appendix H). Each panelist was asked to record his evaluation by making a verticle line across the horizontal line at the point that best reflected his perception of the magnitude of that property. Panelists were also asked to describe the flavor of each sample.

Breads were tested over a two-day period under red lights by semi-trained panelists. Ten panelists were used the first day. Five more panelists were added to the group on the second day. Each panelist was given one-half slice of each test loaf identified by a three digit random number. Breads were divided into two groups. Half the breads were tested

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[1] $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$

in the morning and half were tested in the afternoon. Whole wheat bread served as a control loaf and was included in both morning and afternoon sessions. Identification numbers were changed and the procedure was repeated the following day. Daily values assigned to whole wheat bread for each sensory property were averaged and the daily values for each fiber-supplemented bread were adjusted accordingly.

Ratings between 0 and 60 were assigned to each evaluation and responses to the questionnaire (Appendix H) were analyzed according to the methods described by Larmond (1977) using analysis of variance and Tukey's Test.

3.6 PHYSIOLOGICAL EFFECTS OF FIBER-SUPPLEMENTED BREAD DIETS

The fiber supplements studied would most likely be consumed by humans in food products such as bread. The physiological effects of ingesting fiber-supplemented breads were compared in weanling rats with the physiological effects of a whole wheat bread diet. The effects on gastrointestinal function and lipid metabolism were studied. Diets were nutritionally adequate (Warner and Breur, 1972) and the level of dietary fiber corresponded to the 5% cellulose addition found in many purified rat diets.

3.6.1 Experimental design

The experimental design used was a completely randomized experiment with ten replications. Means were compared by Duncan's multiple range test.

3.6.2 Preparation of bread

The same fiber supplements used in the breadmaking study were used for this investigation. Flour blends formulated by Pearson's Square to contain 7.5% NDF were incorporated into breads prepared by the sponge-dough procedure described in Section 3.5.2 and Appendix G. The bread formulation was scaled up to include 500 g flour blend. A Kitchen-Aid K-45 mixer equipped with a dough hook was used to mix the sponge and dough. Dough was punched and panned by hand, using a rolling pin and baking sheet according to AACC (1962) Method 10-10. The breads were baked at 220°C for 25 min, cooled, freeze dried and ground in a Jacobson hammermill Model 160-D through a $1/32$ in screen prior to diet formulation.

3.6.3 Chemical analysis of bread

The proximate composition of the breads was determined by standard AACC (1962) and AOAC (1975) procedures as described in Section 3.2.1. Gross energy was determined with a Parr Oxygen Adiabatic Bomb 1241 Calorimeter equipped with a Parr 1655 Digital Thermometer. NDF was determined by the Mongeau and Brassard (1979) modification described in Section 3.4.5 and Appendix F.

3.6.4 Diet formulation

The fiber-supplemented breads comprised two thirds of the diet (Table 3.7). The remaining third of the diet was similar to diets formulated in Section 3.4.2. Diets were formulated to contain 16% protein and 8% fat. Protein and fat not supplied by the fiber-supplemented bread were supplied by casein and corn oil, respectively. Commercial vitamin and mineral supplements from United States Biochemical Corporation (Appendix E) were

Table 3.7 Formulation of bread diets (dry basis)

		Fiber supplement						
	Whole wheat	Flax hulls	Wheat bran		Sunflower hulls		Pea hulls	Cellulose
			Coarse	Fine	Coarse	Fine		
		(g/100 g diet)						
Bread[1]	58.14	66.67	66.67	66.67	66.67	66.67	66.67	66.67
Casein	12.61	11.85	12.24	11.63	12.40	13.33	12.75	13.49
Corn oil	5.67	4.97	5.81	5.81	5.87	5.94	6.18	5.74
Corn starch	16.68	9.61	8.38	8.99	8.16	7.16	7.50	7.20
Vitamin mixture[2]	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Mineral mixture[3]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1] % fiber supplement in flour blend (14.0% mb): flax hulls 19.51, pea hulls 12.84, coarse wheat bran 11.24, fine wheat bran 16.23, coarse sunflower hulls 5.88, fine sunflower hulls 5.83, cellulose 5.74

[2] U.S. Biochemical Corporation. Vitamin supplement

[3] U.S. Biochemical Corporation. Draper 4164