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CEREAL STRUCTURE AND ITS RELATIONSHIP TO NUTRITIONAL QUALITY

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Abstract

Factors that determine the digestibility of carbohydrates and minerals in cereals are examined. Most carbohydrates and minerals in cereals are structurally bound, either surrounded by or associated with cell wall components not easily digested by non-ruminant animals and humans. Treatments such as mechanical grinding and heat improve the digestibility of nutrients. Further processing and cooking result in structural and physicochemical changes of cereal starch, phytate, and dietary fiber. Such changes greatly influence the physiological and metabolic effects in animals and humans. The digestive breakdown of most nutrient components is also dependent on the activities of enzymes in cereals and in the mammalian digestive system. However, starch, phytate, and dietary fiber are not entirely and readily degraded by enzymes. Undegraded components reduce both the caloric value of the food and the availabilities of other nutrients by interacting with them in the gastrointestinal tract. Studies on availabilities of carbohydrates and minerals in cereal foods are conducted in humans and rats or under in vitro conditions, using various analytical methods including microscopy. The advantage of applying light microscopy and scanning electron microscopy coupled with energy dispersive X-ray microanalysis to study the digestive breakdown of structural components in cereal foods is highlighted by demonstrating the capabilities of the techniques to reveal both structural and microchemical information.

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Introduction

Cereals are good sources of carbohydrates and minerals important for sustaining the energy and growth requirements of humans and animals. Cereals also contain dietary fiber. Increased consumption of dietary fiber has been associated with various health benefits (Trowell, 1976; Anderson and Chen, 1979).

Information concerning factors that affect the nutritional quality of cereal food derives from studies conducted in humans, or animals such as rats, fed diets containing various cereal products (McCance and Widdowson, 1935; Jenkins et al., 1975; Ismail-Beigi et al., 1977; Simpson et al., 1981; Navert et al., 1985; Heaton et al., 1988). In vitro conditions, simulating gastrointestinal environments, have also been used (Snow and O'Dea, 1981; Holm et al., 1985, 1988; Platt and Clydesdale, 1984, 1987). Oat and wheat brans are commercially available products and most frequently studied because of interests in the physiological and metabolic effects of phytic acid and dietary fiber (Promare and Heaton, 1973; Reinhold et al., 1975, 1981; Davies et al., 1977; Anderson et al., 1984; Moak et al., 1987; Shinnick et al., 1988). Results obtained from many nutritional studies indicate that the structures and physical forms of cereal food components greatly affect the availability and utilization of the nutrients (Snow and O'Dea, 1981; Van Soest, 1984; Jenkins et al., 1986; Heaton et al., 1988; Holm et al., 1988).

Most cereal carbohydrates and minerals are associated with microscopically distinct structures (McMasters et al., 1971; Fulcher and Wong, 1980). Microscopic studies reveal much detail concerning the morphological organization and nutrient composition of oat and wheat grains before and after cereal processing (Pomeranz and Shellenberger, 1961; Buttrose, 1978; Fulcher and Wong, 1980; Fulcher, 1986; Lookhart et al., 1986; Yiu, 1986; Yiu et al., 1987). Using oat and wheat products as examples, the present review demonstrates how microscopy, particularly fluorescence and other light microscopy, has contributed to the understanding of relationships between cereal structures and the availability of carbohydrates and minerals in cereal foods. More specifically, the review examines factors such as

processing, cooking, and enzymes that influence the structures and digestibilities of starch, phytate, and dietary fiber. Some of the effects of undigested fiber and phytate on the absorption of other nutrients in the mammalian gastrointestinal tract are also discussed.

Starch

Structure and Distribution

Starch is located inside the cells of the endosperm and is rarely found in the germ and aleurone tissues in mature grains. Starch occurs in the form of colorless translucent bodies, identified as starch granules. Reviews by Evers (1979), Hood and Liboff (1982), and Fulcher (1986) gave detailed descriptions of the structures of cereal starches. Cereal starch granules vary in size and morphological appearance, depending on the species of the cereal grain. For example, wheat starch consists of both small (2 - 10 μm) spherical and large (20 - 40 μm) lenticular granules (Fig. 1). Unlike wheat starch, oat starch occurs chiefly as compound granules which are aggregates of sub-granules (Fig. 2). The oat starch granules range from 20 to 100 μm in size.

Various techniques of light microscopy are suitable for studying the distribution of starch in cereal grains. For instance, staining procedures using iodine - potassium iodide, the periodic acid-Schiff reagent, or fluorescein-coupled plant lectins such as Lens culinaris agglutinin and Concanavalin A, are appropriate for revealing the location and structural organization of starch in a variety of cereals and cereal foods (Jensen, 1962; Fulcher and Wong, 1980; Miller et al., 1984; Yiu, 1986). The staining procedures provide both convenience and speed for detecting starch content in cereals. Scanning and transmission electron microscopy are also useful for investigating the complex structure of starch (Gallant and Guilbot, 1969; Yamaguchi et al., 1979). Most cereal starches exhibit birefringence under polarized light (Wivinis and Maywald, 1967; Greenwood, 1979). Both oat and wheat starches show the characteristic 'maltese cross' pattern under a polarized light microscope (Fig. 3). Birefringence is lost when a starch granule undergoes physical changes associated with gelatinization (Sandstedt, 1961; Lineback and Wongsrikasem, 1980; Varriano-Marston, 1982).

Processing and Cooking

During the milling of wheat, endospermic cells of the subaleurone layer are more resistant to the force of grinding, and are reduced in size less readily than cells of the inner starchy endosperm (Kent, 1966; Pomeranz, 1982). The coarse fraction of wheat flour, which derives primarily from the centre of the endosperm, has higher starch content than the finer flour fraction, which contains more fragments of the protein-rich subaleurone layer (Pomeranz, 1982). Endosperm cells in soft wheat varieties contain starch granules embedded in a friable protein matrix which is susceptible to the grinding force, resulting in the release of intact starch granules with little damage (Kent, 1969). On the other hand, endosperm cells of hard wheat varieties tend to shatter rather than powder due to the continuous protein matrix, resulting in breakage of both starch and the protein matrix (Moss et al., 1980; Pomeranz, 1982). Starch damage in the flour increases the water binding capacity and susceptibility to α-amylase degradation (Jones, 1940; Pomeranz, 1982).

Figure Captions

Unless otherwise stated, all micrographs show 3% glutaraldehyde-fixed, glycol methacrylate-embedded sections of oat or wheat grain tissues. Numbers at scale bars are in μm. Photographed using fluorescence exciter/barrier filters set for maximum transmission at 365 nm/>418 nm (FCI) or 490 nm/>520 nm (FCII).

Fig. 1 A section of wheat kernel showing structures of starch granules (arrows) after staining with F-LCA (fluorescein-labelled Lens culinaris agglutinin, 1.2 mg/ml in 0.01M sodium phosphate buffer, pH 7). FCII.

Fig. 2 A section of oat kernel showing the structures of compound starch granules (*) and cell walls (arrows) after staining with F-LCA and 0.01% Congo Red. FCII.

Fig. 3 An unstained, frozen section of oat kernel viewed under polarized light to reveal the pattern of birefringent starch granules (arrows). Photographed using polarizing optics.

Fig. 4 A section of quick-cooking rolled oats stained with F-Con A (fluorescein-labelled Concanavalin A, 1.2 mg/ml in 0.01M sodium phosphate buffer, pH 7) showing broken compound starch granules (arrows). FCII. (Yiu, 1986).

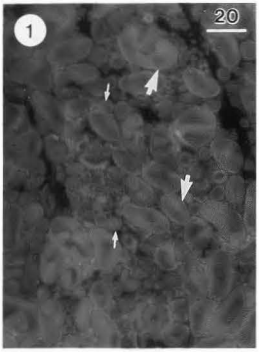
Fig. 5 A section of cooked rolled oats stained with F-Con A, showing the structure of cooked starch (arrows). FCII. (Yiu, 1986).

Fig. 6 A section of digesta removed from the small intestine of a rat fed a diet containing wheat bran, showing structures of wheat starch granules (arrows). Photographed using bright-field optics.

Fig. 7 A section of rat digesta prepared and stained the same way as in Fig. 6, demonstrating the presence of partially digested corn starch granules (arrows).

Fig. 8 An elemental profile of oat phytin globoid. A 2 μm thick, glycol methacrylate-embedded, carbon coated section of rat colonic digesta examined under a scanning electron microscope at 20 kV, and analyzed with an energy dispersive X-ray microprobe for 100 s/site. Probe current: 5×10^{-9} A. Probe size: 180 nm. K: potassium, Mg: magnesium, P: phosphorus.

Fig. 9 A section of wheat kernel stained with 0.1% Acriflavine HCl to show the distribution of phytin globoids (small arrows) within the aleurone layer with cell walls (large arrows) of high phenolic contents. FCI.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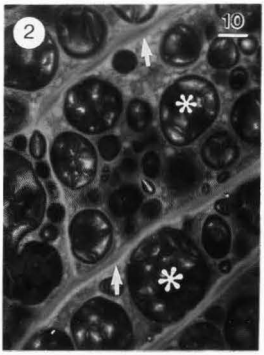
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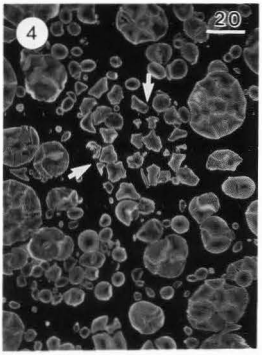
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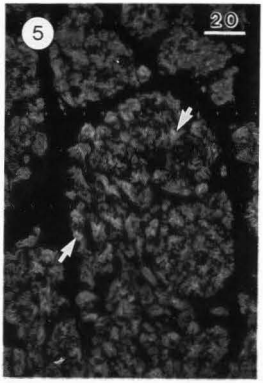
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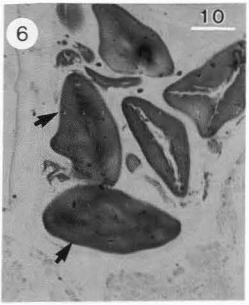
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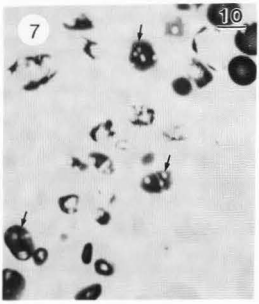
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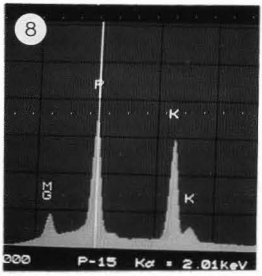
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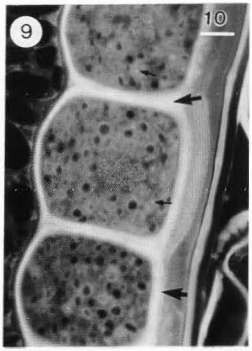
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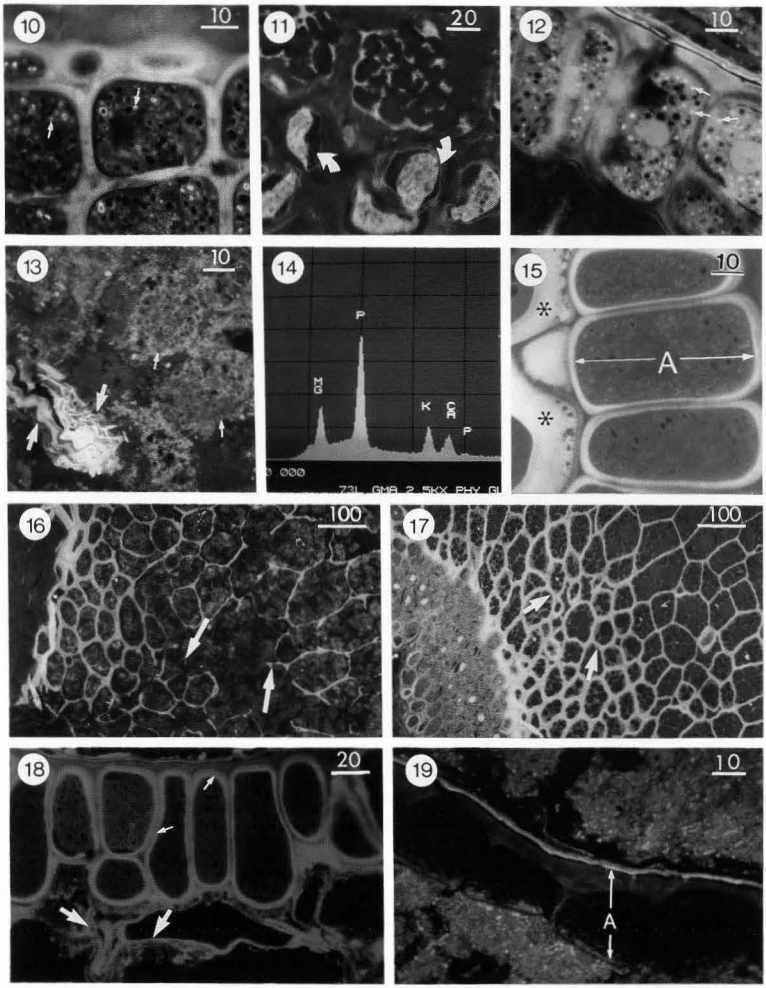
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Figure Captions

Unless otherwise stated, all micrographs show 3% glutaraldehyde-fixed, glycol methacrylate-embedded sections of oat or wheat grain tissues. Numbers at scale bars are in µm. Photographed using fluorescence exciter/barrier filters set for maximum transmission at 365 nm/ >418 nm (FCI) or 490 nm/>520 nm (FCII).

Fig. 10 A section of wheat bran stained with 0.1% Acridine Orange, showing the presence of phytin globoids (arrows). FCI.

Fig. 11 A section of puffed wheat stained with 0.1% Acriflavine HCl, demonstrating changes in the aleurone cell structure (arrows). FCII.

Fig. 12 A section of ileo digesta removed from a rat fed a diet containing oat bran, and stained with 0.1% Acridine Orange, showing the presence of phytin globoids (arrows) within the aleurone cells. FCII. (Yiu and Mongeau, 1987).

Fig. 13 A section of rat colonic digesta stained with 0.1% Acriflavine HCl to show the presence of undigested phytin globoids (small arrows) and cell wall fragments of high phenolic contents (large arrows). FCI. (Yiu and Mongeau, 1987).

Fig. 14 An elemental profile of an undigested phytin globoid. A 2 µm thick, glycol methacrylate-embedded, carbon-coated section of rat colonic digesta examined under a scanning electron microscope at 20 kV, and analyzed with an energy dispersive X-ray microprobe for 100 s/site. Probe current: 5×10^{-9} A. Probe size: 180 nm. Ca: calcium, K: potassium, Mg: magnesium, P: phosphorus.

Fig. 15 A section of oat kernel stained with 0.01% Calcofluor White in 50% ethanol, showing the distribution of β-glucan-rich aleurone (A) and sub-aleurone (*) cell walls. FCI.

Fig. 16 A section of instant rolled oats stained with fluorescein-labelled Lens culinaris agglutinin (1.2 mg/ml in 0.01M sodium phosphate buffer, pH 7) and 0.01% Congo Red to show the extent of cell fracture (arrows) after processing. FCII.

Fig. 17 A section of regular rolled oats stained and photographed the same way as in fig. 16 to demonstrate the intact cell wall structures (arrows).

Fig. 18 A section of rat ileo digesta stained with 0.01% Cellufluor in 50% ethanol, showing the partially digested sub-aleurone (large arrows) and relatively intact aleurone (small arrows) layers. FCI. (Yiu and Mongeau, 1987).

Fig. 19 A section of rat colonic digesta stained with 0.1% Acridine Orange to show the partially digested aleurone cell walls (arrows). FCII. (Yiu and Mongeau, 1987).

Mechanical grinding, e.g., rolling and flaking, tends to induce the breakdown of compound starch granules in oats (Lookhart et al., 1986; Yiu, 1986). The thinner the oat flake, such as those of quick-cooking rolled oats, the more the breakdown occurs (Fig. 4). However, the structural integrity of starch sub-granules remains unchanged (Yiu, 1986).

When starch is heated in the presence of water, the granule swells as a result of water absorption. The swelling is initially hampered by the rigidity of the cell wall, resulting in many distorted and convoluted starch structures (Fig. 5). The integrity of the granule is lost when starch becomes completely gelatinized. The expanded structure of starch provides greater accessibility to enzymes, resulting in an increased rate of starch digestion (Wursh et al., 1986; Yiu et al., 1987). Furthermore, the rate of increase of glucose and insulin concentrations in blood is directly related to the percentage of starch gelatinized (Holm et al., 1985, 1988; Ross et al., 1987).

Processing methods such as extrusion cooking, explosion puffing, and instantization hydrate the starch granules or disrupt the native structures, similar to but surpassing the result of conventional heating (Brand et al., 1985). Such processing conditions appear to change starch digestibility and elicit different glycemic responses (Holm et al., 1985; Jenkins et al., 1986; Ross et al., 1987).

Digestibility

Cereal starches are readily digested by humans and animals. Starch-specific hydrolytic enzymes are abundant in most cereal grains, microorganisms, and mammalian salivary and pancreatic secretions (Jones, 1940; Marshall and Whelan, 1979). Detailed mechanisms involving the enzymatic hydrolysis of starch are described by Manners (1985). Briefly, α-amylase randomly hydrolyzes amylose and amylopectin to maltosaccharides which are degraded by α-glucosidases to glucose. The present review mainly examines factors that influence the digestive breakdown of cereal starch.

Particle size reduction of the starch-bearing matrix increases the digestibility of starch. Greater accessibility to enzymatic reactions, makes starch of finely ground flour more readily digested than starch of unprocessed cereal grains (Snow and O'Dea, 1981; Heaton et al., 1988). Damaged starch is more susceptible to amylase degradation than intact granules (Jones, 1940).

The presence of α-amylase inhibitors in cereals reduces starch digestibility (Shainkin and Birk, 1970; Rea et al., 1985). Alpha-amylase inhibitors can be removed through milling and cooking (Snow and O'Dea, 1981; Rea et al., 1985). Results of in vitro and in vivo studies demonstrate that interactions can take place between starch and other food components like lipids (Larsson and Miezis, 1979; Holm et al., 1983), proteins (Anderson et al., 1981; Jenkins et al., 1987), polyphenols (Thompson et al., 1984; Bjorck and Nyman, 1987; Knudsen et al., 1988), or phytic acid (Yoon et al., 1983; Thompson, 1986),

resulting in the formation of complexes that resist enzymatic degradation.

A fraction of starch ingested from processed cereals has been identified as resistant to breakdown by α-amylase both in vitro and in the small intestine of man (Levine and Levitt, 1981; Englyst and Cummings, 1985). Processing procedures, particularly freezing and thawing, cause retrogradation of the starch and increase resistance to amylolytic action (Englyst et al., 1983). Starches that resist digestion are available for microbial fermentation in the lower gut, but the generated energy is reduced (Waslien, 1988). Resistant starches can cause inaccuracy in quantifying the amount of dietary fiber in food products. Methods which rely on gelatinization in water and enzymatic removal of starch prior to the quantification of dietary fiber are affected by the presence of resistant starches. A method has been developed to determine the amount of starch in processed cereals resistant to amylolytic enzymes used for dietary fiber determination (Englyst et al., 1983). However resistant starches constitute only a small fraction of starch that escapes in vivo digestion (Englyst and Cummings, 1985). Hence, alternative additional methods are required to assess the content and digestibility of starch in cereal foods.

Microscopy serves as a practical tool for detecting and analyzing the digestive breakdown of starch in cereal foods. For example, light microscopy using iodine - potassium iodide as a staining reagent, can be used to detect starch in the small intestine of the rat (Fig. 6). The structural appearance of starch present in rat digesta reflects the extent of starch breakdown (Fig. 7). According to Sandstedt (1955) and Evers et al. (1971), α-amylase-digested starch has a hollow centre linked to the surface by a few radial channels, whereas amyloglucosidase-digested starch has a surface covered with shallow pits.

Phytate

Structure and Distribution

Phytate (myo-inositol hexaphosphate) accounts for 70-90% of the total phosphorus reserve in most mature cereal grains (Ashton and Williams, 1958; O'Dell et al., 1972; Lolas et al., 1976; Frolich and Nyman, 1988). Chemical data (O'Dell et al., 1972) indicate that the majority of the phosphorus reserve is contained in the bran and germ fractions of cereal grains like wheat. Microscopic studies contribute to knowledge of the occurrence and distribution of phytate in cereal grains. Phytate-containing particles can be identified and located in the aleurone and scutellum tissues of most cereal grain kernels by electron microprobe X-ray analysis coupled with scanning electron microscopy (Tanaka et al., 1974), transmission electron microscopy (Ogawa et al., 1975), and energy dispersive X-ray (EDX) microanalysis (Liu and Pomeranz, 1975; Buttrose, 1978). The phytate-containing particles are electron-dense inclusions embedded in the protein matrix of the aleurone grains, and are referred to as phytin globoid crystals or phytin globoids (Lott and Spitzer, 1980). Ranging from 1 to 2 μm in diameter, phytin globoid crystals are mostly spherical in shape and contain high concentrations of phosphorus (P), potassium (K), and magnesium (Mg) (Lott and Ockenden, 1986). When subjected to EDX microanalysis, the globoid crystals emit X-rays characteristic of their elemental composition. A typical EDX spectral profile of oat phytin globoids is composed of three major element peaks, P, K, and Mg (Fig. 8). A small quantity of other elements is also present (Buttrose, 1978). The concentrations vary depending on grain varieties and locations of growth (Buttrose, 1978; Batten and Lott, 1986).

Rapid detection of the distribution of phytin globoids in cereals and cereal foods can be achieved using optical light microscopy. Polarized light microscopy effectively locates the birefringent structures of phytin globoids in hand-prepared or glycol-methacrylate embedded materials without any staining (Fulcher, 1982; Yiu et al., 1982). For confirmation, other types of light microscopy are often used. Cationic stains such as Acriflavine HCl, Acridine Orange, and Toluidine Blue are suitable microscopic markers for phytin inclusions (Yiu et al., 1982; Fulcher, 1982; Yiu, 1986; Yiu and Mongeau, 1987). Fig. 9 illustrates the distribution of phytin globoids in the aleurone cells of wheat as revealed by fluorescence microscopy.

Processing and Cooking

Milling reduces the phytate content in wheat (Nayini and Markakis, 1983) by removing the bran and germ fractions from the flour, but milling does not dissociate the structural attachment of phytin globoids from bran and germ fractions (Fig. 10). Vigorous processing methods like extrusion cooking and puffing induce structural changes in cereals to such an extent that components within the aleurone cells are no longer identifiable by phytin-specific staining (Fig. 11). Extrusion cooking alters the physicochemical properties of phytate, reduces phytate degradation in the intestine (Sandberg et al., 1986) and eliminates endogenous activities of phytate-specific enzymes (phytase) in cereals (Sandberg et al., 1987). The decrease in phytate degradation is associated with decreased absorption of zinc, phosphorus, and magnesium in the human small intestine (Kivisto et al., 1986). Milder heat treatment like domestic cooking reduces the phytate content in cereals such as wheat and rye, but not oats (Sandstrom et al., 1987). During bread making, the presence of additional phytases from yeast and the baking process significantly reduce the phytate content in bread (de Lange et al., 1961; Nayini and Markakis, 1983). Phytate-reduced bread has less effect on in vitro and in vivo absorption of minerals than phytate-containing bread (Reinhold et al., 1974; Navert et al., 1985).

Digestibility of Cereal Phytate and Nutritional Implications

Early metabolic studies indicated that phytate phosphorus is not readily available for digestive absorption by humans and animals (McCance and Widdowson, 1935; Mellanby, 1949). Dietary

deficiency of phosphorus is unlikely since phosphorus is readily available from other dietary sources. However, when cereals constitute a large portion of the diet, the degree to which humans can utilize phytate may become important.

The degradation of dietary phytate chiefly depends on the hydrolytic activities of phytases (Nayini and Markakis, 1986). Phytases, or myo-inositol hexaphosphate phosphohydrolases, are enzymes that break down phytic acid to myo-inositol and inorganic phosphate via intermediate myo-inositol phosphates (penta- to mono-phosphates).

Phytase activities exist in the endosperm of wheat (Peers, 1953), and in the aleurone cells of rice (Yoshida et al., 1975), barley (Tronier et al., 1971), sorghum (Adams and Novellie, 1975), and corn (Chang, 1967). Phytase activity increases during germination (McCance and Widdowson, 1944; Bartnik and Szafranska, 1987) resulting in a decrease in the phytate content of the grain (Nayini and Markakis, 1986). By comparison with wheat and rye, oats have lower phytase activities both before and after germination (McCance and Widdowson, 1944; Bartnik and Szafranska, 1987). Recent results based on ^{31}P-nuclear magnetic resonance spectroscopy confirm that oat phytase is inactivated by the heat treatment received during commercial oat processing (Frolich et al., 1988). Other studies conclude that processing such as extrusion cooking impairs phytase activity in wheat bran (Sandberg et al., 1986 & 1987). Hence, low or reduced activities of phytases account for the relatively high phytate contents and low phytate digestibilities in certain oat and extruded wheat products (Mellanby, 1949; Sandberg, et al., 1987; Yiu and Mongeau, 1987).

Phytase activities are located in the mucosal tissues of most mammalian intestines (Nayini and Markakis, 1986). Some of the phytate present in the small intestine is likely hydrolyzed by mucosal phytase. The extent of the enzymatic reaction depends on the presence and concentration of dietary minerals like Ca and Zn (Wise, 1986). The significance of the involvement of mucosal phytase in phytate degradation is not clear. One feeding study shows that mucosal phytases and enzymes such as alkaline phosphatase do not play important roles in phytate digestion, as close to 95% of the ingested phytate from phytase-deactivated wheat bran can be recovered in human ileostomy contents (Sandberg and Andersson, 1988). Another study reports that much of the ingested phytate, present in oat bran fed to rats, remains undigested in the small intestine (Yiu and Mongeau, 1987). Microscopic examination of the rat digesta shows that many of the ingested phytin globoids are structurally associated with the aleurone tissues (Fig. 12). Microscopic observations also provide direct evidence that the majority of the phytate breakdown takes place in the lower gut of the animal (Yiu and Mongeau, 1987). Phytases originating from the microflora, which usually populate the large intestines of animals and humans, seem to play a key role in phytate degradation. However, despite the presence of microbial phytase, phytate degradation is significantly influenced by phytase activities endogenous to most cereals (Bartnik and Szafranska, 1987; Sandberg and Andersson, 1988), and certain processings and baking (de Lange et al., 1961; Reinhold et al., 1974; Nayini and Markakis, 1983; Navert et al., 1985).

Microscopic examination of dietary phytate in colonic contents of rats revealed intact oat bran phytin globoids (Yiu and Mongeau, 1987). The undigested phytin globoids not only retained morphological and staining characteristics (Fig. 13) but also the elemental contents as revealed by EDX-microanalysis (Fig. 14). Undigested phytin is a cause of concern since the cation-binding activities may impair mineral bioavailability (Mellanby, 1949; Erdman, 1979; Oberleas and Harland, 1981).

Mineral deficiencies have been noted in humans and monogastric animals whose diets consist predominantly of whole grains of high phytate content (Mellanby, 1949; Erdman, 1979). The presence of six ortho-phosphate moieties in the phytic acid molecule provides the compound with a potential for complexing cations such as zinc, iron, magnesium, and calcium (O'Dell, 1969; Morris, 1986). Interactions between phytic acid and cations result in the formation of insoluble complexes, thereby reducing the availability of minerals (Erdman, 1979; Platt and Clydesdale, 1987). In addition, interactions can occur between protein and phytic acid, protein-cation and phytic acid, or starch and phytic acid (Cheryan, 1980; O'Dell and de Boland, 1976; Thompson, 1986; Wise, 1986). However, the deleterious effect of phytate on mineral metabolism in humans and animals is avoidable when diets are well balanced, especially in mineral contents (Morris, 1986; Moak et al., 1987).

Dietary Fiber

The definition of dietary fiber is still a debatable subject (Trowell, 1976; Cummings, 1976; Southgate, 1978; Selvendran, 1983; Englyst et al., 1987; Asp et al., 1988). However, it is generally accepted that indigestible plant materials are the main constituents (Trowell, 1988). This review examines only cereal brans that are known to be associated with the major physiological effects of dietary fiber and are of major commercial interest (Anderson and Chen, 1979; Anderson, 1985; Schneeman, 1987). Cereal bran is a product of commercial processing, the outer part of a grain kernel isolated through mechanical grinding and sieving (Deane and Commers, 1986). Bran is composed of several layers of fibrous tissues, including the pericarp and seed coat, and parts of the endosperm which include the aleurone and subaleurone cells (Figs. 10 & 15).

Physiological and Metabolic Effects of Oat and Wheat Brans

Oat and wheat brans attract public interest because of known physiological and metabolic effects believed to be beneficial. Oat and wheat brans can bind water, bile salts, and other substances in the intestinal tract (Promare and Heaton, 1973; Eastwood and Mowbray, 1976; Eastwood et al., 1980; Spiller et al., 1986; Anderson and Chen, 1986), resulting in various potential health benefits (Trowell, 1976; Anderson and

Chen, 1979; Anderson, 1985; Anderson and Tietyen-Clark, 1986; Schneeman, 1987; Burkitt, 1988). However, oat and wheat brans differ in colonic and metabolic functions. Oat bran is effective in reducing serum cholesterol levels and slowing glycemic responses, and wheat bran in increasing fecal weight and decreasing transit time, thereby decreasing the incidence of diverticulosis and colorectal cancer (Kritchevsky et al., 1984; Anderson, 1985; Anderson and Chen, 1986).

Fiber Composition of Oat and Wheat Brans

Dietary fiber has been divided into two categories, soluble and insoluble (Anderson and Chen, 1979). The rationale for this division is based on solubility in hot water. Soluble cereal fiber includes polysaccharides referred to as gums and some hemicelluloses, whereas insoluble fiber includes cellulose, some hemicellulosic polysaccharides and lignin (Southgate, 1978; Anderson and Chen, 1979; Southgate and Kritchevsky, 1981). Physiologically based definitions, such as adopted by the Canadian Expert Committee on Dietary Fiber (Health and Welfare Canada, 1985), refer to plant materials not digestible by man. Such materials consist of nonstarch polysaccharides and lignin, and may include associated substances.

Oat and wheat brans differ in their fiber contents and compositions. According to Frolich and Nyman (1988), commercial oat bran has less than half the amount of total dietary fiber of wheat bran, but its soluble fiber content is greater, approximately 35%, as compared to wheat bran which has about 2%. Furthermore, the majority of the soluble oat fiber is present in the form of (1→3)(1→4)-β-D-glucan, generally known as oat gum (Wood, 1986), whereas arabinoxylans are the major soluble fibers in wheat (Selvendran, 1983).

Structures of Oat and Wheat Brans

Cereal cell walls, particularly cell walls present in the bran, are major sources of dietary fiber (Cummings, 1976; Southgate, 1978; Selvendran, 1983). Chemical studies of isolated cell wall fractions provide detailed information on the chemical composition of cereal cell walls. Most cereal cell walls are composed of cellulose microfibrils embedded in a matrix of hemicelluloses, some of which are cross-linked by lignin and phenolic esters, and/or proteins (Mares and Stone, 1973; Bacic and Stone, 1981; Selvendran, 1983).

Differences in fiber composition between oat and wheat brans are best revealed by fluorescence microscopy which provides both structural and microchemical information. When stained with dyes such as Congo Red or Calcofluor White (Wood et al., 1983), oat bran is characterized by its β-D-glucan content located chiefly in the inner aleurone and subaleurone cell walls (Fig. 15). Wheat bran, on the other hand, does not have the same histochemistry; its aleurone cell walls are dominated by the relatively high phenolic content best revealed by fluorescence microscopy when viewed under short wavelength (<365nm) excitation (Fig. 9). Intense autofluorescence is detected in the wheat aleurone cell wall because of its ferulic and p-coumaric acid contents (Fulcher et al., 1972; Fulcher, 1982). Other structural components of the bran, including the outer pericarp and seed coat layers, which have high lignin and cutin contents (Ring and Selvendran, 1980; Schwarz et al., 1988) as well as the subaleurone starch granules, can also be revealed with fluorescence microscopy (Fig. 9).

Processing and Cooking

Mechanical processing breaks down the endosperm cell walls of oats and wheat, reducing particle size (Schultze and MacMasters, 1962; Moss et al., 1980; Yiu, 1986). The extent of processing affects the degree of cell wall breakdown. For example, instant rolled oats have considerably more cell wall fractures (Fig. 16) than regular rolled oats (Fig. 17), as the former are subjected to more processing steps than the latter (Deane and Commers, 1986). Particle size reduction through grinding collapses the physical structure of wheat bran and alters its physicochemical properties to such an extent that the water-holding (Van Soest, 1984; Cadden, 1987) and bile salt-binding capacities of wheat bran (Mongeau and Brassard, 1982) are reduced. On the other hand, processing wheat bran of low moisture content by extrusion cooking at high temperature and pressure and short duration of time increases not only the soluble fiber content but also the digestibility of wheat bran in the rat (Bjorck et al., 1984).

Domestic cooking reduces the water-holding capacity of wheat bran (Wyman et al., 1976). Extensive heating and chemical treatments, such as isolation and purification of fiber components like delignified cellulose, decrease the hydration capacity of the fibers, slow down the rate of degradation in the colon (Van Soest, 1984), and modify mineral-binding activity (Frolich et al., 1984). In rolled oats, cooking facilitates the release of β-D-glucan from the cell wall (Yiu et al., 1987). The amount of the β-D-glucan released is greater in porridge prepared by cooking rolled oats gradually from room temperature than by cooking rolled oats rapidly in boiling water (Fig. 20). Microscopic examination indicated

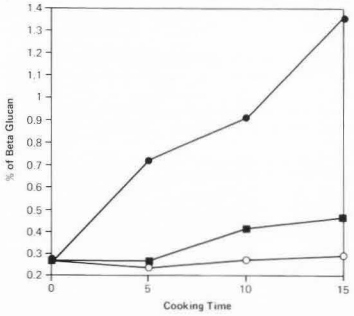
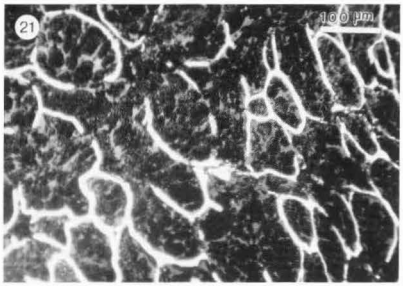
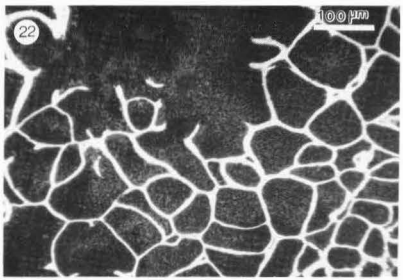


Fig. 20 Effect of different preparation methods on β-glucan release from rolled oats. Gradual cooking: (●-●), Rapid cooking: (■-■), soaking: (o-o). Cooking time = simmering time. (Yiu et al., 1987).

that gradually cooked rolled oats have considerably more cell wall disruption (Fig. 21) than rapidly cooked rolled oats (Fig. 22).





Figs. 21 & 22 Glycol methacrylate-embedded sections of rolled oats prepared by gradual cooking and rapid cooking, respectively, and stained with 0.01% Calcofluor in 50% ethanol to reveal differences in cell wall breakdown in the inner oat endosperm. (Yiu et al., 1987).

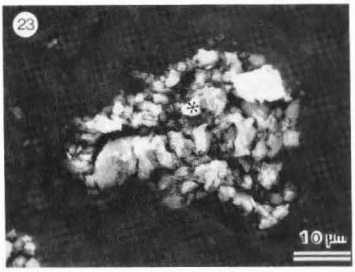
Degradation and Nutritional Implications

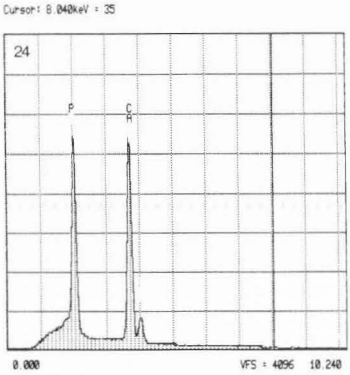
The degradation of cereal fibers is dependent on the enzymatic activities provided by the microflora normally present in the colon where absorption of the products of fiber fermentation occurs (Hungate, 1976; Cummings, 1976; Cummings and Englyst, 1987). The products are mostly volatile fatty acids including acetic, butyric, and propionic acids and carbon dioxide, hydrogen, and methane gases. Activities of cellulases, β-glucosidases, cellobiase, and β-glucanases are reported in the human colonic microflora (Salyers et al., 1976; Bacon, 1978). Chemical and metabolic studies of individual fiber components reveal that about 56%-87% of hemicelluloses are digestible, and about 40% of ingested cellulose is degradable (Cummings, 1976; Anderson and Chen, 1979; Nyman et al., 1986). However, highly lignified cell walls and the presence of phenolics, as well as substances such as cutin and silica greatly reduce the digestibility of cereal fibers (Van Soest and Jones, 1968; Cummings, 1976). Many cell walls remain structurally intact after passing through the colons of humans or rats (Dintzis et al., 1979; Yiu and Mongeau, 1987).

Fluorescence microscopy is a useful tool to study the digestive breakdown of cereal cell walls by animals (Fulcher and Wood, 1983; Yiu and Mongeau, 1987). Microscopic observation provides direct evidence of differences in digestibility among the various structural components of cereal bran subjected to digestive processes (Yiu and Mongeau, 1987). For example, the β-D-glucan-rich subaleurone cell wall of oat bran is susceptible to the digestive environment of the small intestine of the rat (Fig. 18), whereas degradation of the aleurone cell wall does not take place until the bran reaches the colon (Fig. 19). On the other hand, most of the pericarp and seed coat layers (Fig. 13) as well as the trichomes remained undigested (Yiu and Mongeau, 1987). Trichomes, which are hair-like tubular structures found on the surface of most oat grains, have a high silica content.

The detection of undigested cell wall components in the colonic digesta indicates unavailable food materials. Not only are carbohydrates of cereal cell walls unavailable, but structurally associated trace minerals, such as silicon, chromium, manganese, and cobalt likewise are not absorbed (Jones, 1978). Furthermore, certain fiber components of oat and wheat brans, such as lignin, cellulose, and hemicellulose, have affinities for minerals including calcium, iron, zinc, copper, and magnesium (Reinhold et al., 1975; Jones, 1978; James et al., 1978; James, 1980; Gillooly et al., 1984; Platt and Clydesdale, 1984; Moak et al., 1987). The main functional groups in the organic components of cereal cell walls that may be involved in mineral binding include the carboxyl and hydroxyl groups of phenolic compounds, lignin, and certain polysaccharides (Jones, 1978; James et al., 1978). Results of several metabolic studies indicate that long-term intake of high-fiber food increases fecal mineral excretion. However, the excretion has no deleterious effect on mineral balance in humans due to abilities of the human body to adapt to changes in dietary conditions (Ismail-Beigi et al., 1977; Van Dokkum et al., 1982; Morris and Ellis, 1985).

Both phytate and fiber present in the bran are in close proximity to one another. Hence, it is often difficult to assess the individual effect of the bran components on mineral binding by analytical methods involving chemical extraction and determination (Davies et al., 1977; Platt and Clydesdale, 1987). Microscopy, however, can provide such information. Microscopic evidence suggests that indigestible remnants of wheat bran, mostly pericarp tissues, are associated with increases in the excretion of calcium and iron in humans (Dintzis et al, 1985). The structural (Fig. 23) and elemental (Fig. 24) contents of some of the minerals defecated by rats fed a diet rich in oat bran can be analyzed using fluorescence microscopy and scanning electron microscopy coupled with EDX-microanalysis. Structural association between the minerals and oat bran components is not evident. However, the microscopic





Figs. 23 & 24 A glycol methacrylate-embedded, carbon-coated section of rat colonic digesta examined under a scanning electron microscope at 20 kV, and analyzed with an energy dispersive X-ray microprobe for 100 s/site, showing (Fig. 23) the structures of unabsorbed minerals and (Fig. 24) an elemental profile of one of the structures (identified by an * in Fig. 23). Probe current: 5×10^{-9} A. Probe size: 180 nm. Ca: calcium, P: phosphorus.

study on excreted materials demonstrates the analytical capability of microscopy combined with other detection methods such as EDX-microanalysis. EDX-microanalysis in conjunction with microscopy may serve as useful techniques for studying direct association between minerals and individual bran components.

Summary

Examples given in this review demonstrate how microscopy can be used to study cereal microstructures. The nutritional quality of cereal food is affected by the organization of structural components associated with starch, phytate, and dietary fiber. Processing and cooking, as well as specific enzymes are factors which can alter cereal structures to such an extent that nutrient availabilites are affected.

The availabilities of most cereal carbohydrates and minerals for digestive absorption are affected by biological structures associated with cereal foods. Some structures are not readily accessible to the digestive enzymes present in the gastrointestinal tract. Mechanical grinding and heat are used to improve their digestibilities by reducing particle size, breaking down cell walls, inducing starch gelatinization, destroying α-amylase inhibitors and activating phytase in cereals. Excessive processing and high heat, on the other hand, may alter the morphological and physicochemical properties of starch, phytate, or cereal fiber to such an extent that digestibility is reduced. Undigested phytate and dietary fiber have the potential to adversely influence the bioavailability of minerals in humans and animals. However, repeated metabolic studies demonstrate that, with sufficient intake of dietary minerals, mineral balance can be maintained on diets high in phytate and fiber contents.

Used in conjunction with other analytical methods, particularly EDX-microanalysis, microscopy is an important tool which has the ability to obtain not only structural but also microchemical information pertinent to the nutrient composition of cereal foods.

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Discussion with Reviewers

B.G. Swanson: What evidence can you provide to suggest that microscopy can serve as an accurate analytical tool?
Author: With the aid of specific staining reagents such as iodine - potassium iodide and acid Fuchsin, microscopy can be used to accurately differentiate starch granules from protein bodies in most cereal grains.

B.G. Swanson: How do you perceive microscopy can quantitatively determine starch digestibility?
Author: Microscopic observation can reveal structural changes of starch granules subjected to enzymatic digestion, but cannot quantify how much starch is digested.

B.G. Swanson: What is your conclusion regarding importance of phytate to digestibility, mineral absorption and the nutritional quality of cereals?
Author: The major concern of phytate in relation to the nutritional quality of cereals is the mineral-binding property. However, phytate contents in most cereal grains are reduced as a result of processing, baking, and mild heat treatments. Feeding studies indicated that the deleterious effect of phytate on mineral metabolism can be avoided by maintaining diets that are well balanced in mineral contents.

B.G. Swanson: Can you compare observation of dietary fiber by fluorescence and scanning electron microscopy?
Author: While scanning electron microscopy has better resolving power than fluorescence microscopy, it does not reveal any chemical information of a fiber structure. The distributions of fiber-associated substances, such as phenolic acids and components such as β-D-glucans in cereal cell walls, can be easily detected using fluorescence microscopy.

L.U. Thompson: Will you please further clarify how the appearance and composition of the crystalline minerals illustrated in Figures 23 and 24 may reveal nutrient interactions?
Author: Figures 23 and 24 are included to demonstrate the analytical capability of SEM and EDX microanalysis. Such techniques have the ability to provide both structural and elemental information. Hence, any changes in the elemental composition of phytin globoids after they have passed through the gastrointestinal tract can be detected using the above techniques. Differences in the composition should reflect the mineral-binding activity of the globoids, provided that artifacts such as the migration of soluble elements in and out of the globoid structures during sample preparation are taken into account or eliminated.

L.U. Thompson: Since phytase in cereal foods may affect the breakdown of phytic acid in the gastrointestinal tract, have you or others tried estimating its location and concentration by microscopic techniques? How?
Author: Tronier et al. (1971) localized phytase activity in the aleurone cell of barley using transmission electron microscopy. Other microscopic techniques such as immunofluorescence or enzyme-linked immuno-staining are suitable for detecting and quantitating enzyme activities in animal or plant tissues.

Contribution No. 804