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## FOOD MICROSCOPY AND THE NUTRITIONAL QUALITY OF CEREAL FOODS

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### Abstract

The nutritional quality of cereal foods is directly related to the nature of nutrient storage in cereal grains. Most cereal nutrients, such as carbohydrates and minerals, are structurally bound. Processing alters the structural organization of the cereal grain. Results obtained from many nutritional studies indicate that the structure and physical form of a cereal food greatly influence the availability of its nutrients.

Using oats and wheat as examples, this review demonstrates how microscopy contributes to understanding the relationship between cereal structures and the availability of nutrients in cereal foods. Various forms of food microscopy play important roles in revealing structural changes of cereal foods that result from processing, cooking and enzymatic reactions. These changes directly affect the digestibility of starch, phytate and dietary fiber in oats and wheat. The present review also examines the effects of undigested fiber and phytate on the absorption of minerals in the mammalian gastrointestinal tract. Food Microscopy is a potential tool for studying the mineral binding property of cereal bran components.

**Key Words:** Light microscopy, scanning electron microscopy coupled with energy dispersive X-ray microanalysis, confocal laser scanning microscopy, cereal structures, processing effect, nutritional quality.

### Introduction

Microscopy is used as an analytical tool for various aspects of food analysis. These include locating storage nutrients in unprocessed and processed cereal grains (Yiu, 1986, 1989), studying interactions between food components (Offer *et al.*, 1989; Ma *et al.*, 1991), and identifying food-borne contaminants in various food products (Stasny *et al.*, 1981; Holley *et al.*, 1985).

Different types of microscopy are available, each requiring specific operating techniques and offering unique analytical capabilities. This paper focuses on the applications of five different types of microscopy that are suitable for studying the nutritional quality of cereal foods. The objective is to show the important role which food microscopy plays in linking food processing technologies and analysis and with nutritional sciences. The five types of microscopy include bright-field, fluorescence, and polarized light microscopy, confocal laser scanning microscopy, and scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) microanalysis.

### Light Microscopy

Except SEM-EDX microanalysis, all of the techniques mentioned previously belong to the class of optical microscopy. They operate on the same basic principle by which an image is formed when electromagnetic radiation is transmitted through or reflected from an object mounted on the specimen stage of a light microscope. The types of light microscopy vary, depending on the methods of illumination and image detection.

### Bright-field microscopy

In conventional bright-field microscopy, transmitted light is brought to focus in the plane of the object via a condenser lens. The microscope objective forms a tiny real (but inverted) image of the object in the focal plane of the eyepiece lens. The eyepiece magnifies that image to a point where it can be resolved on the retina of the eye. Object specimens can be stained with color reagents to improve the contrast of the image and the detection specificity. Bright-field microscopy is the most

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commonly used microscopic detection method. It requires a simple or compound microscope to conduct food structure analysis.

### Fluorescence microscopy

The principle of fluorescence microscopy is based on an optical phenomenon in which light of a certain wavelength is absorbed by a substance and re-emitted as light of a longer wavelength. Some fluorescent substances occur naturally. For example, ferulic acid, a phenolic compound present in high concentrations in cereal aleurone cell walls, imparts blue autofluorescence upon ultraviolet light excitation (Fulcher *et al.*, 1972). Fluorescence can be induced in some food structures by staining with fluorescent reagents (fluorochromes). Some of these reagents have known chemical specificity. For example, Calcofluor White binds (1→3)(1→4)- $\beta$ -D-glucan, a cereal cell wall polysaccharide, and induces bluish-white fluorescence on the bound cell wall (Wood *et al.*, 1983). Certain fluorescein-labeled plant lectins, such as *Lens culinaris* agglutinin and Concanavalin A, are suitable fluorescent markers for cereal starches (Miller *et al.*, 1984).

A fluorescence microscope is a compound microscope equipped with a set of light filters (also known as exciter and barrier filters) to allow fluorescence excitation and detection of the emitted light at appropriate wavelengths. In addition, the microscope normally requires a high intensity light source and a set of special objectives for examination of fluorescent specimens.

### Polarized light microscopy

Polarized light microscopy is used to detect anisotropic substances which have internal crystalline structures and display more than one refractive index. When viewed under polarized light, these substances exhibit birefringence. Birefringence is an optical phenomenon which will be described in the following section.

The polarized light microscope differs from a conventional bright-field microscope by having two Nicol prisms, also known as the analyzer and the polarizer. When the two prisms are placed with their transmitting axes parallel to each other, light transmitted by the polarizer is also transmitted by the analyzer. When the prisms are crossed and their axes are at right angles to each other, no light is transmitted. When, however, a birefringent object is introduced into the light path between the crossed prisms, a variable amount of light passes at the site of the object. The polarized light beam is split into two rays oscillating at right angles and traveling through the specimen object at different velocities. The plane of light oscillating parallel to the analyzer is transmitted by the analyzer, thus producing a bright image of the object on a dark background. Examples of food substances that exhibit birefringence under polarized light include most starches (Wivinis and Maywald, 1967), crystalline fats (Kalab *et al.*, 1987) and some crystalline salts (Yiu, 1985).

### Figure Captions

Unless otherwise stated, all micrographs show 3% glutaraldehyde-fixed, glycol methacrylate-embedded sections of oat or wheat samples.

**Figure 1.** A wheat kernel showing structures of starch granules (arrows) after staining with fluorescein-labeled *Lens culinaris* agglutinin photographed using fluorescence exciter/barrier filters set for maximum transmission at 490 nm / > 520 nm. Bar = 50  $\mu$ m.

**Figure 2.** A fluorescence micrograph of quick-cooking rolled oats stained with fluorescein-labeled Concanavalin A, showing the structures of intact (asterisks) and broken (arrows) compound starch granules (exciter/barrier filters set for maximum transmission at 490 nm / > 520 nm. Bar = 20  $\mu$ m).

**Figure 3.** An unstained, frozen section of oat kernel viewed under polarized light to reveal the pattern of birefringent starch granules (arrows). Bar = 20  $\mu$ m.

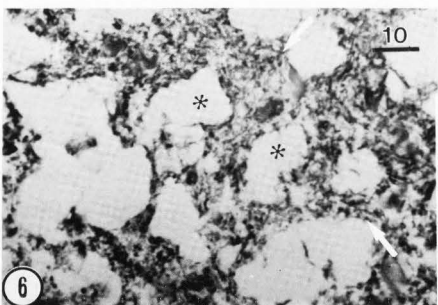
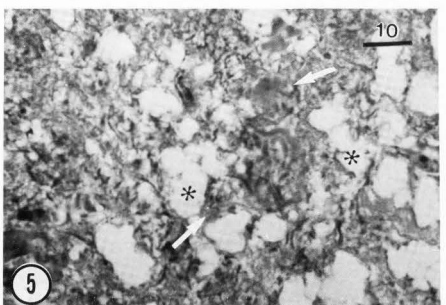
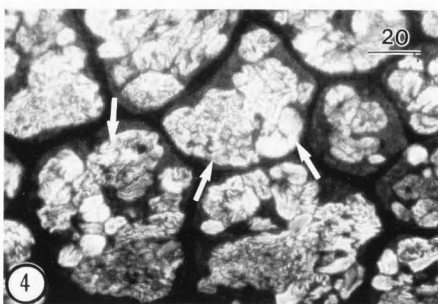
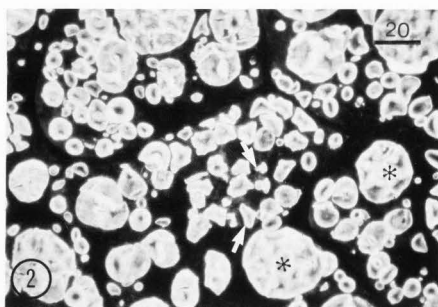
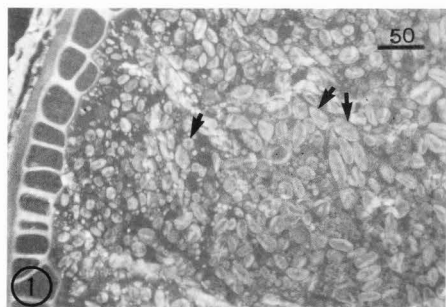
**Figure 4.** Cooked rolled oats stained with fluorescein-labeled Concanavalin A, showing the structures of cooked starch (arrows). Exciter/barrier filters set for maximum transmission at 490 nm / > 520 nm. Bar = 20  $\mu$ m.

**Figure 5.** Rapidly cooked rolled oats stained with iodine potassium iodide and examined by bright-field microscopy to show the structures of cooked starch (arrows) and small void spaces (asterisks). Bar = 10  $\mu$ m.

**Figure 6.** Gradually cooked rolled oats stained with iodine potassium iodide and examined by bright-field microscopy, revealing the expanded structures of cooked starch (arrows) and large void spaces (asterisks). Bar = 10  $\mu$ m.

### Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) represents one of the latest developments in fluorescence detection technology. A confocal laser microscope differs from a fluorescence microscope in its illumination and image detection systems. The illumination system of a confocal microscope contains a laser light beam which is focused on the specimen by conventional microscope optics. This point of focus is the first of the two related points necessary for confocal microscopy. The high energy laser light interacts with the specimen, and low energy light (fluorescence) is emitted. This light passes back through the microscope optics to a simple lens which focuses the emitted light on a detector pinhole. The pinhole forms the second focal point. A detector (usually a photomultiplier tube) picks up the light. The signal is amplified and processed electronically to form an image of the scanned area.



An image of the specimen is generated from the points of illumination gathered by the detector during the scanning cycle. Both illumination and detection are directed at the same point on the specimen. The confocal system excludes all light reflected from out-of-focus objects, thus producing an image of higher contrast and better resolution than conventional fluorescence microscopy (Miller and Foster, 1991).

Furthermore, the confocal microscope enables the examination of food samples at different focal points (depths). Consequently, it generates optical sections which can be electronically stored and processed to provide three-dimensional images of the specimen. The above technique has been used to study the microstructures of mayonnaise, cheese and dough (Heertje *et al.*, 1987), as well as the functionality and microstructure of meats (Offer *et al.*, 1989).

### SEM Coupled with EDX

SEM uses accelerated electrons focused by electromagnetic or electrostatic lenses to form an electron beam. The focused beam initiates several interactions when it impinges upon a target specimen. One of the phenomena that occur is the inelastic scattering of electrons. These electrons lose energy by interacting with the specimen. SEM-EDX microanalysis is concerned mainly with two types of energy loss, namely the secondary electrons and X-ray emission.

Secondary electrons resulting from the electron and specimen interaction are detected and amplified to form an SEM image of the scanned area of the specimen. The image provides information on the surface topography and three-dimensional structure of the specimen.

Energy in the form of X-rays is generated as an incident electron strikes the specimen. With sufficient energy, the incident electron can dislodge an electron from the inner shell of an atom of an element present in the specimen. The atom is in its excited (ionized) state. Upon returning to its original state by transition of an outer electron into the vacancy in the inner shell, this atom emits energy in the form of an X-ray photon. The frequency of the X-rays emitted from the specimen is a function of the atomic number of the elements which constitute the specimen. The intensity of the X-rays is proportional to the concentration of these elements. An energy dispersive spectrometer detects and measures the emitted X-rays. It also converts X-ray intensities into qualitative and quantitative data. These data provide information on the elemental composition of the specimen in the area under examination.

SEM-EDX microanalysis is successfully adapted to various mineral studies. These include the study of mineral composition in cocoa solids (Brooker, 1990), mineral distribution in cereal grains (Buttrose, 1978) and mineral migration in wheat during milling (Saleh *et al.*, 1984). Charbonneau (1988) used the same technique to detect corrosion in food cans.

### Food Microscopy and Cereal Structures

This section will show how the five different types of microscopy are used to study the relationship between food microstructures and the nutritional quality of cereal foods. Cereal grains are rich sources of starch, dietary fiber, and minerals. Most of these nutrients are stored within microscopically distinct structures. Oat and wheat products will be used as examples for this review.

#### Starch

**Structure and distribution.** Oat and wheat starches occur in the form of colorless translucent bodies, identified as starch granules. Wheat starch consists of both small (2-10  $\mu\text{m}$ ) spherical and large (20-40  $\mu\text{m}$ ) lenticular granules (Fig. 1). Unlike wheat starch, oat starch occurs chiefly as compound structures which are aggregates of individual starch granules (Fig. 2). The compound starch granules range from 20 to 100  $\mu\text{m}$  in diameter whereas the diameter of individual starch granules is 4-8  $\mu\text{m}$ .

Both oat and wheat starches are located within cells of the starchy endosperm of their respective grain kernels. Both display birefringence, in a 'Maltese cross' pattern, when examined under the polarized light (Fig. 3).

**Effects of processing and cooking on starch structure.** Mechanical grinding breaks up the compound structure of oat starch (Fig. 2), but appears to have no damaging effect on the integrity of individual starch granules (Yiu, 1986). When starch is heated in the presence of water, the granules begin to swell because of water absorption. During the cooking of rolled oats, swelling of the starch granules is initially hampered by the rigidity of the oat cell walls, resulting in markedly distorted and convoluted starch structures (Fig. 4). The structural integrity and birefringent property of the granules are ultimately lost when the starch becomes completely gelatinized.

Bright-field microscopy was used to compare the structures of cooked starch (Yiu *et al.*, 1987). The starch from rolled oats cooked rapidly in boiling water was compared with the starch of rolled oats cooked gradually from room temperature. The rapidly cooked starch (Fig. 5) contained smaller water-holding spaces and was less convoluted in structure than the gradually cooked starch (Fig. 6). Image analysis of the microstructures provided a quantitative assessment of the difference in hydration between the two cooked products (results not shown). This difference contributes to the textural variation in rolled oats prepared by the two cooking methods (Yiu *et al.*, 1987). Gradual cooking resulted in a preparation of porridge that was creamy and smooth in texture. Rapid cooking, on the other hand, resulted in a grainier and less sticky product.

**Digestibility of raw and cooked starch.** Particle size reduction enhances the digestibility of starch-bearing oat and wheat grains. Raw starch is less readily

digested by human salivary  $\alpha$ -amylase than cooked starch (Fig. 7). During cooking, the expanded structure of gelatinized starch provides greater accessibility to digestive enzymes, resulting in an increased rate of starch digestion. The structural appearance of partially digested starch is revealed by bright-field microscopy in Fig. 8 which shows the appearance of spherical lesions on the surface of the enzyme-digested starch granules.

Processing methods, such as extrusion cooking, puffing and instantization, hydrate the starch granules. They disrupt the native structures of starch granules and alter starch digestibility (Holm *et al.*, 1985). Freezing and thawing induce retrogradation of starch and increase its resistance to amylolysis (Englyst *et al.*, 1983).

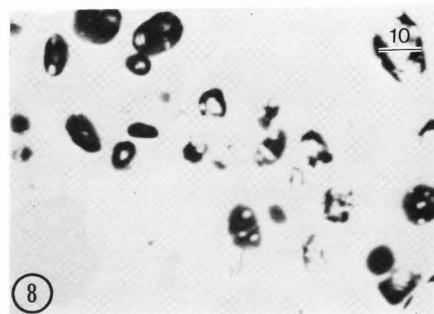
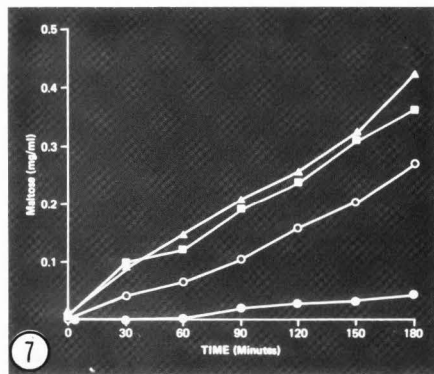
#### Dietary fiber

**Structure and distribution.** Cereal cell walls, particularly those of oat and wheat bran, are major sources of dietary fiber. Oat bran and wheat bran differ in their fiber content and composition. Frolich and Nyman (1988) estimated that commercial oat bran has less than half the amount of total dietary fiber of wheat bran. However, the former contains more soluble fiber than the latter, approximately 35% as compared to 2% found in wheat bran. Furthermore, most of the soluble fiber in oats is present as (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan, generally known as  $\beta$ -glucan.

Fluorescence microscopy is a useful tool to study the difference in fiber composition between oat bran and wheat bran. When stained with a specific fluorochrome (e.g. Calcofluor White) and viewed under appropriate excitation (< 365 nm), oat bran is characterized by its  $\beta$ -glucan content. The glucan is found in the endosperm cell walls, particularly those of the inner aleurone and subaleurone layers (Fig. 9). Wheat bran, on the other hand, does not have the same histochemistry. Its cell walls contain large amounts of phenolic substances. The latter autofluoresce when viewed under short wavelength (< 365 nm) excitation (Fig. 10).

**Effects of processing and cooking on cell wall structures.** Mechanical processing breaks down the endosperm cell walls of oats and wheat (Moss *et al.*, 1980; Yiu, 1986). The degree of the breakdown depends on the impact of the mechanical force applied. For example, during the production of rolled oats, thick oat flakes are produced as previously conditioned whole oat kernels are passed through a roller mill. Thin oat flakes result from subjecting the whole kernels to a cutting step before rolling (Deane and Commers, 1986). Thin oat flakes have more fractured cell walls than thick oat flakes (Yiu, 1986).

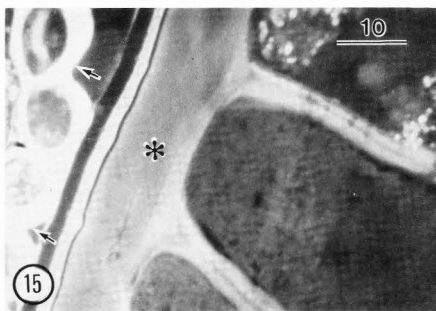
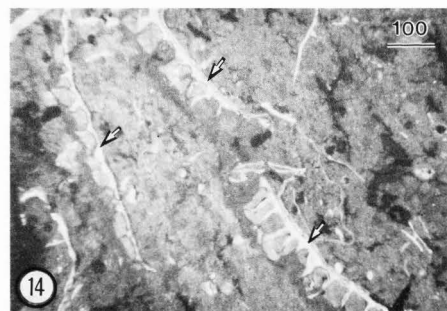
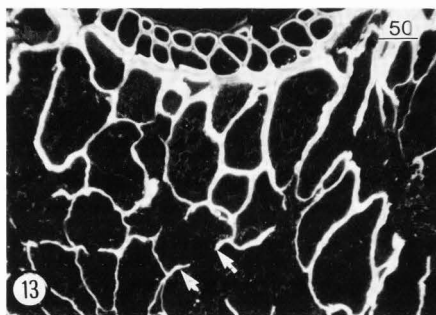
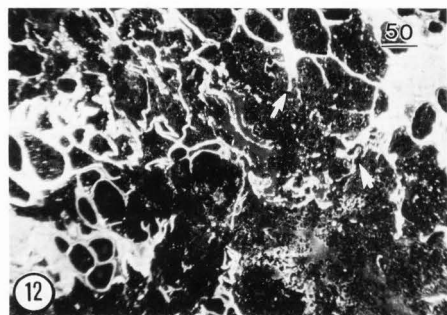
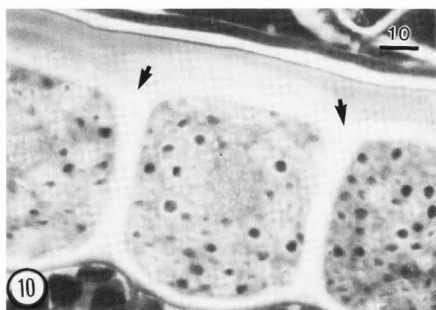
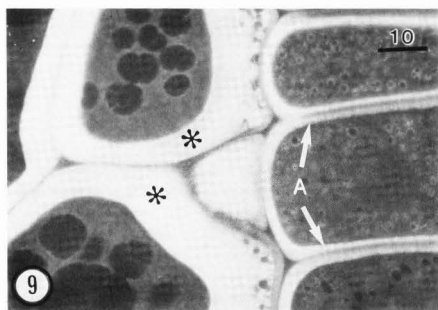
Thick oat flakes require a longer cooking time than thin oat flakes. The former cooked product is also less mushy in texture and has a grainier mouthfeel than the latter cooked flakes. Cooking enhances the release of  $\beta$ -glucan from the oat cell wall. The amount released is closely associated with the duration of cooking (Fig. 11). The release is also related to the extent of cell wall disruption (Yiu *et al.*, 1987, 1991).



**Figure 7.** Rate of starch digestion of rolled oats prepared by soaking (o - o), rapid cooking ( $\blacktriangle$  -  $\blacktriangle$ ), gradual cooking ( $\blacksquare$  -  $\blacksquare$ ) and incubated with human saliva. The control contained a gradually cooked sample incubated with heat-inactivated human saliva ( $\bullet$  -  $\bullet$ ).

**Figure 8.** Human saliva-digested rolled oats stained with iodine potassium iodide and examined by bright-field microscopy to reveal residues of the starch granules. Bar = 10  $\mu$ m.

Cooking methods also influence the amount of  $\beta$ -glucan released from the oat cell wall. Oat flakes prepared by the conventional, top-of-the-stove method have considerably more cell wall disruption (Fig. 12) and more  $\beta$ -glucan release than oat flakes prepared by microwave cooking (Fig. 13).



Note Figure 11 is on the facing page.

**Figure 9.** An oat kernel stained with Calcofluor White, showing the distribution of  $\beta$ -glucan-rich aleurone (A) and subaleurone (asterisks) cell walls. Exciter/barrier filters set for maximum transmission at 365 nm / > 418 nm. Bar = 10  $\mu$ m.

**Figure 10.** A wheat kernel stained with Acridine Orange and viewed under short wavelength excitation (365 nm / > 418 nm) to show structures of the aleurone cell walls of high phenolic contents (arrows). Bar = 10  $\mu$ m.

**Figure 12.** A rolled oat sample prepared by conventional (20 minutes) cooking and stained with Calcofluor to demonstrate the extent of the cell wall breakdown (arrows) in the oat tissues. Exciter/barrier filters as in Figure 9. Bar = 50  $\mu$ m.

**Figure 13.** A rolled oat sample prepared by microwave (20 minutes) cooking and stained with Calcofluor to reveal the structures of the oat cell walls (arrows). Exciter/barrier filters as in Figure 9. Bar = 50  $\mu$ m.

**Figure 14.** The colonic digesta of a rat fed a diet containing oat bran. The digesta were stained with Acridine Orange to reveal the remnant (arrows) of the bran cell walls. Exciter/barrier filters set at 490 nm / > 520 nm. Bar = 100  $\mu$ m.

**Figure 15.** The colonic digesta of a rat fed a diet containing wheat bran. The digesta were stained with Acridine Orange, showing the partially digested aleurone (asterisk) and pericarp (arrows) tissues. Filters set as in Figure 14. Bar = 10  $\mu$ m.

Table 1

Fermentative Characteristics of Cereal Bran\*

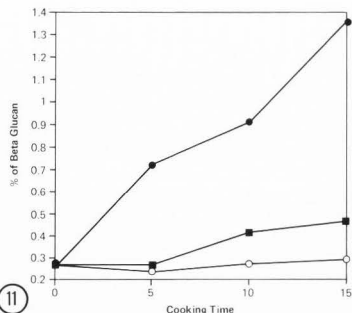
	Oat Bran	Wheat Bran
% NDF Digestibility	60.50	43.55
SCFA (mmol/g DM)	5.08	4.02
H <sub>2</sub> (mmol/g DM)	495.47	254.31
CH <sub>4</sub> (mmol/g DM)	29.38	114.45

\*Cereal bran samples were first incubated with pepsin at pH 2.2 for 3 hours, followed by a pancreatic-bile mixture at pH 7.5 for 9 hours, and a human fecal inoculum under anaerobic conditions for 24 hours.

NDF: Neutral detergent fiber.

SCFA: Short chain fatty acids.

DM: Dry matter.



**Figure 11.** Effect of different cooking methods on  $\beta$ -glucan release from rolled oats. Soaking (o - o), rapid cooking (■ - ■) and gradual cooking (● - ●).

#### Cereal fibers and their nutritional implication

Data from metabolic studies suggest that soluble fiber like  $\beta$ -glucan influences the rate of nutrient absorption in the human small intestine and consequently affects the metabolism of carbohydrates (Wood *et al.*, 1990) and fats (Anderson and Chen, 1986). The predominance of  $\beta$ -glucan in oat bran marks the difference in the metabolic and physiological functions between oat and wheat bran. Oat bran has hypocholesterolemic properties (Anderson and Chen, 1986), whereas wheat bran is effective in fecal bulking and reducing the incidence of colorectal cancers (Burkitt, 1988).

In humans, most of the fiber degradation takes place in the large intestine by microbial fermentation. Oat and wheat bran also differ in their fiber fermentability (Mongeau *et al.*, 1991). They two have different fermentative characteristics (McBurney and Thompson, 1990) which are measurable by the different amounts of the products generated from the above fermentation. These products include short chain fatty acids, hydrogen gas and methane gas (Table 1).

Fluorescence microscopy is a useful tool to provide a visible comparison of the structural breakdown between oat and wheat cell walls. The  $\beta$ -glucan-rich aleurone and subaleurone cell walls of oat bran are more susceptible to the fermentation process (Fig. 14) than the aleurone and the subaleurone walls of wheat bran (Fig. 15).

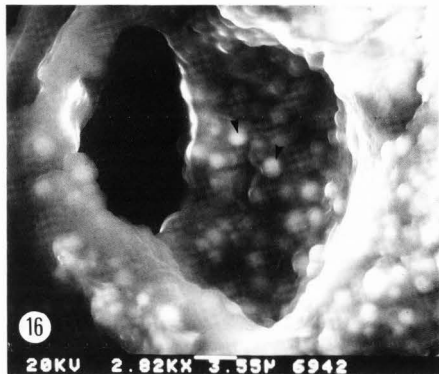
The detection of undigested bran fragments in the colon suggests that some carbohydrate constituents of the bran cell wall are unavailable as nutrients. Results of mineral binding studies show that oat and wheat bran had affinity for certain minerals (Moak *et al.*, 1987; Platt and Clydesdale, 1987). Dintzis *et al.* (1985) found that the occurrence of the indigestible remnants of wheat bran, mostly pericarp tissues, in the feces was associated with an increase in calcium and iron excretion in humans. However, the excretion did not result in mineral imbalance.



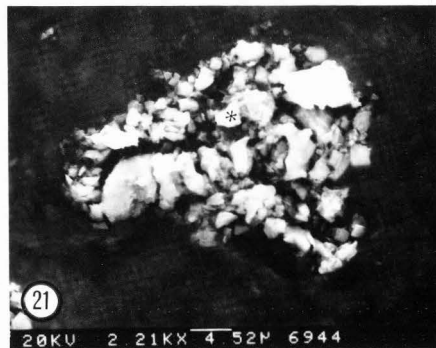
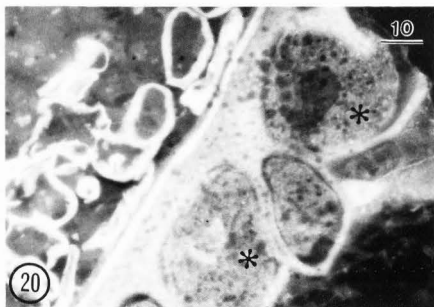
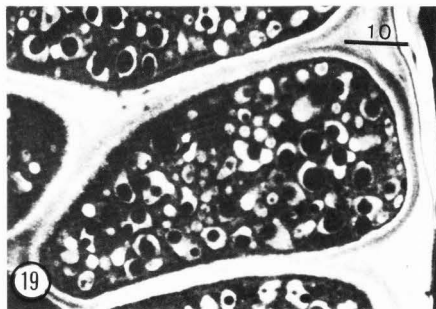
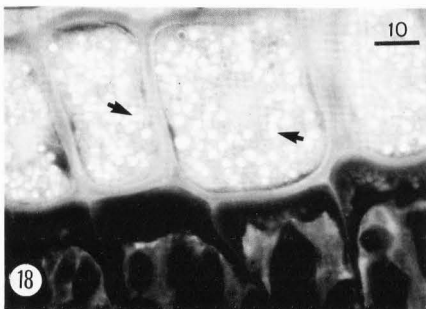
## Minerals

**Structure and distribution.** Chemical studies on the distribution of minerals in cereal grains show that most of the mineral reserves is contained in the bran and germ fractions of the grains (O'Dell *et al.*, 1972). It occurs as phytate, a mixed salt of potassium and magnesium *myo*-inositol hexaphosphate, which accounts for 70-90% of the total phosphorus reserve in oats and wheat (O'Dell *et al.*, 1972; Frolich and Nyman, 1988).

Studies using SEM-EDX provide valuable information on the distribution, structural organization and elemental composition of phytate-containing particles in these cereals (Buttrose, 1978; Lott and Spitzer, 1980). The above particles, also known as phytin globoids, are found in the aleurone and scutellum tissues of the grains. Under an electron beam, they appear as electron-dense spherical inclusions embedded in a protein matrix (Fig. 16). These particles range from 1 to 2  $\mu\text{m}$  in diameter. When subjected to EDX microanalysis, the globoids emit X-rays characteristic of their elemental composition. A typical EDX spectral profile of an oat phytin globoid is composed of three major element peaks, phosphorus (P), potassium (K), and magnesium (Mg) (Fig. 17).



Note Figure 17 is on the facing page.



**Figure 16.** Oat bran carbon-coated for SEM-EDX microanalysis and examined at 20 kV, revealing the electron-dense phytin globoids (arrows) within an aleurone cell. Bar = 3.55  $\mu\text{m}$ .

**Figure 17 (at right).** An EDX spectral profile of one of the oat phytin globoids observed in Figure 16. X-ray signals were collected and analyzed with an EDX microprobe for 100 seconds per site. Probe current:  $5 \times 10^{-9}$  A, probe size: 180 nm.

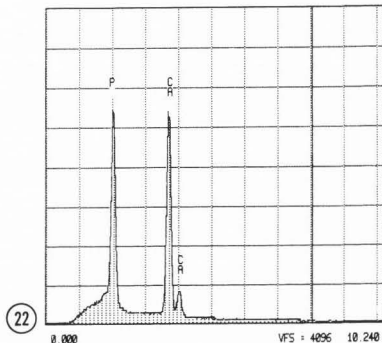
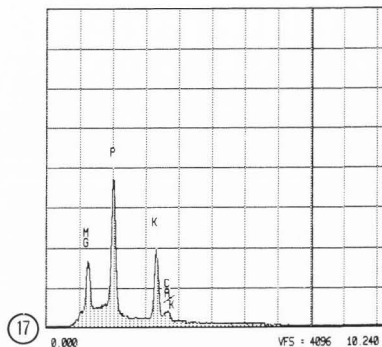
**Figure 18.** An oat kernel stained with Acriflavine, showing the presence of phytin globoids (arrows) within the aleurone cells. Filters set at 546 nm / > 590 nm. Bar = 10  $\mu\text{m}$ .

**Figure 19.** A computer-enhanced micrograph of a hand-cut section of oats examined unstained by confocal laser scanning microscopy under short wavelength excitation (365 nm / 418 nm), revealing structural details of the aleurone cells. Bar = 10  $\mu\text{m}$ .

**Figure 20.** Puffed wheat stained with Acriflavine, demonstrating changes (asterisks) in the aleurone cell structures. Filters set at 490 nm / > 520 nm. Bar = 10  $\mu\text{m}$ .

**Figure 21.** A carbon-coated section of rat colonic digesta examined at 20 kV showing the structures of unabsorbed minerals. Asterisk marks a structure analyzed for elemental composition (see Figure 22). Bar = 4.52  $\mu\text{m}$ .

**Figure 22 (at right).** An elemental profile of one of the structures shown in Figure 21 (identified by an asterisk). X-ray signals were collected and analyzed with an EDX microprobe for 100 seconds per site. Probe current:  $5 \times 10^{-9}$  A, probe size: 180 nm.



The crystalline structures of phytin globoids are detectable using polarized light microscopy (result not shown). For confirmation, however, the detection is often carried out with fluorescence microscopy, using Acriflavine as a fluorescent probe (Fig. 18), and/or bright-field microscopy, using Toluidine Blue as a staining reagent. For high resolution work, confocal laser scanning microscopy provides excellent contrast and detailed structural information on the morphology of phytin globoids and their surrounding subcellular structures (Fig. 19). More studies will be required in order to explore the potential of CLSM as an analytical tool to study nutrient storage in cereal grains.

#### Processing and cooking

Milling reduces the phytate content of wheat (Nayini and Markakis, 1983) by removing the bran and germ fractions from the flour. However, milling does not dissociate the structural attachment of phytin globoids from these fractions.

Mild heat treatment, such as domestic cooking, reduces the phytate content in wheat but not in oats (Sandstrom *et al.*, 1987). The difference in the phytate content between the two cooked cereals is related to the activities of a specific enzyme (phytase) present in their cereal grains. Results based on  $^{31}\text{P}$ -nuclear magnetic resonance spectroscopy confirm that oat phytase, which breaks down phytic acid, is inactivated by the heat treatment used during commercial oat processing (Frollich *et al.*, 1988). The low phytase activity is linked to the relatively high phytate content of oats and oat products.

During bread-making, the presence of additional phytases from yeast and the baking process itself significantly reduce the phytate content in bread (Nayini and Markakis, 1983). Vigorous processing methods like extrusion cooking and puffing induce structural damage to the bran fraction. Some of the damage is observable microscopically as in Figure 20 which shows that components within the aleurone cells are no longer identifiable by phytin-specific staining (Fig. 20).

**Phytate digestibility and its nutritional implication.** Early metabolic studies showed that cereal phytate was not readily available for digestive absorption by humans (McCance and Widdowson, 1935). Much of the ingested phytate present in oat bran fed to rats remained undigested in the small intestine (Yiu and Mongeau, 1987). Microscopic examination of the rat digesta revealed that many of the phytin globoids remained structurally associated with the aleurone tissue of the bran. Furthermore, data from SEM-EDX microanalysis showed that many undigested globoids retained their structural identity and elemental composition upon reaching the distal end of the large intestine.

Low or reduced phytase activities were associated with low phytate digestibility in certain oat and extruded wheat products (Sandberg *et al.*, 1987). Mineral deficiency was linked to low phytate digestibility. Decreases in phytate degradation resulted in parallel reductions of zinc, phosphorus, and magnesium absorption in humans (Kivisto *et al.*, 1986).

SEM-EDX microanalysis is also a useful tool to investigate the fate of some dietary minerals present in the diet containing cereal bran. Structural features and elemental contents of feces of rats fed a diet rich in oat bran are presented in Figs. 21 and 22. Such data provide valuable information on the mineral binding activity of the bran components. However, more studies will be required in order to fully explore the analytical capability of SEM-EDX microanalysis in nutritional studies.

### Summary

Most carbohydrate and mineral constituents of oat and wheat grains are stored as distinct microstructural components. Some of these components include starch granules, cereal cell walls and phytin globoids. Their structure and chemical composition can be determined using microscopy. This paper reviews the application of various forms of light microscopy and scanning electron microscopy in cereal structure studies.

Through the study and analysis of cereal structures, the impact of processing can be related with chemical and structural changes of some cereal components. The present review shows how food microscopy functions as an analytical tool for studying these changes. It also shows the usefulness of light microscopy in determining factors that influence the texture and digestibility of cereal foods. In addition, the review provides examples to illustrate the use of SEM-EDX microanalysis in investigating the nutrient availability of oat and wheat bran components. This technique has an analytical potential for studying the interactions that involve phytate, dietary fiber and dietary minerals.

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