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EFFECTS OF PROCESSING AND COOKING ON
THE STRUCTURAL AND MICROCHEMICAL COMPOSITION OF OATS

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Abstract

Fluorescence microscopy was used to analyze the structural and microchemical organization of oat constituents in cooked and uncooked products of whole grain and three commercially available rolled oats. Results of the microscopic examination indicated that proteins and lipids in the endosperm tissue were most susceptible to processing. Instead of being individually packaged in distinct structural units like those found in unprocessed groats, both proteins and lipids appeared as aggregated masses after processing. Cooking induced further aggregation. Starch grains in the uncooked rolled oat samples still retained their granular, polygonal structures, although some compound grains were broken up into individual starch granules in quick rolled oats. Partial gelatinization of starch was observed in instant rolled oat samples. Cooking resulted in many starch granules losing their original structural organization and their anisotropic characteristics. Most endospermic cell walls in the rolled oat samples were fractured due to the impact of mechanical processing; cooking released some of the β -glucans. Not all the β -glucans in rolled oats were dispersed by cooking; most of them remained associated with the fragmented cell walls. The aleurone and sub-aleurone walls were relatively resistant to processing and cooking. Phytin and phenolic compounds were abundant in the sturdy aleurone layer of old fashioned rolled oats. Additional flaking, as in the production of quick and instant rolled oats, induced more cell wall breakage in the aleurone layer, leading to the exposure of its cell contents. Cooking reduced the detectable number of phytin globoids in rolled oat samples whereas phenolic compounds remained strongly bound to the aleurone wall.

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Introduction

Oats are used mainly as a livestock feed and only a small portion of the crop (about 8%) is used for human consumption (Thomas, 1978). Recent observations that rolled oats and oat bran have hypocholesterolemic effects on humans (Anderson et al., 1983) have renewed interest in oats as an important food source. Oats are a good source of proteins of high biological value, complex carbohydrates, polyunsaturated fats, minerals and vitamins (Weaver et al., 1981). Furthermore, the crop requires minimum labor in handling and producing and is most suitable to grow in the North American climate (Stanton, 1953). In the past, most oat research was focussed on the technological aspects of processing, such as extraction and characterization of oat constituents (starch, proteins and gums) and determination of their functionalities (Paton, 1977; Wood et al., 1978). Apart from a few published data (Lookhart et al., 1986), relatively few studies have examined the effects of processing and cooking on the structural organization of oats.

Oats have been implicated in regulating certain physiological functions, such as cholesterol reduction. It is believed that at least one of the constituents in oats, the oat gum, is responsible for the hypocholesterolemic effect (Chen et al., 1981). Hence, the retention of constituents in oats and their products after commercial processing and cooking may indirectly determine the extent of their physiological action. Many factors govern the availability of constituents in oats and other cereal products: some of these include intrinsic factors, such as the structural and chemical organization of their storage reserves as well as extrinsic factors, such as processing and cooking. The present study aims at studying the effects of these factors on the composition of constituents in oat products.

Fluorescence microscopy is a useful tool for studying the structural and chemical composition of seed constituents in cereals (Fulcher and Wong, 1980; Earp et al., 1983) and oil seeds (Yiu et al., 1982). It is also an analytical tool for revealing the effects of processing on food constituents (Yiu et al., 1983 and Yiu, 1985). The specificities of some fluorochromes, such as

Key Words: Rolled oats, Structures, Microchemical composition, Processing effects, Cooking effects.

Congo Red and Calcofluor White for β -glucans (Wood et al., 1983), Acriflavine HCl for phytin (Yiu et al., 1982 and Fulcher, 1982), and Nile Blue A for cereal lipids (Hargin et al., 1980) as well as fluoresceinated lectins for cereal starch (Miller et al., 1984) are well established. Many of these fluorescent reagents can be used as specific microscopic markers. Furthermore, the relatively rapid and simple techniques of fluorescence microscopy allow rapid screening of a large number of samples to be conducted. These techniques can also be used in conjunction with bright-field and polarizing microscopy.

Materials and Methods

Oat samples

Three kinds of rolled oat products were examined: old fashioned (or regular), quick (or one-minute), and instant rolled oats. All three products were purchased commercially. Technical details for the production of these products are not available. In general, old fashioned rolled oats are produced by steaming and rolling whole, dehulled grains (groats). Quick rolled oats are produced by rolling previously cut groats (3-4 pieces cut from a whole groat) after steaming. The production of instant rolled oats is similar to that of quick rolled oats except that a higher temperature is used during steaming.

Rollled oats were soaked or cooked in boiling water according to methods recommended by the manufacturers. Cooked oat flakes (about 20 pieces) were removed immediately from the rest of the mixture and were rinsed in cold water.

Mature kernels of oats (*Avena sativa* L., cultivars: Scott and Hinoat) were obtained from Plant Gene Resources of Canada, (Agriculture Canada, Ottawa, Ont.) and were cut transversely into 1-2 mm thick pieces.

All cooked and uncooked oat samples were separately fixed in 5% glutaraldehyde in 0.01 M sodium phosphate buffer, pH 7.0, at 4°C for 24-48 hours.

Frozen sections

Frozen sections were used for lipid and some starch studies. Fixed oat samples were embedded in a support medium for frozen sectioning (Histo Prep., Fisher Scientific Co., Fair Lawn, N.J.), mounted on cold object discs and frozen immediately. Sections were cut 6-8 μ m thick using a cryo-microtome (Reichert-Jung Scientific Instruments, Belleville, Ont.) and affixed to glass slides for subsequent microscopic examination.

Glycol methacrylate (GMA)-embedded sections

Fixed samples were dehydrated through a series of changes of alcohol, from methyl cellosolve, ethanol, n-propanol to n-butanol according to the method described by Fulcher and Wong (1980). The samples were then infiltrated with GMA monomer for 3-5 days prior to polymerization at 60°C or at room temperature under ultraviolet light. Sections 2-4 μ m thick were cut using glass knives in an ultramicrotome (Sorvall Inc., Newtown, CT.) and were mounted on glass slides for subsequent microscopic examination.

Microscopic examination

Sections were examined using a Zeiss Univer-

sal Research Photomicroscope (Carl Zeiss Ltd., Montreal, Quebec) equipped with both a conventional bright-field illuminating system and a III RS epi-illuminating condenser combined with an HBO 100 W mercury-arc burner for bright-field or fluorescence analysis. The III RS condenser contains three fluorescence filter systems with a dichromatic beam splitter and an exciter/barrier filter set for maximum transmission at 365 nm/>418 nm (FC I), 450-490 nm/>520 nm (FC II) and 546 nm/>590 nm (FC III). Photomicrographs were obtained using 35 mm Kodak Ektachrome 400 Day-light film. Sections were photographed after staining using one or more of the following procedures.

Proteins GMA-embedded sections were stained with an aqueous solution of 0.01% (w/v) 1-anilino-8-naphthalene sulfonic acid (ANS) (Sigma Chem. Co., St. Louis, MO.) or 0.1% (w/v) Acid Fuchsin (Fisher Scientific Co., Fair Lawn, N.J.) according to the method by Fulcher and Wong (1980). Stained sections were rinsed with distilled water, air-dried and mounted in non-fluorescent immersion oil under a coverslip. These sections were then examined microscopically using filters FC I for sections stained with ANS and FC II for those with Acid Fuchsin.

Starch The method described by Miller et al. (1984) was used with slight modifications. Sections were incubated with 1.2 mg/ml of fluorescein-labelled *Lens culinaris* agglutinin (F-LCA) or Concanavalin A (F-Con A) (Cedarlane Lab. Ltd., Hornby, Ont.) in 0.01M sodium phosphate buffer, pH 7, at room temperature for 1-2 min. Stained sections were rinsed thoroughly with distilled water, air-dried, mounted in oil and examined under the microscope using filter FC II. Stained or unstained sections were also examined under polarized light (through two crossed polarizers) to observe birefringence in starch.

Lipids Frozen sections were stained with 0.01% (w/v) aqueous Nile Blue A (CI 51180, Eastman Kodak Co., Rochester, N.Y.) for 1 minute. Stained sections were rinsed and mounted in water under a coverslip and examined microscopically using filter FC II.

Beta-glucans GMA-embedded sections were stained with aqueous 0.01% (w/v) Calcofluor, (Polysciences, Inc., Warrington, PA.) or 0.01% (w/v) Congo Red (CI 22120, Fisher Scientific Co., Fair Lawn, N.J.) for 1-2 min. After rinsing and drying, mounted sections were examined under the microscope using filters FC I for Calcofluor and FC II or FC III for Congo Red.

Phytin GMA-embedded sections were stained with 0.01% (w/v) aqueous Acriflavine HCl (BDH Chem. Ltd., Poole, England) for 1-2 min, according to the method described by Yiu et al. (1982). After rinsing and drying, sections were mounted in immersion oil and examined microscopically using the FC I or FC III system.

Phenolic compounds Many phenolic compounds emit autofluorescence under short wavelength excitation (Fulcher and Wong, 1980). Sections were mounted in non-fluorescence immersion oil and examined microscopically using filter FC I.

To detect simultaneously the presence of more than one of the above components, some sec-

tions were stained with two fluorochromes. This was done by allowing the sections to react sequentially with the appropriate reagent, with thorough rinsing between each staining. Stained sections were then examined microscopically using filter system FC I for the combined staining of Acid Fuchsin and Calcofluor, FC II for F-LCA and Congo Red, and FC I for Acriflavine HCl and Calcofluor.

Results and Discussion

The structure of the groat has been described in detail by Fulcher and Wong (1980), and only a brief description is presented here. Most rolled oat products are produced from whole groats which contain three fractions that differ in milling properties, cell structures, and chemical contents. They are the bran, the starchy endosperm, and the germ. Commercial bran is composed of several outer layers of fibrous tissues. These include the aleurone and the sub-aleurone layers which are part of the starchy endosperm. They tend to separate from the rest of the tissue during most mechanical processing. Oat bran is rich in proteins, lipids, β -glucans, minerals, and vitamins (Weaver et al., 1981). It is also rich in phenolic compounds, most of which are associated with the primary cell wall of the aleurone layer. The starchy endosperm, which occupies a major part of the groat, is where most of the storage reserves of oats are located. These include proteins, starch, lipids and gums. The germ occupies only 2-3% of the total groat area. It comprises several distinct tissues, including the scutellar parenchyma where high concentrations of proteins, lipids, minerals, and phenolic compounds are located (Fulcher and Wong, 1980).

Proteins

Oat storage proteins occur primarily as protein bodies within various tissues of the groat. Their presence can be easily demonstrated using appropriate fluorescence markers such as Acid Fuchsin or ANS (Fulcher and Wong, 1980). Most oat protein bodies are spherical in shape and their size ranges from 0.2 to 6.0 μm in diameter (Fig. 1). In comparison with protein bodies of the groat, those present in rolled oats were no longer recognizable as individual structures. Instead, they appeared as amorphous aggregates, some of which still remained confined, even after processing, within structurally well defined cell walls (Fig. 2). Cooking and boiling in water caused more aggregation of the protein bodies which remained detectable using the above fluorescence markers (Fig. 3). These findings indicate that processing and cooking have altered the structural organization of oat storage proteins but have little impact on changing their affinity for Acid Fuchsin or ANS.

Starch

Oat starch occurs mostly as compound or aggregated grains, each of which is composed of two to several polygonal granules. These starch grains range from 20 to 100 μm in diameter while individual granules are 4-8 μm in diameter. Using F-Con A and F-LCA as markers (see Materials

and Methods for details), microscopic examination of GMA-embedded sections obtained from the uncooked rolled oat varieties revealed the presence of many intact as well as broken starch granules (Fig. 4). The structural integrity of the compound starch grain was affected by the extent of processing: there were more broken starch grains in quick rolled oats than in old fashioned rolled oats. However, many of these broken starch granules still retained their characteristic birefringence when the samples were examined under polarized light, indicating that processing did not greatly induce gelatinization of the oat starch. On the other hand, most starch granules found in instant rolled oats had partially lost their birefringence, indicating that the related processing method resulted in partial gelatinization of the oat starch. Hot water soaking, a recommended method for preparing instant rolled oats, did not drastically change the structural composition of the starch granules. Cooking rolled oats in water (3 min) not only completely eliminated the birefringence of the starch but also altered its basic structure. The cooked and completely gelatinized oat starch was no longer granular in structure but appeared as convoluted folds (Fig. 5), much different from that observed in uncooked rolled oats (Fig. 4). The longer the rolled oats were cooked, the less recognizable their starch became and detection of the amorphous starch matrix in well cooked (20 min) rolled oat samples (Fig. 6) depended mainly on the specificity of the fluorescent marker. In general, the structural change of starch in the cooked old fashioned rolled oat samples was not as drastic as that observed in the quick or instant rolled oat samples, even though all were cooked under the same conditions, for the same duration of time. This finding indicates that the thickness of the oat flake probably contributes to a slower rate of water penetration, and consequently, a slower rate of starch gelatinization.

Lipids

The lipid content of oats ranges from 5 to 9% depending on the variety. Most of the great lipid is stored in the endosperm in the form of oil droplets which can be detected by simple staining using Nile Blue A (Fulcher and Wong, 1980). Frozen sections were used for examining oat lipid reserves in rolled oat samples to avoid using organic solvents which were used as dehydrating reagents for the GMA-embedding procedure. Sections from cooked or uncooked rolled oats were stained with Nile Blue A and were examined under the fluorescence microscope. Most of the lipid content still remained within the cells that had intact cell walls even after cooking but individual lipid bodies were no longer detected. Instead, large pools of coalesced oil droplets were observed throughout the entire groat structure (Fig. 7). This finding indicates that the loss of lipids in rolled oats due to processing and cooking is insignificant.

Beta-glucans

The majority of oat fibre is water soluble, mainly in the form of a gum. A large portion of the oat gum is mostly (1+3)(1+4)- β -D-glucan

(β -glucans) (Wood et al., 1978). The presence of β -glucans in the oat kernel can be detected microscopically using specific markers, such as Congo Red, and Calcofluor White (Wood et al., 1983). Most oat β -glucans occur in the cell wall of the endosperm, along the inner wall of the aleurone layer, and are particularly concentrated in the sub-aleurone endosperm cell walls (Fig. 8). Microscopic examination of cooked and uncooked rolled oat samples revealed that most endospermic cell walls were altered by processing (Fig. 9). Old fashioned rolled oats had relatively less and instant rolled oats had more endospermic cell wall breakdown than quick rolled oats. On the other hand, cell walls of the sub-aleurone and the aleurone layers were less affected by processing as many of them remained relatively intact. They still reacted with Calcofluor even after cooking (Fig. 9). The above findings show that the loss or dispersion of β -glucans due to processing and cooking was minimized by the strong, structural association between β -glucans and the two cell walls which were relatively resistant to mechanical forces and heat.

Phytate and phenolic compounds

Most oat phytate and phenolic compounds are localized within structures that are in close proximity to each other, and both have adverse effects on the bioavailability of minerals and proteins in cereal foods. Due to their relative abundance in cereal grains and oilseeds, both phytate and phenolic compounds are of major concern to food processing technologists and nutritionists.

Some phenolic compounds are naturally fluorescent (autofluorescent); they occur in cell walls of many tissues and are readily detectable under short wavelength excitation (Fulcher et al., 1972). On the other hand, the detection of phytin-containing structures, mostly in the form of protein inclusions, depends on appropriate staining and related microscopic methods (Yiu et al., 1982; Fulcher, 1982). The fluorescence microscopic technique, using Acriflavine HCl as the staining reagent, was used in this study to simultaneously examine phytate and phenolic compounds.

In oats, most phytin and phenolic compounds are stored in the aleurone and the scutellar tissues. The structural organization of these tissues can be revealed by viewing a section of the groat under short wavelength illumination (FC I). Both tissues have autofluorescent cell walls surrounding numerous protein bodies within which dark, spherical inclusions are visible (Fig. 10). The presence of phenolic acids, although their exact identities are not established, accounts for the blue autofluorescence emitted by the aleurone and the scutellar walls (Fulcher et al., 1972). Using Acriflavine HCl as a marker and FC I for appropriate fluorescence excitation, the presence of phytin globoids can be demonstrated among other protein inclusions. The above method facilitated the rapid screening of cellular contents in both aleurone and scutellar tissues of various rolled oat samples. Most cell walls of these tissues in old fashioned rolled

oats were structurally intact, many remained autofluorescent, and orange-red fluorescent inclusions were visible, indicating the presence of phytin-containing globoids (Fig. 11). There was slightly more cell wall breakdown in the aleurone and the scutellar tissues of the quick and the instant rolled oat samples due to increased processing. Many of the fragmented cell walls still retained their autofluorescence after cooking. The broken cell walls led to the exposure of their cellular contents where some protein inclusions remained visible, but only a few reacted with Acriflavine HCl (Fig. 12). The above findings indicate that there is a potential loss of phytin due to processing and cooking. Phenolic compounds, on the other hand, are less affected due to their strong structural association with the cell wall.

Conclusions

The present study demonstrated that certain oat structures were most strongly affected by the processing of rolled oats. These included the protein and the lipid bodies; both appeared in aggregated forms after processing (Figs. 2, 3 and 7). However, since the two could still be detected in cooked and uncooked rolled oats by specific microscopic markers, their values as food constituents are retained. They were made more available as nutrient sources by the breakdown of the endospermic cell wall which encloses most of the oat storage reserves including starch. The basic structure of starch granules was not affected by processing, although increased milling, e.g., more flaking during the production of quick rolled oats, induced more breakage of compound starch grains (Fig. 4). Cooking completely altered the starch structure (Figs. 5 and 6) and its birefringent characteristic. Increased milling also induced more cell wall breakage including those of the resilient aleurone and sub-aleurone layers. Beta-glucans and some phenolic compounds are structurally bound to the cell wall tissues. Processing, cooking, and their combined impact did not completely remove these two groups of compounds from the oat cell walls (Figs. 3, 6, 9 and 12). The phytin-containing globoids, however, were more affected as they were less distinct in the cooked rolled oats (Fig. 12).

Processing and cooking improve the digestibility of plant (cereal) cell walls and related materials which are known to be digested with difficulty. The present study demonstrates such a case: the oat constituents are made more available as nutrients, since processing induces cell wall breakage, reduces particle size, induces the release of cell contents, and increases the surface area subjected to cooking and subsequent enzymatic digestion.

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Discussion with Reviewers

C.F. Earp: You stated that the large pools of coalesced oil droplets were observed throughout the entire groat structure. Was this an even distribution throughout the endosperm or did some areas appear to have more lipids than others?

Author: The distribution was not even: the aleurone layer had more aggregated oil droplets per cell than the rest of the endosperm.

Y. Pomeranz: The various oat products should be defined as to processing (size, temperature, length of heat treatment).

Author: Details of the processing procedures were not available because the oat products were obtained commercially. Although the manufacturers were contacted for more information, their company policy prevented them from revealing technical details.

Reviewer III: Heat lability of proteins: are both aleurone and sub-aleurone proteins equally susceptible or did you see any differences in their damage/susceptibility to heat?

Author: Results of the microscopic examination indicated that protein bodies in the sub-aleurone layer were more susceptible to commercial processing than the aleurone layer. However, more studies would be required in order to determine their degree of susceptibility to heat.

Reviewer III: Did you find differences in the 'intensity' of the stained β -glucan in processed and unprocessed sections? If so, could they be due to some changes in β -glucan molecules?

Author: The fluorescence intensity emitted by the stained cell walls of unprocessed oats was greater than that of the processed products. The difference could be due to many factors including changes in the binding property between the fluorescence marker and β -glucan.

Reviewer III: In the filter combination of FC I, why don't the β -glucan deposits appear 'red' (Fig. 10), as they should, with the Congo Red stain?

Author: The above filter combination facilitates the simultaneous observation of phenolic compounds and β -glucans. However, the absorption

and emission spectra of β -glucan-bound-Congo Red are not at their maximum at such wavelengths (365nm/>418nm). The maximum transmission for bound Congo Red is at 470nm/588nm (Wood et al., 1983).

Reviewer III: Have you looked for "flavonoid" type compounds in the endosperm?

Author: No, flavonoid compounds in oats were not investigated in the present study.

Figure Captions

Unless otherwise stated, all fluorescence micrographs show GMA-embedded sections of whole grain or rolled oats. Scale bars are in μ m. Abbreviations: al = aleurone layer, end = starchy endosperm, F-Con A = fluoresceinated-Concanavalin A, F-LCA = fluoresceinated Lens culinaris agglutinin, sub = sub-aleurone layer.

Fig. 1. A section of the oat (Avena sativa L. cv. Hinoat) endosperm stained with Acid Fuchsin and Calcofluor showing individual protein bodies (arrows). Photographed using FC II.

Fig. 2. A section of uncooked rolled oats stained with Acid Fuchsin and Calcofluor demonstrating aggregated protein structures (*) surrounded by the intact cell wall where β -glucans were detected. Photographed using FC I.

Fig. 3. A section of cooked rolled oats stained as in Fig. 2 showing a mixture of aggregated protein structures (*) and cell wall fragments (arrows). Photographed using FC I.

Fig. 4. A section of uncooked rolled oats reacted with F-Con A revealing the presence of both intact (*) and broken (arrows) starch grains in the endosperm tissue. Photographed using FC II.

Fig. 5. An F-Con A stained section of cooked rolled oats showing gelatinized starch in the endosperm tissue. Photographed using FC II.

Fig. 6. A section of cooked rolled oats stained with F-LCA and Congo Red demonstrating the mixture of gelatinized starch (*) and broken cell wall fragments (arrows). Photographed using FC II.

Fig. 7. A frozen section of cooked rolled oats stained with Nile Blue A showing large pools of lipids (yellow fluorescence) within aleurone and endosperm cells. Photographed using FC II.

Fig. 8. A section of the bran region of oats (Avena sativa L. cv. Hinoat) stained with F-LCA and Congo Red revealing the presence of β -glucans in the inner aleurone layer (arrows) and the thick sub-aleurone cell walls (*). Photographed using FC II.

Fig. 9. A section of cooked rolled oats stained with Calcofluor showing the extent of cell wall breakdown in the endosperm. Photographed using FC I.

Fig. 10. A Congo Red-stained section of the oat (Avena sativa L. cv. Scott) aleurone layer showing the presence of phenolic compounds on its unstained but autofluorescent cell walls encompassing visible protein globoids (arrows) and the adjacent stained sub-aleurone cell wall (*). Photographed using FC I.

Fig. 11. A section of cooked old fashioned rolled oats stained with Acriflavine HCl revealing phytin containing globoids (arrows) in the aleurone layer. Photographed using FC I.

Fig. 12. A section of cooked quick rolled oats stained with Acriflavine HCl and Calcofluor showing the extrusion of aleurone cell contents (*). Photographed using FC I.

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