Introduction

Scanning electron microscopy (SEM) in the conventional and cold-stage modes is well suited for studies of the microstructure of milk products. In this study, "conventional" SEM is characterized by the examination of metal-coated dried samples at ambient temperature using secondary electrons at an accelerating voltage between 5 and 30 kV.

Aggregation of casein micelles during the gelation of milk (16), protein matrices in yoghurt (29, 30) and curd (13), and the development of the microstructure in cheese (22, 33, 37, 41) have all been studied by SEM. However, with the exception of milk powders (8, 24, 25), SEM has not been employed with milk products to the extent which would fully exploit this technique. The reasons for this may be due to the very fine microstructure of some milk products and relatively difficult sample preparation required to obtain micrographs free from artefacts.

The corpuscular microstructure of milk is based on casein micelles, which are protein globules approximately 100 nm in diameter (32). Fat is also present in the form of globules, the dimensions of which depend on whether the milk has been homogenized or not (17). In the production of most milk products, the basic step is the coagulation of milk characterized by the aggregation of casein micelles into chains and clusters. Because of the small dimensions of the casein micelles, medium high magnifications are used to study them and their aggregates. The low content of structural solids (~6%) in gelled milk and the resulting high porosity of the protein matrix make the samples fragile and increase the probability of the occurrence of artefacts.

Techniques for the preparation of milk products were reviewed earlier (24, 26). In most published papers care is taken to minimize artefacts. For this reason it is not possible to present a collection of artefacts from the literature, although occasionally some have been published unintentionally. Artefacts in SEM are defined as products of sample preparation and imaging procedures which alter the subject under study contrary to the interest of the microscopist. This means, for example, that although the extraction of fat from a cheese sample alters the initial microstructure, it is not considered to be an artefact.

Abstract

Artefacts develop due to changes in the microstructure of the sample under study because of inappropriate preparatory steps and/or due to distortion of the images of properly prepared samples during operation of the microscope. Of a wide variety of possible artefacts, only those occurring most frequently have been selected and illustrated with micrographs. In milk powders, the most common artefacts are the recrystallization of lactose in a humid atmosphere, "line" and "dark-area" charging artefacts, and electron beam damage. In moist milk products, artefacts may arise at any preparatory step, such as sampling, fixation, dehydration and critical-point drying or freezing and freeze-drying, mounting, metal coating, and microscopical examination. Images of the same subjects with artefacts either present or reduced to a minimum are compared and measures to avoid the development of the artefacts are suggested.


KEY WORDS: Artefacts; Charging; Conventional scanning electron microscopy; Defects; Electron beam damage; Microscope operation; Milk products; Sample preparation techniques.
artifact if the intent of the microscopist is to study the cheese protein matrix free of fat. If, however, some fat is left in the sample due to incomplete extraction, which is not the intention of the microscopist, the image obtained is artefac-tual. Artefacts arise either from improper sample preparation or from improper operation of the microscope. There are many papers describing the appropriate execution of sample preparation as well as microscope operation techniques (4, 38-40) and the food scientist intending to study the microstructure of milk products should be familiar with such techniques. The objective of this paper is to describe specific artefacts most commonly encountered in conventional SEM of milk products, explain their origin and nature, and suggest corrective measures in order to avoid them.

Materials and Methods

Milk products (spray-dried skim milk and buttermilk, Cottage cheese, Cheddar cheese, and yoghurt containing pregelatinized starch) of commercial origin as well as products made in the laboratory (yoghurt containing 10% nonfat milk solids and the same yoghurt containing 20% sugar or 0.2% pregelatinized Col-Flo 67 modified starch) (29) were used in this study.

Preparation of the samples for electron microscopy was varied to produce specific artefacts. Cambridge Stereoscan Mark II and AMR 1000 scanning electron microscopes operated at 20 kV unless mentioned otherwise were used to obtain micrographs, which were taken on 35-mm film.

Results and Discussion

The development of artefacts was studied separately from two different points of origin, namely as produced by sample preparation and also as produced during the operation of the scanning electron microscope. Some artefacts, such as "charging", may develop as a result of both factors.

The artefacts are described in the order as they may develop during the preparation of the sample for SEM (see the simplified flow chart of sample preparation).

Sampling

In general, samples are taken from beneath the surface, where the food product is not affected by external forces such as pressure of packaging or evaporation from an exposed surface unless the interest of the microscopist is focussed on the surface layer, for example, on Cottage cheese granules or the surface of mold-ripened cheese. Even when a sharp blade is used when sampling, sample components are smeared on the cut surface and obscure the true microstructure of the sample under study (Fig. 1A); true microstructure is revealed by fracturing at a later stage of sample preparation to expose the undamaged interior (Fig. 1B).

Powdered milk products destined for SEM are maintained in a dry atmosphere to avoid changes in their appearance and microstructure due to humidity. In lactose-containing spray-dried milk powders, exposure to air having a relative humidity of 40% or more (43) leads to the hydration of amorphous lactose anhydride, which is uniformly distributed throughout the powder particles, and crystallization of the α-hydrate on the particle surface (Fig. 2); this results in the agglomera-tion of the spray-dried particles which otherwise occur as separate entities.

Fixation

Samples of milk products are usually fixed in a glutaraldehyde solution to stabilize the protein matrix and to facilitate the removal of soluble constituents such as lactose and whey proteins. Fixation of samples with glutaraldehyde, however, does not fix the fat component. Fat in the form of globules present in low-fat milk products which have a porous matrix such as yoghurt or Cottage cheese may be stabilized by postfixation with osmium tetroxide, particularly in the presence of imidazole (Fig. 3). However, in experiments aimed at preventing the fat from crystallization (1), fixation in imidazole-buffered OsO₄ at an elevated temperature of 40°C resulted in ruptured fat globule membranes and leakage of the fat (Fig. 4).

When the fat in the milk product is not protected by fat globule membranes, for example, in process cheese, conventional postfixation with osmium tetroxide stabilizes only the part of it, which contains unsaturated fatty acids. The rest of the fat is extracted from the sample during dehydration in ethanol and during critical-point drying from Freon or carbon dioxide. Remnants of
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**Fig. 1.** Microstructure of Cheddar cheese.
(A) The surface of the cheese particle was smeared with protein during cutting with a blade thus obscuring the microstructure of the sample. (Sample fixed with glutaraldehyde, washed, freeze-dried, and defatted in chloroform). (B) Fracturing reveals the internal microstructure of the sample. (Fixed sample dehydrated in ethanol, defatted in chloroform, freeze-fractured, and critical-point dried. Extraction of fat reveals cavities (arrows) in the protein matrix).

**Fig. 2.** Particles of spray-dried skim milk following exposure to a humid atmosphere. Exposure for 1 h resulted in the crystallization of lactose α-hydrate on particle surfaces and agglomeration of the particles. Insufficient electric contact between the agglomerate and the metal support resulted in a charging artefact in the form of a vertical line (arrows).

**Fig. 3.** Fat globules in whole-milk yoghurt preserved by postfixation with imidazole-buffered osmium tetroxide. Fat was revealed by freeze-fracturing (arrow). Other fat globules (f) are seen unfractured below the fracture plane; b = lactobacillus.

**Fig. 4.** Fat globule membrane ruptured during postfixation of whole-milk yoghurt at 40°C (stereo pair, 12° separation). Fat leaked out through openings (arrows) developed in the membrane at the elevated temperature. Two light circles in the micrographs are provided to facilitate focusing of the eyes.
stabilized fat are the source of an artefact characterized by the presence of fat residues in cavities, which had initially been filled with the fat before extraction (Fig. 5A). Unless the fat is destined for complete preservation (1), for example, by trypsin etching of the protein matrix (15, 48), it is better not to use OsO₄ and to extract the fat with chloroform or n-hexane (26, 27). However, even in a cheese sample which had not been postfixed with osmium tetroxide, incomplete extraction may leave residual fat in the form of droplets (Fig. 5B). After all the fat is removed, its initial distribution and the dimensions of the fat particles are determined indirectly from the distribution and the dimensions of the resulting void spaces (cavities) (Fig. 18). Freeze-drying preserves fat in fixed and postfixed cheese samples, but this fat causes problems during mounting and metal-coating, melts easily when exposed to the electron beam, and obscures the protein matrix (Fig. 6) unless the sample is handled and examined at a low temperature (44, 45).

The removal of water-soluble constituents from the sample due to exposure to aqueous preparatory solutions means that even subjects of interest to SEM may be lost, for example, crystals of melting bacteria, fat globules, or casein micelles (26, 41). Structures resembling the original crystals are, in fact, their imprints in the protein matrix after the crystals are dissolved and extracted (Fig. 7).

Freezing

Freezing is the prerequisite in the preparation of samples destined to for freeze-fracturing. Depending on which preparatory procedure follows, the sample is frozen with the aqueous phase present to be freeze-dried, or the aqueous phase in the sample is replaced with a cryoprotective medium such as 30% glycerol or with absolute ethanol and the sample is freeze-fractured, melted, and critical-point dried.

This frequent artefact seen in frozen samples is the distortion of the protein matrix due to ice crystal development (Fig. 8). This artefact is caused by slow freezing and develops when the sample is too large or when a small sample is surrounded by excess water, when an inappropriate freezing agent (such as liquid nitrogen, dry ice with acetone etc.) is used, or when the speed, at which the sample is plunged into the appropriate coolant such as nitrogen slush (49) or freon cooled to its freezing point with liquid nitrogen (26, 27), is too slow.

Freeze-drying

Freeze-drying is one of two drying techniques most frequently used, the other being critical-point drying. In conventional SEM, the specimen is examined at ambient temperature. It must be dry in order to minimize generation of volatile vapours in vacuo inside the electron microscope. Problems associated with drying techniques were reviewed by Boyde (6).

The advantage of freeze-drying is that it preserves most food components including fat. In fixed samples not washed thoroughly with distilled water, the residual dissolved substances such as buffer salts or lactose may appear in the form of a fine efflorescence on the surface of the resulting freeze-dried sample; this efflorescence is usually noticeable under a dissecting microscope during the mounting of the samples and warrants disposal of the sample in question. Although freeze-drying distorts the gelatinized starch and alginites which are sometimes used as thickening agents in yoghurt (29), the distortion is less extensive than that caused by critical-point drying (9); this is evidently associated with the dehydration of unfixed polysaccharide gels in organic solvents.

An improperly functioning freeze-drier is a potential source of serious artefacts. Recrystallization of the frozen aqueous phase in the sample during freeze-drying is a serious risk to which the sample is exposed, particularly if the temperature of the sample rises above -80°C before all the ice has sublimed off; such artefacts are similar to those caused by slow freezing of the specimen. Contamination of the sample with an oil mist from the vacuum pump is another potential artefact; in this laboratory samples are kept from being contaminated in this way by installation of an activated alumina trap between the pump and the specimen chamber; the trap is freshly charged before each run.

Freeze-fracturing

Freeze-fracturing exposes the internal structure of the specimen. A specimen may be freeze-fractured while impregnated with a cryoprotective agent such as 30% glycerol or after it is dehydrated in absolute ethanol. Both media decrease or even eliminate the risk of ice crystal development. There are, however, differences in the appearance of the fractured specimens: with 30% glycerol, the fracture planes run through lactic acid bacteria, fat globules, or casein micelles whereas with absolute alcohol, the fracture planes run between bacteria, fat globules, and casein micelles (26). This indicates that the sample reacts differently under different conditions and that this behaviour should be considered when the micrographs obtained are evaluated.

Dehydration

In general, the term "dehydration" means any removal of water from the specimen, but here it is used more specifically to mean substitution of organic solvents for water. The objective of dehydrating the fixed specimen is to prepare it for critical-point drying or for freeze-drying from alcohol. The aqueous phase in the food specimen is first replaced with an organic solvent miscible with water, for example, ethanol or acetone. Being less efficient in extracting fat than acetone, ethanol in the form of a graded series of concentrations is more frequently used to dehydrate fixed fat-containing milk products. The final step consists of impregnating the sample with 100% ethanol (absolute alcohol). Incomplete removal of water may be a source of artefacts, although dehydration shortened to only 10 min of total exposure to the dehydrating agent failed to demonstrate any apparent artefacts.

Chemical dehydration in acidified 2,2-dimethoxypropane is a rapid, single-step technique, in
Fig. 5. Residual fat in process cheese. (A) Postfixation of the sample with osmium tetroxide prevented a part of the fat (arrows) from extraction with chloroform. (B) In a cheese sample which had not been postfix with OsO4, incomplete extraction of the fat with chloroform has left residual fat in the form of droplets (arrows).

Fig. 6. Cheese protein matrix obscured with fat. A fixed (glutaraldehyde) cheese sample was freeze-dried and dry-fractured; fat was retained in the sample.

Fig. 7. Crystals of melting salt in process cheese. Water-soluble crystals of sodium citrate were removed during fixation in an aqueous glutaraldehyde solution; their imprints were left in the protein matrix (arrows). Globular void spaces (f) indicate the presence of fat before extraction.

Fig. 8. Yoghurt sample frozen in liquid nitrogen. (A) Ice crystal (arrows) development towards the centre (asterisk) of a fixed and washed yoghurt sample during slow freezing distorted the casein micelle matrix. (B) Detail of the damage to the yoghurt matrix caused by the ice crystal development. Protein was compacted in the form of ridges (arrows).
which water is quantitatively consumed to hydrolyze the reagent into methanol and acetone; the hydrolysis is catalyzed by hydrogen ions (35). A comparison was made between the results obtained with the dehydration of cells in culture using ethanol and 2,2-dimethoxypropane (21) but no systematic studies have been carried out with milk products; no differences were noticed when the two dehydrating agents were used with yoghurt in this study.

Defatting

It is preferable to remove fat from the sample unless it can be adequately retained for SEM (1, 15, 48). Dehydration and defatting may be combined in one operation by using acidified 2,2-dimethoxypropane. Because the combined resulting molarities of methanol and acetone in the dehydration medium correspond to the total amount of water in the sample at the completion of dehydration, 2,2-dimethoxypropane which exists in great excess is practically the only lipophilic solvent affecting the sample.

Another defatting procedure consists of transferring the sample, dehydrated in absolute alcohol or acetone, into chloroform. Following several changes of the latter solvent, the extracted sample is returned to absolute alcohol for subsequent critical-point drying.

Micrographs of cheese samples chemically dehydrated and defatted in 2,2-dimethoxypropane and samples of the same cheese conventionally dehydrated in a graded alcohol series and defatted in chloroform are virtually indistinguishable from each other. It was already mentioned in the section dealing with fixation that it is preferable to omit postfixation with osmium tetroxide if the sample is destined for defatting.

Critical-point drying

Principles and practical use of critical-point drying were described elsewhere (2, 10, 26, 27). In general, the sample impregnated with an organic solvent such as ethanol, acetone, or amyl acetate is placed in a pressurized cell where the organic solvent is replaced at a low temperature with a transitional fluid (Freon or carbon dioxide). Heating of the cell contents above the critical temperature (31.3°C for CO₂) converts the transitional fluid into gas; after the pressure is reduced, the sample is dry without having passed through any phase boundary.

In spite of this advantage, critical-point drying is not free from producing artefacts (6). This technique was reported to cause shrinkage of some fine biological structures such as animal tissues (5), but no measurements have yet been made on this effect with regard to milk products. The effects of critical-point drying on milk products which contain gelatinized starch and alginites as thickening agents have been reported (29). These thickeners are not fixed by glutaraldehyde and undergo structural changes (shrinkage and distortion) in the organic solvents used to dehydrate the specimen for subsequent critical-point drying.

A set of micrographs (Figs. 9-12) demonstrates differences in the images of a yoghurt consisting of 10% milk solids and 2% pregelatinized Col-Flo 67 modified starch. The starch was not of "instant" quality and the granules were not completely gelatinized during the heat treatment.

Legends to figures on facing page:

Fig. 9. Freeze-drying of an unfixed yoghurt (10% milk solids) containing 2% pregelatinized starch. The microstructure of starch (large arrow) was severely distorted whereas the protein matrix was affected to a lesser extent (small arrow).

Fig. 10. Yoghurt containing 2% pregelatinized starch, fixed with glutaraldehyde, dehydrated in ethanol, freeze-fractured, and critical-point dried. Arrows point to starch particles; b = lactic acid bacteria.

Fig. 11. Yoghurt containing 2% pregelatinized starch, fixed with glutaraldehyde, postfixed with osmium tetroxide, dehydrated in ethanol, freeze-fractured, and critical-point dried. Arrows point to starch particles.

Fig. 12. Yoghurt containing 2% pregelatinized starch, fixed with glutaraldehyde, freeze-fractured, and freeze-dried. (A) Starch particles (s) appear to be in a good contact with the protein matrix (p) but suffer from the characteristic ice crystal damage; b = lactic acid bacteria. (B) Detail of contact between pregelatinized starch (s), protein matrix (p), and lactobacilli (l) is shown at a higher magnification.

Fig. 13. Commercial yoghurt containing pregelatinized starch treated in the same way as the laboratory-made yoghurt shown in Fig. 10. Arrows point to starch granules. Protein matrix (p) is considerably more compact than in the laboratory-made yoghurt (Figs. 10-12) which contained only 10% total milk solids; b = lactic acid bacteria.

Fig. 14. Yoghurt containing 10% total milk solids and 10% sucrose. Crystals (arrows) of unknown composition developed in fixed yoghurt samples dehydrated in ethanol and critical-point dried; c = lactic acid bacteria (streptococci).

Fig. 15. Yoghurt (10% total milk solids) dried from Freon 113 in a desiccator at a reduced pressure. The sample was fixed, dehydrated, freeze-fractured, and melted in ethanol; Freon 113 was substituted for ethanol and the sample was dried in a desiccator at a reduced pressure. Local compaction of the casein micelle matrix is apparent in comparison with another sample of the same yoghurt which had been critical-point dried (Fig. 16).

Fig. 16. Critical-point dried yoghurt sample (10% total milk solids). The image is assumed to be free of artefacts; 1 = lactic acid bacteria (Lactobacilli).
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of the milk used. However, the appearance of the starch was the same in this laboratory-made yoghurt and commercial yoghurt when the same SEM techniques were used (Fig. 13). Fig. 9 shows the former yoghurt frozen and freeze-dried without any preliminary preparatory steps. Artefacts caused by the development of ice crystals and by the presence of soluble constituents render the micrographs unsuitable for any study. Fig. 10 was obtained with a sample fixed in glutaraldehyde, dehydrated in alcohol, freeze-fractured, and critical-point dried. The casein micelle network is interspersed with irregularly shaped starch particles; there are large void spaces between starch and casein indicating that the micrograph does not reflect the original microstructure. In yoghurt which was impregnated with 30% glycerol following fixation in glutaraldehyde, freeze-fractured, melted in 30% glycerol, dehydrated in alcohol, and critical-point dried, the starch particles were severely shrunken and diffused into the protein matrix at the same time. Postfixation with OsO4 followed by dehydration in alcohol, freeze-fracturing, and critical-point drying resulted in a lesser distortion (shrinkage) of the starch granules as judged from smaller void spaces between starch and casein (Fig. 11). The tightest microstructure of this yoghurt was found when it was fixed with glutaraldehyde, washed with water, and freeze-dried (Fig. 12A); the starch itself, however, appeared porous, apparently because of the development of fine ice crystals inside the gelatinized particles during freezing and their subsequent sublimation during freeze-drying. Otherwise, the initial dimensions of starch particles were to be understood and their contact with casein micelles and lactic acid bacteria preserved (Fig. 12B). Freeze-fracturing of the original unfixed yoghurt followed by replication with platinum and carbon and examination of the replicas in a transmission electron microscope is needed to identify the SEM preparatory technique which produces the minimum of artefacts with this yoghurt. Fig. 13 shows a commercial yoghurt with the declared presence of gelatinized starch, which was fixed in glutaraldehyde, dehydrated in alcohol, freeze-fractured, and critical-point dried; thus, it corresponds to Fig. 10 except that the casein matrix in the commercial yoghurt is considerably denser than in the yoghurt made in the laboratory.

Yoghurt made with 10 and 20% sucrose, fixed in a 3.5% glutaraldehyde solution, dehydrated in ethanol, and critical-point dried from carbon dioxide appeared to contain crystal-like aggregates in the protein matrix (Fig. 14), the formation of which was reproducible. Crystals were also found by Katsaras and Stenzel (31) in beef muscles dehydrated in ethanol and critical-point dried; the crystals were not present either in yoghurt or in beef muscles when ethanol-dehydrated samples were dried in vacuo or when acetone, isopropanol, or 2,2-dimethoxypropane were used to dehydrate the samples. The chemical nature of the crystals is not known.

Drying in air
Drying of fixed biological samples in air without preliminary dehydration is not recommended. Acceptable but not good results have been obtained, however, by air-drying samples impregnated with organic solvents such as ethanol or acetone.

An alternative method to freeze- and critical-point drying, designed to retain fat, consists of postfixing the sample in an osmium tetroxide solution, dehydrating in ethanol, substituting Freon 113 for ethanol, and drying the sample at a reduced pressure (3, 34) in a desiccator. In meat emulsions dried by this technique (3), preserved fat globules were found to be displaced from their original positions in the protein matrix, which should be considered to be an artefact.

A similar procedure was tested with nonfat yoghurt; it consisted of fixing the specimen, dehydrating it in ethanol, freeze-fracturing, thawing in ethanol, substitution with Freon, and drying in a desiccator. The casein micelle matrix appears to be more compacted (Fig. 15) than the matrix of freeze-dried or critical-point dried specimens (Fig. 16). This technique cannot, therefore, be recommended for yoghurt; its usefulness in drying more compact milk products such as cheeses has not been explored.

Mounting
Mounting on a metal stub prepares the sample for coating with a thin metal layer and for subsequent insertion of the sample into the electron microscope.

Preparatory methods for SEM of various powders were reviewed by Johari and DeNee (20) and by DeNee (11). They are also useful in preparing food samples. Mounting of powders and solid particles for SEM was recently reviewed in great detail by Murphy (38) who has described many very useful techniques. Their use in food microscopy is highly recommended. Powdered foods have routinely been mounted on stubs covered with a double-sided sticky tape (27, 28), the edges of which had been painted with a silver cement to improve conductivity between the tape and the stub. To minimize the development of charging artefacts, particularly if gold coating will be done by vacuum evaporation, the powder particles should be in a single layer. If too many particles are present, some of them lack electric contact with the stub and this leads to an imaging disturbance called "charging" (Fig. 17A) (46). Areas which appear considerably lighter on the screen than other properly mounted structures at low magnifications, usually cause severe imaging problems when examined at a higher magnification. When studying particle size distribution, it is better to apply only a small amount of the milk powder to the stub and retain all the particles than to start with too much powder and to remove the larger particles preferentially remove the larger particles. It is relatively easy to obtain good images of thinly mounted powder particles. However, the charging artefact is less likely to develop in densely packed particles provided that sputter coating rather than vacuum evaporation is used (Fig. 17B).

Solid samples are mounted directly on SEM stubs. Low-mass stubs, for example those made of aluminum or carbon are best because they produce minimal interference with the sample during SEM.
Fig. 17. Scanning electron microscopy of spray-dried skim milk particles.
A = Charging artefact in the form of streaks (arrows) developed due to insufficient electric contact between the milk particles and the metal stub (gold coating by vacuum evaporation). B = Sputter coating of densely packed skim milk particles with gold resulted in artefact-free images.

Fig. 18. Example of a yoghurt particle properly mounted for SEM. The particle walls were painted with silver cement up to the freeze-fracture plane (arrows) to aid conductivity in the porous sample.

Fig. 19. Yoghurt matrix collapsed by the penetration of thin silver cement. Arrows point to the collapsed matrix, asterisks show an area less affected by the cement. Intact yoghurt matrix is shown in Fig. 18.

Fig. 20. Freeze-fracture plane of a yoghurt sample coated with a portion of gold evaporated at a very low angle. Light streaks (arrows) indicate areas which were insufficiently coated with gold due to the evaporation angle and would suffer from more severe charging artefacts if examined at a higher magnification.

Fig. 21. Charging artefact caused by insufficient gold coating. Some areas in the smooth fracture plane of this Cottage cheese sample are excessively light (arrows); this artefact is not as easy to recognize as the streaks in Fig. 17.
Although beryllium was mentioned by Brown and Teetsov (7) as a suitable material for the stubs, it cannot be recommended for general use because of its toxicity.

The appropriate adhesive (cement) selected to mount the sample should have the characteristics listed by Murphy (36). The characteristics most important for the mounting of milk product samples are: adequate viscosity to prevent the collapse of porous samples; sufficient tackiness to provide proper bonding of the sample to the stub; resistance to heat generated during metal evaporation or sputtering and by the electron beam without altered surface morphology of the adhesive; a resultant smooth surface upon drying; and ease of application with reasonable drying time and a low vapour pressure after drying in order not to contaminate the vacuum system of the microscope.

Suitable cements have been listed by Murphy (36). Porous samples, such as coagulated milk or yoghurt, require special handling for best results. Dried freeze-fractured fragments are mounted with their fractured planes facing up. The cement is extended up the sides of the specimens as close to the fracture as possible (Fig. 18). Four to six particles can easily be mounted on a stub in this way. Inspection of each particle for proper mounting with the stub tilted and a few finishing touches with the cement will contribute to successful SEM.

Improper mounting may be the source of several artefacts. Failure to provide good electrical contact between the sample and the stub leads to charging artefacts (46). The reason may be an insufficient amount of the adhesive used, or its improper consistency (viscosity): a cement too thick does not adhere properly to porous particles whereas a cement too thin penetrates the particle and alters its microstructure (Fig. 19). Instruments used to manipulate the sample leave their imprints especially on porous samples (23). It is also advisable to remove any debris, resulting from fracturing, which may adhere to the surfaces due to static electricity generated during the mounting of the samples; this cleaning may be accomplished with a gentle stream of dry air.

Coating with metal

This procedure renders the specimen electrically conductive. It is accomplished by vacuum evaporation or sputter coating but other techniques are also available (12). In biology, effects of the different coating techniques on specific subjects have been studied and published (18, 19, 42). Triode sputter coating, which employs a water-cooled stage, may be better suited for coating fat-containing specimens than vacuum evaporation, during which the specimen may be excessively heated. A comparison of both techniques has not yet been made with milk products. Micrographs of yoghurt obtained in this study appeared to be similar irrespective of the coating technique used.

Gold or a gold-palladium alloy is used alone with compact samples such as cheese but preliminary coating with carbon reduces the risk of charging artefacts in porous samples such as milk gels. The reason for this behaviour is still a point of discussion. However, if carbon is allowed to sputter even briefly during evaporation because an excessively high electric current is used, minute carbon particles may contaminate the sample surface, and are clearly seen upon observation in the microscope. If carbon sputtering is a problem, it can be prevented by using indirect evaporation in a gas at a very low pressure of approx. 130 mPa (1.3x10^-3 mbar or 1x10^-3 torr).

For metal coating by vacuum evaporation in the author's laboratory, the gold is divided into two portions deposited sequentially on the sample at two different angles while the sample is rotated. If the fracture plane of the specimen runs parallel to the stub base, evaporation of gold at a very acute angle (≤20°) cannot be recommended for particles, which had been thoroughly painted with the mounting cement. Most of the gold would be deposited on the sides of the particles, which is unnecessary, whereas very little of the gold would be deposited on the fracture faces at such low angles, and charging would still result. In fact, this coating may be the source of a shadowing artefact when the fracture is uneven. SEM examination at low magnification indicated that gold evaporated at a very low angle did not coat depressions in the fracture. A difference in the amount of gold deposited in the shaded and exposed areas is noticeable even after the other portion of gold was evaporated at a greater angle (Fig. 20).

Similar to improper mounting, insufficient coating leads to charging artefacts (Fig. 21); samples such as milk gels, which have a large surface area due to their porosity, require more gold to be evaporated onto the fracture face to achieve proper coating than do compact samples, such as cheese, which have a smaller surface area. However, excessively large amounts of metal obscure surface details and result in overcoating artefacts. The depth of coating in conventional SEM is approximately 20 nm.

During evaporation in vacuo, metal atoms travel in straight lines. When several samples are coated at the same time using a rotary table, the metal is deposited according to a certain pattern.
which does not ensure that crevices in porous structures will be coated. One way to improve the uniform coating is to rotate the sample around two axes at the same time. Another way is to coat the sample in a somewhat inferior vacuum of 13 to 133 mPa. At this pressure, there is a higher incidence of collisions between gas molecules present in the evaporation chamber and the vaporized metal atoms leading to a deflection of the latter ones from their straight line path and their deposition on the sample in areas which otherwise would remain uncoated (26).

SEM examination

Operation of scanning electron microscopes has been described in many handbooks, some of which may vary the accelerating voltage, their straight line path and their deposition on the sample may be examined. Assuming that images of milk products, which are produced, are due to secondary electrons, the electron microscope may vary the accelerating voltage, working distance, tilt, beam current, aperture, lens currents, and microscope contrast. When taking micrographs, additional conditions must be selected, for example, brightness, camera contrast, line density, and scan rate on the monitor and format and sensitivity of the film used. All these and additional factors were reviewed by Pfefferkorn et al. (40) in a highly informative paper. The authors have provided valuable advice on how to obtain optimum results. Their emphasis is on adjusting the variables depending on the nature of the sample and on the objective of the study.

Accelerating voltage

It is believed, in general, that a higher accelerating voltage gives better resolution. High accelerating voltage (20 kV and higher), however, is not only unnecessary when samples such as milk powder or cheese are examined at low magnifications (<1,000X), but it may even produce inferior micrographs if the specimen is susceptible to charging. Electrons penetrate deeper into the specimen at higher accelerating voltages and images of structures located below the sample surface may interfere with images from the surface, producing a somewhat inferior and less informative image. Differences in the images obtained at a low (5 kV) and a high (30 kV) accelerating voltage with two different samples at two different microscope magnifications are shown in Figs. 22 and 23. At the low magnification (120 X), the low accelerating voltage produced good images of spray-dried milk powder particles (Fig. 22 A), whereas at the higher magnification (6,000 X), the image of a yoghurt sample taken at the low accelerating voltage was of unacceptable quality (Fig. 23A).

As the kinetic energy of the electrons is increased with increasing accelerating voltage, the risk of electron beam damage to the sample is increased (4, 47). Milk powders are very susceptible to this kind of damage (8, 25, 28) which is evident particularly when the focussing at a higher magnification (>2000 X) takes too much time, i.e. several seconds (Fig. 24). The electron beam may conveniently be focussed by operating the microscope in the TV-mode using a small selected area on the screen. This kind of focussing also may reveal deficiencies in astigmatism correction. Another way of focussing uses a line scan: the oscilloscope pattern of the beam line is adjusted with the focussing control to produce the sharpest peaks. This way of focussing is rapid but does not provide information about astigmatism. It may cause beam damage artefacts in the form of cracks in the sample (Fig. 24C) or may imprint lines on its surface, which subsequently show up on the micrographs (Figs. 24C and 25). The severity of the electron beam damage can be reduced by lowering the beam current, by focussing as rapidly as possible, by scanning the sample during photography at a shorter frame time or in the TV-mode, if possible, or by reducing the accelerating voltage.

Working distance

Working distance affects both the resolution and the depth of focus. If the design of the microscope allows the working distance to be adjusted by moving the specimen, that distance should be as short as possible for best resolution whereas it should be as long as possible for maximum depth of focus. The dimensions of the sample may limit the usable range of working distance. Because working distance affects magnification, it should be maintained constant if the
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25  2μm

28  5μm

29A  2μm

26  10μm

29B  2μm

29C  2μm

27A  100μm

27B  100μm

30  10μm

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resulting micrographs are destined for comparison, unless the same magnification is adjusted by the zoom, and using the rotation unit to scan parallel to the x-axis after changing the working distance of the tilt axis.

Tilt
Tilting the sample makes it possible to change the angle of the incident electron beam to the sample surface. Undistorted images are obtained if the angle is 90° (27). Otherwise, dimensions perpendicular to the axis of tilt are shortened in the micrographs approximately according to the formula:
\[ d_\perp = d_\parallel \times \tan \alpha \]
where \( d_\parallel \) is the length of the image, \( d_\perp \) is the real length, and \( \alpha \) is the angle of tilt.

This formula does not take the effect of changed working distance into consideration: that distance is either shorter or longer for structures located at opposite sides of the axis of tilt. This all makes the interpretation of the micrographs more difficult (compare Fig. 26 with Fig. 16); in addition, tilting moves different parts of the sample in and out of focus.

Samples examined for microstructure in cross fractures immediately below the surface usually need to be tilted. The tilt, which positions the sample at a 90° angle to the incident electron beam, however, produces an artefact characterized by an excessively light edge of the sample (Fig. 27A). This impedes examination of the subjects of the greatest interest, for example, the hypothetical "skin" on Cottage cheese granules (13, 24); a slight decrease in the angle of tilt prevents the "edge phenomenon" (Fig. 27B). Similar excessively light edges are also encountered when cracks or cavities are present in the sample (Fig. 28); characteristic examples are void spaces in yoghurt developed by the action of lactic acid bacteria and cavities in cheese caused by the extraction of fat droplets or crystals of melting salts. This phenomenon can be sometimes caused by defects in electric conductivity and consequent charge build-up.

Astigmatism correction
Magnetic lenses in the electron microscope do not have a perfect symmetry. If the symmetry is elliptical rather than circular, a single point focusses, as the lens current is varied, to two separate line foci instead of to a single point. This means that underfocussed and overfocussed spheres (such as casein micelles below and above the focus plane) appear elongated at right angles to each other (Fig. 29). To correct this defect, the magnetic field of the final lens is brought to the desired symmetry. The objective is to correct the primary beam with a stigmator to have the smallest diameter possible and to have a circular cross section, in order to obtain the best resolution in all directions. Two major controls are usually used: the stigmator magnitude and orientation are adjusted alternately and repeatedly until the sharpest image is obtained. This correction is possible only for the asymmetry in the magnetic field of the final lens but not for effects having another origin such as an incorrect alignment of the filament, dirty aperture etc. (14).

Lens current
Lens current is related to the signal-to-noise ratio. The optimal ratio can be obtained by manipulating the controls and examining the effects of that manipulation. Adjustment can be learned only through experience (40) and differs for each accelerating voltage value.

Microscope contrast
Fractured milk product samples can usually be examined in the SEM without any change in the contrast setting. However, very flat samples, for example, ultrafiltration membranes, may show details better at an increased contrast setting, particularly when artefacts may emerge. An excessively high contrast setting eliminates halftones and converts the micrographs into black-and-white two-dimensional patterns. Such micrographs (compare Figs. 30 and 16) do not properly represent the three-dimensional structure of the sample. However, because the depth of focus is suppressed and the sample structure is highlighted, such micrographs may be used in digital image analysis, for least for comparative purposes, since regular SEM micrographs are not considered to be suitable for this analysis (50).

Conclusion
Electron microscopy is well suited for studies of microstructure in milk products. Scanning electron microscopy, in particular, is capable of providing solutions to some problems quite rapidly. The usefulness of the SEM results depends to a great extent on a proper preparation of the samples and a correct operation of the microscope. The possibility that artefacts may develop needs to be considered and measures aimed at reducing their incidence have to be implemented. Modern instruments and accessories (high-resolution microscopes, sputter coaters, charge neutralizers) as well as new preparatory techniques (procedures rendering the specimens electrically conductive) may diminish the incidence of artefacts in conventional SEM but may also produce new artefacts.

Even if all efforts are made to reduce the introduction of artefacts of any origin, it is advisable to use other microscopical techniques, for example, transmission electron microscopy, to confirm the findings made using SEM.

Acknowledgments
Skillful technical assistance provided by Mrs. Paula Allan-Wojtas is acknowledged. The author thanks Mr. Ernest F. Bond and Dr. David N. Holcomb for useful suggestions and for reviewing the manuscript. Electron Microscope Centre, Research Branch, Agriculture Canada in Ottawa provided facilities. This paper is Contribution 595 from the Food Research Institute.

References
SEM ARTEFACTS IN MILK PRODUCTS


47. Short JM, Fernquist RC. (1976). Dynamic studies of electron beam heating in the SEM.


Discussion with Reviewers

T. Makita: What concentration of imidazole combined with osmium tetroxide is suitable for the preservation of fat?
Author: The postfixative used with whole-milk yoghurt (I) was prepared by dissolving 0.5 g of crystalline OsO4 in 50 mL of a 0.05 M veronal-acetate buffer (pH 7.4) and mixing this stock solution with an equal volume of a 0.2 M imidazole solution adjusted to pH 7.4 with 1 N HCl.

T. Makita: What combination:

Freon \[\rightarrow\] Ethanol
Carbon \[\rightarrow\] Acetone
Carbon dioxide \[\rightarrow\] Amyl acetate

or which reagents in critical-point drying do you recommend best for milk products?
Author: I use ethanol and carbon dioxide.

T. Makita: What combination of metal coating (gold + carbon; palladium + carbon; platinum + carbon) would be the best for milk products?
Author: In conventional SEM, gold alone or in a combination with carbon produces good results. In high-resolution SEM, platinum should be used.

T. Makita: As far as the charging artefacts are concerned, you appear to regard the electric contact of the specimen to the stub to be the most important factor. Moisture or the presence of gas as you have mentioned, would also be key factors. In this respect, you have emphasized too much to shorten the dehydration ("... although dehydration shortened to only 10 min of total exposure to the dehydrating agent failed to demonstrate any apparent artefacts")? Can you recommend a minimal required dehydration time for a given size of the specimen? As you have mentioned, dehydration is the substitution of organic solvents for water, can you explain how important it is to remove the organic solvents before metal coating, because it is also a key factor in charging?
Author: Metal coating is carried out at a reduced pressure. If a volatile substance such as water or an organic solvent is present in the sample, the desired low pressure (<13 mPa) is achieved only after that substance has been evaporated.
T. Makita: The magnification attainable by SEM has been increased to 100,000-300,000 X. Preparation of the specimens, especially metal coating, has to be adjusted to the higher resolution. Sooner or later, milk products will also be subjected to such high magnifications. Thus, more information in this respect would be appreciated.

Author: The question is, what the very high magnifications are expected to reveal in milk products, in which casein micelles (100 nm in diameter) are the structural protein units and fat globules (>100 nm in diameter), lactic acid bacteria, salt crystals etc. are present in addition. At present, the mutual relations of all these components are of interest and the magnifications used have not exceeded 24,000 X.

S.H. Humphreys: The preservation of fat with imidazole-buffered OSO₄ would seem to involve formation of different complexes as "traditional" understanding of OSO₄ fixation does not invoke participation of the buffer in fixing. This striking preservation of fat is well worth a precise protocol, especially as the reference cited does not give a precise protocol.

Author: The reference (1) provides sufficient information on the preparation and use (time and temperature) of the fixatives. It has been stated that fat globules postfixed with an imidazole-buffered 0.5% OSO₄ solution for 24 h at 22°C were well preserved, that a 2% OSO₄ solution emphasized details in the fat globule membrane but led to an excessive deposition of Os in casein micelles present in the sample, and that postfixation of the samples at 4°C resulted in the disintegration of the fat globules. The study indicates that the conditions for optimal postfixation of other milk products may differ from those established for whole-milk yoghurt.

S.H. Humphreys: What does it mean that chemical dehydration of samples in acidified 2,2-dimethoxypropane is a "rapid" technique?

Author: There is no need to carry out the dehydration with 2,2-dimethoxypropane in steps and change the dehydrating agent such as ethanol or acetone several times in order to remove all the water present. Water reacts chemically and quantitatively with acidified 2,2-dimethoxypropane and produces methanol and acetone. This procedure is less laborious and is accomplished within a shorter period of time than dehydration using ethanol or acetone.

W. Buchheim: Chemical fixation (e.g. in glutaraldehyde) is a common preparatory step in order to stabilize protein aggregates and matrices. How do you estimate the degree of shrinkage induced by this treatment, especially with samples like yoghurt?

Author: No studies have been reported on this subject, although a combined effect of fixation and dehydration on Cheddar cheese (overall linear shrinkage of 19.5%) has been mentioned (52).

W. Buchheim: Please comment on the folded surface of the two unfractured fat globules in Fig. 3. Is this an artefact?

Author: Compared to the images of fat globules in milk which you have obtained by freeze-fracturing and freeze-etching (51), fat globules in Fig. 3 appear as if covered with wrinkled fat globule membranes. Adsorbed proteins and the gold coating may be factors contributing to this appearance but may not explain it fully. If the wrinkled surface is an artefact, its origin and nature have yet to be explained.

W. Buchheim: Could the openings in the fat globule membranes (Fig. 4) perhaps represent locations of previously adsorbed casein micelles which desorbed during the dehydration step?

Author: In the absence of experimental proof, such a hypothesis would be unfounded.

W. Buchheim: It appears unlikely that Fig. 13 represents the true original structure of a yoghurt sample because of the high water content (~90%). Could it perhaps represent an atypical particle of highly aggregated protein within this yoghurt?

Author: The appearance of that commercial yoghurt certainly is surprising and the study of its microstructure could produce interesting results provided that the composition and manufacturing procedure are known. However, this is usually not the case with commercial products. The microstructure shown in Fig. 13 was consistently obtained with samples irrespective of the sampling site and is not related to highly aggregated protein areas such as the one mentioned earlier (24).

G.E. Pfefferkorn: The possibility of backscattered electron (BSE) images at a low magnification is not mentioned; the method of Volbert (53), who subtracts the BSE image from the normal secondary electron image to show only the topography of the surface should be discussed.

Author: Signal mixing techniques using a 2-detector system make it possible to show the "true" surface topography in micocompositional specimens without any artefacts. However, there are no reports on their being used to examine electron microscopy of milk products and I have no practical experience with them.

Additional References

THE EFFECT OF SALT AND PYROPHOSPHATE ON THE STRUCTURE OF MEAT

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Abstract

Our objective was to determine whether or not salt and pyrophosphate have the same effect on the structure of pieces of meat as they have on isolated myofibrils. Blocks of pig M. longissimus dorsi were incubated in solutions of sodium chloride at pH 5.5 or sodium chloride plus sodium pyrophosphate at pH 5.5 or 8.0. The blocks were obtained from fresh (24h post-mortem) or aged (72h post-mortem) muscle and incubated for 5 or 24h with minimal agitation. There was considerable uptake of water by the tissue especially at the higher pH and longer times. Electron microscopy of the meat incubated in salt plus pyrophosphate at pH 8.0 revealed complete or nearly complete extraction of the A-band to a depth of at least one fibre from the surface. In meat incubated in salt plus pyrophosphate at pH 5.5 the extraction of the A-band was less complete and appeared to occur only near the surface. In salt alone no extraction of the A-band occurred.

Swelling of myofibrils close to the surface could be detected either by a reduction of density or by a greater separation of filaments. Break-up of the Z-line, probably due to mechanical disruption imposed by swelling of myofibrils, was a common feature of the salt treatments. Mitochondria near the surface were grossly swollen, especially with salt plus pyrophosphate at pH 8.0.

At low pH amorphous material was observed inside and outside the cell membrane, but at high pH filamentous material was present in these areas.

Introduction

Sodium chloride has a widespread use in the manufacture of meat products. It has been used as a preservative to facilitate long-term storage and it imparts a characteristic flavour that remains greatly in demand. An addition of sodium chloride results in an increase in the water-holding capacity of meat, the uptake of additional water causing swelling of the tissue. In the presence of polyphosphate this occurs at a lower concentration of sodium chloride. The presence of sodium chloride, especially in combination with polyphosphates, also assists the retention of water during cooking. The literature on the water-holding properties of meat has been reviewed by Hamm (1960, 1975, 1981) and Offer and Trinick (1983).

Mechanical agitation, such as massaging or tumbling, of meat pieces or particles in the presence of sodium chloride and polyphosphate gives rise to the formation of a sticky exudate on the surface (Theno et al., 1978a,b,c). This exudate contains myofibrillar proteins, in particular myosin, and is responsible for the binding together of meat pieces in cooked meat products. The conditions under which these proteins are extracted are thus of considerable importance. Recent work by Offer and Trinick (1983) describes the behaviour of single myofibrils from rabbit M. psoas in different concentrations of sodium chloride in the presence or absence of sodium pyrophosphate at pH 5.5. They observed that myofibrils swell very substantially in salt solutions resembling those used in meat processing, and concluded that the myofibrils were the major sites of water uptake in meat. In the absence of pyrophosphate a concentration of 0.8M sodium chloride was required for swelling. Under these conditions there was partial extraction of myosin from the centre of the A-band. In the presence of pyrophosphate, a much lower concentration of sodium chloride (0.4 M) was required for swelling and extraction of the A-band was complete or nearly complete. Removal of the A-band progressed from the ends towards the central M-line.
The findings of Offer and Trinick (1983) are valuable in suggesting the mechanism by which water is taken up by meat. However, it will be appreciated that in such experiments the myofibrils were exposed to very large volumes of solutions and therefore any solubilized protein, such as myosin, would be removed from the vicinity of the myofibril. Hence, any tendency for the myofibril to disaggregate under the salt conditions would be exacerbated. Furthermore, since myofibrils are sub-cellular structures, no cell membrane or endomysium was present to act as a barrier to the diffusion of solutes or a constraint on swelling of the myofibril. Finally, the experiments of Offer and Trinick were performed at room temperature, rather than the low temperature at which meat products are commonly manufactured.

Commercial practice is concerned with bulk tissue with a consequential restriction of access to those elements that are below the surface. In order to obtain information about the fate of muscle cells in whole tissue when treated with salt, a model system consisting of blocks of meat of standard dimensions may be used. Such a system will provide information about the depth of penetration of the salt solution used, the extent to which swelling of myofibrils occurs, the extent to which proteins, particularly the myosin of the A-band, are solubilized and any other structural changes that occur. It is particularly important to know the extent of extraction of the A-band under such conditions since myosin is a better binder of meat pieces than actomyosin (Macfarlane et al., 1977), and myosin extraction was a feature in the observations made by Offer and Trinick (1983).

Lewis and Jowett (1972, 1975) examined the surface of blocks of pig muscle soaked in solutions of sodium chloride with or without tripolyphosphate. Using light microscopy they detected little or no change in structure compared with fresh, untreated meat. Changes were observed using electron microscopy, however. In meat treated with salt and polyphosphate these consisted mainly of disorganization of filaments in the H-zone, an observation which did not seem to fit with those made on extracted myofibrils by Offer and Trinick (1983). A re-examination of the structural changes produced by salt and polyphosphate was therefore desirable, particularly when using well defined ionic conditions and meat of well defined post-mortem history. In this paper we shall describe the changes observed in blocks of porcine M. longissimus dorsi exposed to a limited volume of salt solution, with and without polyphosphate, at a pH (5.5) close to the final pH of the muscle in rigor. Since brines of alkaline pH are frequently used in commercial meat processing the effect of a similar solution at a high pH (8.0) was also observed. The length of time for which the tissue was exposed to these solutions was varied, as was the post-mortem age of the tissue prior to incubation. In order to avoid the complications presented by mechanical damage to the exposed surfaces of the blocks of tissue, minimal agitation was applied, serving only to mix the solution surrounding the tissue. The following report should be regarded as a preliminary communication, as a full analysis of the often quite complicated structural changes has yet to be made. In this study we have concentrated on the effect on fibres at the surface of the block of tissue, although we have attempted to see whether the structural changes altered with depth.

Materials and Methods

The M. longissimus dorsi from one side of a commercial hybrid pig (86.5 kg carcass weight) was used for these experiments. The animal was electrically stunned and slaughtered by sticking. Blood drainage occurred as rapidly as possible, the carcass was suspended head downwards, from the Achilles tendon of one leg. In order to limit shortening of the fibres of the muscle to be used, the hind leg on the side from which the M. longissimus dorsi would be removed was tied at approximately 900 to the spine.

After slaughter the carcass was held for 5 h at ambient temperature (18°C) and then transferred to a chill room at 0°C. The M. longissimus dorsi was removed from the 'stretched' side 24 h after slaughter. The muscle was divided longitudinally; one half, designated 'fresh', was processed immediately while the other half was wrapped and held for a further 48 h at 4°C and was designated 'aged'. These post-slaughter holding periods are comparable with current commercial practice in the United Kingdom.

Samples of approximately 1 g were removed for pH determination. These were homogenised using a laboratory mixer-emulsifier (Silverson Machines Ltd., Chesham, Bucks., UK), in 10 cm3 of a solution containing 150 mM potassium chloride and 5 mM iodoacetic acid neutralised to pH 7.0. The pH of each extract was determined with a PIM63 Digital pH meter (Radiometer, Copenhagen, Denmark) using a combined glass electrode (Russell pH Ltd., Auchtermuchty, Scotland). The final pH was 5.4.

Blocks of tissue

Six blocks of tissue, each measuring 3 x 2 x 1 cm were cut from the fresh or aged portion of the M. longissimus dorsi so that the longitudinal orientation of the fibres coincided with the long (3 cm) axis of the tissue block. Each block was weighed after removal of superficial moisture by gentle blotting until there was no further sign of moisture transfer on clean absorbent paper. These blocks were incubated in the salt solutions described below before preparation.
Salt, pyrophosphate and meat structure

for microscopical examination. Additional blocks of tissue were removed from the fresh or aged portion of the muscle but were not incubated in the salt solutions. These were fixed immediately and served as controls.

Salt solutions

Three solutions were used for incubation of the tissue blocks, as follows:

A. 0.6 M sodium chloride (3.5%)
   10 mM sodium acetate
   1 mM magnesium chloride
   adjusted to pH 5.5 with hydrochloric acid

B. Solution A plus
   10 mM tetra sodium pyrophosphate
   adjusted to pH 5.5 with hydrochloric acid

C. Solution A plus
   10 mM tetra sodium pyrophosphate
   adjusted to pH 8.0 with hydrochloric acid

The solutions were stored at 4°C.

The concentration of sodium chloride shown above was chosen on the basis of experiments with myofibrils (Offer and Trinick, 1983). In the presence of pyrophosphate this concentration would be expected to be amply sufficient to cause swelling and A-band extraction. In the absence of pyrophosphate it would be on the threshold required for swelling. It should also be borne in mind that dilution of the irrigating solution will occur on incubation.

Incubation of tissue blocks

Each block of tissue was placed in a glass specimen tube 25 mm in diameter. 18 cm³ of one of the solutions, A, B or C were added to give a tissue to fluid volume ratio of 1:3. The tissue was supported on a stainless steel spring so that the entire block was surrounded by salt solution which, under gentle agitation, was able to circulate freely. The loaded tubes were placed radially in a rack inclined at 45° to the vertical and revolving at 4°C for 5 or 24 h. The tubes were sealed to prevent loss of fluid during inversion or by evaporation.

When the incubation period was complete, the tissue blocks were removed from the specimen tubes, drained into the tubes and gently blotted prior to weighing. From the weights obtained before and after incubation, the gross weight gain was determined.

Analysis of solutions after incubation

Protein The biuret method of Gornall et al (1949) was used to measure the protein content of the solutions.

Chloride After dilution with deionised water the chloride content of each solution was determined. A Radiometer CMT 10 chloride titrator was used to make these measurements.

Preparation for microscopy

On completion of the incubation period samples measuring approximately 5 x 5 x 2 mm were removed from one end of the block of tissue as shown in Figure 1. One 5 mm dimension coincided with the longitudinal axis of the fibres. The samples were fixed in 2.5% glutaraldehyde buffered with 0.1M sodium cacodylate at pH 6.8 in accordance with the common practice of using fixatives in the region of neutrality. After 2h fixation the samples were trimmed to form smaller blocks, approximately 5 x 2 x 2 mm, which were returned to the fixative for a further 22h. Thus the final samples incorporated parts of two of the faces of the original block of tissue - one in longitudinal orientation, the other being transverse. Dehydration through graded alcohols was followed by impregnation and embedding in LR white acrylic resin - medium grade (London Resin Co., Basingstoke, Hants., UK) in polypropylene capsules (TAAB, Reading, Berks., UK). The embedments were polymerised at 60°C for 24 h.

Sections, 30-50 nm thick, were cut using a glass or diamond knife on an LKBIII ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 400T electron microscope.

Results

Changes in the block of tissue

Change in weight An increase in the weight of the blocks of meat after incubation in the salt solutions was detected under all the conditions applied. The increases, expressed as a percentage of the initial weight, are listed in Table 1.

Table 1. Percentage increase in weight of blocks of meat following incubation.

<table>
<thead>
<tr>
<th>Post mortem</th>
<th>Incubation time</th>
<th>NaCl pH 5.5</th>
<th>NaCl plus pyrophosphate pH 5.5</th>
<th>NaCl plus pyrophosphate pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>age of tissue (hrs)</td>
<td>at 4°C (hrs)</td>
<td>% Increase in weight</td>
<td>% Increase in weight</td>
<td>% Increase in weight</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>5.7</td>
<td>9.3</td>
<td>12.1</td>
</tr>
<tr>
<td>72</td>
<td>5</td>
<td>14.5</td>
<td>16.2</td>
<td>20.0</td>
</tr>
<tr>
<td>24</td>
<td>18.0</td>
<td>23.2</td>
<td>22.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Concentration of protein and chloride following incubation in different media.

<table>
<thead>
<tr>
<th>Incubating Medium</th>
<th>Incubation time at 4°C (hours)</th>
<th>NaCl pH 5.5</th>
<th>NaCl plus pyrophosphate pH 5.5</th>
<th>NaCl plus pyrophosphate pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post mortem age of tissue (hours)</td>
<td></td>
<td>Protein Chloride Protein Chloride Protein Chloride</td>
<td>mg/cm³ M</td>
<td>mg/cm³ M</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>7.1 0.52</td>
<td>8.6 0.56</td>
<td>12.5 0.53</td>
</tr>
<tr>
<td>72</td>
<td>24</td>
<td>15.8 0.58</td>
<td>15.5 0.53</td>
<td>22.5 0.49</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>11.3 0.49</td>
<td>9.1 0.49</td>
<td>9.3 0.54</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>15.1 0.49</td>
<td>15.7 -</td>
<td>19.8 0.43</td>
</tr>
</tbody>
</table>

Table 3. The incidence of various structural alterations following incubation in different media containing salt and pyrophosphate.

<table>
<thead>
<tr>
<th>Incubating medium</th>
<th>NaCl pH 5.5</th>
<th>NaCl + pyrophosphate pH 5.5</th>
<th>NaCl + pyrophosphate pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post mortem age (hours)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>72</td>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td>Incubation time at 4°C (hrs)</td>
<td>5 24 5 24</td>
<td>5 24 5 24</td>
<td>5 24 5 24</td>
</tr>
<tr>
<td>Marked swelling of peripheral myofibrils</td>
<td>+ + + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Partial extraction of A-band to stubs</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Total extraction of A-band: loss of M-line</td>
<td>+ + + (+) (+) +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Z-line break-up</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Region of low density close to Z-line</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Filamentous deposit of extracted protein near to cell membrane</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Mitochondrial swelling</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Definite change in structure observed. (+) The Z-line shows reduced electron density but is still intact.

Changes in appearance: After incubation for 5 h the blocks of tissue became paler and more translucent to a depth of about 1 mm from the surface. This increased to 2 - 3 mm after 24 h incubation but the boundary of the translucent zone was not well defined. There were no apparent differences in the effects on appearance caused by the different incubating media. Although the translucent zone presumably corresponds to a region in which marked structural changes, especially swelling, are occurring, the relationship between this and the depth of penetration of sodium chloride into the tissue remains to be explored. Zones of 1 or 2 mm in depth from the surface would occupy fractions of 0.33 or 0.59 respectively of the total volume of the meat blocks. If these zones represent regions where swelling has occurred then the water uptake in these zones is as much as 16 - 37%, depending on conditions.

Changes in the salt solutions: Appearance. The solution at high pH became straw-coloured after incubation, due, presumably, to the extraction of myoglobin. The solution remained clear. The low pH solutions varied in their ultimate appearance. Short incubation of fresh or aged tissue gave rise to a clear straw-coloured solution, but after long incubation the solution became cloudy; a flocculent precipitate was eventually formed.
Analysis of the salt solutions

The total protein and chloride concentrations in each solution after incubation are listed in Table 2. This shows that a long incubation results, as expected, in an increase in protein extracted, compared with the short incubation. The presence of pyrophosphate at pH 5.5 had no effect on the amount of protein extracted, but at the higher pH there was increased extraction in the fresh tissue.

The amount of protein extracted from the blocks of meat was considerable; 23% of the total protein of the block of fresh meat was extracted in sodium chloride at pH 5.5 in 24 h at 4°C, and in the presence of pyrophosphate at pH 8.03% was extracted. The values for aged tissue also fell within this range. After incubation, the chloride ion concentration in the brine fell substantially (Table 2).

As explained above we know that there was net water uptake by the meat blocks. The decrease in chloride ion concentration must therefore be due to uptake of this ion beyond that taken up with the water. If all the water originally in the meat blocks (assumed to be 75% by weight) became equilibrated with the external salt solution, the concentration of chloride in the medium would fall to 3/3.75 of the original, that is 0.48 M. Although for each condition of incubation in Table 2 only one block of meat was used, and confirmation of these data is necessary, there is a strong suggestion that chloride ions have penetrated much or all of the water in the meat.

Changes in structure

The various structural changes and their observed incidence are listed in Table 3. As this is a preliminary report of work in progress, it will be appreciated that more examples of the phenomena listed may be revealed by more extensive examination.

In both fresh and aged controls, Z-lines were observed in electron-dense structures with thin filaments attached to them (Fig. 2). Nine of the twelve treatments produced break-up of the Z-line along its length, as illustrated in Fig. 3. This phenomenon was detected at a distance of at least 0.15 µm beneath the surface of the tissue block. A similar but less definite change in appearance was observed in two other treatments, with the Z-lines being less dense than in the controls. Change in Z-line structure was the most common of the phenomena related to the action of salt with or without pyrophosphate at high or low pH.

In these experiments we were concerned to determine whether there was any evidence of swelling in the myofibrils. It was of great interest, therefore, to discover that frequently there was a loss of density in these structures at the surface. In fresh tissue incubated in sodium chloride at low pH, myofibrils at the edge of the block showed considerably reduced density (Fig. 4). The appearance of myofibrils situated more deeply in the tissue resembled that of the controls.

The plane of the section illustrated in Fig. 4 was perpendicular to the exposed surface of the block of tissue. Since loss of density in the appearance of myofibrils is a feature which was commonly observed, it is important that possible interpretations are considered. First, the A-band is intact but increased separation between the filaments due to swelling has occurred. Such a reduction in packing density would give rise to loss of density in the electron image. The second possible interpretation is that the thick filaments of the A-band have been extracted and the spaces between the thin filaments are more clearly seen. This will also have the effect of reducing the density of the image. In Fig. 4 the reduced density of the uppermost myofibril is likely to be due to the first of these possibilities, since even with isolated myofibrils, this concentration of sodium chloride does not extract the A-band (Offer and Trinick, 1983).

Swelling was also observed in myofibrils in which the long axes were perpendicular to an exposed surface of a treated block of tissue. Fig. 5 illustrates this phenomenon. The exposed surface is represented by the cut ends of the myofibrils on the right-hand side. Hells these myofibrils have a lower density, the filaments being more clearly visible with greater separation between them, than those in the sarcomeres in the left half of the field.

It was of special interest to determine the extent to which the A-band was extracted. The removal of myosin from the A-band in fresh and aged samples incubated in the presence of salt and pyrophosphate at high pH. Under these conditions extraction occurred at the ends of the A-band, leaving shortened filaments with the M-line conspicuously visible. Figs. 6 and 7 illustrate an example of these changes in myofibrils from aged meat incubated in sodium chloride at high pH. Trinick and Cooper (1980) described a similar effect with thick filaments of rabbit M. Psoas exposed to increasing concentrations of potassium chloride. In our experiments shortened filaments were found to vary in length from about 1.0 µm down to 0.2 µm, the lower end of the range corresponding to the stubs described by Trinick and Cooper (1980). In Fig. 6 thick filament stubs and M-line are clearly visible, while in Fig. 7 a further loss of A-band material has resulted in a reduction in the length of the stubs. The M-line, however, is still preserved. Further removal of A-band material, including the M-line is shown in Fig. 8 which depicts strings of I-bands in which the thin filaments are somewhat irregularly arranged. Fig. 9, which is a view of many myofibrils at low magnification, shows that the extent of extraction of A-band material, that is whether the extraction was complete or produced stubs, is a variable phenomenon with the distribution of shortened thick filaments being random. In the area
Fig. 2 Fresh muscle

Fig. 3 Fresh muscle-incubated in sodium chloride, pH 5.5 for 5h. Note the break up of Z-lines (arrows).

Illustrated it appears that A-bands have been completely extracted from most sarcomeres. Extraction of the A-band was detected at a distance from the surface of the tissue of at least the width of a fibre after 5 h incubation in salt and pyrophosphate at pH 8.0.

With salt plus pyrophosphate at pH 5.5 extraction of A-band material still occurred (Fig. 10). In the top right of the figure there is an area at the surface where the myofibrillar structure seems to have been completely eroded. In the sarcomeres in the left hand part of the figure however, the extraction of the A-band is incomplete and the shortened A-bands are wider than seen in Figs. 6 and 7 and have very irregular edges. Examination at low magnification suggested that extraction of the A-band occurred in myofibrils close to the surface of the meat block but could not be detected at greater depths. This point requires further investigation because, particularly in shortened sarcomeres, it is not always possible to tell whether thick filaments are present or absent. With salt alone no extraction of the A-band was seen, even at the surface of the meat blocks.

Fig. 4 Fresh muscle - incubated in sodium chloride, pH 5.5 for 24h. The myofibril at the top of the micrograph is adjacent to the cell membrane. Note the reduced density in this myofibril and the break-up of Z-lines.

Fig. 5 Aged muscle - incubated in sodium chloride, pH 5.5 for 5h. Myofibrils near exposed surface of meat (right hand edge) show loss of density, probably due to swelling.

Fig. 6 Aged muscle - incubated in sodium chloride plus pyrophosphate, pH 8.0 for 5h. Thick filaments are reduced in length but myofibrils are still recognisable. Beneath the cell membrane (top) is array of dissociated filaments with occasional collagen fibril.
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Fig. 7 Same sample as Fig. 6. Myofibrils have undergone further extraction to form shorter thick filaments - 'stubs'.

Fig. 8 Fresh muscle - incubated in sodium chloride plus pyrophosphate, pH 8.0 for 24h. Note the loss of thick filaments and M-line. Z-line is indistinct with detachment of thin filaments.

Fig. 9 Aged muscle - incubated in sodium chloride plus pyrophosphate, pH 8.0 for 5h showing scattered distribution of 'stubs' (arrows).

Fig. 10 Aged muscle - incubated in sodium chloride plus pyrophosphate, pH 5.5 for 5h. Note reduction in length of thick filaments and disorganised structure on the right.

Fig. 11 Fresh muscle - incubated in sodium chloride, pH 5.5 for 24h. Note the regions of low density between broken Z-lines and I-bands.

Fig. 12 Fresh muscle - incubated in sodium chloride plus pyrophosphate, pH 5.5 for 5h showing accumulation of amorphous material near the cell membrane. A similar but less extensive deposit was observed in the control.
C.A. Voyle, P.D. Jolley and G.W. Offer

In normal vertebrate skeletal muscle there is a continuity between the thin filaments of the I-band and the Z-line, as illustrated in Fig. 2. A region of very low density, of width 0.2 μm on either side of the Z-line, was commonly observed in fresh meat incubated for 24 h in sodium chloride at low pH (Fig. 11) and also in fresh and aged meat incubated in salt and pyrophosphate at high pH (Figs. 6 and 8). The area in Fig. 4, also illustrating fresh meat incubated for 24 h in sodium chloride at low pH, did not show this phenomenon. Occasional filamentous material could be seen in the region of low density. A similar phenomenon was illustrated by Walcott and Ridgway (1967) in Fig. 1 of their paper describing the ultrastructure of myosin-extracted striated muscle fibres. No discussion of the nature of this region was offered by Walcott and Ridgway (1967).

A possible explanation of this phenomenon is the weakening of the attachment of thin filaments to the Z-line, resulting eventually in the detachment of the thin filaments. This explanation is suggested by the appearance shown in Fig. 8 where a clear demarcation may be observed between the thin filaments and the region of low density adjacent to the rather poorly defined Z-line. However it is difficult to explain why the regions should be of constant width. Alternatively, it is possible that thin filaments are still anchored in the Z-line but loss of density is due to the selective extraction of part of a third kind of filament (Wang, 1982) in this region.

In view of the presence of a sticky exudate on the surface of salt-treated meat subject to mechanical agitation, it was of interest to determine whether any material was present close to the sarcolemma. Amorphous material, often in large quantities, was observed inside as well as outside the cell membrane in fresh tissue incubated in salt and pyrophosphate at low pH (Fig. 12). Similar material was also seen in the controls but it was present in smaller amounts; none was observed after incubation in salt only at low pH.

Incubation at high pH favoured the release of structures from myofibrils nearest to the exposed surface of the block of tissue. These structures retained a morphology resembling that of thick filaments (Figs. 6, 13 and 14). In Fig. 13 it can be seen that there is a gradient of structural change from the left to the right of the micrograph. Compact structure is maintained in the deep myofibrils. Near the surface there is increasing evidence of swelling, with greater separation between the filaments. At the surface some filaments are no longer part of the organized structure. In another area a considerable disarray of filaments was observed at the end of a muscle fibre in a block of tissue incubated in salt and pyrophosphate at high pH (Fig. 14). This is an interesting contrast with the area shown in Fig. 6 in which the same treatment but for shorter time results in a sharp demarcation between myofibrils exhibiting A-band extraction, producing short lengths of thick filaments, and a clearly localized region containing dissociated filaments in disarray.

The phenomenon in these experiments was the appearance of large vacuoles close to the surface of the meat blocks, especially those incubated in salt and pyrophosphate. Closer examination showed that the vacuoles were grossly swollen mitochondria since cristae could be recognized. Some were swollen to the point of rupture (Fig. 15), having increased in diameter nearly twofold. The swelling was greatest in areas of tissue nearest to the exposed surface of the block of meat; further in towards the centre of the meat block the mitochondria were not so enlarged (Fig. 16). Swelling was, however, detected to a depth of about 1 mm from the surface of the tissue. Swollen mitochondria were present in all treatments containing pyrophosphate and also in fresh tissue incubated with salt only for 24 h, but none were observed in the controls. Our colleague, Dr K.S. Cheah, (personal communication) has also observed the phenomenon in bacon.

Discussion

Extraction of A-band

Our objective in examining the structure of meat blocks incubated in salt with or without pyrophosphate was to determine whether the structural changes observed with isolated myofibrils also occurred with pieces of meat.

We have shown that with salt plus pyrophosphate at high pH the A-band is either completely extracted or nearly completely extracted to at least the depth of a fibre. At lower pH with salt plus pyrophosphate the A-band was only partially extracted near the surface of the meat and extraction did not occur at deeper levels. In the absence of pyrophosphate no extraction of the A-band could be observed. These observations are broadly consistent with those of Of fer and Trinick (1983) on isolated myofibrils. They reported that in the presence of pyrophosphate at pH 5.5, 0.4 M NaCl was sufficient to extract protein from the end of the A-band. The extraction was either complete or nearly complete leaving a region close to the M-line. In the absence of pyrophosphate the A-band was either not extracted or extracted very incompletely at its centre, a substantially higher concentration of NaCl (0.6 - 0.8 M) being required to effect extraction. Pyrophosphate was presumed to act as an analogue of ATP and by dissociation of actomyosin to allow the thick filaments to depolymerise at a lower salt concentration.

It may be readily understood why there should be differences between the present results and those of Offer and Trinick (1983)
with regard to the degree of extraction of the A-band. In the experiments of Offer and Trinick the myofibrils were treated with a large volume of salt solution so that if there was any appreciable dissociation of thick filaments, the myosin molecules would be removed and eventually the thick filaments would be extracted. In the present experiments the volume of brine was limited so that only if the position of equilibrium lay strongly in favour of dissociation would extraction be observed. It should also be noted that the salt concentration fell during the incubation to a level below the threshold required for extraction even in the myofibril experiments.

The difference between the depths to which extraction occurred in salt plus pyrophosphate at high and low pH may be explained in terms of the highly co-operative equilibrium that exists between myosin molecules and filaments (Josephs and Harrington, 1966). Below a certain critical protein concentration, which depends on the ionic conditions, myosin exists entirely in the form of molecules; any myosin in excess of this critical concentration exists as filaments. The extent of depolymerisation of thick filaments is thus highly dependent on the protein concentration; salt conditions effecting complete depolymerisation in a dilute suspension of myofibrils may achieve only a small degree of depolymerisation in a more concentrated system. When salt diffuses into the meat block the thick filaments will tend to depolymerise. The myosin molecules produced will diffuse out into the salt solution and a gradient of concentration of myosin molecules will be established in the block. But as myosin molecules diffuse away from the surface of the meat they will be

Fig. 13 Aged muscle - incubated in sodium chloride plus pyrophosphate, pH 8.0 for 24h. Structures resembling thick filaments have been released from peripheral myofibrils.

Fig. 14 Aged muscle - incubated in sodium chloride plus pyrophosphate, pH 8.0 for 24 h. Sarcomeres at the ends of myofibrils have been destroyed leaving filaments in disarray.

Fig. 15 Fresh muscle - incubated in sodium chloride plus pyrophosphate, pH 5.5 for 5h. Note swollen mitochondria (arrows).

Fig. 16 Fresh muscle - incubated in sodium chloride plus pyrophosphate, pH 5.5 for 5h. Swollen mitochondria were observed in peripheral fibres in nearly all treatments (see Table 3).
replenished by depolymerisation of more thick filaments; this reduces the depth over which the gradient of myosin molecules occurs. The effect is that the myosin molecule concentration is maintained constant at the critical concentration over a substantial part of the meat block and only in a region near the surface does the myosin molecule concentration fall below this level; in this region the thick filaments must all be depolymerised. The extent of this region will be dependent on the equilibrium constant for the depolymerisation and will therefore be highly dependent on the ionic conditions. Since the thick filament is more easily depolymerised at higher pH (Josephs and Harrington, 1966) the greater depth to which the A-band is extracted can be qualitatively explained.

Extraction of myosin from the A-band depends not only on the depolymerisation of a substantial part of the myofibril but also on the fragmentation of myosin from the actin filaments. It is therefore of interest that despite the very high local concentration of actin filaments present in the meat blocks, extraction of the ends of the A-band to give stubs occurred under ionic conditions not greatly different from those used by Trinick and Cooper (1980) who were examining the depolymerisation of individual thick filaments.

**Swelling**

The most important observation from the work on isolated myofibrils (Offer and Trinick, 1983) was that the myofibrils swelled very substantially either in salt alone or with pyrophosphate. It is technically much more difficult to detect swelling in the meat blocks. In part this is because there is shrinkage of specimens during the preparation for microscopy; in part it is because the longitudinal sections sample the hexagonal lattice randomly rather than along a fixed crystallographic plane so that the interfibrillar spacing cannot be measured; in part it is because in the meat blocks one cannot observe a myofibril before, as well as after, the salt incubation. Nevertheless, we have observed a reduction in density in myofibrils very close to the surface (Fig. 4) which was probably due to swelling, and in other specimens we have observed a large spacing between filaments in such peripheral myofibrils (Figs. 13, 15). There are strong grounds for supposing that moderate swelling occurred at much greater depths. The fall in concentration of the salt solutions suggests that the sodium chloride penetrated a substantial part of the meat blocks. Furthermore, the blocks exhibited a zone, extending a substantial distance (1-2 mm) from the surface, that was markedly more translucent than the remainder of the block. In view of the substantial water uptake of the blocks, resulting in increases in weight of up to 25%, the most reasonable explanation is that the translucent zone is a region where the muscle fibres, and presumably the constituent myofibrils, have swollen to a maximum extent of 37%. This water uptake could be accounted for by only a 17% increase in the filament lattice spacing in this zone which we could not expect to detect in the present work.

**Changes in Z-line**

Offer and Trinick (1983) noted in their experiments on myofibrils that as a result of salt treatment the Z-line of most myofibrils swelled as much as the A-band and became very weak. In some myofibrillar preparations, however, although the A-band swelled, the Z-line did not and the myofibrils were pinched-in at each Z-line. Such observations suggest that the Z-line tends to resist myofibrillar expansion. It seems likely that the fragmentation of the Z-line we have observed is a structural change imposed on the Z-line when it is forced to swell in response to the swelling of the rest of the myofibril. It remains a possibility, however, that the fragmentation is due to partial extraction of the Z-line material.

**Mitochondrial swelling**

We observed very marked swelling of the mitochondria, particularly in the presence of pyrophosphate at pH 8. We are not aware of this phenomenon being described before and it raises the question of whether such swelling contributes substantially to water uptake. The swelling was marked only near the surface of the meat blocks and even in examples such as is shown in Fig. 16 the fraction of the area occupied by sectioned mitochondria is only of the order of 5%. It therefore seems unlikely that more than a small fraction of the water uptake could be due to this source.

**Material near cell membrane**

In commercial practice massaging and tumbling are processes used in the manufacture of meat products. A sticky exudate is formed on the surface of the meat pieces that serves to bind them together. It was therefore of interest that we observed material deposited inside as well as outside the cell membrane under most treatments. It is of particular interest and possible practical importance that the morphology of extracted material was influenced by pH. At low pH this material was amorphous but we observed filaments after incubation in salt plus pyrophosphate at high pH. These latter conditions would be expected to be those showing the best binding between meat pieces according to the experience of our own and other laboratories. The nature of these filaments awaits investigation.

**Comparison with previous work**

Our results may be compared with those of Lewis and Jewell (1975) who studied the effect of incubating meat blocks with salt and/or polyphosphate although there are some differences in the treatments. Lewis and Jewell mainly employed brine to meat ratios of 1:1; they used tripolyphosphate rather than pyrophosphate and the pH of their brine was not defined. In 4% salt alone they observed extraction of the A-band and commented on a loosening of structure. Our results are thus...
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very similar. With 4% salt plus 1% triphosphate they concluded that the largest change was in the H-zone where the filaments became disorganised. This conclusion disagrees with our observations, where in the presence of pyrophosphate the A-band was depolymerised from its ends. However, their Figure 5 shows myofilaments in which the thick filaments have been extracted leaving strings of I-bands. This result, rather than their conclusion, is in agreement with our own results.

Future objectives

The observations we have recorded here represent only the start of an investigation of the effect of salt on meat structure. In future work we plan to examine more fully the changes in structure between the exposed surface and the deeper parts of the tissue in order to establish the relation between the gradients of sodium chloride concentration, of pyrophosphate concentration, of A-band extraction, of Z-line fragmentation and of swelling. We intend to determine the extent of swelling of the filament lattices at various depths by examination of transverse sections cut from a transverse slice through the centre of the block. Finally we intend to explore the relation between the deposits of material at the surface of the block and the sticky exudate.

Acknowledgements

The authors are grateful to D.J. Restall for drawing their attention to swollen vacuoles which he observed by light microscopy in salt-treated muscle. They also wish to acknowledge the skilled technical assistance of A. Cousins, Anne Phillips and R. Angell in the preparation of material for electron microscopy and in chemical analysis. The authors wish to thank Dr P.J. Knight for discussion and helpful comment.

References


Discussion with Reviewers

R.G. Cassens: Do the authors believe that the solutions used preclude completely the action of proteolytic enzymes?

S.H. Cohen: Could you very briefly discuss the roles of CAF (calcium-activated factor) and cathepsin enzymes in what might be a synergistic action with salt and pyrophosphate on myofibrillar degradation?

Authors: We would certainly not suppose that proteolysis was absent in our experiments. There are two stages at which proteolytic activity may occur. The first is during the post-slaughter period, before incubation of the meat pieces in brine. The second is during the incubation period. In our controls with no salt treatment the Z-line was intact after 24h, although a small amount of degradation occurred after 72h. Therefore the very considerable fragmentation of the Z-line which we observe must arise during the salt treatment. This fragmentation could result from (a) mechanical damage due to swelling of the myofibrils, (b) extraction of Z-line material by salt, (c) enhanced proteolytic activity in the presence of salt as the Reviewers suggest. However, for the following reasons we think (c) is unlikely.

First, the calcium-activated neutral proteinase (CANP or CAF) is active in the pH range 6.5 - 9.0 but pyrophosphate acts as a calcium binding agent and therefore the activity of this proteinase would be expected to be much reduced.

Second, cathepsins are active below pH 5.5, cathepsin's B and D degrading myosin at pH 5.5. Lysozomes are stable at high salt concentrations (personal communication - Dr O.J. Etherington). Therefore, under the high salt conditions used in this experiment it is unlikely that there will be more lysosomal damage than in the untreated controls and the level of released catheptic activity would not be expected to be elevated.

R.G. Cassens: I have noted that the authors plan considerable future work but no consideration is to be given to nitrите. This is rather surprising since nitrіte is very often used in combination with salt in the meat industry. What is the rationale?

Authors: Nitrite is added to meat products in order to enhance flavour and colour and to minimise microbiological activity. It has been reported by Fox et al. (1980) that nitrite in the presence of sodium chloride brings about changes in the interfacial spaces, particularly involving the endomysium. Our present concern is with water uptake and extraction of protein. So far as we are aware, nitrite is not involved in these processes. Our aim is to work with model systems and we prefer to keep the conditions as simple as possible.

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closely than did the myofibril experiments, they differ from commercial conditions in using a much greater brine to meat ratio (3:1).

Magnesium chloride was included in the brine to avoid excessive dilution of the magnesium ions in the meat. Acetate was included as a buffer.

D.F. Lewis: Do you consider it a possibility that the region of low staining density on either side of the Z-line, described in several of your micrographs, represents loss of thin filaments, these remaining intact where they overlap with thick filaments in the A-band?

Authors: The production of the regions of low staining intensity is something of a puzzle and our current interpretation of these regions can only be tentative. Figure 11 shows the dark bands present. These dark staining bands are of a similar width to the A-bands of untreated meat (Figure 2) and we therefore assume that they are in fact A-bands. If this is correct the regions of medium staining intensity between these dark bands and the regions of low staining intensity are presumably regions of non-overlapped thin filaments. The reviewer suggests that the lightly stained regions represent regions where the thin filaments have been extracted. While we cannot exclude this interpretation, we do not favour it for several reasons. Firstly, it is not clear why the entire length of the thin filaments not overlapped by thick filaments is not extracted. In Figure 8 all the A-band is removed; there is a gap on either side of the Z-line but clear arrays of unextracted thin filaments may be seen. Secondly, using SDS polyacrylamide gel electrophoresis on supernatant and residue of extracted myofibrils exposed to 0.5M sodium chloride at pH 5.5, Offner and Trinick (1983) did not detect any loss of actin from the residue, nor its presence in the supernatant.

D.F. Lewis: Figure 7 shows some myofibrils where the M-line and pseudo H-zone have been loosely extracted as seen, for example in the upper and lower myofibrils in the micrograph. In these myofibrils the filaments that are seen occupy the region between the H-zone and the I-band. In untreated meat this region contains both actin and myosin filaments. The range of thicknesses of the filaments in this region is consistent with both actin and myosin still being present. Why do you not even consider the possibility that both types of filament are present?

Authors: The evidence of SDS gel electrophoresis indicates that salt plus pyrophosphate readily extracts myosin. The greatly reduced density of the region that contained the A-band in Figure 7 also shows that most of the myosin in these myofibrils has been extracted. We suggest therefore that the residual filaments are thin actin-containing filaments and that these may be clumped, as described by Lewis and Jewell (1975), thus presenting a greater thickness than that associated with discrete thin filaments. But we would not want to discount the possibility that some myosin remains in molecular form attached to actin filaments.

D.F. Lewis: Some of the "dissociated fibres" on the right hand side of Figure 6 show distinct collagen-type banding (view at 2X0 to see). This indicates that they are outside the cell membrane. Is it possible that the other "dissociated fibres" are derived from connective tissue proteins?

Authors: Dr Lewis is right to point out that in the region shown in Figure 6 there are a few collagen fibres. Some are in longitudinal orientation and a few are transverse. Those displayed in longitudinal section exhibit a very fine banding pattern, characteristic of collagen. These fibres are of uniform width with a sharp outline and resemble endomysial collagen. The majority of the filaments in this region are less wide, have a frayed outline and do not exhibit periodicity so we do not think they are collagen. It is likely that in rigor the cell membrane is ruptured. Therefore it is not surprising to find extracellular collagen mixed with filaments of myofibrillar origin.

D.F. Lewis: Sarcoplasmic proteins constitute around 30% of the proteins in the meat cells. What effects do you think your treatments have on sarcoplasmic proteins?

Authors: Sarcoplasmic proteins are soluble in water or dilute salt solutions. They represent a complex mixture of about fifty components, many of which are glycolytic enzymes (Lawrie, 1979). It is likely that much of this material is washed out of the muscle fibres, as indicated by the presence of myoglobin in the brine after extraction. At least one sarcoplasmic protein (phosphofructokinase) precipitates at pH 5.5 but the amount of this protein is not large and precipitation would already have occurred before the salt treatment. Ref: Lawrie R.A. (1979) Meat Science, Pergamon Press, Oxford, 3rd Edn. Chapter 4.

D.F. Lewis: Siegal, Theno and Schmidt (1978) examined the composition of exudate from tumbled hams using brines containing 3% salt with and without 0.5% of a commercial polyphosphate preparation. They reported similar proportions of myosin and actin in the exudate and found little change in these values in the presence of polyphosphate. If your interpretation of your micrographs is correct would you not have expected them to have found an increased proportion of myosin when phosphate was present in the brine?

Authors: In their experiments Siegel et al centrifuged the sticky exudate and washed the pellet several times. Unfortunately, they discarded the supernatants and the material they examined by SDS gel electrophoresis was therefore not the soluble proteins present in the sticky exudate but the insoluble residue. Not surprisingly, therefore, this material contained a high proportion of actin. This result is misleading in terms of observed structural changes. If the soluble proteins in the supernatant had been subjected to analysis we would have expected a higher proportion of myosin and a lower proportion of actin to be detected.

D.F. Lewis: To what factors do you attribute the difference in structural behaviour between your 24 h and 72 h post-mortem meat? In particular did you notice a pH change or any signs of autolysis in the unsoaked meats?

Authors: In our observations the differences in structural behaviour between 24 h and 72 h post-mortem meat are linked with the pH of the incubating medium. As shown in Table 3 total extraction of A-band material occurs only in 72 h post-mortem meat at pH 5.5 whereas this effect is observed in both 24 h and 72 h post-mortem meat at pH 8.0. We have already discussed the possible role of cathepsins and CAF in the changes described but further observations need to be made in order to obtain a fuller understanding of the mechanisms involved.

We did not observe any substantial signs of autolysis in the unsoaked meats and we do not have any data on changes in pH in the brine or the meat.

D.F. Lewis: Lewis and Jewell (1975) described work with constant ionic strength systems but varying the proportions of polyphosphate and chloride. In your work the total ionic strength of each system is different. What effect do you think this has on the meat structure?

Authors: It has long been known (Bendall, 1954; Hamm, 1960) that both polyphosphate and chloride ions have specific effects. In such a system the total ionic strength is of lesser importance than the individual concentration of these ions.

From the pH values of pyrophosphoric acid at high ionic strength (van den Oord and Wesdorp, 1978) we can calculate that for the medium with pyrophosphate at pH 5.5 the total chloride concentration was 0.62M and the ionic strength \( \mu = 0.68 \). For the medium with pyrophosphate at pH 8.0 the corresponding values were 0.60 M and 0.71, and for the medium without pyrophosphate, 0.60 M and 0.61. Therefore, in any case, the difference in chloride concentration and ionic strength between these media was small.


D.F. Lewis: Lewis and Jewell (1975) described work with animals of different ages (from 18 to 24 weeks) of different breed and sex and at post-mortem storage times of 24 and 48 h. Their main finding was that after heating meat soaked in salt and polyphosphate, the region of the sarcomere that was least dispersed was the region where the A and I band overlap. They noted some variability in the behaviour of the M-lines between different samples. As a result of your studies of meat of a "well defined age", which factors do you consider contribute most to the variation in M-line behaviour?

Authors: This question raises a number of issues. First, our phrase "well defined age" refers to the post-mortem history of the meat rather than the physiological age of the animal. Second, we also observed variations in the behaviour of M-lines in our samples from a single animal. In order, therefore, to determine whether this variation depends on age, sex or breed we would need to examine many more samples.

D.F. Lewis: Regarding your analysis of the incubating media, a) does the precipitated protein in the brine contribute to the protein level measured by the biuret method or is only soluble protein measured? b) do the calculations on theoretical chloride levels include any allowance for chloride added as hydrochloric acid to adjust the pH? c) is chloride bound to the precipitated protein in the brine included in the measured chloride value?

Authors: In removing aliquots for measuring protein levels the incubating medium was well mixed. It is therefore to be assumed that any precipitated protein or the chloride in the sample analysed will contribute to the measured protein concentration.

With regard to the chloride levels expected for complete equilibration, the additional chloride contributed by the hydrochloric acid required for neutralising the pyrophosphate is, as mentioned above, very small (0.012 M for the pH 5.5 medium, 0.001 M for the pH 8.0 medium). Thus the calculated chloride concentration of 0.48 M expected for complete equilibration with the medium lacking pyrophosphate would be essentially unchanged with the pyrophosphate-containing medium. In this medium the chloride concentration would be 0.49 M at pH 5.5 and 0.48 M at pH 8.0.
SOME FACTORS INFLUENCING ANTE-MORTEM CHANGES IN MUSCLE:
A BRIEF REVIEW

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Abstract

Ante-mortem changes to muscle microstructure are influenced in many ways. During pre-natal cell differentiation and growth, myoblasts develop and mature into cells with quite different characteristics. Incorporated into the genome of these cells is the ability to synthesize a wide variety of filaments which occupy specific niches within each myofibril. During post-natal development, depending upon the particular precursor cell lines, different fiber types are produced. These are especially important in contributing to the ultimate palatability of meat. In this paper several factors which influence ante-mortem changes to muscle microstructure are discussed. While some of these are better understood than others, all of them, nevertheless, are important.

Initial paper received January 21 1984.
Final manuscript received November 18 1984.
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Key Words: Growth, muscle cells, intermediate fibers, enzymes, fiber types.

Differentiation and Growth of Muscle Cells

In early stages of prenatal growth, when tissues are differentiating, presumptive myoblasts may undergo a "proliferative" cell cycle, yielding two replicating myoblasts, or may undergo a "quantal" cell cycle which yields one or two postmitotic myoblasts (Holzer et al., 1973). In tissue differentiation, mitosis is the primary mechanism of cell proliferation, and two types of mitosis can occur (see Fig. 1). The first type is proliferative mitosis where presumptive myoblasts replicate. The second type is quantal mitosis where postmitotic myoblasts are formed; i.e., daughter cells may be different from parent cells and cell (tissue) differentiation is initiated.

During early prenatal development, when hyperplasia occurs, mesenchyme cells which are relatively free of thick and thin filaments (Fig. 2), undergo proliferative mitosis. They can also undergo quantal mitosis to form either fibroblasts, the primitive cells of connective tissue, or myoblasts, which are characterized as being mononucleated, but still lacking striations from the precursors of thick and thin filaments (Fig. 3) or, for that matter, striations from the precursors of several classes of intermediate filaments (keratin, desmin, vimentin, etc.) described by Lawson (1983).

Fig. 1. Differentiation of muscle cells.
Following quantal mitosis, myoblasts interact with one another and fuse to become myotubes (Fig. 4) which ultimately develop into myofibrils and then muscle (Ishikawa et al., 1968). Shortly after undergoing quantal mitosis, the mononucleated myoblasts have synthesized enough myosin, actin and tropomyosin for these proteins to be detected in thick and thin filaments (Holzer et al., 1973).

According to Fischman (1967), the cytoplasmic fusion of mononucleated myoblasts leads to multinucleated skeletal muscle fibers. The fusion is mediated by the Ca$^{2+}$ binding protein, calmodulin, which regulates cyclic nucleotide metabolism, protein phosphorylation, microtubule assembly, cell motility, and Ca$^{2+}$ flux across membranes (Bar-Sagi and Prives, 1983). Cells which eventually become myotubes become segregated from surrounding mesenchymal or connective tissue compartments by the acquisition of external lamellae which envelop groups of presumptive muscle cells (Kelly, 1969).

In early myotube development, thick and thin filaments begin to align, then the nuclei from fused myoblasts begin moving to the periphery of the muscle cell (Fig. 5). At this point the entire structure is considered a muscle fiber.

**Differentiation of Myofibrillar Filaments**

Development of myofibrils precedes formation of the sarcoplasmic reticulum and T-system (Fischman, 1967); however, during myofibrillogenesis two groups of filaments can be found (Kelly, 1968). One is a group of 100 Å diameter filaments with no orientation, the other is a group of 50 Å diameter filaments which aggregate in parallel skeins along the cell membrane. In more mature cells, 130-150 Å filaments, which are probably myosin, can be seen.

Gomer and Lazarides (1983) found that when myoblasts fuse to form myotubes, several proteins including α-actinin, desmin, light and heavy chain myosin, troponin and troponin-T begin to be synthesized. They also found an association of filamin...
with the Z line and that the periphery of the Z line also contains actin and intermediate filament proteins such as desmin, vimentin and synemin. α-actinin and another intermediate filament protein—vinculin—are two actin-related proteins which Auvur et al. (1983) found to be located close to the area of microfilament-membrane association.

Several intracellular proteins with apparent organizational roles have been identified and are currently under study. Some of these include (1) fibronectin (Gardner and Fambrough, 1983) which is necessary for the attachment of myoblasts to collagen; (2) C-protein (Offer, 1973) which may be responsible for bridging thick filaments and for myosin regulation; (3) epinemin (Lawson, 1983) which is associated with vimentin filaments in non-neural cells; (4) vinculin (Geiger et al., 1980) which makes up the cortical lattice in skeletal muscle; (5) vimentin (Bennett et al., 1978; Granger and Lazarides, 1978, 1979) which, with desmin, interlinks adjacent myofibrils at the Z line; (6) α-, β- and ε-actinins (Goll et al., 1972; Maruyama, 1976; Kuroda et al., 1981) which are associated with the Z line; (7) M-protein (Masaki et al., 1968) which is a constituent of the M line; (8) synemin (Granger and Lazarides, 1980) which is associated with desmin and vimentin; (9 and 10) titin (Wang et al., 1979) and nebulin (Wang and Williamson, 1980) which are the major components of the longitudinal filaments which connect the Z lines.

The study of the antemortem synthesis of these proteins is essential for a thorough understanding of the conversion of muscle to meat.

Postnatal Development of Muscle Cells

Hypertrophy characterizes postnatal development, with muscle fibers growing by increasing in both diameter and length. The rate of size increase slows as an animal approaches maturity. Forrest et al. (1975) found the diameter of individual muscle fibers is increased as myofibrils proliferate by longitudinal splitting of larger myofibrils into smaller ones. This causes the number of myofibrils within a fiber to increase by 10–15 times during an animal's lifetime.

The length of a muscle is increased by two mechanisms; firstly, by the fusion of myogenic cells with the existing muscle fiber; and secondly, by the generation of new contractile units, the sarcomeres, which are inserted into myofibrils where the fiber ends attach to connective tissue (Moss and LeBlond, 1971).

Muscle Fiber Types

There is evidence that differentiation into diverse fiber types occurs after the formation of muscle fibers, not before (Stockdale, 1982). Some unknown process, either endogenous or exogenous, causes the embryonic muscle fiber to change its genomic programming which leads to the differentiation of individual fiber types (Toyota and Shimada, 1981).

Red fibers are usually the ones present at birth and transform into intermediate and white fibers (Rowe and Goldspink, 1969). Tomanek (1976) reported that, in postnatal development, red fibers differentiate first into white and finally into intermediate fibers. There is a gradual transition from red to white fibers as an animal matures (Dreyer et al., 1977).

Physiologically, according to Squire (1981), mammalian muscle fibers can be distinguished by whether their energy supply is glycolytic or oxidative. Glycolytic fibers are fast contracting and appear white; oxidative fibers have a slow contractile response and appear red. These slow and fast fibers are referred to as Type I and II respectively and the Type II fast fibers are subdivided into fast oxidative/glycolytic (IIA) and fast glycolytic (IIB).

Ultrastructurally, there are three types of fibers which are recognized in fast twitch muscle. They are: white fibers which have narrow Z lines and few mitochondria; intermediate fibers which have narrow Z lines and many mitochondria; and red fibers which have both wide Z lines and many mitochondria (Gauthier, 1979). Some of the characteristics of red and white fibers are summarized in Table 1.

Fig. 6 shows the three fiber types based on their NADH redox content. The red fibers stain dark, white fibers stain white and intermediate fibers stain less intensely than the red fibers. In addition to differing enzymatically, the ultrastructural differences are seen in Figs. 7, 8 and 9, which are electron micrographs of red, intermediate and white fibers, respectively. The graded concentration of mitochondria and Z line differences can also be distinguished.

### Table 1. Characteristics of Red and White Muscle Fibers

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Characteristic Details</th>
</tr>
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<tbody>
<tr>
<td>Red Fibers</td>
<td>Contains more myoglobin; Sudan black A positive (more lipids); More mitochondria; Smaller in diameter; In clumps that are surrounded by white fibers (pig); Surrounded by many capillaries; Rich in sarcoplasm; Less soluble protein; Less connective tissue; Aerobic metabolism; High oxidative enzyme activity (TCA cycle activity); Contains less glycogen; Slow contraction; Muscles: Psoas; Soleus; Trapezius; Sartorius</td>
</tr>
<tr>
<td>White Fibers</td>
<td>Contains less myoglobin; Sudan black A negative (less lipids); Fewer mitochondria; Larger in diameter; In periphery of bundle (pig); Surrounded by fewer capillaries; Less sarcoplasm; More soluble protein; More connective tissue; Anaerobic metabolism; High glycolytic enzyme activity (glycogen and glucose activity); Contains more glycogen; Twitch contraction (fast); Muscles: Longissimus; Brachioradialis; Gastrocnemius</td>
</tr>
</tbody>
</table>

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Fig. 6. Light micrograph of three fiber types (strong, weak and intermediate staining) based on NADH-tetrazolium reductase. Bar = 50 μm. Reproduced from Dubowitz, V. 1970. Differentiation of fiber types in skeletal muscle. In “The Physiology and Biochemistry of Muscle as a Food, 2.” Ed. by EJ Briskey, RG Cassens, BB Marsh. Page 90 by copyright permission from the University of Wisconsin Press.

Fig. 7. Electron micrograph of a red muscle fiber. The Z line (Z), large mitochondria with closely packed cristae (m) and a portion of paired mitochondria (arrow) can be seen. Bar = 1 μm. Reproduced from Gauthier, GF. 1970. The ultrastructure of three fiber types in mammalian muscle. In “The Physiology and Biochemistry of Muscle as a Food, 2.” Ed. by EJ Briskey, RG Cassens, BB Marsh. Page 112 by copyright permission from the University of Wisconsin Press.

Fig. 8. Electron micrograph of an intermediate muscle fiber. The intermediate fiber is similar to the red fiber in Fig. 7 except the mitochondria (m) are somewhat smaller and the Z lines are narrower. Bar = 1 μm. Reproduced from Gauthier, GF. 1970. In “The Physiology and Biochemistry of Muscle as a Food, 2.” Ed. by EJ Briskey, RG Cassens, BB Marsh. Page 111 by copyright permission from the University of Wisconsin Press.

Fig. 9. Electron micrograph of a white fiber. Note the paired mitochondria (M) and relatively thin Z lines (Z). Bar = 1 μm. Reproduced from Gauthier, GF. 1970. In “The Physiology and Biochemistry of Muscle as a Food, 2.” Page 112 by copyright permission from the University of Wisconsin Press.

Factors Influencing Muscle Mass

Since the number of fibers is the most important factor in limiting ultimate muscle size (Goldspink, 1974), and since the genetically determined number is achieved at or near the time of birth, increase in muscle mass is accomplished only by enlargement of muscle fibers (Luft and Goldspink, 1967). All muscle fibers enlarge, but particularly by the conversion of red fibers to larger white fibers.

Spindler et al. (1980) reported that as cattle aged, mean cross sectional area of fibers doubled overall, while the cross sectional area of white fibers increased slightly, and red fibers decreased slightly. Ashmore et al. (1972) evaluated growth patterns in piglet, lamb and calf muscles histochemically and concluded that transformation of intermediate fibers to white fibers increased at the expense of red fibers without alteration in the total number of fibers.

Reducing food intake leads to a reduction in muscle mass, since starvation causes a decrease in fiber size and, at the same time leads to a reduction in the number of myofibrils (Van Linge, 1962). Decrease in muscle bulk is directly related to a decrease in mean fiber diameter in cattle (Robertson and Baker, 1933) and in pigs (McMeekan, 1940).

Biochemical control of protein metabolism in relation to starvation is not completely understood; however, it appears that
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not only is the rate of protein degradation increased during starvation, but the rate of synthesis of new protein is decreased as well (Young, 1970). This may result from a shortage of one or more essential amino acids in the muscle fibers or it may be due to a reduction in the protein synthesizing capacity of polyribosomes.

Moody et al. (1980) postulated that the nature of the available source of energy in lamb rations caused a shift from intermediate to white muscle fibers. As the protein content of rations increased, percentages of intermediate and white fibers decreased quadratically (Johnston et al., 1975).

In general, there is no difference in the total number of muscle fibers in the same anatomical muscles of males and females; however, even though fiber size is greater in males, Brooke (1970) reported fiber type percentage difference is similar for both sexes. But, he found white fibers are smaller than red fibers in females and larger in males. On the other hand, Dreyer et al. (1977) reported that bulls had a higher percentage of red fibers and a lower percentage of white fibers than steers. This contrasts with results obtained by Bass et al. (1971) who found that significant growth occurred in guinea pig temporal muscle following administration of testosterone, and, this growth was accompanied by a change from intermediate to white fibers.

Miscellaneous Factors

At the present time, selection of meat animals is based upon the appraisal of such characteristics as muscle size and growth rate (Swatland, 1973). Being blind to all the factors contributing to muscle size and growth rate, it is not surprising that intensive selective breeding can produce or reveal anomalies such as stress susceptible pigs or double muscled cattle. Stress susceptible pigs have a higher white to red fiber ratio compared to normal pigs (Didley et al., 1970) and double muscled cattle have larger and more abundant white fibers than red, compared with normal cattle (Holmes and Ashmore, 1972).

Several researchers have related fiber diameter and muscle bundle size to meat quantity. Joubert (1956) and Hegarty (1971) reported that fiber diameter increases with age in cattle. Miller et al. (1975) found the total number of muscle fibers was more closely related to muscle mass than was fiber diameter. In addition, the authors found that faster growing pigs appeared to possess more, but smaller muscle fibers than slower growing pigs. Castle and Gregory (1929), Smith (1963) and Staun (1963) found just the opposite to be the case. They reported that animals possessing large muscle fibers are often rapidly growing and muscular.

Calkins et al. (1981) related palatability to fiber type and reported that the presence of white fibers correlated poorly with marbling and tenderness ratings; however, the presence of red fibers correlated well. The authors concluded that, since the oxidative capacity of a muscle is related to marbling and tenderness, fiber type could possibly be used to predict both marbling and tenderness.

Conclusion

Ante-mortem changes in muscle obviously affect the ultimate palatability of meat; however, it is not just one event occurring prior to slaughter which determines how tough or tender meat will be. Many factors have to be considered as being potentially responsible for determining ultimate acceptability. The variations in a given muscle between individual animals, sexes and species exacerbate the problem. There are just so many factors, a solution awaits further research.

References


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PRELIMINARY EVALUATION OF LECTINS AS FLUORESCENT PROBES OF SEED STRUCTURE AND COMPOSITION

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Abstract

Several commercially available fluorescein isothiocyanate and rhodamine isothiocyanate-conjugated plant lectins have been applied to cereal and oilseed tissues to permit identification and localization of specific structures and carbohydrates by fluorescence microscopy. Ulex europeaus Agglutinin I (UEA I) and Ricinus communis Agglutinin I (RCA I) showed specificity for the amyloids in rapeseed cotyledonary cell walls. Wheat Germ Agglutinin (WGA) bound to rapeseed coat mucilage, as well as fungal hyphae in infected wheat. Lens culinaris Agglutinin (LCA) bound only to starch in cereal sections, and at higher magnifications of isolated starch granules, the annular structure was clearly visible.

Introduction

Lectins are sugar-binding proteins or glycoproteins of non-immune origin which agglutinate cells and/or precipitate glycoconjugates (Goldstein et al. 1980). Although most lectins have been isolated from plants, they have also been found in bacteria, fungi, lichens, invertebrates, and vertebrates. The carbohydrate specificity of lectins is usually directed to a single monosaccharide or structurally related monosaccharides, and lectins may bind to their complementary monosaccharides whether they occur as free sugars, or as terminal groups on various types of glycoconjugates (Clarke and Hoggart 1982). Interest is increasing in the use of lectins as microscopic probes to investigate the nature and distribution of carbohydrate-containing components of both plant and animal tissues and cells (for examples see Rougier et al. 1979, Vermeer and McCully 1981, Pena et al. 1981, Holthofer et al. 1981, Baldo et al. 1982 a,b,c, and Sato and Spicer 1982).

The chemical nature of polysaccharides is often difficult to determine in situ. Solvent extraction frequently yields mixtures which require complex purification procedures (for example, see Siddiqui and Wood 1977b), and often yields little information about the original location of the extracted components within the tissues. The availability of purified lectins provides a potential tool for identifying and determining where carbohydrates and/or carbohydrate-containing components are situated within tissues and cells. In addition, the specificity of lectins can extend beyond sugar residues, to include the glycosyl linkages in an oligosaccharide. For example, Lens culinaris agglutinin is specific for α-glucosyl groups, while Ricinus communis agglutinin is specific for α-galactosyl groups.

Commercially available labelled lectins include several, which are useful in bright field, fluorescence, and electron microscopy (EM). We have chosen fluorescent-labelled lectins in our study because of the sensitivity of fluorescence microscopy as compared to bright-field methods,

Key Words: Lectins, fluorescence microscopy, seed structure, cell walls, carbohydrates, amyloid, mucilage.
and the ease of preparation and examination of samples as compared to EM. The range of lectin specificities is quite broad (Clarke and Hoggart 1982) and their availability is increasing. As such, it may become possible to select lectins with specific affinities to detect and localize components of particular interest in raw materials, and follow the fate of these components as materials are processed and consumed. This approach could aid in evaluation of the availability of carbohydrates in processed materials from a nutritional standpoint. The use of lectins in routine laboratory examination of foods is not practical due to their cost, but their potential as probes in fundamental investigations of carbohydrates in food materials, both raw and processed, is considerable. This preliminary examination of various lectins demonstrates clear structural specificities, for which the underlying chemical specificities are proposed.

Materials and Methods

Samples

Mature seeds of Brassica campestris L. cultivars Echo, Candle, and Sarson R500 were used to examine lectins with binding specificities for rapeseed. Mature kernels of oats (Avena sativa L. cv. Hinoat) and wheat (Triticum aestivum L. cv. Fredrick) were used for investigation of cereal starch-binding lectins. Grains of Triticum cv. Concord were naturally contaminated with the head blight fungus Fusarium graminearum Schwabe were also used to assess the affinity of various lectins for fungal hyphae in cereals. Although the level of Fusarium infection varied from kernel to kernel in the sample tested, on average the level of contamination within the sample was very high, as indicated by the unusually high levels (7 ppm) of the fungal metabolite deoxynivalenol (vomitoxin). Infected material was used in a milling and baking study described earlier (Young et al. 1984).

Sectioning

Frozen Sections. Whole, unfixed rapeseeds were soaked in cold water for at least 30 minutes, mounted in Tissue Tek II O.C.T. Compound (Lab Tek Products, Miles Laboratories Inc., Naperville, Illinois), and frozen on dry ice-cooled mounting blocks. Frozen sections 10 µm thick were cut using steel knives in a cryostat set at -20°C and mounted on glass slides pre-coated with Albumin Fixative (Fisher Scientific Company, Fairlawn, New Jersey).

Glycol Methacrylate (GMA) Sections. For higher resolution, samples were fixed and embedded in glycol methacrylate plastic as described by Fulcher and Wong (1980). Briefly: samples were fixed in 6% glutaraldehyde in 0.025 M Na phosphate buffer pH 7.0 at 4°C for 72 hours. Samples were dehydrated sequentially in methyl cellosolve, ethanol, n-propanol, and n-butanol, infiltrated with GMA monomer (Feder and O'Brien 1968) for 72 hours, and polymerized at 60°C (overnight) or at room temperature under U.V. light (3 to 4 days). Sections 2-4 µm thick were cut using glass knives and mounted on slides.

Lectins

Purified lectins labelled with fluorescein isothiocyanate or rhodamine isothiocyanate were obtained from Cedarlane Laboratories (Hornby, Ontario). The following lectins were tested: Wheat Germ Agglutinin (WGA), with a reported specificity for N-acetylgalcosamine and its oligosaccharides, Lens culinaris Agglutinin (LCA), with reported specificities for α-D-glucosyl groups, Ulex europaeus Agglutinin I (UEA I) which is specific for L-fucosyl groups, and Ricinus communis Agglutinin I (RCA I), with a reported specificity for α-D-galactosyl groups (Clarke and Hoggart 1982).

For staining, sections were incubated with 1.0 mg/ml of lectin in 10 mM HEPES buffer containing 0.15 M NaCl and 0.04% sodium azide at pH 6.1-8.5 (depending on the lectin used, as recommended by the supplier) for 30 minutes in a moist chamber. Sections were then washed twice for 15 minutes each in a solution containing 0.001 M each of Ca2+, Mg2+, and Mn2+ (Baldo et al. 1982a) at pH 7.0 and monitored microscopically to ensure that all excess lectin had been removed. Slides were air-dried and mounted in non-fluorescent immersion oil for microscopic examination. Some sections were counter-stained after lectin binding for 5 minutes in Fast Green (0.01% in 5% acetic acid, pH 2.5) or Evans Blue (0.01% in distilled water, pH 7.0).

Tests for inhibition of binding of UEA I and RCA I were performed by adding L-fucose, D-galactose, or D-glucose (to 0.2 M) to the lectin solution and pre-incubating for 30 minutes before applying to sections.

Microscopic Examination

Sections were examined using a Zeiss Universal Research Microscope (Carl Zeiss Canada Ltd.) equipped with a III RS epi-illuminating condenser for fluorescence analysis. The condenser contained fluorescence filter combinations (FC's) with dichromatic beam splitters, and exciter/barrier filters with maximum transmission at 450-490 nm/520 nm (FC I) for examination of fluorescein-labelled material, and 546 nm/590 nm (FC II) for examination of rhodamine-labelled material (Fulcher and Wong 1980). Photomicrographs were obtained using 35 mm Kodak Tri-X pan film, ASA 400.

Results and Discussion

Of the lectins investigated, several exhibit specificities for carbohydrate-containing components, making them potentially useful for identifying and locating particular components in foods. Preliminary studies on rapeseed and cereal grains are presented here as examples of the possible uses of lectins in determining the distribution of specific carbohydrates in raw materials.

Rapeseed cotyledonal cell walls have been described previously using iodine/potassium iodide...
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and the fluorochromes Calcofluor White M2R New and Congo Red (Yiu et al. 1983). While these reagents and dyes have proved to be useful in the examination of whole rapeseed and its products, their specificity is somewhat limited. Iodine/potassium iodide, often used to localize amyloid (Koornman 1957, Johansen 1940) which is the carbohydrate found in rapeseed cotyledonary cell walls, also stains a number of other polysaccharides, including starch, glycogen, hydrolyzed cellulose, and chitin (Johansen 1940). Congo Red has also been shown to have an affinity for mixed-linkage β-glucans in cereal cell walls (Wood and Fulcher 1978, Fulcher and Wong 1980, Fulcher et al. 1984), but in rapeseed the dye binds to the seed coat mucilage as well as to the cotyledonary cell walls. Calcofluor is similar to Congo Red in its staining affinities, and binds to a wide range of materials in addition to rapeseed cell walls, including cellulose, chitin, and a variety of other β-linked polymers (Maeda and Ishida 1967, Hughes and McCulley 1975, Fulcher and Wong 1980, Wood et al. 1983).

Of the lectins tested, UEA I and RCA I, with reported specificities for L-fucose and D-galactose respectively (Clarke and Hoggart 1982), showed significant specific binding to rapeseed cotyledonary cell walls. (Figs. 1, 2, 3). In thin GMA sections (Fig. 1), binding of UEA I occurred only in the cotyledonary cell walls, and distribution of the fluorescent lectin was not uniform throughout the cell wall. Whether this was due to incomplete binding of the lectin (perhaps due to interference by the GMA embedding), or to variation within the cell wall itself, has not yet been determined. More uniform distribution of fluorescence was observed in the thicker, unembedded cryostat sections treated with either RCA I (Fig. 2) or UEA I (Fig. 3). No other cell walls within the seeds had any affinity for these lectins, suggesting that fucosamyl and amylody are both confined to cotyledonary cell walls.

L-fucose and D-galactose occur as terminal residues in fucosamyl and amylody isolated from rapeseed meal (Siddiqui and Wood 1971, 1977a, 1977b). The suggestion that UEA I was in fact binding primarily to the fucosamyl is supported by the significant decrease in lectin binding to the cell wall after the lectin was pre-incubated with L-fucose for 30 minutes prior to staining (Fig. 4). No such decrease was observed when the lectin was preincubated with D-glucose or D-galactose. In comparison, Fig. 3 shows the very intense fluorescence observed when pre-incubation of the UEA I lectin with L-fucose is omitted. Similar inhibition studies on RCA I show loss of binding ability when the lectin was pre-incubated with D-galactose while cell wall fluorescence was still visible when pre-incubated with D-glucose or L-fucose.

Mucilage, a carbohydrate of some rapeseed and most mustard seed coats or hulls (Weber et al. 1974, Vose 1974, Yiu et al. 1982, Van Caeseele and Mills 1983) exhibits a different lectin affinity than the cotyledonary cell walls. When applied to rapeseed or mustard sections, WGA, which has a reported specificity for N-acetylglucosamine (Clarke and Hoggart 1982), showed a pronounced affinity for the seed coat mucilage, with no other binding observed, as shown in Fig. 5. Mucilage is an acidic polysaccharide consisting primarily of cellulose and uronic acids, notably galacturonic acid (Weber et al. 1974, Vose 1974), as well as glucose, arabinose and xylose. Galactose and an uncharacterized glycoprotein have also been detected (Aspinall 1976, Aspinall and Krishnamurthy 1976). Whether this protein is in fact present in the mucilage, and whether it contains N-acetylglucosamine, a common saccharide in plant glycoproteins (Sharon 1974), remains to be determined.

When applied to sections of cereal grains, LCA stained only the starch granules, as shown in the oat section in Figure 6. This marked specificity of LCA for starch suggests that it may provide a potentially useful probe into the nature and distribution of starches in processed (especially cooked) foods, where it can no longer be identified on the basis of morphology or birefringence.

The internal structure of starch has been examined frequently using both light and electron microscopy (Wivinis and Maywald 1967, Moss 1976, Gallant and Sterling 1976, Hood and Liboff 1983). Although the superior resolution of electron microscopy provides a very detailed structural picture of the starch granule, extensive pretreatments are usually used to visualize "growth rings", including enzyme or acid hydrolysis, oxidation with periodic acid, and/or treatment with thiourea-carbazide (Gallant and Sterling 1976, Evers 1979, Hood and Liboff 1983). In contrast, the binding of fluorescein-conjugated LCA to starch is a simple, fairly rapid procedure - clearly shows the annular organization, as shown in the large wheat starch A granules in Fig. 7. The smaller B granules also show intense staining of a central core, but do not exhibit the ring structure with this method. An envelope or halo is also visible around many of the granules. The chemical nature of this envelope is as yet unclear, and may in part be due to refraction artefacts at the granule surface. Because the two major components of starch, amylose and amylopectin, both contain α-D-glucosyl chains, one of the reported specificities of LCA (Clarke and Hoggart 1982), it is likely that both starch polymers are capable of binding the lectin to some extent. Possible differences in the LCA binding specificities of the two polysaccharides remain to be determined, but the pronounced affinity of LCA for starch emphasizes its potential utility in examining the distribution and fine structure of starch in both raw and processed materials.

Fungal infection is a common problem in food systems, especially where raw materials such as grains or oilseeds are stored in bulk for extended periods of time. Determination of fungal infection is usually accomplished using isolation and cultural techniques, or by assay of fungal metabolites such as chitin (Tuite and Foster 1979) or ergosterol and deoxynivalenol (Young et al. 1984). These techniques can, however be quite expensive and time consuming, and often yield little information about the actual extent of fungal penetration into the material.
Similarly, low levels of fungal penetration of a grain are also frequently difficult to detect microscopically although this problem has recently been addressed in rapeseed by Schans et al. (1982) and Van Caeseele and Mills (1983) using the fluorochrome acridine orange to visualize fungal hyphae with the fluorescence microscope. Although not exclusively specific for fungal hyphae, the high sensitivity of the fluorochrome offers improved detectability. In comparison, WGA showed specificity only for fungal hyphae in Fusarium-infected wheat tissues (Figs. 8, 9, 10). In Fig. 8, the fluorescein-labelled hyphae are visible within the pericarp, and in a packed mass between the two cuticular layers of the testa in the early stages of infection. In this case, the infection appears to have been superficial, involving only the outer layer of the grain. In Fig. 9, infection has progressed into the subaleurone region of the grain, and individual hyphae are also readily detected using WGA as a fluorescent probe. In this case, the hyphae have penetrated into individual starch granules, presumably aided by the high amylolytic activity in this particular kernel which was selected from a sample of partially sprouted (germinated) grains. In Fig. 10 however, there was no initial indication of sprouting and the WGA-labelled hyphae are clearly visible in the central endosperm as they have invaded the storage protein matrix between the starch granules.

N-acetylglucosamine, one of the reported specificities of WGA (Clarke and Hoggart 1982), is a common cell wall polysaccharide of many fungi (Sturgeon 1974) and the affinity of WGA for fungal hyphae has been demonstrated by Mirelman et al. (1975). The utility of WGA in detection of cereal-contaminating organisms is clearly demonstrated in Figs. 9-10. The binding is quite specific, very sensitive, and obviously capable of detecting individual hyphae. N-acetylglucosamine-binding lectins may also prove useful in examining fundamental processes of food contamination and perhaps in assessing related processes such as mold-ripening of cheeses.

Although the cost of lectins renders them impractical for routine (e.g., quality control) analyses, the results presented here are but a few selected examples of their many potential applications in fundamental investigations of the distribution of carbohydrates in food materials. Use of these probes should lead to a greater understanding of the relationships between composition, structure and function in cells and tissues, and they should also allow monitoring of changes in morphology and distribution of carbohydrates during processing of raw materials. As fluorescent probes, lectins are both highly sensitive and specific markers which should find many future applications in food science.

Figure Captions

All figures are fluorescence micrographs. With the exception of Figures 3 and 4, all were stained with fluorescein-labelled lectins, and were photographed using FC I. Figures 3 and 4 were stained with rhodamine-labelled lectin, and were photographed using FC II. Scale bars in mm.

Figure 1. UEA I treated GMA section of Sarson rapeseed showing fluorescent staining of cotyledon cell walls (arrow). Autofluorescent protein bodies (p) are visible inside the cells.

Figure 2. RCA I treated cryostat section of Echo rapeseed showing fluorescent staining of cotyledon cell walls.

Figures 3 and 4 illustrate the inhibition of UEA I binding by L-fucose. When the lectin was pre-incubated for 30 minutes with 0.2 M L-fucose before applying to the section, significantly less lectin was bound (Fig. 4) than when lectin alone was applied to the section (Fig. 3). Both figures are cryostat sections of Echo rapeseed.

Figure 5. WGA-treated cryostat section of Candle rapeseed, counterstained with Fast Green, showing fluorescent lectin binding to the seed coat mucilage (arrow). No specific fluorescence is visible in the cotyledon (C).

Figure 6. LCA-treated section of oat showing lectin binding to starch granules (S). No fluorescence is visible in the embryo (E) or in the endosperm matrix surrounding each compound starch granule.

Figure 7. LCA-treated GMA section of starch granules from wheat showing growth rings (arrowhead) in the large type A granules. Lectin binding also occurs in the smaller type B granules (arrow) but no growth rings are visible. "Halos" are discernible among both types of granules.

Figure 8. WGA binding to fungal hyphae penetrating the pericarp (arrow) and packed between the cuticular layers of the testa (arrowhead) of wheat. Fluorescence in the aleurone cells (A) is auto-fluorescence, and is not due to lectin binding.

Figure 9. GMA section of sprouted wheat showing the fungal hyphae (arrow), after WGA treatment, penetrating the non-fluorescent starch granule (arrowhead). Auto-fluorescence is visible in the aleurone layer (A).

Figure 10. WGA binding to fungal hyphae (arrows) between starch granules (S) in the starchy endosperm of wheat. Individual hyphae are readily distinguishable.
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Acknowledgments

The patience and technical assistance of Mr. F. Wong are gratefully acknowledged. This research was supported in part by the Council of Canada CUP grant no 854.

References


Lectins as probes of seed structure

F.W. Sosulski:

Do well retinal small starch granules as well intensely as with large starch granules? Would there be differences in LCA binding reflect variations in molecular packing within the granule?

Authors: We cannot comment on differences in intensity, assay methods mean these are not available. We can only note differences in staining patterns.

J.M. Faubion:

Do you think it is possible to label with two lectins simultaneously, fluorescein-labeled WGA and rhodamine-labeled LCA for example?

Authors: Simultaneous staining should be possible, providing the appropriate lectins are different.

J.M. Faubion:

Have you examined any waxy or high amylose cereals for the interaction of their starches with LCA as well?

Authors: Yes...

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*Available from Canola Council of Canada, 301-433 Main Stree, Winnipeg, Manitoba, Canada R3B 1B3
Acknowledgements

The patience and expert technical assistance of Mr. F. Wong are gratefully acknowledged. This research was supported in part by Canola Council of Canada CUAP grant no. 83-42.

References


Lectins as probes of seed structure


*Available from Canola Council of Canada, 301-433 Main Street, Winnipeg, Manitoba, Canada R3B 1B3

Discussion With Reviewers

F.W. Sosulski: Does LCA react with small starch granules as intensely as with the large starch granules? Would these differences in LCA binding reflect variations in molecular packing within the granule?

Authors: We cannot comment on differences in intensity, as methods to measure them are not available. We can only note differences in staining patterns.

F.W. Sosulski: The authors emphasize the potential for using LCA affinity as a tool in the investigation of the structure of starch in processed foods. Could the authors comment on the nature of LCA binding with gelatinized or retrograded starch?

Authors: We have not yet examined processed starches except in noting that gelatinized starches appear to retain an affinity for the appropriate lectin.

J.M. Faubion: Do you think it feasible to label with two lectins simultaneously, fluorescein-labelled WGA and rhodamine-labelled LCA for example?

Authors: Simultaneous labelling should be possible, providing the affinities of the two lectins are different.

J.M. Faubion: Have you examined any waxy or high amylose cereals for the interaction of their starches with LCA?

Authors: Yes. LCA binds to these starches as well.
MORPHOLOGICAL DEVELOPMENT IN SORGHUM GRAIN

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¹Sorghum Beer Unit, ²National Food Research Institute, CSIR, P O Box 395, PRETORIA, South Africa, 0001.
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Abstract

Immature sorghum grain was harvested at various stages of maturity and its development followed by transmission and scanning electron microscopy. This was done to study the developmental morphology of the sorghum grain. The period immediately following fertilization is a time of rapid development in the sorghum caryopsis. The endosperm expands crushing the nucellus and in the nonbird-resistant sorghums the inner integument is also crushed during this expansion. The cells of the ovary wall expand and elongate to form the pericarp. By the soft dough stage the endosperm has gained most of its storage material and thereafter there is a considerable loss of moisture. During the early stages of development the endosperm cell walls were extensively pitted which could allow for translocation. However, once the period of translocation was over the cell walls became intact.

Introduction

Sorghum (Sorghum bicolor (L.) Moench) is the major cereal grain consumed in the semi-arid tropics. In Africa, sorghum is germinated and used as malt in the brewing of a traditional beer. This brewing is now carried out on an industrial scale. To improve the final product it is important to understand the initial ingredients. Sorghum differs somewhat from barley so it is important to study the structural changes which occur during sorghum malting. Because of this and other uses of sorghum for food, an understanding of the whole grain structure is important. To further aid in an understanding of this structure, this study of sorghum's developmental morphology was undertaken.

To describe the morphology of the sorghum kernel, a standard terminology is now widely recognized and will be used here (Dogget, 1970; Morrall et al., 1981; Earp and Rooney, 1982). The main parts of the caryopsis are the embryo with its scutellum which together make up the germ, the endosperm which is a storage tissue and the pericarp which encases the kernel.

Most of the studies reported so far on sorghum grain structure have been concerned with the grain as regards its utilization (Hoseney et al., 1974; Sullins and Rooney, 1975). Insufficient information is available in the literature on the development of immature sorghum to be meaningful. Although limited work has been reported (Subramanyam et al., 1980 a & b) this study was undertaken to show the origin and development of some of the more important structures as relates to changes in the kernel during malting. As the embryology of sorghum grain has already been covered (Artschwager and McGuire, 1949) the present study does not cover embryo development.

KEY WORDS: Sorghum bicolor, grain developmental morphology, endosperm development, pericarp development, cell wall.
Materials and Methods

A non-bird-resistant sorghum variety, NK 283, was grown by the Plant and Seed Control Division, Roodplaat Experimental Farm, South African Department of Agriculture. The different stages of maturity agreed with those described by Vanderlip and Reeves (1972) and samples were collected at the intervals listed in Table 1.

Table 1. Stages of development and times of harvest of immature NK 283 sorghum grain (1982/83 crop)

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Days post-anthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-fertilization</td>
<td>7</td>
</tr>
<tr>
<td>Milk stage</td>
<td>14</td>
</tr>
<tr>
<td>Soft dough</td>
<td>21</td>
</tr>
<tr>
<td>Hard dough</td>
<td>28</td>
</tr>
<tr>
<td>Physiological ripe</td>
<td>35</td>
</tr>
<tr>
<td>Time of harvest</td>
<td>63</td>
</tr>
</tbody>
</table>

The panicles were collected when the kernels in the middle of the panicle had reached the correct stage of maturity. Kernels were taken from the middle of these panicles and fixed within an hour of harvest.

Specimens were prepared for scanning electron microscopy by cutting or fracturing through the middle of the kernel and then fixing in 5% glutaraldehyde buffered to pH 7.4 at 4°C. The samples were then dehydrated through an ethanol series and critical-point dried from amyl acetate- CO2. Samples were mounted on metal stubs with double-sided cello­ tape and sputter coated with gold-palladium. They were then examined with a JEOL JSM 135 Scanning Electron Microscope with an accelerating voltage of 20 kV. Material for transmission electron microscopy was prepared as previously described (Glennie et al., 1983). Approximately six to eight separate samples were examined for each developmental stage.

Total polyphenols were determined using a modified Jerumanis method (Dabber, 1975). Starch was determined by enzymic hydrolysis and measured as glucose by the glucose oxidase method (Fleming and Pegler, 1963).

Results and Discussion

Post-anthesis in sorghum was found to be a period of rapid development. Developmental stages will be used when discussing the age of the caryopsis. The age in days is presented in Table 1 but age and developmental stage do not always coincide, depending on season and geographic location.

Post-anthesis was the first stage where the endosperm could be observed expanding into the nucellus (Figure 1a). At this stage the immature sorghum endosperm had a more rigid cellular structure than the coencticy endosperm of the developing wheat caryopsis (Bechtel et al., 1982). The collapse of the sorghum nucellus was probably caused by sample preparation for scanning electron microscopy. When similar samples were prepared for transmission electron microscopy by embedding, this collapse was not observed. It is probable that the nucellus cells had insufficient contents and insufficiently strong cell walls to withstand preparation for EM without embedding. In contrast to Figure 1a, grain at the hard dough stage appeared reasonably mature (Figure 1b). The glumes no longer remained attached, the ovary wall had developed into a pericarp, the endosperm had completely crushed the nucellus and the germ was well developed.

The ovary wall of the immature kernel developed into the pericarp. This development took the form of cell enlargement rather than cell division (Sanders, 1955). Seven days after anthesis the ovary wall was composed of fairly regular almost rectangular cells (Figure 2a). The cell walls of these cells appeared to be regularly pitted. Since these cells develop into a pericarp by expansion it is possible that these pits could be areas of cell wall expansion. A common feature of these cells was plastids containing small, presumably immature, starch granules (Figure 2b). In this respect, sorghum is similar to maize.

As the endosperm expanded, the cells in the pericarp came under pressure which caused them to elongate. Fourteen days after anthesis, at the milk stage, the pericarp cells had both elongated and filled with starch (Figure 3). Although the starch granules in Figure 3 were much larger than those in Figure 2a those starch granules found in the pericarp were smaller than those found in the endosperm. The starch granules in the pericarp are probably fully developed at the milk stage as the cytoplasm of the pericarp cells starts to degenerate about 14 days after anthesis.

There appears to be some confusion in the literature about what is the testa in sorghum grain. Definitions vary from the seed coat which adheres to the outer edges of the inner integument (Hoseney et al., 1981) to that layer whose presence is controlled by the B1 and B2 genes (Rooney et al., 1979). In the work described here, reference will be made to the polyphenol containing layer which can confer bird-resistance on the grain and is developed from the inner integument (Morrall et al., 1981). The polyphenol containing layer is usually absent in non-bird-resistant sorghums, the inner integument simply being crushed by the expanding endosperm. The micrographs in Figure 4 show the sequential collapse of the inner integument in a non-bird-resistant sorghum.

The inner integument, which at the time of anthesis surrounded the nucellus, was two cells thick (Figure 4a). The cells were full of cytoplasm and there appeared to be no lateral stretching of the cells. The inner integument was separated from the nucellus by a layer of cutin. Sanders (1955) referred to a cutin layer covering the inner integument but in this study as well as another (Morrall et al., 1981), a cutin layer was found between the integument and...
Morphological Development in Sorghum Grain

Fig. 2. Sorghum pericarp at different stages of development.
a) Scanning electron micrography of ovary wall at anthesis. Bar = 100 μm.
b) Transmission electron micrograph of plastid in mesocarp cell at post-fertilization. SG = starch granule; arrow = thylakoid. Bar = 0.5 μm.

Fig. 1. Scanning electron micrographs of sorghum grain at different stages of development.
a) Developing endosperm (arrow) in ovary at post-fertilization. Bar = 100 μm.
b) Grain at hard dough stage. Bar = 1,000 μm. E = endosperm; Em = embryo; FE = floury endosperm; HE = horny endosperm; N = nucellus; OW = ovary wall; P = pericarp; S = scutellum.

Fig. 3. Transmission electron micrograph of the mesocarp at milk stage. Starch granules (SG) are present. Bar = 2.0 μm.
nucellus. At the milk stage it was located between the residual inner integument and the aleurone layer of the endosperm (Figure 4b).

At the milk stage the endosperm had completely crushed the nucellus and was still expanding thus causing the cells of the pericarp to elongate. The cell contents of the inner integument disappeared during this period and by the soft dough stage the endosperm had expanded sufficiently to stretch the inner integument into a narrow band (Figure 4c). The inner integument was completely crushed at the hard dough stage (Figure 4d). The next distal layer of cells (tube cells) now appeared to contain small amounts of electron dense material which could be polyphenol as well as some granules which resembled starch granules.

The polyphenol content of the grain at different stages of development is listed in Table 2. Kernels at the milk stage contained no detectable polyphenol but from this stage onward the polyphenol content increased until at the time of harvest it contained 1.50 mg/100 kernels.

Table 2. Starch and polyphenol content in sorghum grain at different stages of development reported on dry weight basis

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Starch g/100 kernels (%)</th>
<th>Polyphenol mg/100 kernels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk stage</td>
<td>0.56 (33.07)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Soft dough</td>
<td>1.38 (48.19)</td>
<td>1.02 (0.02)</td>
</tr>
<tr>
<td>Hard dough</td>
<td>1.42 (51.77)</td>
<td>1.06 (0.03)</td>
</tr>
<tr>
<td>Physiological ripe</td>
<td>2.24 (59.42)</td>
<td>2.13 (0.05)</td>
</tr>
<tr>
<td>Time of harvest</td>
<td>2.18 (70.75)</td>
<td>1.50 (0.04)</td>
</tr>
</tbody>
</table>

Even at the milk stage, a bird-resistant cultivar was found to contain considerable amounts of polyphenol (Glennie et al., 1981). Although the polyphenol content of the nonbird-resistant cultivar used in this study is low when compared to that of the bird-resistant sorghums, it could account for the electron dense areas in Figure 4d.

The development of the endosperm is one of the most important areas of grain development. The kernel gains most of its dry matter during the early stages of development while the later stages are marked mainly by a large reduction in moisture (Figure 5). As shown in Figure 5 the kernels have an early period of very rapid dry weight gain. This gain can be accounted for mainly by the deposition of starch (Table 2).

The subaleurone cells of the endosperm contain both starch granules and protein bodies. Even at the soft dough stage, starch and protein are beginning to be close-packed (Figure 6a). At the hard dough stage the starch granules are more closely packed and will soon become indented by the protein bodies (Figure 6b). At this stage the starch granules contain sufficient water to be susceptible to enzyme attack (note pits). This would probably mean that they are sufficiently plastic to be easily indented by the protein bodies.

At the hard dough stage, starch granules in the central or floury endosperm were still round and relatively soft (Figure 7a). At the physiological ripe stage, the starch granules were packed closely together and were forced to take up polygonal shapes (Figure 7b). Subramanyam et al., (1980a) proposed that the round and relatively soft starch granules are forced into polygonal shapes as water is lost from the kernel. The present study supports this as there is sufficient loss of moisture during this period to
cause the starch granules to pack closely (Figure 5). Also, starch is still being deposited (Table 2) to reinforce this close packing. Just as the immature panicle contains grains of different degrees of maturity the endosperm of a single immature kernel contains cells of varying physiological ages. It appears that there is a gradient in maturity from the peripheral area near the aleurone layer to the central floury endosperm. The peripheral cells were found to be the more mature. This supports the observation made by Sanders (1955) that the outermost layer of the endosperm was the first area to mature. A similar pattern was reported for immature Zea mays L. kernels where a variability in the starch content of the different cells was reported (Shannon, 1974). During germination, the reverse pattern was found. Endosperm modification began in the central floury endosperm adjacent to the scutellum (Glennie et al., 1983).

The cell walls of the endosperm enclose the storage products deposited there. Yet, during the developmental stages, cell walls must allow for the translocation of the precursors. During the milk stage the endosperm was in an active state of filling and the cell walls contained large numbers of pits (Figure 8a). Although translocation can easily occur through cell walls, the large number of pits shown in Figure 8a would also allow for rapid translocation. Also, the pits could represent areas of cell wall synthesis as the cell walls expanded with the expanding endosperm.

By the hard dough stage the filling of the endosperm was virtually complete and the cell walls contained only a few random pits as shown in Figure 8b. The cell walls appeared intact at the physiological ripe stage with no visible pitting (Figure 8c). At this stage the kernel had reached its maximum starch content (Table 2) and translocation had probably ceased. It appears that the more starch that was deposited in the endosperm, the fewer pits that appeared in the endosperm cell walls.

During germination it was found that the reverse pattern of cell wall pitting occurred (Glennie, 1984). The entire cell walls that were present in the mature grain were found to be extensively pitted after six days germination. Besides translocation through the cell walls, the pits would allow for translocation of storage materials out of the endosperm. Unfortunately, the micrographs from both of these studies were unable to demonstrate that the pits formed during germination were in the same location as the pits which were present during development.

Conclusions

In the immature sorghum caryopsis a period of rapid development occurred immediately following fertilization. The expanding endosperm crushed the inner integument of the nonbird-resistant sorghum. At the same time, the ovary wall developed into a pericarp. The grain had acquired most of its storage products by the soft dough stage and from this stage onwards development was marked by a considerable loss in moisture. Up to the hard dough stage the endosperm cell walls exhibited extensive pitting but when this period of translocation ceased the pits disappeared and the cell walls became intact.

References


Earp CF, Rooney LW. (1982). Scanning electron microscopy of the pericarp and testa of several sorghum varieties. Food Microstructure 1, 125-134.


Morphological Development in Sorghum Grain

Fig. 7. Scanning electron micrographs of the central floury endosperm.

a) Hard dough - starch granules are very loosely packed and debris is probably cell contents involved in starch deposition. Bar = 10.0 µm.
b) Physiological ripe - starch granules are more closely packed with very little debris. Bar = 2.0 µm. CW = cell wall; SG = starch granule.

Fig. 8. Scanning electron micrographs of endosperm cell walls.

a) Milk stage with many pits (P). Bar = 1.1 µm.
b) Hard dough with few pits. Bar = 1.1 µm.
c) Physiological ripe with no visible pits. There is much debris as well as the protein matrix (PM) which surrounds the starch granules in the horny endosperm. Bar = 1.1 µm.


Discussion with Reviewers

L.W. Rooney: What conditions existed in the field during sorghum growth and development? Moulds can and do affect kernels during development. Could they account for the "holes" in the cell walls?
Authors: Sorghum is normally grown in Africa under semi-arid conditions with the grain being left in the field to dry naturally under the sun before harvest. Moulds are rarely a problem. The sorghum for this experiment was grown in 1982/83 in the middle of a severe drought. These conditions are hardly favourable for mould growth. Many sections of grain were examined by both SEM and TEM and not a trace of fungi was found.

L.W. Rooney: What physiological studies exist that support your contention that holes are present in the cell walls? On the basis of a single sample grown in a single season, it is very dangerous to draw the conclusions you have made. What other data support your conclusions?
Authors: We prefer the term "pits" to "holes". Over the last few years we have been isolating the cell walls of sorghum grain as well as sorghum malt. The cell walls of sorghum become extensively pitted during malting but they do not breakdown completely as they do during barley malting. Since pits are usually associated with translocation (e.g., in tracheids in the xylem) and they appeared in the sorghum endosperm cell walls at a time when large amounts of reserve material was being mobilized, we assumed that they could act as areas of translocation.

L.W. Rooney: The objective of your work was to study the sorghum kernel during its development. Why were photos not presented for each of the various sampling periods after anthesis? Why are not photos presented for zero days (at anthesis)?
Authors: Photomicrographs of the corn Photomicrographs of the caryopsis at fertilization as well as other photomicrographs at some stages of development have already been published (Sanders, 1955; Subramanyam et al., 1980 a and b).

L.W. Rooney: Sorghum kernels within a panicle vary in maturity from top to bottom by as many as 5-7 days. Did you account for this in your sampling procedures?
Authors: Samples were collected by experienced horticulturists and every effort was made to obtain material which fitted the correct stage. Only material from the middle of the panicle was taken in an effort to avoid variation in maturity from top to bottom. The samples were fixed in 5% glutaraldehyde within an hour of collection.

C.F. Earp: When you state variation in starch granule size - what range do you see in pericarp and parts of endosperm?
Authors: Although there is wide variation in starch granule size within any area we find that those in the pericarp are approximately 2-4 μm in diameter while those in the floury endosperm are approximately 10-15 μm (those in the horny endosperm appear to be slightly smaller). For comparison, see Discussion with Reviewers in Earp and Rooney, 1982.

E. Varriano-Marston: Do you think that the debris on the cell walls in Fig. 7a and Fig. 8 is possibly caused by the electron microscopic preparation method (fixation, critical point drying)?
Authors: It is quite possible that this debris is precipitated by fixation and critical point drying. However, what is important is that when the different stages of development are treated in a similar fashion some stages of development produce more debris than others (e.g., Figs. 8a, b and c).

E. Varriano-Marston: Why weren't the samples freeze-dried rather than solution fixing and critical point drying? If the grains were fairly low moisture content then solution fixing undoubtedly caused dislocation of some of the cellular components and large voids in some of the cells, e.g., Fig. 7a.
Authors: It was found that critical point drying preserved more of the structural detail than did freeze drying. However, freeze drying was used to prepare grain samples for energy dispersive X-ray analysis. (Glennie et al., 1981).

Additional reference

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MINERAL MIGRATION IN THE WHEAT KERNEL DURING MILL CONDITIONING

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Unité Microscopie
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Abstract

The structure and histology of the kernel govern migration of water during conditioning or drying. Studies by the energy dispersive x-ray system under the SEM have shown that during an increase of water content from 11.5 to 16.5 percent, soluble elements migrated from the peripheral bran, accumulated in the aleurone cells and passed through its walls to the endosperm of the kernel if the water content was above 14.5 percent. Results of study were compared with analyses of milling fractions obtained under the same conditions.

Introduction

The structure of the mature wheat kernel is well adapted to a progressive water absorption. Nevertheless, during the kernel development (Jenkins et al., 1975; Sinvendis, 1974) teguments form a water-repellent protective zone. During the early stages of kernel development the first product of photosynthesis in the testa-pericarp is the C4 acid malate which could be a direct consequence of the close impermeability of its outer surface, even limiting the diffusion of atmospheric carbon dioxide (Duffus, 1979).

In the mature kernel, the bran contains a second cuticular wax which forms an impermeable and elastic layer during conditioning, the testa. It was demonstrated by Fulcher and Wood (1983) using Nile blue under blue light illumination (excitation 450-490 nm; transmission greater than 520 nm) showing strong yellow fluorescence, and by Hinton (1955) using a capillary tube in close contact with different layers of the bran. Water penetration was very low through the hyaline layer and lowest through the testa.

The threshed mature kernel is not quite impermeable. The cutinized seed coat is missing at the level of the tunicular scar where there is an area of pigmented strand type of cells. The inner layers of pericarp consist of these walled cells containing intercellular spaces through which water can move rapidly (Pomeranz, 1982).

Stenvert and Kingswood (1976) have shown, using autoradiography of wheat tempered by up to 15.5% moisture with tritiated water, that penetration of water was especially rapid near the top of the germ region where there could be seen natural lines of cleavage between the embryo and the endosperm and the embryo and the bran. The authors noted a preferential movement of water in the dorsal region and delayed movement into the central and crease regions.

Water absorption during wheat conditioning has been reviewed recently by Pomeranz (1982). Nuret and Willm (1961) noted that conditioning involved addition or release of water according to the intended use and a rest time to allow judicious distribution of water in the kernel. Conditioning results in a physical separation of the bran from the endosperm. In addition, physicochemical transformations modify the storage
protein matrix from vitreous to floury (Moss et al., 1980).

Total minerals in parts of the wheat kernel have been calculated by MacMasters et al. (1971) according to the proportions of each tissue. About 61% of the total minerals are located in the aleurone layer, 20% in the endosperm, 7% in the pericarp and testa, 8% in the scutellum and 4% in the embryo. Not all the elements are distributed in the same way as the total minerals; thus, for example, 70% of Mg are present in the aleurone layer, 10 to 12% in the scutellum, and less than 10% in the endosperm; 50% of Ca and Na are in the endosperm and 25 to 30% in the aleurone layer.

Mineral material is often expressed by the ash content of samples. Buré (1938) has drawn a representative diagram showing the ash content (%) of the tissues of the soft wheat kernel (Fig. 1). The encircled number represents the ash ratio for each tissue.

Fig. 1. Distribution of ash in the wheat kernel (from Buré, 1938). Ash (%) is shown as function % of the tissue. Circled numbers represent, for each tissue the ash ratio as function of total kernel ash. Histograms width represent the % of tissue in the whole kernel; histogram heights are proportional to the tissue ash concentrations (% DM).

The shift was followed by microanalysis (Energy Dispersive System) of sectioned kernels and confirmed by analyses of milling fractions.

Materials and methods

Wheat kernels
Soft wheat (var. Capitole, 1978) harvested at 14.3% moisture was desorbed to 11.1% at 20°C with P₂O₅, under vacuum, conditioned with water in closed bottles, and mixed in a conditioned room (4°C) for one week to produce series with water contents of 13.5 to 16.5%. Hydration was by sorption. Water content were determined using the French standard method NF - V/03-701.

Kernel hardness determination
Kernel hardness was determined by the Pohl grain cutter. At each water level, 100 kernels were sectioned to determine the rate of mealy, intermediate and vitreous kernels.

Microanalysis of kernel cross sections
Sections (1 mm thick) were placed into similarly sized cavities drilled into pure carbon cylinders (Fig. 2a) in such a way that they were horizontal and at the level of the cylinder plane. Spaces between the periphery of the sections and the carbon holder were carefully filled with a thick carbon glue, slightly diluted with ammonia. A very thin layer of carbon was deposited onto the grain using the JEOL JEE 4B evaporator.
Mineral migration in wheat kernel

Fig. 2. Preparation of samples for microanalysis: a) wheat kernel cross section in pure carbon cylinder; b) milling fraction or ash in pure carbon cylinder.

Fig. 3. Microanalysis spectra of the teguments (1 to 5), aleurone layer (6 to 10) and mealy endosperm (11 to 15) as function of the water content of the wheat kernel; 1, 6 and 11: 12.5%; 2, 7 and 12: 13.5%; 3, 8 and 13: 14.4%; 4, 9 and 14: 15.4%; 5, 10 and 15: 16.5% water content.

Analyses were performed using the JEOL SEM 50A. For analyses, homogeneous tissue parts were selected for scanning.

Milled fractions
Conditioned kernels were milled on a Chopin-Dubois experimental mill; 4 fractions were obtained: i) semolina with coarse particles including part of the ground germ; ii) flour, fine particles from the inner endosperm; iii) bran from the outer parts of the kernels; iv) shorts containing reground and twice bolted bran.

Ash of the milling fractions was determined at 550°C according to the official method NF-V-03-760.

Microanalysis of milled fractions and ash
Samples were pressed into small cavities (Fig. 2b) drilled into pure carbon cylinders as described previously for the preparation of kernel samples.

Conditions of microanalysis
Analysis were performed using the EDAX System 711F. The detector pipe angle was tilted to 45°. The resolution was 150 eV for Mn (Kα) at 20 keV. Time of analysis was 200 seconds. The scanned areas at 1000X magnification were 150 square micrometers for the tissues and 20,000 square micrometers at 300X for all fractions and ash samples. SEM accelerating voltage was 20 keV and absorbed current 1 to 5X10^-11A. A computer was used for the ZAF corrections. Angle of sample tilt was 0°; X-ray emergence angle was adjusted to 34.3°. Quantitative analyses were performed using comparative spectra of BaCl₂. 2H₂O crystals obtained each time under the same conditions of analyses as for the samples.

Results and Discussion
Influence of hydration on the kernel structure
Hardness tests showed (Table I) a decrease of the vitreous and an increase of the mealy kernels as the water content increased. The percentage of intermediate kernels remained almost constant. Grain of intermediate texture had small, dot-like floury zones at the lowest water content (12.5%). As the water content increased,
Table 1: Influence of hydration on kernel texture

<table>
<thead>
<tr>
<th>Water content (%)</th>
<th>mealy %</th>
<th>intermediate %</th>
<th>vitreous %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>11</td>
<td>84</td>
<td>5</td>
</tr>
<tr>
<td>13.5</td>
<td>12</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>14.4</td>
<td>13</td>
<td>86</td>
<td>1</td>
</tr>
<tr>
<td>15.4</td>
<td>15</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>16.5</td>
<td>16</td>
<td>84</td>
<td>-</td>
</tr>
</tbody>
</table>

floury areas enlarged and finally merged. There were no completely vitreous kernels in grain with 16.5% moisture.

Mineral changes during damping

Analyses were conducted by scanning five areas (tomentum, aleurone layer, mealy and vitreous endosperms, scutellum) of the intermediate kernels. Figure 3 shows EDS spectra for the accumulated in the aleurone layer until a maximum of 4.3% at 14.4% water content, and decreased to 1.8% at 16.5% water.

In the vitreous and starchy endosperms, concentration of potassium was stable (about 0.06%) for 11.1 to 14.4% water content. After that, potassium increased to 0.13%. In the scutellum, changes were very irregular at the beginning of
Mineral migration in wheat kernel

Fig. 5. SEM and potassium X-ray images of wheat kernel sections also function of the grain water content. a: 11.1%; b: 12.5%; c: 13.5%; d: 14.4%; e: 15.4% and f: 16.5%.

wetting; from 14.4% water content, concentration of potassium increased.

Phosphorus: is the second major mineral component. Phosphorus was high in the aleurone layer and in the scutellum. It changed in a manner that was similar to that of potassium, a decrease in the tegument, and an increase until 14.4% water content followed by a decrease in the aleurone. The changes were inverted in the endosperm. In the scutellum, changes paralleled those of potassium.

Sulfur: sulfur decreased in the tegument as the water content increased. Concentration of sulfur in the aleurone cells decreased to half at 13.5% water and increased to the original level at higher water contents. The decrease of sulfur took place earlier in the mealy endosperm than in the vitreous one, probably due to the facility of water to move faster into the mealy endosperm which is porous and whose protein matrix is discontinuous. Changes in sulfur in the scutellum were similar to the other components.

Magnesium: traces were observed in the tegument until 13.5% water content; it could not be detected at higher levels. The concentration in the aleurone layer was very high (between 0.3 to 0.6%). As with potassium and phosphorus, concentration of magnesium increased between 13.5 and 14.4% water content and decreased to around 0.1% at higher levels. Migration of magnesium was confirmed by its increase in the mealy and vitreous endosperms.

Chlorine: at the 11.1% water content, chlorine was detected only in the scutellum and endosperm. In the scutellum, concentration of chlorine, gradually decreased until 13.5% water content, could not be detected as in the aleurone layer and was present at the 16.5% water content. This scheme was inverted in the endosperm where after 13.5% water content, chlorine gradually decreased. Chlorine concentration was higher in the mealy than in vitreous endosperm.

Figure 5 shows the potassium X-ray images obtained under the same detection conditions for different water contents. Proteins of the aleurone cells showed particularly strong emission; they were more diffuse in the tegument below 13.5% water content and were weaker in the subaleurone layer. The emission increased in the aleurone until 14.4% water content. Subsequently the emission was stronger in the subaleurone layer. No emission was shown in the thick cell walls of the aleurone layer below 15.4% water content.

According to Butcher and Stenvert (1973), the rapid movement of water through the grain occurs initially in the germ area, the remainder of the kernel being protected by the tegument cuticular layers which act as physical barriers against rapid hydration. Still, the aleurone layer is a barrier to water migration, probably due to water binding by the high protein content of this layer.

Hemicellulose also contributes to the rate of water penetration in the tegument. At the beginning of dampening, tissues rich in open porous or hydrophilic structures are also rich in water.
Fig. 6. Longitudinal section of the wheat kernel: a) its tissue content (after Buré, 1938), b) SEM of peripheral part of the kernel, and c) corresponding milling fractions.

Fig. 7. Microanalysis spectra of the bran (1 and 5), shorts (2 and 6), semolina (3 and 7) and flour (4 and 8) as function of two water levels of the wheat kernel: 1 to 4 (12.5%) and 5 to 8 (16.5%).

content. At the end of a long rest time, there is an equilibrium in moisture content. Differences in the rate of water penetration between mealy and vitreous endosperms could be explained by the absence of open structures and high concentrations of proteins in the vitreous endosperms which exhibit slow rates of water movement (Butcher and Stenvert, 1973). During wheat conditioning free water is absorbed fast; subsequent moisture movement is by diffusion from both the tegument layers and the germ region. If potassium can be considered as a marker for water migration, hydration takes place in the tegument layers during damping up to about 14.5% water
content. During such hydration, mineral components accumulate outside of the aleurone cell walls which act as a barrier. When hydration is high, water passes into the subaleurone layer and the starchy endosperm and carries along the soluble elements.

**Mineral changes in milling fractions**

Milling yields: Figure 6 depicts a longitudinal section of the wheat kernel and indicates the corresponding milling fractions. Theoretically, semolina and white flour are obtained from the starchy endosperm, bran includes the pericarp and the aleurone layer, and shorts are obtained by reground and twice bolted bran and include the inner part of the bran, the aleurone layer and some subaleurone layer. We milled 500 g of wheat for each moisture level. Resulting fractions are described in Table 2. As the water content increased, there was a progressive increase in the flour extraction from 26 to 36% and the percentage of mealy kernels increased from 11 to 16% (Table 1). At the same time, percentage of semolina decreased from 48 to 38 and the amount of highly vitreous kernels gradually decreased. The amount of kernels with intermediate texture was unchanged but areas of mealy endosperm increased gradually in size and merged. Bran yields after bolting remained basically constant and amounts of shorts increased from 0.3 to 0.9%. The yield of bran plus shorts remained fairly constant (25.3 to 27.0%) and was not affected consistently by the increase in moisture content from 14.4 to 16.5%.

Sections of bran fractions showed us expected release of the subaleurone layer; occasionally we saw a separation between the epiderm and

<table>
<thead>
<tr>
<th>Kernel moisture (% DM)</th>
<th>Flavour %</th>
<th>Semolina %</th>
<th>Bran %</th>
<th>Shorts %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>26.0</td>
<td>48.3</td>
<td>25.2</td>
<td>0.3</td>
</tr>
<tr>
<td>13.5</td>
<td>28.7</td>
<td>45.0</td>
<td>25.6</td>
<td>0.6</td>
</tr>
<tr>
<td>14.4</td>
<td>30.7</td>
<td>42.4</td>
<td>26.0</td>
<td>0.7</td>
</tr>
<tr>
<td>15.4</td>
<td>33.0</td>
<td>40.2</td>
<td>25.8</td>
<td>0.8</td>
</tr>
<tr>
<td>16.5</td>
<td>35.9</td>
<td>37.9</td>
<td>25.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

![Fig. 8. Histograms of the K, P, S, Mg and Cl concentrations (% DM) in milling fractions (semolina, flour, bran and shorts). Numbers under histograms correspond to the water content of the samples (1: 12.5%; 2: 13.5%; 3: 14.4%; 4: 15.4%; 5: 16.5%). Chlorine is represented by hatching.](image-url)
Analysis of milling fractions: Figure 7 shows spectra of bran, shorts, semolina and flour for the 12.5 and 16.5 water contents. There were considerable decreases of minerals in the bran and the shorts in agreement with the spectra of bran and aleurone in Figure 4.

Figure 8 shows that the release of elements in the peripheral fractions was not compensated by their recoveries in fractions from the central part of the kernel, especially at the 16.5% water content where all the elements showed a deficit. The decrease was particularly conspicuous in the shorts.

Some fluctuations were noted for potassium in the bran fractions, which were compared to the aleurone layer. About half of the potassium in the aleurone layer was present in the bran, because of the heterogeneity of the sample and resulting dilution of the X-ray emission. Histograms of semolina and flour, even though two times smaller, were comparable to those of the mealy and the vitreous endosperms.

Some of the discrepancies between the results of testing tissues and milling fractions can be attributed to heterogeneity of the milling fractions, loss and migration of water during milling, differences between the crystalline structure of the BaCl₂ reference crystal and the porous structure of the powdered milled particles (which may disperse the X-ray emission).

Analysis of ash: An experiment was also conducted on milling fractions and ash of Capitol wheat harvested from 1981 (Figs. 9 and 10). Potassium was highest in bran, but there were inexplicable variations and decreases in the other fractions. Similar variations were observed for the other elements. There were significant decreases in sulfur and especially chlorine, duringashing.

Total ash and individual minerals were higher by up to one-third than those reported by Pomeranz and Dikeman (1983) using atomic absorption spectrometry (Dikeman et al., 1982). The differences could be due to the analytical methods and/or extraction rates of the flours (Peterson et al., 1983).

Conclusions

EDS, while not as precise as in atomic absorption spectrometry or EDXRF, is well suited for localization of minerals in various tissues. Migration and distribution of mineral components are affected by the texture of the wheat, water level, length of conditioning and degree and type of milling.
Mineral migration in wheat kernel

References


Discussion with Reviewers

R. Moss: How were the grain samples dried prior to carbon coating?

Authors: Dehydration using acetone series and critical point or freeze-drying in such cases of very low moistures (no free water) seemed unadaptable and able to cause artifacts of mineral relocation.

R.G. Fulcher: Is there any possibility that changes in moisture levels during preparation may have influenced the apparent relocation of minerals?

Authors: It may be that changes in moisture levels during preparation influence the apparent relocation of minerals. We discussed that possibility before this study. In fact, moistures of samples were very low and migrations took a long time. On the periphery of the sections, cells were cut and opened towards the top, limited by their cell walls. The probability for horizontal shift from cell to cell was then negligible. In contrast minute vertical shifts could probably occur, which cannot be detected according to the area scanned for microanalyses.

R. Moss: Do the authors feel that this work has commercial relevance? In milling, lying times are much shorter than the seven days used here (usually times range from 8 to 24 hours). Milling behaviour is sensitive to lying time, is mineral distribution?

Authors: As written earlier, such migrations have been described by Rohrlrich and Hopp (1961). The present study was to prove this and precisely what happened at the tissular level. Results show that in the regulating of laws care of possible shifting must be taken. For example, an increase in the ash content may not always be due to the presence of peripheral parts in flour.

Actually, conditioning and lying time take usually one day or less. But in that case heterogeneity in moisture at the tissular level can be seen. We considered that 7 days lying time gave more uniform moisture and then more confident results in mineral distribution.

R. Moss: An indication of whether the differences in mineral composition are statistically significant should be given. How many samples were examined and what level of significance (e.g. p < 0.991)?

Authors: The study was mainly exploratory and the aim was to show the evolution of some mineral changes. Procedure of analysis took a very long time and the difficulty was to analyse under the same conditions a long series of samples in a few days. Data were the mean of 2 areas of each tissue for 2 kernels of the same moisture. When data differed, a third and fourth kernel were also analysed. So, it cannot be said that mineral
composition was statistically significant because only a few kernels were investigated but, in total, results were homogeneous.

On the other hand, analysis of a scanned area, which represented the analyses of very high number of individual points, was statistically significant.

A.T. Marshall: You state that the detector angle was 45° and that the specimen tilt angle was 0°. It is not clear how you arrive at an X-ray emergence angle (take off angle) of 34.3°. Please, explain in more detail what is meant?

G.M. Roomans: The description under "Microanalysls conditions" is not clear. If the sample tilt is 0°, and the X-ray emergency angle was 34.3° then the detector must have been tilted 34.3° too.

Authors: This take off angle has been calculated according to geometry data of the specimen stage of the microscope (JEOL 50A) and using the "Take off angle program" published in Edax Editor 1978, 8, (4) p. 15.

Note that our samples were 3.5 mm higher than the goniometer stage, which explains this data.

G.M. Roomans: A ZAF correction is no longer considered the best method for analysis of biological bulk specimens (Boekestein A., Stols A.L.H., Stadhouders A.M. (1980). Quantitation in X-ray microanalysis of biological bulk specimens. SEM 1980; II: 321-334; Boekestein A., Stadhouders A.M., Stols A.L.H., Roomans G.M. (1983). Quantitative biological X-ray microanalysis of bulk specimens: an analysis of inaccuracies involved in ZAF-correction. SEM 1983; II: 725-736) where it is demonstrated that the EDAX ZAF programs are not accurate for analysis of low-Z specimens. To make matters worse, the authors have used a standard that does not resemble the specimen in any respect. This will increase the inaccuracies in the ZAF correction and, in addition, differences in mass loss between specimen and standard are introduced. Although this does not affect the relative values of the measured concentrations, the absolute values may not be correct.

A.T. Marshall: You present your data as concentrations by weight percent and refer to the Edax program with ZAF corrections for your particular matrix. Does the ZAF correction program allow you to insert C, O and N concentrations? What did you use for standards for the other elements and what is the significance of the BaCl₂ standard? Is the program a no standards program?

Authors: When the study was done, 3 years ago, ZAF program was used as a useful and routine program. Since then, we have also heard of the new program for analysis of biological bulk specimens, by Boekestein et al.

Different tissues of the wheat kernel are heterogeneous in structure and composition, some being porous (bran, aleurone layer), others being more compact, as the endosperms. The best standard to be used has never been described and seems still unknown for such a case. We have chosen the BaCl₂, crystals being flat and clean, stable under electron beam and because they did not provoke shifts. Note that the ZAF program allows us to insert C, O and N concentrations, except for the ash where the program allows us to insert O only. It is possible that the BaCl₂ was not the best standard to be used for this study. Maybe this explains the differences we noted with AAS (atomic absorption spectrometry).

G.M. Roomans: Are the differences in dot density (X-ray maps) not due to differences in local mass?

A.T. Marshall: In showing X-ray maps, a map of continuum counts should also be shown, since it is possible to obtain the type of maps you show in Fig. 5 simply by virtue of an increase in background counts under the K peak. This increase could be a result of an increase in density. It is therefore important to show that this is not the case by including a map using a peak free band of continuum.

Authors: A map using a peak free band of continuum shows very weak, scarce and uniform dot density.

The map of potassium shows high dot density at the aleurone layer level. So, the dots show differences in potassium concentration and not differences in local mass or density.
The crumb of bread baked from wheat flour, rye flour, and rye meal was examined by light- (LM) and scanning electron-microscopy (SEM). Whereas in the wheat bread the crumb is held together by a matrix of denatured protein, in the rye bread crumb highly expanded starch granules fulfill that role. Fracturing freeze-dried crumb provided different information than sectioning prior to freeze-drying. In the first case, little damage was caused to components of outer surfaces of vacuoles. In the second case, the protein matrix and starch granules were broken. At the same time, the presence of micropores in the material surrounding the vacuole was observed and confirmed the findings from LM of sections of the bread crumb. Examination by SEM of residues of bread crumb macerated to wash out soluble starch demonstrated the presence of a residual coherent structure of apparently denatured gluten proteins in wheat bread. In rye bread there were only few similar, less coherent, structures.

Material and Methods

Bread

Formulations and procedures used in preparation of the wheat and rye flours and meal bread were described elsewhere (Pomeranz et al., 1984a, b).
Light Microscopy

Bread crumb was sectioned with a freezing microtome table, (Leitz Co., Inc.) with cooling aggregate for object table and knife cooling. Pieces, about 5 mm long, were cut from freshly baked bread and frozen without fixation, in an embedding medium for frozen tissue specimens (O.C.T. compound) at -20°C on the object table of the microtome and sections 10 µm thick were prepared. The sections were glued to glass slides painted with a thin layer of glycerol-gelatin, stained for protein with Xylidin Ponceau (Pomeranz and Shellenberger, 1961) or for starch with iodine, and observed under a Zeiss light microscope.

Scanning Electron Microscopy

Two methods of sample preparation were used:

A. Chunks of crumb were removed from freshly baked bread cooled to room temperature, frozen at -20°C in a Leibold Heraeus GT 3 freezer, and freeze-dried. Small pieces of the freeze-dried bread were mounted on stubs with a special glue (Leit C) in such a manner that the original surfaces of the freeze-dried crumb removed from the freshly baked bread could be examined. The mounted pieces were sputter-coated with gold. The preparations were viewed and photographed in a Leitz AMR 1,600 T scanning electron microscope at an accelerating voltage of 20 kV.

B. Slices of freshly baked bread were frozen at -20°C, freeze-dried, and broken. The newly broken surfaces were examined by SEM as in Method A.

Results and Discussion

Examination under the light microscope makes it possible, through staining, to determine semiquantitatively the distribution of protein and starch. Figures 1 and 2 indicate that distribution of protein in the crumb of both the wheat and rye bread is not uniform. There were considerably more protein stained for starch in the wheat bread crumb than in the rye bread crumb. In addition, the wheat bread crumb had several areas of high protein concentration distributed at random throughout the crumb. The general impression is that whereas in the wheat bread crumb the protein matrix holds the crumb together, in the rye bread crumb no such coherent matrix is present. The rye starch granules are more modified and expanded than the wheat starch granules, probably because of the lower gelatinization temperature of rye starch under baking conditions. The proximity of highly expanded starch, even in the micrograph of the rye bread crumb, does not prevent the formation of well-delineated, individual starch granules. To the extent that protein is present, it surrounds the starch granules in the rye bread crumb and forms a matrix in which rye starch granules are embedded. A similar conclusion was reached during observation under the LM (not shown) of iodine-stained preparations. Protein comprised the major matrix in the bread crumb of wheat bread and starch in the crumb of rye bread. The results were, however, not as clear-cut due to the non-specific staining by iodine of non-starchy components, especially in the rye bread. In the ease of bread made from whole grain rye meal, an additional contributor to the crumb is the presence of chunks of starchy endosperm and bran (not shown).

Scanning electron micrographs of the wheat and rye bread crumb are shown in Figs. 3-10. The greater depth of field observed in scanning electron micrographs presents a good overview of the structure of the bread crumb in wheat (Fig. 3) and rye (Fig. 7) bread. In samples prepared by Method A (surfaces cut before freeze-drying), one can see in areas surrounding the vacuoles the arrangement and distribution as well as the modification-expansion of individual starch granules and compare them with, apparently, less modified starch granules (Figs. 3, 5, 7, and 9). In agreement with the micrographs from LM (Figs. 1 and 2), the starch is substantially more modified-expanded in rye than in wheat bread crumbs. In wheat bread (Fig. 5-10), the starch granules are clearly embedded in a protein matrix. On the other hand, it is more difficult to discern the outline of the individual starch granules in the inner structure of the vacuole in rye bread (Fig. 9 - high magnification). This is due to the more extensive extension-modification of the granules, but also to the fact that they may be covered by a layer of gums, proteins, and soluble starch.

Samples prepared by Method B (surfaces cut after freeze-drying) differ substantially from samples prepared by Method A. Whereas the outer surfaces of the vacuoles remain relatively intact, considerable damage was caused to the starch, proteins, and other components inside the vacuole walls (Figs. 4, 6, 8, and 10). A comparison of the micrographs of crumb prepared by Method A (Figs. 3, 5, 7, and 9) with the micrographs of crumb prepared by Method B (Figs. 4, 6, 8, and 10), indicates that the former show little and the latter show considerable amount of micropores. Those micropores are the result of minute vacuoles in the walls surrounding the larger vacuoles. They can be seen only after the walls of the vacuoles are fractured. They can be seen also in the preparations examined under LM. The possibility that some of the micropores are the result of shrinkage during freeze-drying cannot be excluded. Thus, the information obtained by the two methods of sample preparation compliments the other. Useful information can be obtained by examination of micrographs at high magnification. Method B shows the folding and layer-like stratification of starch in the walls of the vacuoles as well as a partial cover of a protein matrix. The results of micrographs for Method B, as expected, parallel those for LM of their sections from frozen material. This information is particularly useful in examining the vacuolar wall structure of rye bread. Whereas it is difficult to conclude from Fig. 9 about the manner in which the starch granules are held together, Fig. 10 demonstrates the "spot welding" interaction at fairly regular intervals, that is responsible for the structure of the crumb of rye bread.
The findings were also confirmed by scanning electron micrographs of bread crumb macerated with water to remove the soluble starch prior to mounting. In the case of wheat flour bread (Fig. 11), even after the soluble starch was washed out there remained a residual coherent matrix of the denatured gluten. In the case of rye bread, a less coherent structure was left as the main components were washed out (Fig. 12).

References

Discussion with Reviewers
R. Moss: "Freshly" baked bread--some indication of whether or not the bread was cooled would be useful in helping the reader assess likely artefacts associated with processing i.e. hot or warm bread is difficult to sample without causing artefacts on the cut surface. Also, more details of the freezing process--this is also not clear in the Cereal Chemistry paper i.e. air frozen, blast or still air?
Authors: Chunks of crumb were removed from freshly baked bread, cooled to room temperature. They were frozen in a Lellobd Heraeus GT 3 freezer. The freezer is equipped with an air-suction device.
R. Moss: The term "spot welding" is not very informative. The comment re Fig. 9 seems equally applicable to Fig. 10 except that Fig. 10 is at higher magnification i.e. no information is given as to the nature of the components responsible for the 'spot' welding.
Authors: The magnifications in Figs. 9 and 10 are approximately the same.
R. Moss: No mention is made of the protein content of the flours used in these experiments. If they are the same as used for the 1984(a) Cereal Chemistry article, the appreciably lower protein content of the rye flour compared to the wheat flour may be responsible for the markedly different protein matrix in the LM of rye bread (Fig. 2) i.e. very little protein is apparent. In the reviewer's experience, the accumulations of protein seen in Fig. 1 are very dependent on protein content (as well as degree of development) and breads from low protein wheat flour have an extremely fine and delicate protein matrix.
Authors: The protein contents were 12.1% and 9.4% (both dry matter basis) in the wheat and rye flours, respectively. A decrease in protein content will decrease the extent of staining with Xylinid Ponceau. We do not believe, however, that the differences in protein distribution (Figs. 1 and 2) and in coherence of the protein matrix (Figs. 11 and 12) are due to differences in protein content, only.
R. Moss: The maceration experiments are interesting but why do the authors feel that the differences they observed are due to the washing out of soluble starch (as stated in the abstract), rather than a difference in the fragility of the crumb? The latter would be more related to the different nature of the protein matrix.
Authors: The differences were not due to maceration; they were made visible as a result of washing out of starch.
R. Moss: Are the artefacts associated with Method B due to shrinkage during freeze drying or due to shrinkage during freezing? Did the authors investigate other, more rapid freezing methods (e.g. isopentane cooled liquid N2 or N2 slush)? Freeze-fracturing the samples might also have provided helpful information.
Authors: We have not tried various freezing temperatures and have cited work of others (Chabot, 1979 and Varriano-Marston, 1977) in this respect. We do not believe that Methods A and B differed in their effects on shrinking.
Reviewer No. 2: The only difference I could see between Method A and Method B in the scanning electron microscopy was that in A original surfaces were viewed, and in B freeze-dried-fractured surfaces were seen. While I would predict some differences in these two surfaces, I would not expect the type of differences illustrated. Also, freeze-dried bread is very fragile, so keeping track of original versus fractured surfaces can be difficult. I thought that this was the reason for two separate protocols, because in fact both original crumb surface and fractured dried crumb surface can be revealed in one freeze drying step.
Authors: We presented only a small part of micrographs. Practically all wheat bread samples treated by Method B produced micrographs represented in Figs. 4 and 6; all rye bread samples
Figure 1. LM of a cross section through the wall of a vacuole of wheat bread crumb. Bar = 100 μm.
Figure 2. LM of a cross section through the wall of a vacuole of bread crumb from rye flour. Bar = 100 μm.
Figure 3. SEM of crumb of wheat bread, Procedure A. Bar = 100 μm.
Figure 4. SEM of crumb of wheat bread, Procedure B. Bar = 100 μm.
Figure 5. SEM of crumb of wheat bread, Procedure A. Bar = 10 μm.
Figure 6. SEM of crumb of wheat bread, Procedure B. Bar = 10 μm.
Figure 7. SEM of crumb of rye bread, Procedure A. Bar = 100 μm.
Figure 8. SEM of crumb of rye bread, Procedure B. Bar = 100 μm.
Figure 9. SEM of crumb of rye bread, Procedure A. Bar = 10 μm.
Figure 10. SEM of crumb of rye bread, Procedure B. Bar = 10 μm.
Figure 11. SEM of water-macerated crumb of wheat bread. Bar = 10 μm.
Figure 12. SEM of water-macerated crumb of rye bread. Bar = 10 μm.
treated by Method B produced micrographs represented in Figs. 8 and 10.

Reviewer No. 2: Examination of the micrographs showed very great differences between the two protocols, but I did not understand why the B method produced such different images, with loss of detail of starch granules. It appeared that these images looked more like a freeze dried gel than like bread. Was it possible that in Method B the bread had become wet at some time? In rereading the light microscopy section, had the imbedding medium O.C.T. (which I am not familiar with) been used for scanning electron microscopy preparation as well?

Authors: It is our belief that the matrix and starch structure in samples prepared by Methods A and B differed significantly and consistently, irrespective of starch fracture. The samples were not imbedded for SEM. It is unlikely that the samples picked up large amounts of water during freezing.

Reviewer No. 3: Why do you think freezing at -20°C will not damage the matrix and swollen starch granules during freezing?

Authors: Artefact formation as a result of ice crystal formation during freezing at -20°C cannot be excluded. It is possible that use of lower freezing temperatures (-40 or -60°C) should be investigated.

Reviewer No. 3: What were the relative volumes of the different breads? What was the relative amount of air cell structure in the breads? Could these differences account, in part, for the amount of matrix found/unit area of crumb observed? (This discussion is useful even when published earlier.)

Authors: The specific volumes of the rye bread were 1.9 to 2.4 g/cm³ and of wheat bread 3.3 to 3.7 g/cm³ (See Bruemmer, J. Getreide, Mehl, Brot. [1971], 26, 125-128; [1972], 26, 234-236).

Reviewer No. 3: Can the authors describe the differences in starch size, shape, swelling temperatures? Can one or two micrographs be included from previous work?

Authors: Das Getreide, Part 1. Verlag Paul Parey, Berlin, (1966), pp. 28-30: small starch granules (up to 7.5 μm in wheat and rye) comprise about 90% of the total number; wheat starch granules are up to 45 μm and rye starch granules up to 60 μm in diameter. The beginning average gelatinization temperatures are 60°C for wheat and 56°C for rye starch and the average final gelatinization temperatures are 80°C for wheat and 62°C for rye starch.
ULTRASTRUCTURE OF QUINOA FRUIT (Chenopodium quinoa Willd)

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Abstract

The structure of quinoa (Chenopodium quinoa) fruit before and after germination was studied using electron microscopy. Protective coverings include a perianth consisting of loosely adhering cells which are readily removed by washing, a pericarp and two seed coat layers. Starch granules fill the perispem cell and are arranged in 18 to 20 \( \mu \text{m} \) oblong aggregates. Limited hydrolysis of the starch occurs after 24 hrs of germination, with amylolytic erosion of large granules occurring at the hilum and periphery of the granules. Ungerminated embryo cells contain protein bodies with phosphorus-containing globoid inclusions. Essentially complete hydrolysis of the embryo protein bodies occurs within 24 hrs of germination leaving large central vacuoles within the cells.

Introduction

Quinoa (Chenopodium quinoa, Willd) is a major staple food of people in the Andes. The fruit is used for porridges or ground into flour for preparing breads and cakes. Quinoa is a drought resistant crop and can be produced on land that will not support the growth of common cereals (White et al., 1955, Simmonds, 1965) so it is often used as a substitute for cereal grain in food preparations. The yield of fruit is 840 to 3000 kg/hectare (Simmonds, 1965).

The protein content of quinoa fruit ranges from 9 to 15% (Etchevers, 1980; Aguilar et al., 1979; Sanchez-Marroquin, 1983; Clavijo et al., 1973; Quiros-Perez and Elvehjem, 1957). Fat content is about 4% (Simmonds, 1965). Starch comprises about 60% of quinoa (Wolf et al., 1950). Quinoa starch has a diameter of 1–2.5 \( \mu \text{m} \), a gelatinization temperature range of 57–64°C, an amylose content of 11%, and an average amylpectin chain length of 27 (Atwell et al., 1983). Quinoa starch pastes do not gel on standing (Wolf et al. 1950).

No reports have been published on the structural characteristics of quinoa other than a description of aggregated starch granules isolated from the fruit (Atwell et al., 1983). The anatomy of quinoa fruit is important from a processing standpoint as well as to increase our knowledge of its structural characteristics. The objective of this study was to describe the ultrastructure of quinoa fruit and the changes in structure as affected by germination.

Materials and Methods

Materials

Four quinoa varieties were studied: Blanca, Rosada, Pasankallo and Koito. The samples originated from Bolivia.

Methods

Scanning Electron Microscopy (SEM). The fruit was fractured with a dull razor blade, mounted on Al stubs, and coated with Au/Pd. Some samples were washed in distilled water and lightly abraded between the fingers to remove the loose outer layer of the fruit prior to SEM observations. Other samples were germinated by soaking in distilled water for 24 hrs. and then freeze-dried. Samples were viewed in an ETEC U-1 SEM operated at 10 to 20 kV or in a JEOL 35C.
Qualitative X-ray microanalysis of fractured, carbon coated samples was done using a Link X-ray Energy Dispersive Microanalyzer attached to a JEOL 35C SEM. Counting rates were 2500–3000 cs/sec and analysis time was 100 sec. at 20 kV.

**Transmission Electron Microscopy (TEM).** Some quinoa fruit were fixed for TEM following the simultaneous glutaraldehyde-OsO₄ fixation schedule of Franke et al. (1969). Other samples were fixed sequentially, first in glutaraldehyde and then in OsO₄ after buffer rinsing. Fixed samples were dehydrated in a graded acetone series (30% to 100%), embedded in Mascorro’s resin (Mascorro et al., 1976), and sectioned with a glass knife on a Reichert OM–2 Ultra-microtome. Sections (60 nm thick) were stained with 5% uranyl acetate in 50% ethanol followed by lead citrate (Reynolds, 1963), and viewed in a Philips 201 transmission electron microscope at 60 kV.

Small fragments of moistened perisperm of ungerminated and germinated quinoa fruit were placed in copper specimen holders and frozen in liquid freon. Freeze-fracturing and Pt shadowing at an angle of 35° were done at −170°C in a Balzers Freeze Etch Unit. Replicas were cleaned by sonicating in Chlorox for 5 min., held in Chlorox for an additional 3 hr., washed in water, and collected on uncoated 300 mesh copper grids. Micrographs were taken on a Zeiss EM-10 transmission microscope at 60 kV.

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Figure 1. SEM of quinoa fruit: (a) and (b) enclosed in the perianth. Gross internal anatomy of quinoa shows pericarp and perianth (P), embryo (E), and perisperm (PE). Fracture done parallel to the plane of the cotyledon.

Figure 2. Scanning electron micrographs of quinoa: (a) perianth is removed by washing to reveal the pericarp, (b) the hilum, and (c) tracheids at the hilum.
Results and Discussion

Quinoa fruit are disc-shaped and range in diameter from 1 to 3 mm (Fig. 1a). The major anatomical parts of the fruit (Fig. 1b), the outer covering (perianth and seed coats), the perisperm, and the embryo, are described below.

Outer Coverings

Often when quinoa is harvested, the fruit fall off the plant still enclosed in the perianth (Fig. 1a). The weakly adhering cells of the perianth are easily removed by washing and scrubbing in water to expose the smooth surface of the pale yellow pericarp (Fig. 2a). The perianth of some varieties is magenta colored by a water soluble pigment that has a λmax of 530 nm which is characteristic of betacyanins (Harborne and Simmonds, 1964).

The hilum, the scar left from the attachment of the fruit to the placenta, is located at the center of the fruit (Fig. 2b). Tracheid structures involved in the transport of water and nutrients from the plant to the fruit can often be seen at the hilum (Fig. 2c).

Fractures perpendicular to the plane of the cotyledons (perpendicular to the disc structure, Fig. 3a) reveal starchy perisperm covered by a pericarp and seed coat structures. In this case, the perianth has been removed, but when present it consists of a layer of cells loosely attached to the pericarp (Fig. 3b). The pericarp layer consists of a dense, compact layer of cells about 10 μm thick (Fig. 3b and c). There are two seed coat layers beneath the pericarp. One layer is about 20 μm thick and contains polygonal starch granules and electron dense bodies (Fig. 3c). A second seed coat structure is cemented to the perisperm (Fig. 3d). This 3 μm thick structure may be the cuticle.
Figure 4. Qualitative X-ray energy dispersive analysis spectra of the perianth (a) and the pericarp after washing away the perianth (b). The Cu peak is from instrument parts. Both spectra printed at the 4 k vertical scale.
Ultrastructure of Quinoa Fruit

It is a common practice among quinoa consumers to wash and scrub the fruit in water prior to consumption (Simmonds, 1965). This washing removes the bitter saponins that are apparently located in the outer coverings (Aguilar et al., 1979). Washing removes the perianth which contains high K and Cl contents and minor or trace levels of Mg, Al, Si, P, S and Ca (Fig. 4a). It is doubtful that hand washing is vigorous enough to remove the pericarp. Like the perianth, unwashed pericarp contains high concentrations of K and similar low contents of S and Ca (Fig. 4b). However, there is less Cl in the pericarp than in the perianth.

Perisperm.

Quinoa fruit differs from cereal grains in that the storage reserves for the developing embryo are found in the perisperm rather than the endosperm (Wolf et al., 1950). The perisperm is located in the center of the fruit (Fig. 1b).

Starch granules in perisperm cells are polygonal and range in size from 0.4 to 2.0 μm (Fig. 5a and b). Transmission electron micrographs indicated that two populations of starch granule sizes exist in the perisperm: one population centers around a granule diameter of 0.5 μm; the other centers around a granule diameter of 1.3 μm (Fig. 5c). Atwell et al. (1983) reported a particle size distribution of 0.63 to 8.0 μm for a pure quinoa starch preparation with the median diameter being about 1.5 μm. Our data indicate that the size range is smaller and that a bimodal distribution exists. Some cells appear to contain only the larger granules while other cells contain mainly small granules (Fig. 5c). Starch granules are found as single entities within the cells (Figs. 5c and d) or compound structures consisting of spherical or oblong aggregates (Fig. 5a). As many as 14,000 starch granules may comprise an aggregate about 18 to 20 μm in size (Seidemann, 1966).
The compact spherulitic texture of the starch granules was revealed in both freeze fracture replicas (Fig. 6a) and thin sections of samples that were stained sequentially with glutaraldehyde and then OsO₄ (Fig. 6b). The reasons why quinoa starch granules strongly react with OsO₄ during sequential fixation and not during a simultaneous fixation procedures are unknown. Unlike starch granules of many cereal grains (Buttrose, 1960; Gallant et al., 1972), no concentric rings were observed in quinoa granules.

Matrix protein surrounds the starch granules and interconnects them within the cells (Figs. 5c and d). In most TEM preparations, the starch granules pulled away from the surrounding protein which may suggest weak bonding between those two components. No obvious protein bodies were observed. Perisperm cell walls appear about 1 μm or less in thickness in Figure 5c but less than 0.5 μm thick in Figure 5d.

X-ray energy dispersive analysis of the perisperm did not reveal high concentrations of any one element (Fig. 7). Low levels of K, S, Cl, and Si predominated.

**Embryo**

The embryo surrounds the quinoa perisperm (Fig. 1b). The mature embryo is a dicotyledon. Transmission electron micrographs (Fig. 8a and b) of the cotyledonary cells show a complex structure consisting of lipid bodies, protein bodies, nucleus and other organelles necessary to carry out the degradative and synthetic functions involved in the transformation of the seed into a plant. The protein matrix of some protein bodies is granular suggesting that water imbibition during fixation may have initiated cytoplasmic changes normally associated with incipient germination. It is impossible to chemically fix dry seeds without cell hydration during fixation. The protein bodies usually contain two or more electron transparent globoid crystals which, in turn, contain electron dense globoid crystals or voids caused by the loss of globoid crystals during thin-sectioning. Lott and Buttrose (1977) have shown that globoid crystals of many seeds are rich in phytin (a salt of myoinositol hexaphosphate).

**Effects of Germination**

During germination, the embryo unwinds and the cotyledons separate (Fig. 10a). Since quinoa is essentially nondormant (Simmonds, 1965), germination was complete within 24 hrs. The structure of the perisperm did not change dramatically after germination. The starch granules were more loosely packed within the cells than in the perisperm of ungerminated fruit, and the matrix protein was retracted from the starch granules (Fig. 10b). No evidence of significant erosion of starch granule surfaces due to amylolysis was observed by SEM. Some large granules (1 μm or greater) did, however, show evidence of amylolytic degradation at the hilum (Fig. 10c) and the periphery of the granules in transmission electron micrographs (Fig. 10d). Surface digestion holes were 40 nm to 120 nm in diameter (Fig. 10d). Preferential digestion at the hilum suggests that this area is less crystalline than the starch granule periphery.

**Figure 7.** Qualitative X-ray energy dispersive analysis spectrum of the perisperm. Cu and Zn peaks are from instrument parts. Vertical scale is 2 k.

**Figure 8.** Embryo of ungerminated quinoa. Transmission electron micrographs in (a) and (b) show protein bodies (pb), globoid inclusions (G), lipid bodies (L), nucleus (N) and nucleolus (Nu).

**Figure 9.** Qualitative X-ray energy dispersive analysis spectrum of the embryo, showing high content of P and K. Vertical scale is 8 k.

**Figure 10.** Germinated quinoa. After 24 hrs. the embryo of the seed unwinds and the cotyledons separate (a). Perisperm cells show retraction of protein from the starch granules (b) and amylolytic degradation (arrows) at the hilum (c) and periphery (d) of the granules.
Ultrastructure of Quinoa Fruit
Quinoa embryo cells (Fig. 11) showed structural changes typically observed in germinated seeds (e.g. Swift and O'Brien 1972). After 24 hr. germination, most of the protein bodies were hydrolyzed and the remaining organelles and cytoplasm stained more faintly than in the ungerminated embryo cells (Fig. 11a). Cell organelles are surrounded by ribosomes, and many of the cells contain large central vacuoles resulting from the hydrolysis of lipid and protein bodies (Fig. 11b). More lipid bodies remain in the cytoplasm than protein bodies which suggests that during germination utilization of protein is more rapid than lipid.

Summary and Conclusions

The fruit of Chenopodium quinoa is consumed in some areas of South America similarly to our consumption of cereal grains in the United States. In fact, all of the literature refers to quinoa as a grain. However, quinoa fruit has some unique chemical constituents and anatomical characteristics that clearly differentiate it from cereal grains.

Quinoa contains saponins which are bitter and possibly toxic. Unlike tannins in sorghum, quinoa saponins are readily removed by washing in water and lightly abrading the fruit. Although the saponins are known to be present in the covering layers of the fruit (Simmonds, 1965), it is unknown whether they exist primarily in the perianth or the pericarp. Such information would be useful if large scale production and processing of this food were considered.

Like cereal grains, starch is the major constituent of quinoa fruit. This storage carbohydrate is located in the perisperm rather than the endosperm. The small polygonal granules in the cells form compound structures with well-defined oblong shapes. Only two articles have been published on the physicochemical characteristics of quinoa starch (Wolf et al., 1950; Atwell et al., 1983). The authors report different gel characteristics and amyllose contents for quinoa starch. Discrepancies may be a reflection of varietal differences. Further studies are warranted.

Quinoa fruit is essentially nondormant (Simmonds, 1965). As it imbibes water, germination rapidly ensues, and a dramatic reduction in embryo subcellular organization occurs within 24 hr. Unlike many cereal grains, amylolytic action towards quinoa starch granules is not extensive during germination. Their polygonal structure, small size and aggregation may deter enzyme hydrolysis. Data on cereal starches suggest that some of these factors affect rates of amylolytic degradation (Sandstedt, 1955, Lineback and Pompipom, 1978; Beleia and Varriano-Marston, 1981). Additional knowledge concerning the susceptibility of quinoa starch to amylase action would be helpful in understanding the role of these enzymes in germination as well as providing information of nutritional significance.

Acknowledgements

The authors thank D.A. Fellers, Western Regional Research Center, Berkeley, California, for the quinoa samples.

References


Figure II. Embryo cells after germinating quinoa for 24 hr. Some lipid bodies (L) still remain but many are partially hydrolyzed (a). Free ribosomes and mitochondria (m) are present; intact protein bodies are rarely seen. Large central vacuoles (v) are present in many cells (b). CW – cell wall.
Ultrastructure of Quinoa Fruit


Discussion with Reviewers

C.F. Earp: What is the approximate proportions of dry weight of each of the major parts of quinoa?

Authors: No data are available and since the fruits are very small, special dissecting techniques would be necessary to obtain meaningful data on the relative composition of the component parts.

C.F. Earp: What is the difference between a perisperm and an endosperm?

Authors: The endosperm is formed within the embryo sac. The perisperm is derived from the nucellus.

D.B. Bechtel: Since the perianth is so thin, delicate and apparently non-continuous, how can you be sure that the X-ray pattern observed in Fig. 4a is truly the perianth rather than the pericarp “showing” through? How were you able to eliminate the irregular surface effects on the X-ray pattern?

Authors: A spot X-ray analysis was done on the perianth which involves analyzing areas the size of the electron beam. Since the perianth is structurally distinct from the pericarp, it was easy to differentiate the two structures. The perianth is about 2 microns thick. It is quite unlikely that X-ray photons generated at depths greater than 2 microns would be detected. Surface roughness does contribute to variability in X-ray photon collection. This is implicit in all spectra that are generated from unpolished surfaces which is why we call it qualitative X-ray analysis.

D.J. Gallant: What kind of breads are made from quinoa?

Authors: No references gave details of the bread preparations.
ULTRASTRUCTURAL ASPECTS OF SPUN PEA AND FABABEAN PROTEINS

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Abstract

The ultrastructure of pea and fababean spun proteins has been studied by SEM and TEM as a function of dope pH and washing bath salt concentrations. The textural properties (mechanical resistance, moisture content) and diameter of the fibres have been determined.

Spinning was only possible when dope pH was higher than 11. An increase in dope pH from 11.5 to 13 induced a shear strength increase whereas the moisture content and the diameter of the fibres decreased. The structure of the fibres became more compact and changed from an aggregate of spherical particles to a tridimensional network. When dope pH was equal to 12.6, the increase in washing bath salt content from 2 to 10% NaCl produced more compact fibres.

At high dope pH, the protein aggregates were dissociated and the polypeptide chains were unfolded, which favoured the lining up of the macromolecules during the spinning process and increased the protein-protein interactions in the fibres.

High salt concentration in the washing baths produced a salting out effect which probably also enhanced the chain-chain interactions. Contrary to previous studies, protein strand orientation along the fibre axis and a double cortex-core structure have been demonstrated.

Introduction

According to Flint (1979), the spinning technique for making edible spun protein fibres is the exact imitation of a technology already used in the manufacture of textile fibres such as rayon. This technology has been applied to the processing of all kinds of food proteins (Young and Lawrie, 1975; Culioli et al., 1981; Tuoby et al., 1983) and the vocabulary has even been taken directly from the textile industry. Although the U.S. patent of Boyer (1954) for spinning proteins is not very recent, edible spun protein fibres are still not produced on a large scale. This is why spun proteins have been presented by De Man (1976) as very new textured protein food, used to replace existing meat products. The process as it was outlined by Culioli (1981), consists (Fig. 1) of pumping a dope (alkaline solution of protein isolate) through a spinneret (a die containing many hundreds of pin-holes, from 50 to 250 μm in diameter) into a coagulation bath containing acid (acetic, phosphoric or lactic, at various concentrations) and salt (most often 2 to 20% NaCl) to form insoluble protein fibres. Coagulated fibre bundles are removed under tension to improve the rheological fibre properties. They are then neutralized and washed in various baths, spin-dried and immersed in binding agents (ovalbumin, gluten, soya bean proteins or polysaccharides). All these agents are coagulated either by heat, pH changes or evaporation. Acceptable simulated meat products, with added fats, coloring and flavors, are cut and either frozen or dried.

Culioli (1981) has hypothesized the molecular arrangement during protein spinning as schematized by Ziabicki (1967) in the case of the synthetic spun polymers (Fig. 2). The native globular structure of isolate protein is unfolded and denatured in the alkaline dope medium. Then molecular orientation results from the shearing flow in the spinneret where the velocity profile assumes a parabolic distribution (Fig. 2a). Just after emerging from the spinneret, the protein molecules become disorganized by the expansion of the dope flow; expansion results from restitution of the elastic energy stored during spinneret
flow. Then the macromolecules are again orientated by streaming in the elongational flow (Fig. 2b). Precipitation under stretching increases the orientation parallel to the axis of the fibre (Fig. 2c), intensifying the intra- and intermolecular protein bonds (hydrogen, ionic or covalent).

The structure of spun proteins has generally been studied either with light microscopy or SEM (De Man, 1976; Sato, 1979 and 1981; Wolf and Baker, 1980). Fibres were thus shown to be analogues of the meat or fish muscle fibres: such fibre bundles combined well with unstructured gluten and fats in spun soybean meat analogues (Flint, 1979; Culiolli, 1981). SEM observations were also correlated with physical measurements such as shear strength, diameter and fibre water content (Culiolli et al., 1983) when the dope pH or the salt content of washing baths were varied. The SEM of spun soybean protein isolate showed cylindrical morphology with surface striations running along their length (Wolf and Baker, 1980) that suggests longitudinal orientation of the protein structure.

In TEM studies of spun proteins Tombs (1970), and Young and Lawrie (1975) noted that there was no orientation of protein molecules along the axis of the fibre. In cross sections, the fibre ultrastructure was shown to consist of spherical particles linked together in strands forming a three-dimensional network incorporating pores of various sizes, with random aggregation in some areas.

There were no signs of orientation in TEM micrographs of extruded soybean protein fibre (Smith, 1979). Such observations seem contradictory to the scheme of molecular arrangement in Figure 2.

In order to understand both the spinning process and molecular orientations of the proteins, the ultrastructure of longitudinal sections from the periphery and central core of pea and fababean protein fibres are shown. Structural modifications (protein strand orientation, particle size and alveolation) were studied as a function of dope pH and washing bath salt concentrations.

**Materials and methods**

**Spinning** Pea or fababean protein isolates were prepared at the pilot plant of our Research Center (Food Technology Center - INRA Nantes - France) from hulled pea or fababean flour. The nitrogen was determined to be 15% (pea) and 15.9% (faba-bean) of the isolate dry matter. Protein dopes were prepared at room temperature in a mixer with two horizontal blades. The dope pH was adjusted to the 11.6 to 13.2 range by adding caustic soda. Extrusion was carried out at 20°C through a spinneret containing 300 holes of 100 μm each. The coagulating bath contained 4% acetic acid and 20%
Ultrastructure of spun pea and fababean proteins

Fig. 2. Diagram of molecular arrangement during spinning: a) molecular orientation from shear flow in the spinneret; b) molecular orientation from elongated flow at spinneret exit and c) molecular orientation after deformation of a three-dimensional viscoelastic network (Ziabicki, 1967).

Physical measurements

Water content. The bundles were centrifuged on fritted glass for 15 min at 200 g. The moisture content was then determined by weighing the centrifuged samples before and after drying at 105°C for 12 h.

Fibre Mean Diameter. Determined on wet fibres with a light microscope using an ocular micrometer.

Mechanical Resistance. The fibres were characterized by their shear strength using the method of Laroche and Sale (1976).

SEM

Wet spun fibres were frozen either directly in liquid nitrogen or in isopentane cooled to -150°C with liquid nitrogen, then freeze-dried and cross fractured. The following technique was also used: fixation 2 h in a solution containing 6% glutaraldehyde and 5% NaCl and ethanol dehydration (2 h) with a graded ethanol series. Samples were examined in a JEOL 50A at 20 keV.

TEM

Wet spun fibres were fixed 1 h in 6% glutaraldehyde and postfixed 30 min in 1% OsO₄, the two fixatives being prepared with the washing bath solutions (2, 5 or 10% NaCl in distilled water). Then the bundles were rinsed in distilled water, acetone dehydrated and embedded in Epon 812. Blocks were prepared for longitudinal sectioning as shown in Figure 3. The silver sections, which were cut using a diamond knife on the JEOL UM7 ultramicrotome, were stained for 30 min in 2.8% uranyl acetate (50% methanol) at 48°C and for 5 min in lead citrate (pH 13). Sections were observed in a JEOL 100S at 80 keV.

Results

Physical measurements

Fababean and pea protein spinning is possible only when the dope protein content exceeds 10% and the pH is higher than 11 (Culiolli and Sale, 1981a). When the fababean protein dope pH varied from 11.6 to 13.2, the shear strength of the spun fababean fibres increased, whereas their moisture content and diameter decreased as shown in figure 4a. Similar behaviour was found with spun pea protein isolate. The salt content of washing baths had a great influence (figure 4b). Fibre diameter decreased from 67 to 47 μm, water content decreased from 61 to 48% and shear strength increased from 1.4 to 5.6 x 10⁵ N/m² as the salt content increased from 2 to 10%.

SEM

As observed with spun soybean proteins (De Man, 1976; Wolf and Baker, 1980), spun fababean proteins (Figs. 5 and 6) and spun pea proteins (Fig. 7) showed a cylindrical morphology with surface striations running along their length. Freeze-drying after freezing in isopentane cooled by liquid nitrogen was the best preparation technique (Fig. 5a). On freezing the fibers directly in liquid nitrogen (Fig. 5b) gas turbu-
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Fig. 4. Physical measurements on spun fababean protein fibres: a) evaluation of fibre bundle water content, shear strength and fibre diameter as a function of the dope pH (NaCl content of washing bath: 5%) and b) evaluation of fibre bundle water content, shear strength and fibre diameter as a function of bath NaCl content (dope pH: 12.6).

Fig. 5. SEM preparations of spun fibres: a) fractured spun protein fibre after freezing in isopentane (-150°C) and freeze-drying; b) structural deformation after freezing directly in liquid nitrogen and then freeze-drying and c) fractured spun protein fibre after glutaraldehyde fixation and alcohol dehydration.

(lence caused surface holes and some structural deformations, but the fibre displayed some longitudinal strand-like structure. In Figure 5c, fixation with 4% glutaraldehyde and dehydration in the presence of 5% NaCl, two different parts were apparent: 1) a granular central core; and 2) a more compact, strand-structured cortex.

Figure 6 shows spun fababean protein fibres prepared with increased salt concentration in the washing bath. At low salt concentration (2%), the central core was finely alveolated (Fig. 6a). At intermediate concentration (5%), the fibres appeared granular in structure as was also noted at high concentration (10%) for which the diameter was practically the same (Figs. 6b, 6c). It was noteworthy that NaCl crystals remained on the fibre surface as was demonstrated using microanalysis.

Figure 7 shows spun pea protein fibres prepared from dopes at various pH values. At low pH (Fig. 7a), the cortex was very thin (1 to 3 μm) and the central core appeared granular and very dispersed. At intermediate pH (Fig. 7b), the cortex was thicker (around 5 μm) and the internal structure was porous. At high pH (Fig. 7c), the entire fibre appeared compact with a few large vacuoles in the core. Under the same conditions, pea protein fibre diameters were thicker than those of fababean fibres.
Ultrastructure of spun pea and fababean proteins

TEM

Electron micrographs showed, as observed on spun proteins produced from meat waste (Young and Lawrie, 1975), that the fibres were constituted of spherical protein particles associated together in chains or strands. These chains were arranged in a three-dimensional network entrapping fat globules of various sizes and numerous vacuoles more or less anastomosed.

In the cortex where oblong fat globules and vacuoles were observed, orientation of protein strands along the fibre axis was quite evident (Figs. 8a, 8c, 9a, 9c). This observation is in opposition to those of Young and Lawrie (1975). In the central core of the same fibre (Figs. 8b, 8d, 9b, 9d) strands were randomly oriented. In fibres made from dope at pH 13.2 (Figs. 8e, 8f) protein strands were no longer visible. The structure with minute fat globules and very small vacuoles was very confused. Probably protein-protein interactions were higher at such pH giving hardening to the fibre and lower diameter.

Comparing spun proteins from dopes at pH 12.6, thinner strands were found in 2% NaCl (Figs. 8c, 8d) than in 5% NaCl (Figs. 9a, 9b) and 10% NaCl (Figs. 9c, 9d), the latter showing folded strands penetrating the fat globules and twisted-kinked strands hinting of higher viscosity of the dope. But protein strands alone were not responsible for these different structures.

Young and Lawrie (1975) reported that "poorly defined structures and large pores were found
Fig. 8. TEM of longitudinal sections of spun fababean protein isolate (NaCl content of washing bath : 2%). Sections a, c and e are from the fibre periphery and sections b, d and f are from the central core. Structural changes as a function of the dope pH: a-b) 11.6; c-d) 12.6; e-f) 13.2.


in the fibre giving rise to low mechanical strengths. In fact, the vacuolar system remained a major factor. At a low pH (11.6) the central core and the periphery were very alveolated. At an intermediate pH (12.6) the central core was more alveolated (larger vacuoles) than the cortex. At a high pH (13.2) pores were smaller. The vacuole ratio as a function of total volume of the fibre has been estimated on the TEM pictures (40,000 X) using statistical image analysis. Under the same conditions of salt concentrations (5% NaCl), vacuole ratios of spun fababean protein were 39%, 23% and 16% respectively for corresponding dope pH values of the spinning dopes (11.6, 12.6 and 13.2). At the same dope pH (12.6) vacuole ratios were 40%, 23% and 14% for corresponding values of 2%, 5% and 10% NaCl in the washing baths.

Discussion and Conclusions

Although Tombs (1970) and Young and Lawrie (1975) considered spun fibres as cylindrical gels without any sign of orientated strands, photomicrographs of longitudinal sections of various spun fibres have shown here that the structure is quite different. According to these results, macromolecular strands are orientated along the fibre axis,
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which could explain the surface longitudinal striation previously described (De Man, 1976; Wolf and Baker, 1980). This observation also confirms the hypothesis of Culioli et al. (1983) that molecular orientation results from shear and elongational flows (Fig. 2). The oriented structure of fibre cortex could be explained by: i) during the extrusion through the spinneret by greater lining-up of the macromolecules at the periphery due to higher strain rates near the wall of the pin holes and ii) at the spinneret exit by fast coagulation of the external part of the spun fibre. Under these conditions, the relaxation phenomena due to restitution of elastic energy could be reduced in the external part of the fibre and so, the structures fixed in the orientation induced by the shear flow through the spinneret. Proteins of the central core may then be randomly coagulated under relaxation into the available volume.

Culioli and Salé (1981 a,b) hypothesized about changes that occurred in the dopes. When dope pH was below 11 in the aqueous protein solution, proteins would be hydrated and agglomerated. Under these conditions spinning is practically impossible. When dope pH exceeded 11, increase in dope consistency should be related to the dissociation of protein aggregates into subunits and to the unfolding of polypeptide chains. This unfolding favours lining-up of the chains and protein-protein interactions. Then the structure of the fibres changes from an aggregate of spherical particles (Fig. 6a) to a tridimensional network (Fig. 6b) with longitudinally oriented subunit-like strands. When dope pH is above 12.5, a large decrease in the consistency of dopes is observed. As proposed by Culioli and Salé (1981 a,b) three factors should be responsible for this large decrease in consistency: i) an increase in electrostatic repulsive forces due to the deionization of the guanidyl groups of arginine; ii) the breaking of disulfide bonds; iii) the destruction of hydrophobic areas. Increased unfolding of protein chains is then obtained in the dope which induces more protein-protein interactions during the coagulation in the bath. Resulting fibres appeared more compact, the vacuolar system being three times smaller than at pH 11.6. In the fibres from the most viscous dope, kinked strand appearance is the result of elongated forces.

Concerning the effect of the salt washing baths, it seemed (Figs. 8c, 8d, 9c, 9d) when the salt concentration was too weak that the protein aggregates were less individualized and the protein strands thinner. On the contrary, at higher salt concentrations the orientation of the structure was better seen. It was hypothesized that when salt concentration is high a salting out effect would provoke the protein-protein interactions; conversely, when the salt concentration is too low a salting in effect would give rise to some protein solubilization.

In conclusion, the present study has shown the important influence of dope pH and salt washing bath concentrations on spun protein structure and texture. These results allow one to determine these processing parameters in order to
get the most appropriate texture for the food fibre which, as a meat analogue, must be neither too dry nor hard and the aminoacids must not be modified.

Similar behaviour was noted with both fababean and pea spun proteins. SEM and TEM images were complementary but, although SEM showed a fibre diameter evolution, the diameters could obviously not be measured after conditioning (drying) or TEM fixation, but only on wet fibres with light microscopy.

With image analysis, the great importance of vacuole ratio on texture and fine structure has been shown.

Contrary to previous studies, structural strand orientation along the fibre axis and the double cortex-core structure were proven.

References


Discussion with reviewers

K. Saio: How did you prepare pea or fababean isolates? Did you purchase commercial products?

Authors: Protein isolates were prepared at the pilot plant of our research center from flours of hulled pea (var. Primas) and fababean (var. Ascott). The proteins were solubilized at pH 7 in a slightly alkaline solution. The insoluble parts of the flours were discarded by centrifugation. Then the proteins were precipitated at pH 5.3 and spray-dried after centrifugation and washing.

K. Saio: Fat globules are observed in the TEM micrographs (Fig. 8 or 9). What percentage of fat did you have in the isolates which you used?

Authors: The fababean and pea protein isolates contained 5.9 and 6.5% fat, respectively, on a dry basis.

K. Saio: Did the vacuoles shown in TEM micrographs (for instance, Fig. 7c) correspond to the vacuoles in SEM micrographs?

Authors: Yes, they did. But it is noteworthy that at high pH (13.2) and 5% NaCl content of washing bath, the protein network was more compact with very minute vacuoles (less than 0.1 μm). It was not possible to show that on the TEM micrographs owing to the magnification we used.

K. Saio: The speed of extruding through the die is higher in outer layer of fibre than the inside because of resistance to inner surface of the die whereas the one of expanding and stretching in the coagulating bath is contrary. What do you think about this point? Do you think that the effects of expansion in the coagulating bath are more important than the effects of extrusion through the die for constructing the microstructure of spun fibres?

Authors: The main factor which influences the macrostructural orientation during the flow
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through the spinneret is not the velocity but the strain rate. This factor depends not only on the flow rate but also on the radius of the spinneret holes. It equals zero at the center of the spinneret hole and reaches a maximum value at the wall \( \dot{\gamma} = 4Q/R^2 \), with \( Q = \) flow rate; \( R = \) radius; \( \dot{\gamma} = \) shear strain rate).

Therefore, the orientation of the macromolecules is higher at the periphery than at the center. During the expansion of the dope filament, the macromolecules are disorganized at the spinneret exit. However, at the fibre surface, the coagulation occurs very rapidly which limits the influence of the relaxation while in the core the molecules can relax before the coagulation.

For the orientation developed in the spinneret to be effective in contributing to fibre microstructure, it must be quenched before it is relaxed. So the rate of coagulation must be greater than the reciprocal relaxation time. According to Ziabicki (1967) such is not the case in the textile fibre spinning process except for the thin surface layer. This is confirmed in the case of protein spinning by the double micro-structure observed in the fibre.

W.J. Wolf: Have you examined fibers made without stretching during the spinning process? This may give some information about the importance of stretching as it relates to orientation of protein molecules within the fibers and the diameter of the fibers.

Authors: It is not possible to get fibres without any stretching in the coagulation bath as the liquid filaments associate with each other at the exit of the spin holes and form globules. However, spinning with stretching rates lower than the one used (150%) produces fibres with larger diameter and very low mechanical resistance.

Although we have not realized microstructure observations, these results tend to demonstrate that less orientation is obtained when stretching decreases. During stretching the free fluid jet is subjected to axial tension which: i) limits the relaxation phenomena at the spinneret exit and ii) increases molecular orientation in the elongational flow.

According to Ziabicki (1967) this mechanism can be considered as the most important one in fibre spinning.

J.M. deMan: If there is a difference in structure between cortex and core, it should be of interest to compare fibers produced with spinnerets yielding fibers of different diameter, have the authors done this? If not, is there any information in the literature?

Authors: Although we have used spinnerets of various diameters (from 100 to 150 \( \mu \)m) we have not studied the influence of this parameter on the fibre microstructure. On the other hand, we have not found any information in the literature about relationships between spinneret diameter and the cortex-core structure.

We have emphasized in the previous answers the prominent part played by the shear strain rate in the spinneret, the relaxation at the spinneret exit, the coagulation rate in the acid and salt bath and the elongation strain rate in the macromolecule orientation mechanisms.

A variation in the spinneret diameter induces modifications of these parameters, which can explain some differences in the microstructure. It is likely that spinning with increased spinneret diameter without any modification of the other processing conditions (dope flow rate, fibre take up velocity ...) produces fibres with larger diameters and with a more developed core structure.
EFFECT OF ENVIRONMENT ON THE PHYSICAL STRUCTURE OF THE PEANUT (Arachis hypogaea L.)

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Abstracts

Peanuts produced under the drought conditions of 1980 were marred by off-flavors when processed. Several physical characteristics of these peanuts were noted to be related to these flavor problems. This paper deals with the investigation of these physical peculiarities using scanning electron microscopy. Major findings include previously unreported physical abnormalities such as: (1) tissue damage which appears as spotting on the outer surface of the cotyledon which is a result of cracking and fissuring; (2) tissue damage which appears as a narrow band along the interface of the outer rounded surface and the flattened edge of the inner surface; and (3) a flattened inner surface of the cotyledon.

Introduction

Drought conditions persisted throughout the summer of 1980 in all three of the United States peanut growing regions (USDA, 1981). Temperatures were above normal and rainfall was well below average for the summer months. By August, temperatures were abnormally high setting records in some areas. In December, our peanut laboratory was involved in the evaluation of these peanuts because of a severe off-flavor problem. In the observation of these peanuts, the following abnormalities were noted: (1) tissue damage which appeared as spotting on the outer surface of the cotyledon; (2) tissue damage which appeared as a narrow band along the edges of the inner surface of the cotyledon; and (3) a flattened inner surface of the cotyledon without its characteristic indentation. Similar observations were made with peanuts grown in the ensuing years under similar conditions of growth. Thus, it appears that changes in the structural features may be related to changes in flavor of peanuts.

Peanut seed anatomy and cytology have been investigated by Woodroof and Leahy (1940), Yarbrough (1949), Bagley et al. (1963), Jacks et al. (1967), and Vaughan (1970). More recently, observations were made on the appearance of cell walls and the major subcellular components of both normal and pressed peanuts (Schadel et al., 1983). Also, light and scanning electron microscopy clearly showed pitting of parenchyma cell wall in normal peanut seed.

In accordance with the statement of Chabot (1979) that "the goal of the food scientist is to understand structural features of a material that are important in its functional role in food," we used scanning electron microscopy (SEM) for this study to examine structural differences between normal and environmentally stressed peanuts in an attempt to learn more about the physical defects in stressed peanuts and their possible role in causing flavor defects.

Materials and Methods

The cotyledons of resting peanut (Arachis hypogaea L. cv. Florunner) seed were examined with a dissecting microscope for physical structural characteristics. They were divided into two groups: (1) cotyledons with normal physical characteristics associated with environmentally unstressed peanuts; and (2) cotyledons with abnormal physical characteristics associated

Key Words: Arachis hypogaea, peanut, cotyledon, environment, anatomy.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service, nor criticism of similar ones not mentioned.
with environmental stress. Whole cotyledons, transverse sections of the mid-region of cotyledons, and tissue blocks (2 mm³) from the mid-region and the outer and inner surfaces of the cotyledons were fixed in 4% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.0, for 48 hours. The samples were then rinsed and post-fixed in 1% OsO₄ in 0.05 M sodium cacodylate, pH 7.0 for 2 hours. After washing in 0.05 M sodium cacodylate buffer, the tissue blocks were trimmed slightly to expose the cells for observation for which the cellular contents had not been disturbed by the initial pre-fixation cutting. The samples were dehydrated in a graded series of aqueous ethanol (10, 25, 50, 75, 95, and 100% ethanol) followed by a graded series of ethanol-amyl acetate (10, 25, 50, 75, 95, and 100% amyl acetate). Carbon dioxide was used as the transitional fluid in a Ladd Critical Point Dryer. The tissue was then gold-coated in a Polaron E 5000 Sputtering System. Samples were observed and photographed at 20 kV with an ETEC Autoscan microscope.

Fig. 1. Normal peanut structure: (a) Epidermal cells of the outer rounded surface of the cotyledon; (b) Epidermal cells with stomata (S) on the inner surface of the cotyledon; (c) Transverse section of the isodiametric parenchyma cells; and (d) Transverse section of a single parenchyma cell. Arrows point to the membrane-limited spherosomes (lipid bodies) about 1-2 μm in diameter, among the larger protein bodies and starch grains. Bars = 400 μm.

Results and Discussion

Normal Peanut Structure

The embryo of the resting peanut seed consists of two cotyledons and a small radicle and plumule known as the germ. Processors are primarily concerned with the tissue of the peanut seed cotyledons which constitutes about 96% of the seed weight. Each peanut seed cotyledon consists of epidermal, vascular, and parenchymal tissue. Figures 1 thru 4 depict the morphological and anatomical features of peanut cotyledon structure pertinent to this study. The epidermis is made up of a single layer
of cells which covers the surface of the cotyledon. The epidermal cells of the rounded outer surface are more or less rectangular in outline (Fig. 1a). The epidermal cells of the inner surface are irregular in outline and contain numerous stomata (Fig. 1b).

The vascular tissue of the peanut seed extends through each cotyledon of the embryo. Woodroof and Leahy (1940) described the vascular system as one series of six to eight bundles which follow the curvature of the outer surface and another series of four to six centrally located bundles. This vascular tissue comprises only a small part of each cotyledon.

The majority of the tissue of the cotyledon is made up of rather large, almost isodiametric parenchyma cells (Fig. 1c-1d). The pitted walls of the resting seed parenchyma cells have conspicuous depressions (Fig. 4e). The wall depressions have been described by numerous workers (Woodroof and Leahy, 1940; Vaughan, 1970; Yatsu, 1981; Schadel et al., 1983).

The major subcellular organelles of the parenchyma cells are spherosomes (lipid bodies), protein bodies, and starch grains. The transmission electron microscope has been used by Jacks et al. (1967) and Neucere and Hensarling (1973) to characterize the spherosomes as particles about 1.0–2.0 microns in diameter bounded by a limiting membrane. After OsO₄ fixation, the limiting membranes of the spherosomes (lipid bodies) are observable with the scanning electron microscope (Fig. 1d) and create a “honeycomb effect” which appears around the protein bodies and starch grains.

### Environmentally-Stressed Peanut Structure

One of the physical peculiarities observed in some environmentally stressed peanut cotyledons is tissue damage which appears as a narrow band along the interface of the outer rounded surface and the flattened edge of the inner surface. The unsectioned appearance of this interface in a normal peanut cotyledon is smooth and regular (Fig. 2a). The corresponding tissue has a typical ordered appearance when viewed in a transverse section (Fig. 4a). The unsectioned appearance of this interface in an environmentally stressed peanut cotyledon is rough and irregular (Fig. 2b). The corresponding tissue is characterized by cellular disruption and tissue disorganization when viewed in a transverse section (Fig. 4b).

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**Fig. 2.** Morphology of the inner surface of the cotyledon: (a) Normal cotyledon with smooth and regular edges (arrow); (b) Environmentally stressed cotyledon with rough and irregular edges (arrow) due to tissue damage; (c) Transverse section of the normal cotyledon which reveals the indentation (arrow) which transverses the longitudinal axis; and (d) Transverse section of the environmentally stressed cotyledon which reveals the flattened inner surface (arrow). Bars = 1.0 mm.
Fig. 3. Tissue disruption on the environmentally stressed cotyledon: (a) Outer rounded surface with tissue damage. Bar = 1.0 mm; (b) Fissure on the outer rounded surface with cellular contents extruded. Note the spherical thickened mass of extruded cellular contents (arrow). Bar = 50.0 μm; (c) Transverse section of a fissure on the outer surface. Bar = 50.0 μm; and (d) Transverse section of a fissure revealing amorphous coagulated cytoplasm. Bar = 200 μm.

A second physical peculiarity observed in some environmentally stressed peanut cotyledons is the absence of the characteristic indentation which traverses the longitudinal axis of the inner surface. This structural difference can best be observed in transverse section of the entire cotyledon (Fig. 2c–2d).

A third physical peculiarity observed in environmentally stressed peanut structure is the cracking and fissuring of the cotyledon surface which appears as spotting on the outer surface when viewed with the unaided eye. These physical disruptions are easily observed with the SEM and are characterized by the extrusion of coagulated cellular contents onto the surface of unsectioned cotyledon (Fig. 3a–3b). The cellular contents which have been extruded are referred to as "coagulated" in the sense of a fluid which has changed into a thickened mass. In transverse section, the coagulated cellular contents can be seen to extend to the depth of the fissures (Fig. 3c–3d). This fissuring of tissue and disruption of cellular contents is believed to be the primary source of the off-flavor problems associated with environmentally stressed peanuts. The fissuring is probably
Physical Structure of the Peanut
caused by the lack of water. This would allow air to enter the tissue and promote oxidation of the cell contents, especially unsaturated lipid substances. Further investigations of the extruded cellular contents are necessary to characterize their chemical nature more specifically.

Transverse sections taken from the middle of the inner and outer surface of the normal peanut cotyledon and from identical regions in uncracked areas of environmentally stressed cotyledon revealed no unusual physical characteristics. The cellular contents and tissue organization of the middle of the inner surface in the normal cotyledon with its strong longitudinal indentation (Fig. 4c) were comparable to the cellular contents and tissue organization of the flattened inner surface of environmentally stressed cotyledons (Fig. 4d). Thus, it appears that the lack of the characteristic indentation in the environmentally stressed cotyledon is a manifestation of incomplete morphological development which does not result in cellular and tissue abnormalities. Cellular contents and tissue organization were also comparable in transverse sections taken from the outer rounded surface of normal cotyledons (Fig. 4e) and from uncracked areas of the outer rounded surface of environmentally stressed cotyledons (Fig. 4f).

Acknowledgement

Paper No. 9127 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC.

References


ULTRASTRUCTURAL STUDIES OF RAW AND PROCESSED TISSUE OF THE MAJOR CULTIVATED MUSHROOM, 
AGARICUS BISPORUS

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Abstract

Commercial mushroom processors currently lose approximately 30 percent of the mushroom weight due to shrinkage during processing (blanching and canning), resulting in substantial economic losses. Microscopy was used to assess the extent and type of chemical and structural changes induced by processing mushrooms and causing shrinkage. Scanning electron microscopy revealed that the processing operations including vacuum hydration, blanching, and thermal treatment do not damage the integrity of the tissue. Light microscopy revealed that the morphology of the tissue, shape and spacing of cells, appear similar for raw and processed mushroom tissue. However, the intracellular material remained indistinct for both tissue types, and the processed tissue appeared distorted. Transmission electron microscopy revealed that commercial mushroom processing caused intracellular damage to the tissue. The heat treatment caused the coagulation of cytoplasmic material and the disruption of intracellular membranes, resulting in the loss of water holding capacity of the tissue. Therefore, shrinkage of processed mushrooms results from "denaturation" of the organelles and the associated loss of water holding capacity by those organelles.

Introduction

Shrinkage, the loss of weight during processing (blanching and canning), continues to plague the mushroom processing industry. Weight losses can range as high as 30 percent, which results in substantial economic losses to the processor (Eby, 1975). Shrinkage results from a loss of water and water soluble solids from the mushroom tissue. However, the cause of this loss is not totally understood. Microscopy can be used in determining the severity of mushroom processing or in assessing the effects of processing and/or handling on the quality of mushrooms. Therefore, an ultrastructural examination of raw and processed (blanched and canned) mushroom tissue should provide insight into the causes of mushroom shrinkage.

Agaricus bisporus (Lange) Sing is the major commercially cultivated mushroom in the world (Chang, 1980). Information is available on the structure and ultrastructure of raw mushroom tissue; however, minimal information is available on the ultrastructure of processed mushroom tissue. The general ultrastructure of fungi and the ultrastructure of Agaricus bisporus have been investigated (Bracker, 1967; Beckett et al., 1974; Angel-Papa and Eyme, 1978; Alexopoulos and Mims, 1979). These investigations pertain to the stipe elongation mechanism (Craig et al., 1977a; Craig et al., 1979), the cell wall structure (Novais-Ledieu and Garcia-Mendoza, 1981), basidia and septal pore structures (Craig et al., 1977b; Flegler et al., 1976), cell membrane development (Eyme and Angel-Couvy, 1975), the basidiospore structure (Elliott, 1977; Pest and Holenstein, 1977), and nuclear distribution (Wang and Wu, 1974). However, the relationship of mushroom ultrastructure to processing has not been investigated.

Mushroom processing operations have two major purposes: a) to provide a sterile product and b) to minimize shrinkage of mushroom tissue, to maximize canned product yield. Various processes that limit shrinkage have been reported (Gormley, 1972; Mc Ardle et al., 1974; Beelman and Mc Ardle, 1975; Steinbuch, 1979). Gormley (1972) found that freezing unblanched mushrooms produced a higher quality product than freezing blanched mushrooms. Steinbuch (1979) found that vacuum packing of

KEY WORDS: Agaricus bisporus, Ultrastructure of raw mushroom, Ultrastructure of processed mushroom, Mushroom, Canning, Transmission electron microscopy, Scanning electron microscopy, Light microscopy.
unblanched frozen mushrooms showed promise in improving mushroom quality and reducing shrinkage. Beelman and McArdle (1975) found that post-harvest storage of mushrooms at elevated temperatures (12°C) for 24 hr. to 48 hr., followed by soaking (PSU-3S-Process) or vacuum hydration (SSV-Process) prior to blanching, increased the canned product yield (decreased shrinkage). Increased yields due to storage at elevated temperatures supported the hypothesis that chemical changes within the mushroom tissue caused an increase in water retention by the tissue (Beelman and McArdle, 1975). The higher storage temperatures resulted in an increase in the mushroom tissue's ability to absorb more water before processing and retain more water during processing (Beelman and McArdle, 1975). These results indicate that a loss of water holding capacity or water binding capacity of the mushroom tissue during processing causes shrinkage.

Chemical changes in the protein structure of the mushroom tissue could produce the increase in water holding capacity during storage (Beelman et al., 1973). However, the specific chemical and structural changes that occur during processing to cause shrinkage remain unknown. Therefore, this investigation uses microscopy to assess the extent and type of chemical and structural changes induced by processing (blanching and canning) mushrooms.

Results and Discussion

Typical commercial mushroom processing in the United States (Fig. 1) involves washing the mushrooms, then cold storage for up to 48 hours, and applying a vacuum hydration treatment, which completes the pre-processing steps. The remaining steps of the process include blanching, filling, brining, closing, thermal processing, cooling, and storage. The major goal of this process is for the mushroom tissue to absorb as much water as possible during soaking and vacuum hydration so that the water lost during blanching and thermal processing is mostly the absorbed water. Scanning electron, light, and transmission electron microscopy were used to determine the effects this process had on mushroom tissue.

The scanning electron micrographs of the stem, cap, and gills (Figs. 2-6) show that the absorption of water during vacuum hydration and subsequent loss of water during blanching and thermal processing does not destroy the overall integrity of the tissue. Filaments or hyphae compose the outer surface of the stem (Fig. 2) and the cap (Fig. 4). The longitudinal filaments in the raw (Fig. 2a) and processed stem (Fig. 2b) are bunched together. However, the filaments in the processed tissue appear deflated or shriveled (Fig. 2b). Monacha (1965) described the stem as a combination of wide inflated hyphae and narrow threadlike filaments. The cross-sectional view of the stem (Fig. 3) shows the gills are separated into a trilaminar hyphae. The compact filaments of the processed tissue (Fig. 3b) with gaps between groups of filaments reveal the same distortion seen on the outside surface of the stem (Fig. 2b). Random crossing hyphae compose the cap (Fig. 4). This random crossing leaves spaces wide enough for bacteria to enter and grow in the mushroom tissue. In both cases, the filaments increase in chitin and water contents as the mushroom grows and the cells elongate (Ronner et al., 1956; Webster, 1970).

Individual club shaped basidia cover the outside surface of the gills. Rough, distorted club shaped basidia cover the processed gills (Fig. 6b) while smooth, distinct club shaped basidia cover the raw gills (Fig. 6a). Finally, the processed gills (Fig. 5b) appear more compact than the raw gills (Fig. 5a), which could be a possible indicator of shrinkage.

While certain parts of the mushroom tissue such as the stem and cap appear shriveled and distorted, the overall integrity of the tissue has not been lost as a consequence of

Materials and Methods

Samples

Raw cultivated mushrooms, Agaricus bisporus (Lange) Sing were obtained from The Pennsylvania State University Mushroom Research Center and the commercially processed samples were canned buttons grown and packaged at Pennsylvanian. Waterholding capacity or water binding capacity of the mushroom tissue during processing causes shrinkage.

Chemical changes in the protein structure of the mushroom tissue could produce the increase in water holding capacity during storage (Beelman et al., 1973). However, the specific chemical and structural changes that occur during processing to cause shrinkage remain unknown. Therefore, this investigation uses microscopy to assess the extent and type of chemical and structural changes induced by processing (blanching and canning) mushrooms.
Ultrastructural Studies of *Agaricus bisporus*

**OUTLINE OF COMMERCIAL MUSHROOM PROCESSING OPERATION**

- Fresh Mushrooms
- Wash & Soak
- Cold Storage
- Vacuum Hydration
- Blanching
- Warehousing
- Thermal Processing
- Filling Brining Closing

**Fig. 1.** Flow chart of a typical commercial mushroom processing operation.

**RAW**

**PROCESSSED**

**STEM OUTSIDE SURFACE**

**Fig. 2.** Scanning electron micrograph of the outside surface of raw (a) and processed (b) mushroom stem.

**RAW**

**PROCESSSED**

**GILLS OUTSIDE SURFACE**

**Fig. 4.** SEM micrograph of the outside surface of raw (a) and processed (b) mushroom cap. Arrows indicate gaps large enough for bacteria to enter.

**RAW**

**PROCESSSED**

**STEM CROSS SECTION**

**Fig. 3.** SEM micrograph of the cross-section of raw (a) and processed (b) mushroom stem. Arrows indicate septa.

**RAW**

**PROCESSSED**

**GILLS OUTSIDE SURFACE**

**Fig. 5.** SEM micrograph of the outside surface of raw (a) and processed (b) mushroom gills.

**RAW**

**PROCESSSED**

**Fig. 6.** SEM micrograph of the outside surface of raw (a) and processed (b) mushroom gills.
processing (Figs. 2-6). This indicates that the large amount of water forced into the mushroom tissue during vacuum hydration does not damage the external cellular structure. The loss of water from the tissue during blanching and thermal processing results in the shriveled and distorted appearance of the tissue. The second portion of this investigation used light microscopy to determine if the general morphology changed during processing.

Light microscopy revealed several characteristics present in all sections of the mushroom tissue. First, the morphology of the tissue, shape and spacing of cells, appear similar for the raw and processed mushroom tissue. Sclerotium hypophyseum composed the cap and the stem with random crossing hyphae in the cap and longitudinal hyphae in the stem. The gills contain three layers of cells (Craig et al., 1977b), which include trama cells (T), subhymenial cells (SH), and hymenial cells (H) (Fig. 7). Second, the intracellular material of the raw and processed tissue remained indistinct (Fig. 7). Finally, all of the processed tissue appeared distorted although the organization remained the same as in the raw tissue (Fig. 7).

The transmission electron micrographs (Figs. 8-10) revealed that commercial mushroom processing results in damage to the intracellular material (Figs. 8b,9b,10b). The raw mushroom tissue (Figs. 8a,9a,10a) contained identifiable intracellular material characteristic of basidiozymes (Bracker, 1967; Beckett et al., 1974; Eyme and Angeli-Couvy, 1975; Craig et al., 1977a; Alexopoulos and Mims, 1979; Craig et al., 1979). The structures include cell walls (W), cellular membranes (CM), nucleus (N), nuclear membranes (NM), mitochondria (M), concentric lamellae membranes (CL), electron dense bodies (DB), lipid droplets (D), and vacuoles (V). The damaged processed mushroom tissue lacks identifiable cellular material except for remnants of the cell walls (W), cellular membranes (CM), dolipore septum (S), and vacuoles (V). The remaining cellular material appears as clusters of precipitated electron dense material (D) throughout the cells (Figs. 8b,9b,10b).

Several of the consequences of commercial mushroom processing appear in the transmission electron micrographs (Figs. 8-10). First, as previously mentioned, the intracellular material appears as electron dense clusters rather than as distinct organelles (Figs. 8b,9b,10b). The heat treatment applied during processing caused the coagulation of cytoplasmic material such as proteins and the disruption of the compartmentalizing intracellular membranes (Figs. 8b,9b,10b). The coagulation and the disruption of intracellular membranes result in the loss of water holding capacity of the tissue so that the tissue shrinks to 80 percent of its original weight. However, the intact cell wall and coagulated material traps the remaining water in the tissue.

Two types of tissue are present in the mushroom, the stem and cap, and the gills. The stem and cap contain large vacuoles in the center of the cells (Figs. 8a and 9a), which press the cytoplasmic material near the cell wall (Fig. 9a). Therefore, the majority of coagulated electron dense material remains near the cell wall in the processed tissue (Fig. 8b). The gills contain the greatest amount of intracellular material (Fig. 10a) due to the gills being the reproductive region of the mushroom and these regulate other functions such as stipe growth (Hagimoto and Konishi, 1972). In this case, the electron dense material generally appears in the center of the cell leaving gaps between it and the cell wall (Fig. 10b). These gaps may permit the gill tissue to lose more water than the cap or stem during processing.

Second, the outer layer of the cell wall at the tips of the basidia appear broken and separated from the rest of the cell wall (Fig. 10b, arrows). This phenomenon did not appear in any other section of the mushroom tissue. The broken cell wall may allow more water to leave the tissue.

Finally, mushroom processing causes distortion in the processed tissue (Figs. 8b,9b,10b). The distortion, most apparent in the gill tissue (Fig. 10b), appeared as irregular shaped cells with gaps or channels between cells. Commercial processes allow the absorption of water into the mushroom tissue. However, whether the water is absorbed directly into the cells or into the gaps between the cells has not been determined. In any case, processing releases all of the absorbed water plus part of the original water from the tissue, causing the distortion of cells and channels between cells seen in the processed tissue (Figs. 8b,9b,10b).

Summary and Conclusions

Commercial mushroom processing appreciably alters the intracellular organization of the mushroom tissue causing shrinkage. Scanning electron micrographs showed that processing did not destroy the surface structural features of the mushroom tissue. However, the processed tissue appeared shriveled and distorted, though intact. Light micrographs revealed similar cell walls for raw and processed tissue, but the processed cells appeared distorted. Transmission electron micrographs showed that processing caused intracellular damage to the mushroom tissue. All cellular organelles became coagulated electron dense material resulting in a loss of water holding capacity of the organelles. This results in shrinkage of the tissue due to a loss of water and water soluble solids. Therefore, the loss in canned product yield of processed mushrooms results from "denaturation" of the organelles and the associated loss of water holding capacity by those organelles.

Acknowledgement

Published as Paper No. 6738 Journal Series of The Pennsylvania Agricultural Experiment Station, University Park, PA 16802
Ultrastructural Studies of *Agaricus bisporus*

**Fig. 7.** Light micrograph of the cross-section of the gills for raw (a) and processed (b) mushroom. Trama (T), subhymenial (SH), and hymenial (H) cells are visualized.

**Fig. 8.** Transmission electron micrograph of the longitudinal cut of the raw (a) and processed (b) mushroom stem. Nucleus (N), cell walls (W), mitochondria (M), concentric lamellae (CL), dolipore septum (S), vacuoles (V), and electron dense material (D) are observed.

**Fig. 9.** TEM micrograph of the cross-section of the raw (a) and processed (b) mushroom stem. Nucleus (N), cell walls (W), cellular membranes (CM), mitochondria (M), and electron dense material (D) are observed.

**Fig. 10.** TEM micrograph of the cross-section of the gills for raw (a) and processed (b) mushroom contains nucleus (N), lipid droplets (O), vacuoles (V), concentric lamellae (CL), and electron dense material (D). The arrows indicate partially broken cell wall.

**References**


Craig GD, Newsman RJ, Gull K, Wood DA. (1977b). Subhymenial branching and dolipore septation in...


Discussion with Reviewers

D.A. Wood: Why were only the beginning and end stages of the process examined since the changes observed may have occurred during one of several processes?

Authors: The raw mushroom tissue was impregnated with glutaraldehyde under conditions similar to the vacuum hydration process (see Materials and Methods section). Since all cellular structures were observed in the raw tissue, the vacuum hydration process does not cause the change in the tissue. The thermal treatment accorded to the tissue during blanching is less severe than thermal processing. Also, most of the material lost from the mushroom during blanching was water impregnated into the tissue during vacuum hydration (Beelman and McArdle, 1975). Therefore, we felt that the significant change in the ultrastructure of the tissue results from thermal processing; hence, the reason for examining the beginning and end stages of the process.

D.A. Wood: Could you produce these changes merely by thermal processing?

Authors: Yes.

F. Ingratta: In what way do the authors feel the freezing method of processing mushrooms would affect the cell ultrastructure?

Authors: Integrated Quick Blanching (IQB) and Integrated Quick Freezing (IQF) methods currently used by the frozen mushroom industry, insures the creation of small uniform ice crystals in the frozen product. Assuming minimal thermal shock during storage, we would speculate that the ultrastructure of frozen mushroom tissue would be similar to that of raw mushroom tissue. If thermal shock becomes a factor leading to the formation of large ice crystals, resulting from the migration of intracellular water to intercellular water, the rupture of cell membranes would be expected. However, we would not necessarily expect to visualize the electron dense material that was seen as a result of thermal coagulation.

D.A. Wood: What is the chemical composition of the solids lost from the mushroom tissue during processing?

Authors: The water absorbed by the mushroom tissue during vacuum hydration is the major component lost during processing (Beelman and McArdle, 1975; Steinbuch, 1978). This water contains all of the water soluble proteins originally present in the mushroom tissue (Eby, 1975). However, further chemical analysis of the solids lost from the mushroom tissue during processing was not conducted.
FOOD MICROSTRUCTURE, Vol. 3 (1984), pp. 197-198
SEM Inc., AMF O'Hare (Chicago), IL 60666 U.S.A.

TECHNICAL NOTE

A SIMPLE PROCEDURE FOR THE PREPARATION OF STIRRED YOGHURT FOR SCANNING ELECTRON MICROSCOPY

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Abstract

Stirred yoghurt is aspirated into agar gel tubes having 1.2 mm inner diameter, fixed in glutaraldehyde, dehydrated in ethanol, freeze-fractured under liquid nitrogen, and critical-point dried. Agar gel encapsulation protects the sample and prevents it from disintegration during the preparative steps. Scanning electron microscopy of the mounted fragments reveals the corpuscular microstructure of this type of yoghurt which develops due to stirring and pumping of the product during manufacture.

Introduction

Development of microstructure in set-style yoghurt was studied by electron microscopy (3-6, 8). Scanning electron microscopy (SEM) in particular has been found to be useful to show the porosity of the protein matrix (5), distribution of lactic acid bacteria (8), and the presence of fat globules in yoghurt made from whole milk (1). Preparation of stirred yoghurt for SEM is difficult due to disruption of the rigid gel matrix during manufacture caused by mechanical agitation to produce a smooth flowing viscous product. If a sample of stirred yoghurt is placed in an aqueous fixative, the broken matrix disperses and the sample disintegrates. However, the Salyaev's (7) procedure, initially devised to encapsulate liquid samples, including milk (2), destined for embedding in a resin for sectioning for transmission electron microscopy (TEM) was found suitable to preserve the liquid yoghurt samples during preparative steps for SEM. The objective of this technical note is to describe the use of the encapsulation procedure in SEM and show micrographs of stirred yoghurt.

Materials and Methods

Agar sol (2%) was made using distilled water and was maintained at 40°C with constant stirring. A glass rod (a flame-sealed Pasteur pipet, 1.2 mm in outer diameter) was dipped repeatedly into the agar sol and rotated until the sol gelled and formed a uniform thin (0.3-0.5 mm) layer. The tower and upper parts of the gel tube were trimmed to form a 20 mm long gel sleeve on the glass rod.

Commercial stirred-style yoghurt samples varying in consistency were used in this study. A volume of approx. 3.5 mL of the yoghurt was placed on a glass plate and a small part of it was aspirated into the agar gel tube as shown in Fig. 1. Because of the high viscosity of the yoghurt and the relatively large diameter of the soft agar gel tube, aspiration of the yoghurt was done very slowly at a low angle; a 10 to 12 mm column of the yoghurt was aspirated followed by the aspiration of 1 mm of air. The end of the agar gel tube was gently blotted with tissue paper and sealed with 2 drops of warm agar sol. The gel tube was trimmed at the upper end 1 mm away from the yoghurt column and was also sealed with agar sol.

Yoghurt samples thus encapsulated were fixed in a 3.5% glutaraldehyde solution at 6°C for 24 h, dehydrated in a graded ethanol series, frozen in Freon 12 at -150°C, freeze-fractured under liquid nitrogen, melted in absolute ethanol, and critical-point dried from carbon dioxide. The fragments were mounted on aluminium SEM stubs using silver cement, sputter-coated with gold, and examined in a Cambridge Stereoscan Mark II scanning electron microscope operated at 20 kV.

Results and Discussion

Two steps are essential to this procedure: immobilization of the liquid sample in the agar gel tube and freeze-fracturing to obtain smooth fracture planes suitable for SEM examination. The sample to be examined by SEM is considerably larger than that destined for TEM. Also, because stirred yoghurt is a dense and viscous suspension of casein micelle clusters, it is easier to aspirate it into agar gel tubes of a diameter larger than that used for TEM.

During all the preparatory steps, the agar gel tube remained to be part of the sample. There was no separation of the yoghurt sample from the agar gel (Fig. 2) and the gel appeared to be relatively dense (Fig. 3). The sample fragments remained cohesive and were easy to mount on metal stubs using silver cement. Sputter coating provided sufficient conductivity to examine the sample at 20 kV without encountering charging artefacts. Freeze-fracturing revealed the corpuscular microstructure of the sample (Fig. 4). However, a detail of casein micelle chains and clusters in Fig. 5 is in agreement with images obtained with set-style yoghurt (5, 8).

Thus, this simple procedure makes it possible to prepare stirred yoghurt for SEM and to study the effects of manufacturing conditions on the dimensions and distribution of protein particles. It is probable that other similar foods such as cultured buttermilk can be prepared for SEM using this procedure.

Acknowledgments

The authors thank Dr. H. W. Modler for useful suggestions. Electron Microscope Centre, Research Branch, Agriculture Canada in Ottawa provided facilities. Contribution 623 from the Food Research Institute in Ottawa.

KEY WORDS: Agar gel; Encapsulation; Freeze-fracturing; Scanning electron microscopy; Stirred yoghurt.
Fig. 1. Aspiration of stirred yoghurt into an agar gel tube (A) and its sealing (B and C).


Fig. 2. SEM micrograph of a stirred yoghurt (y) fragment encapsulated in an agar gel tube (a).


Fig. 3. Detail of the dense microstructure of the agar gel tube.


Fig. 4. Overall corpuscular microstructure of stirred yoghurt. Protein particles composed of casein micelles are marked with asterisks.


Fig. 5. Detail of the casein micelle matrix.


References


LITERATURE ABSTRACTS

The purpose of this section is to provide readers of FOOD MICROSTRUCTURE with abstracts of papers on food microscopy, and related subjects. Abbreviations LM, EM, SEM, and TEM stand for light microscopy, electron microscopy, and scanning electron microscopy, respectively. The abstracts are either either author’s own or have been prepared by the editors. FOOD MICROSTRUCTURE invites abstracts from other journals; each abstract should bear the original title, with translation in English where applicable, names of all the authors, address of the senior author, and, in case of an invited paper, the name of the journal, year, volume, issue, and page numbers. Reprints can be supplied to the authors directly for reprints of articles. These abstracts have been compiled by Dr. Nilsos Kalab, with the assistance of Dr. K. K. Bajaj, Dr. Y. Perez, Dr. J. K. Sato, Dr. C. Voyle and Dr. K. P. Woll Original contact of Dr. Kalab if you wish to submit abstracts for FOOD MICROSTRUCTURE. The abstracts are organized as follows:

Milk, Dairy Products

Milk of Northern Furs Seal: Composition, Especially Carbohydrate and Protein


The methods of coagulation were enzyme-set, using 1% lactose culture plus microbial rennin at a rate of 37.5 mL per 454.5 kg milk-and-acid-set using the long-set procedure with 1% lactose culture. The enzyme-set cheese milk developed the desirable coagulum in 12 h whereas coagulation by the acid-set method required 26 h. Gases in each batch were obtained immediately after cutting, immediately after cooking, and immediately after draining. TEM (thin sections) examined the casein micelles, 82.1-86.6 nm for acid-set cheese compared to 175.4-184.3 nm for enzyme-set cheese.

Milk of Bovine Milk: Composition, Especially Carbohydrate and Protein


The methods of coagulation were enzyme-set, using 1% lactose culture plus microbial rennin at a rate of 37.5 mL per 454.5 kg milk-and-acid-set using the long-set procedure with 1% lactose culture. The enzyme-set cheese milk developed the desirable coagulum in 12 h whereas coagulation by the acid-set method required 26 h. Gases in each batch were obtained immediately after cutting, immediately after cooking, and immediately after draining. TEM (thin sections) examined the casein micelles, 82.1-86.6 nm for acid-set cheese compared to 175.4-184.3 nm for enzyme-set cheese.

Milk, Dairy Products

PRODUCTION OF EXTRUSION TEXTURIZED PROTEIN PREPARATIONS


Enzymatically prepared calcium caseinate was extruded at moisture contents of 15 to 30%, preparations of the highest expansion index (1.66) and the lowest density (0.72 Kg/dm³) were obtained when the extrusion was carried out at 140°C using an exhausting nozzle of 3 mm in diameter. The expansion temp. was higher by 15% than the temp. of the heating section at the main screw speed of 90 rpm/s, but the difference between both temperatures was insignificant when the screw speed was 10 rpm/s. SEM showed that the amorphous structure of the protein granulates was transformed by the extrusion process into partly anisotropic structure.

Milk, Dairy Products

COMPOSITION AND ULTRASTRUCTURE OF CALCIUM PHOSPHATE-CITRATE COMPLEXES IN BOVINE MILK SYSTEMS


The study shows that the colloidal calcium phosphate of cows’ milk has a Ca + Mg/P ratio of 1.69 (2.0±0.1; n = 22) and ammonium citrate, Mg, and Zn at molar ratio to Ca averaging 0.05, 0.03, and 0.003, resp. The composition of the natural colloidal calcium phosphate of milk is similar to the precipitate formed by neutralization of ultratilates obtained from acidified milk, and to that of the Ca phosphate-enriched fraction produced by extensive enzymic hydrolysis of the casein micelles in milk. This artificial preparations of milk Ca phosphate revealed in both a very fine and uniform substructure which consisted of granules having an average, true diameter of approx. 2.5 μm. The size and shape of these tiny granules closely resemble the morphology reported for the colloidal phosphate particles in native casein micelles, as well as for the subunits of amorphous Ca phosphate observed during calcification in other biological systems such as mitochondria and bone.

Milk, Dairy Products

INTERPRETATION OF THE MORPHOLOGY OF α-LACTOSE HYDRATE


The morph of the α-lactose hydrate crystal is complicated and cannot be easily predicted from the crystal structure. The normal notations, by which parameters are calculated for the strength of the connected nets or slices, viz ‘g’slice and ‘g’net yield the same morphological order, whereas the older ‘g’nk method leads to a somewhat different result. Both results strongly deviate from the observed morphology and do not take into account the polar character of the crystal.

By considering the blocking part of the intermolecular bonds by α-lactose, a corrected morphology can be derived.
which fits in with the morphological importance of the faces that appear on the crystal. The other faces derived are assumed to be absent because of the non-growing (011) face. 41 references.

EFFECT OF CALCIUM ON THE HYDRATION OF CASEIN. I. WATER VAPOUR SORPTION AND FINE STRUCTURE OF CALCIUM CASEINATES COMBINED WITH THE CHART. CASEIN (011) FACE.


Hydration of Ca and Na caseinates which were prepared from whole casein at different pH levels (range 4.7-8.0) was determined by means of water vapour sorption measurements in the water activity (a) range 0.58-0.85. Water uptake of Ca caseinates was systematically lower than that of Na caseinates prepared at equal pH, the differences increasing with increasing pH and a. Plots of the water content v. the amount of added hydroxide at constant a, revealed a linear relationship between water uptake and cation content, suggesting that the increase in water uptake is mainly determined by the amount and type of cations associated with the casein. In the a, range tested, Ca2+ absorbed about 2·7 and Na+ 3·18 mol water/mol of casein. This implied that replacement of one mol of casein-bound Na+ by Ca2+ is accompanied by a displacement of 1-11 mol water at a, 0·58-0·85. The low cation content is a consequence of the two changes, induced by the chelating and cross-linking effects of Ca2+, which also lead to micelle formation. TEM micrographs of Ca caseinates are presented.

COMPOSITION, STRUCTURE, AND INTEGRITY OF CASEIN MICELLES: A REVIEW.


A review with 106 references consisting of the following sections: Physical properties of casein micelles, thermodynamics of casein micelles, micelle stability, structure, porosity, and surface structure, role of calcium phosphate ester, colloidal calcium phosphate, and micelle synthesis.

CHICKEN PEP SIN AS A BENNET SUBSTITUTE.


Semi-purified chicken pepsin was prepared from provest riculus at a yield of 33% of the original pepsin activity. Cheddar cheese was produced using the chicken pepsin preparation and with calf rennet as a control. The enzymes were standardized to the same milk substrate by determining the Breridge's substrate at pH 6·3; the setting times were a little more than twice as long as with calf rennet. While the yield of curd was not significantly different (P>0.05), observed moisture was higher (1·0%) and moisture-adjusted yields lower (25%) with chicken pepsin. Cheeses were stored under refrigeration and analyzed 3, 6, and 9 months after production. Sensory analysis of pepsin cheese demonstrated the development of bitterness found in a previous study. This change was paralleled by increases in acid soluble N and in stress relaxation, a textural parameter measured by the Instron apparatus. SEM micrographs of the 9 month cheese revealed a breakdown in the matrix of the chicken pepsin cheese compared to the control. It would appear that this level of purity of chicken pepsin might be capable of producing acceptable Cheddar cheese for applications that do not require aging of more than 3 months and use heat to inactivate the enzyme, such as processed cheese spread. Further purification may improve the specificity of the enzyme and reduce delayed proteolysis but would increase its cost considerably.

OBSERVAZIONI SULLA MICROSTRUTTURA DEL FORNACCIO PARMIGIANO-REGGIANO: I GRANULI CASEINICI [Microstructure of the Parmigiano-Reggiano casein. Casein granules].


TEM micrographs show the microstructure of the whole matrix in the title cheese following cooking at 37.5, 50, and 55°C. The casein matrix was denser at the surface than in the interior and of the intergranular space. The crystallization of the curd granules is shown at a low magnification.

UNTERSUCHUNGEN ÜBER VERWENDUNG UND ZELLSTRUKTUR VON MILCH-STÄBCHENKERN IN KEFIRKÖMMERN [Studies on the propagation and cellular structure of lactic acid bacteria present in kefir grains].


TEM indicated that lactic acid bacteria in kefir grains were in the form of round micrococci and streptococci as well as long rods with cross-walls. The development of the bacteria was observed by division and by germination. Depending on the cultivating conditions of the kefir grains, rodlike bacteria gave origin to specifically shaped bacteria and vice versa. The cellular walls in the bacteria were found to be delicate and protected with polysaccharide-protein capsules.

ON THE AGGREGATION KINETICS OF CASEIN MICELLES IN UHT MILK DURING STORAGE.


Rare bulk skim milk was heated directly and indirectly in a UHT pilot plant, cooled, aseptically filled, and stored at 4, 20, or 35°C. During storage, the number, dimensions, and the volume of aggregation particles were quantitatively determined using light scattering techniques. The modified kinetic calculations of the volume growth according to Paasche showed a distinct lag phase at all temperatures, followed by an explosive increase shortly before shelf life, thus indicating the action of more proteolytic enzymes in UHT milk.

MICROCOCOLOBIA FORMATION OF THERMOPHILIC LACTIC ACID BACTERIA IN GRAMA CHEESE.


Formation of thermophilic lactic acid bacteria during the first 48 h of cheese ripening was studied by SEM. The microcolonies consisted of 20-100 cells where thermophiles were present. Microcolonies were formed by lactobacillus helveticus, with a number of cells, bacteria growing at a later stage of the ripening process formed larger colonies, 200-400 μm in diameter.

LOCALIZATION OF ξ-CASEIN ON THIN SECTIONS OF CASEIN MICELLES BY THE GOLD METHOD.


Ultrastructural localization of ξ-casein and sodium caseinate was investigated by the protein A-gold method. Casein micelles, fixed in glutaraldehyde, were embedded in a low temperature embedding agent to improve the sectioning of thin sections. ξ-Casein was found distributed throughout the micelles of all sizes with a higher concentration in smaller micelles. Micelles with a large number of ξ-casein aggregates were observed, even in larger micelles. These results do not agree with 'cost-core' structures proposed for casein micelles. However, they suggested that ξ-casein is distributed uniformly throughout the micelles.

STRUCTURAL CHANGES IN CREAM CHEESE INDUCED BY THROMA, PROCESSING AND EMULSIFYING SALTS.


Cream cheese is composed of fat-casein aggregates and spaces which are filled with whey. The addition of emulsifying salts (phosphate mix) is itself, without heating, caused the casein-fat aggregates to disintegrate; casein micelles also disintegrated to a great extent, into subunits, and dispersed in the whey phase. The disintegration was considerably more severe following the addition of 2.0% of the emulsifying salt than after adding 0.8% of the salt. Heating of the Cream cheese with the emulsifying salt increased the disintegration of the aggregates into fat globules and casein. Heating to 60°C for 10 min with 0.6% emulsifying salt led to the complete disintegration of the casein-fat matrix, although loosely structured casein aggregates were still present. The addition of 0.1% of emulsifying salt with heating to only 70°C led to a largely homogeneous distribution of the fat globules and to a complete disintegration of the casein aggregates into subunits. Heating the cream cheese at 80°C, a temperature which may be necessary to improve the shelf life of the product did not cause additional structural changes with 2% of the emulsifying salt. The Cream cheese was studied by freeze-fracturing and replication with carbon and platinum.
INTERACTIONS OF δ-LACTOGLUBLIN AND δ-LACTALBUMIN WITH LIPIDS: A REVIEW

Although of excellent nutritional quality, whey proteins are considered to have the potential in food products, interactions of δ-lactoglobulin and δ-lactalbumin with lipids are reviewed. Structural properties of membrane proteins and methods for predicting interactions of proteins with lipids are discussed briefly with regard to lipid-protein complexes in foods. Effects of surfactants on conformations of δ-lactalbumin and δ-lactoglobulin are also presented. Studies on the interactions of these proteins with synthetic lipids in monolayers and single bilayer vesicles are described. Implications of these studies on the use of whey protein in food emulsions are considered. Micrographs of interacting whey proteins (negative staining) are presented.

IMPROVED UTILIZATION OF DAIRY PROTEINS: COEXTRUSION OF CASEIN AND WHEAT FLOUR

Coextrudates were produced from mixtures of rennet casein and wheat flour by thermal extrusion using a Creo-Loire BC 45 twin screw extruder. The influence of casein and moisture contents on feed material and processing variables was investigated by response surface methodology. Dependent variables included expansion, Warner-Bratzler shear values, and release of free protein in textural profile panel. All product characteristics investigated, with the exception of flavor, could be predicted from independent variables of the multiple regression equations. Addition of rennet casein did not alter the normally bland flavor of extruded starches. Casein to 10 to 30% of the total solids did not influence significantly any of the extrudate characteristics, which were governed primarily by moisture content. The two major physicochemical reactions thought to be taking place during the extrusion process were starch gelatinization and protein textural changes, and it was observed that starch gelatinization plays a major role in dictating product characteristics. Casein may be used in a protein-fortified, extruded flour-based product at the contents studied without altering the characteristic bland flavor or crisp texture of extruded wheat flour. However, a wide variety of textures may be obtained by altering moisture content and process variables.

RESPONSE SURFACE METHODOLOGY TO STUDY FAT DESTABILIZATION AND DEVELOPMENT OF OVERRUN IN ICE CREAMS PRODUCED WITH POLYUNSATURATED SAFFLOWER OIL AND MILK FAT BLENDS

The effects of safflower oil concentration (0 to 25% of the total fat), emulsifier concentration (0 to 0.35%), and freezing temperature (−0.5 to −2.5°C) on maximum overrun and fat destabilization of ice cream were studied. Experimental ice cream mixes were whipped and partially frozen in a laboratory batch ice cream freezer over 30 min. Semifrozen samples were collected periodically and analyzed for percent overrun and extent of fat destabilization. Fat destabilization was determined by a turbidity test and by LWO examination. A central composite experimental design, analyzed by response surface methodology, was used to elucidate main effects and interactions. Increasing safflower oil tended to depress overrun but with little effect on fat destabilization. The effect of emulsifier concentration and freezing temperature on development of overrun depended upon safflower oil concentration, but the effect of these variables on fat destabilization was independent of the safflower oil concentration. Thus, the changes in the extent of fat destabilization alone were insufficient to explain the whipping properties. To provide a satisfactory fat-containing polysaturated fat ice cream containing high levels of safflower oil can be made with typical overrun through proper formulation and processing changes.

LITERATURE ABSTRACTS

STRUCTURAL CHARACTERIZATION OF SPRAY-DRIED DAIRY PRODUCTS BY SCANNING ELECTRON MICROSCOPY

Samples of 11 different commercially spray-dried products were studied by SEM. Various morphological features were found to characterize each specific product. These features were shown to be useful in identifying the products and to provide a means by which adulteration of spray-dried products with lower price powders could be detected.

CHANGES IN SIZE OF CASEIN MICELLES CAUSED BY GROWTH OF PSYCHROTROPHIC BACTERIA IN RAW SKIM MILK

Raw skim milk was inoculated with a proteolytic psychrotrophic bacterium (γ, v/v) that had previously been isolated from milk. The inoculated skim milk was incubated at 7°C for 0, 3, 5, and 7 days. The pH values of the milk were 6.6, 6.5, 6.4, and 5.95, and the numbers of psychrotrophic species were 1.0x10⁶, 4.x10⁵, 9.x10⁴, and 6.x10³ for days 0, 3, 5, and 7, respectively. Milk samples were negatively stained and examined with TEM, and distribution of the average micelle dimensions was determined. The average (and mode) sizes of the micelles were 849 (789), 1039 (834), 761 (834), and 405 (316) angstroms for milk after days 0, 3, 5, and 7, respectively. Another set of samples was prepared from skim milk immediately after it was subjected to all temperature values of 6.6, 6.5, 6.45, and 5.95; the average (and mode) sizes of micelles were 891 (764), 875 (815), 913 (766), and 840 (815) angstroms, respectively. Changes in micelle size in the incubated samples resulted from bacterial activity other than small changes in pH.

CELEATION PROPERTIES OF MILK PROTEINS, SOY PROTEINS, AND BLENDED PROTEIN SYSTEMS

A review of the chemistry of milk proteins, gelation reactions in milk products and ingredients, chemistry of soy proteins, gelation reactions involving soy proteins, and future implications. Easy references.

STATISTICALLY REPRODUCIBLE EVALUATION OF SIZE OF CASEIN MICELLES IN RAW AND PROCESS MILK

Metal shadowing was found to introduce an error in the measurement of casein micelle dimensions from electron microscopy. The error was calculated as 10% per micelle (for a metal coverage of 20 mg) of the metal deposited on the micelles. In addition, some small micelles were obscured whereas others became evident after the application of the metal. To eliminate this deficiency, one drop of a glutaraldehyde-fixed material was spread on a glass slide and air-dried. The slide was dipped in a 0.01% paraldehyde (collodion)-amyl acetate solution and air-dried. The film was floated on water, placed on Cu grids, and dried. TEM was carried out using the dried film before and after shadowing with C and Pt. By examining the unshadowed film with embedded casein micelles and measuring their diameters, 65% confidence intervals were established and treatment effects on micelle dimensions were analyzed statistically.

EFFECTS OF ULTRA-HIGH-TEMPERATURE PROCESSING ON SIZE AND APPEARANCE OF CASEIN MICELLES IN BOVINE MILK

Effects of ultra-high temperature treatment (up to 134°C for a maximum of 5 s) by falling-film-steam-infection on the size and appearance of casein micelles was investigated. Samples were analyzed by KM. Preheating and homogenization at 85.6 or 82.2°C for approx. 100 s did not alter the mean micelle size when compared to those that were homogenized at 88.9°C with a narrower size distribution and increased tendril formation was in the treated milk. Samples of ultra-high-temperature milk treated for 5 s exhibited larger mean micelle diameters and a
greater tendril formation than those treated for 1.5 or 9 s. Increased numbers of small diffuse particles were in samples treated for 9 s. Samples preheated at 82.2°C and treated at temperatures above 121.1°C showed increasing micelle diameters with increasing temperatures with the exception of those heated at 146.1°C. Initial heat-induced micellar growth was followed by micellar disintegration or disaggregation. It was noted that samples stored at room temperature revealed no general trends. However, the pattern of micellar growth followed by development of a large number of small diffuse particles was discerned.

**STUDY OF THE SURFACE FLORA OF TRADITIONAL CAMEMBERT CHEESE BY SCANNING ELECTRON MICROSCOPY**


From a direct observation of Camembert cheese surfaces and cross sections through the rind during ripening it was possible to follow the successive appearance of different bacterial species. Bacterial microstructure development and regression. Until now, the fate of yeasts and Geotrichum was unknown and yet their presence can be detected in the rind until the end of the ripening. They most likely play a part in the equilibrium of the surface flora. The sigmoid-like organisms which develop from the 3rd week, probably originate from the environment of the cheese factory. Together with lactic acid bacteria and perhaps with the aid of the formation of the aroma components which determine the sensory attributes of these cheeses. The results are complementary to those obtained with the light microscope technique and supply some information about the constitution of Camembert cheese rind.

**VISCOITY AND AGE-THICKENING OF SKIM-MILK CONCENTRATE**


The age thickening of skim-milk concentrate appears to be dependent on the degree of concentration, the storage temperature, and variations in the salt balance. The age-thickening observed is most pronounced with highly concentrated systems. The idea is that age-thickening is due to the loosening of casein micelles. As a consequence of concentration, the pH drops and the ionic strength increases, both of which favour the solubility of κ-casein, which results in increased voluminosity of the casein.

**INFLUENCE OF STABILIZERS AND AN EMULSIFIER ON THE ULTRA-STRUCTURE OF SPRAY-DRIED ICED-CREAM MILK**


The influence of the stabilizers, pregelatinized potato starch, and Na alginate and the emulsifier, Tween-80, on the microstructure of spray-dried ice cream mix was studied by SEM. The particles of powder made without additives were spherical in shape. As had been pregelatinized starch was used, the particles occurred in clusters and each particle was partially surrounded by a thick, viscous layer. The addition of Tween-80 resulted in perfectly separated, particle-like, milk globules on their surfaces. The use of Na alginate usually resulted in a more concave surface with small wrinkles and small pores, and some particles were cracked. The combined use of Na alginate and Tween-80 resulted in particles with crater-like structures on their surfaces.

**KRYSTALLISATIONSERSCHEINUNGEN IN SCHWEIZER-KÄSERN, II. IDENTIFIZIERUNG EINES NEUEN CITRATS**


Crystals are formed on the surface of separately produced slices of process cheese if the foil used to package the cheese is even slightly damaged on its surface. With an intact foil, retardation crystallization occurs within the entire product. In any case, the crystals are very small and are of a typical appearance. They were isolated and characterized by SEM and X-ray crystallography. Identical crystals were isolated from complex solutions and were chemically identified to be a new tertiary NaCa citrate. A suggestion is made to prevent crystallization by spray-drying either the Ca++ or the Na+ concentration in some types of process cheese.

**STABILITY OF WEY PROTEIN UPON HEATING IN ACIDIC CONDITIONS**


The heat stability of whey proteins in very acidic conditions is important in the manufacture of whey-based beverages. Above pH 4, whey proteins coagulated rapidly upon heating to temperatures exceeding 75°C, whereas below pH 3.5, a high resistance to coagulation was observed. It was observed that whey proteins were rapidly coagulated in min. In the vicinity of pH 3.5, a small change in pH resulted in considerable changes of stability, although within a short time of a heated (pH 3.5) whey to pH 4.8, a very soft and slimy sediment was formed whereas upon secondary heating at pH 4.8, rapid coagulation took place. Industrial processes, in which the coagulation of whey proteins upon heating is undesirable should be carried out at pH not exceeding 3.5.

[SCANNING ELECTRON MICROSCOPIC AND POLYACRYLAMIDE GEL ELECTROPHORETIC PROPERTIES OF "POHON LITISU" (WRIGHTIANA CALYSINA)] [In Japanese]


Skin milk was incubated with either "litisu" enzyme or calf rennet at 40°C for 90 min and gels were formed. Under SEM, the structures of lactase particles linked together by short and thin bonds. However, "litisu"-curds prepared with NaCa and at alkaline pH showed the network structure fused and the presence of casein micelle clusters. No appreciable differences in the polyacrylamide patterns were found.


The submicroscopic structure of the gastric coagulum of milk, as examined by freeze-fracturing, depends decisively on the technological treatment of the milk and the digestion period in the stomach. The strongest coagulation of casein occurred with raw and with pasteurized (non-homogenized) milk. With pasteurized and homogenized milk, the incorporation of fat globules in the network limited the aggregation of casein. With ultra-high temperature (UHT) treated homogenized milk, casein and fat globules aggregated at a slower rate in the stomach and the aggregation was limited to smaller areas. Milk that was pasteurized, homogenized, and cultured, formed a very loose coagulum structure which was largely independent of the digestion period in the stomach. A close correlation was found to exist between the submicroscopic structure and the firmness of the coagulum.


Five different batches of skim milk were prepared and fortified with the addition of NaCa citrate (Na-cm), or by concentration using a vacuum evaporator (U), or by centrifugation (CF) and by the addition of similar levels of protein (5.0-5.5%). Yoghurts were made by inoculating the milks with one of 3 commercial yoghurt starter cultures and by inoculating the milk at 42°C for 3 h. The following factors were found to be important in this study to affect firmness of the yoghurts: (1) lactic acid production (acidity); (2) the amount of NaCa lactic acid or more (pH 4.54 or less) were firmer than yoghurts having a lower lactic acid content and a higher pH value. (3) Casein to non-casein protein ratio. Firmer yoghurts were obtained at a ratio of 4.62 than at 3.29-3.49.

The microstructure of the yoghurts as examined by EM was affected by the method of fortification of the milk. SEM-fortified yoghurt had the most dense matrix composed of small micellar chains and small micellar clusters. This was the softest yoghurt. Na-cm-fortified yoghurt had the most open matrix consisting of small casein particle chains and large clusters. This was the firmest yoghurt. 'Appedages' or 'spikes' formed by heat-denatured beta-lactoglobulin or by a complex consisting of beta-lactoglobulin and kappa-casein were attached to casein micelles in all the yoghurts except the one fortified by the addition of Na-cm. Microstructures of both whey and milk proteins and the milk and whey proteins were additional microstructural features observed in the yoghurts under study.
LITERATURE ABSTRACTS

ULTRASTRUCTURAL STUDIES OF MILK DIGESTION IN THE SUCKLING RAT


The structure of milk in the stomach and proximal small intestine of suckling rats at 12 and 24 h, and 5, 10, and 15 days of age was examined by SEM and TEM. The milk curd in the gastric lumen consisted of chains of casein micelles with entrapped milk fat globules. The appearance was similar to that found for bovine milk curds and cottage cheese. The gastric milk curd at 12 and 24 h also contained large masses of granules 4-8 nm in diameter. The relationship of these particles to casein micelles is unknown. Casein micelles in the duodenum appeared to disperse irrespective of age. In the central duodenal lumen milk fat globules lost their encircling membranes and underwent lipolysis, evidenced by progressive peripheral irregularity as they passed between the lateral regions of the villi.

MORPHLOGICAL AND TEXTUAL COMPARISONS OF SOYBEAN MOZZARELLA CHEESE ANALOGS PREPARED WITH DIFFERENT HYDROCOLLOIDS


The morphology and texture of mozzarella cheese analogs prepared from soy protein isolate, gelatin, fat, and imidazole hydrocolloids (gums) were evaluated and compared. The fracturability, hardness, and adhesiveness of the cheese analog gels were found to be proportionally related to the amount of fat and gelatin, and concentration and viscosity of gums. However, the stretchability of the cheese analog gels was not controlled by the viscosity of gums, but by the amount of gum and gelatin in the formulation. Fat content affected the fracturability and hardness, but did not have a significant effect on the other textural parameters. Microstructural studies indicated that gels with a diffuse or stretchable microstructure are related to the gel network which retards the alignment of molecules in the gel structure and, hence, adversely affect the stretching properties of the analog.

[COMPARISON OF THE STRUCTURE OF VARIOUS CHEESE PRODUCTS BY SCANNING ELECTRON MICROSCOPY] [In Japanese]


The chemical composition of Inner Mongolian cheeses, Nepalese cheeses, and some of laboratory-made products was examined. The microstructure of these cheeses and 6 cheeses from Europe was studied by SEM. In general, protein network structures were not found in Oriental cheeses which had low fat and high moisture contents. Such structures were found only in cheeses, in which the ratio of fat-to-protein was about 1:1. The fat content in cheeses contributed to a significant difference in the microstructure.

MILK GEL STRUCTURE. XIV. FIXATION OF FAT GLOBULES IN WHOLE MILK YOGHURT FOR ELECTRON MICROSCOPY


The method for the fixation of lipids in animal tissues by Angenent and Fahlman (Histochim. J. 14, 825-833, 1983) was modified and adapted for use with fat globules in milk products. Whole-milk yoghurt was used as a model system. The samples were fixed in a 1.4% glutaraldehyde solution in 0.1 M cacodylate buffer, pH 7.4, containing 0.05% CaCl2, and were postfixed at 220°C for 24 h in 0.5% OsO4 in a mixture (1:1, v/v) of 0.05 M veronal-acetate buffer, pH 7.4. Immersion fixation significantly facilitated the interaction of unsaturated fatty acids in the milk fat with OsO4. Crystalline fat, supposedly containing saturated fatty acids in the form of triglycerides remained unaltered and stood out in negative contrast against the dark homogenous matrix of the fat. The triglycerides of which consisted mostly of unsaturated oleic and linoleic acids.

FOODS OF PLANT ORIGIN

ELECTRON MICROSCOPIC MORPHOMETRY OF CELL WALL SWELLING IN REHYDRATED CARROTS AND GREEN BEANS: THE INFLUENCE OF WARMING AND STORAGE PARTING TOES


By morphometric analysis of TEM micrographs, the degree of cell wall swelling in rehydrated vegetables (carrots and green beans) was examined, as a function of various blanching, dehydration and storing parameters. The results show a clear correlation between food technol. parameters and cellular morphology of the samples. [Copyright 1982. J.P. Bergmann Verlag].

EVALUATION OF GROUND COFFEE PARTICLES FOR OPTIMUM EXTRACTION


In the extraction process of the soluble elements from infused coffee grounds, the form and dimensions of particles play an important role. Using various analytical techniques, the volume or surface of the particles was measured after separating them into different sizes. For volume measurements, the techniques used (Coulter-counter, porosimeter) give similar results. For surface measurements (image analyser, porosimeter, adsorption of gas), on the contrary, results may be largely different (by a factor of 3). This is explained by large surface developed by the microporosity and by the differences in the measuring thresholds of the techniques. The results obtained by using these values in the model representing the extraction process are compared.

QUANTITATIVE X-RAY MICROANALYSIS OF THE ELEMENT CONCENTRATIONS IN FRESH, BLANCHED BOILED AND REHYDRATED VEGETABLES (CARROTS AND GREEN BEANS)


The contents of K, Ca, Mg, S, P and Cl in cell wall and cytoplasm of fresh and processed green beans and carrots were measured by energy-dispersive X-ray microanalysis in the SEM. Fresh green beans and fresh carrots do not differ in the kind, but in the content of the element that they contain. In the course of blanching and boiling elements are gradually removed from the tissues. The contents measured in the rehydrated tissues (blanched and boiled samples) only K disappears to a very large extent during rehydration.

PHYSICAL PROPERTIES AND STRUCTURE OF HORTICULTURAL CROPS


A review.

ZAHLERN GESTATETE KLINGENAMEN UND SESAMKORNERN ZU DEN KRUNGEN?

[Literatur: 30] [Einleitung und Ergebnisse]


Roasted linseed and sesame seed cannot be used as spices in meat products and their use in sausages is not permitted; their presence can be demonstrated by histological examination. Examples are presented in this paper.

SCANNING ELECTRON MICROSCOPY AND HISTOCHEMISTRY OF OILSEEDS


A review dealing with amylolytic preparation of dry oilseed tissues, aqueous fixation, correlation with TEM, sectioning and other techniques, germinating seeds, and histoch. Fifty SEM micrographs and numerous references.
ULTRASTRUCTURE OF TOMATO FRUIT RIPENING AND THE ROLE OF POLYGLUCONATE REDUCTASE ISOMERASES IN CELL WALL DEGRADATION

CREMEAN, L. E., AND OKUNO, R.

Plant Physiology 72, 1089-1093. [Dept. Physiol. & Environ. Science, School Agric., Univ. of Nottingham, Sutton Bonnington, Loughborough, Leices­
tershire LE12 5RD, U.K.]

Ultrastructural changes (thin-sectioning) in the pericarp of tomato (Lycopersicon esculentum Mill) fruit were fol­

lowed during ripening. Ethylene production was monitored by gas chromatography and samples analyzed at successive stages of the ripening process. Changes in the cytoplasmic ultra­

structure were consistent with the suggestion that ripening is a 'senescence' phenomenon. A large degree of ultrastructural organization, especially of the mitochon­

dria, chromoplasts, and rough endoplasmic reticulum, was retained by ripe fruit.

Striking changes in the structure of the cell wall were noted, beginning with dissolution of the middle lamella and eventual disruption of the primary cell wall. These changes were correlated with appearance of polyglucuronases (EC 3.2.1.15) isoenzymes (PG1). Application of purified tomato PG1 to nature green fruit tissue duplicated the changes in the cell wall noted during normal ripening. Possible roles of the PG1 in cell wall disorganization are discussed.

COTYLEON STRUCTURE OF RESTING PEANUT (ARACHIS HYPOGAEA L. CV. FLORONUNNE) SEED BEFORE AND AFTER HYDRAULIC PRESSING

SHOCHER, V., AND PASTERIS D.

Plant Physiology 97, 1080-1088. [Dept. Physiol. & Environ. Science, School Agric., Univ. of Nottingham, Sutton Bonnington, Loughborough, Leices­
tershire LE12 5RD, U.K.]

Structural characteristics and storage products of plastids in cells of inter-ringed parenchyma of the sugar beet root were studied throughout the 2-year cycle of plant de­

velopment. The dimensions of plastids are measured, de­

veloped of their membrane system is described, and quanti­

tation is performed on the occurrence of plastids with peripheral reticulum and phytoliferous, starch, and lipid inclusions. On the basis of the obtained data, plastids of sugar beet root storage parenchyma are relegated to the category of very small weakly differentiated structures with abundant peripheral reticulum, but weakly developed lipids. Sparse grana are formed in plastids only in the stem part of the root. The abundance of membrane formations in the peripheral system of plastids and the frequency of asso­

ciation of plastids with endoplasmic reticulum apparently solve to facilitate fluxes of substances across the plastid envelope. Plastids of young plants ensure particular deposition of organic substances in the form of starch, which is replaced by lipids at the later stages of plant development. Plastids of individual root showed the observance of starch in root plastids during the period of harvesting is correlated with a lowered level of sucrose in mature cells. The data obtained on the microstructure of plastids and dynamics of intraplasmid inclusions indicate a definite role for plastids in the functioning of sugar beet root storage parenchyma.

There were 2 types of cell damage by biotype C greenbug, Schizaphis graminum (Rondani), in susceptible plants: (1) All identifiable structure was lost by the organelles in a few phloem cells as early as 1 h postinfestation; and (2) a slower degradation of cell organelles occurred in mesophyll adjacent to aphid feeding tracks. Chloroplast membranes were disrupted, leaving enlarged osmophilic granules. Mitochondrial degeneration was slower, with vesicle formation within cristae. Nuclear vesicles occurred at 10 days postinfestation. Dense staining salvaging sheath material occurred near feeding sites. A resistant wheat showed no macroscopic damage at 10 days postinfestation. There were patches of collapsed dried mesophyll in samples 2 days postinfestation; these were surrounded by unaffected cells. Saliva sheath material was observed at 2 days postinfestation, but no later. In susceptible wheat, feeding tracks were primarily intercellular in phloem. Damage in resistance tissue was to mesophyll. Resistance in wheat was physiological and biochemical.


Air-dried pulverized ethanol-insoluble substances and lignocellulose preparations obtained from selected kinds of fruits and vegetables exhibited a relatively high water-binding capacity of 7-18 g water/g powder. The values which were determined by the capillary suction method, were reduced to 2-4 g/g following the destruction of the micro- and ultrastructures (detected by SEM) by vibration grinding. The effect of the grown biological structure on the water-binding capacity is generally considered to be greater than the effect resulting from the quantitative composition of the microfibrillar system in the fruits and vegetables. The results are discussed with regard to food technology and nutrition.


The degree of gelatinization of potato starch was measured by the apparent optical density of starch-water suspensions at 550 nm during isothermal gelatinization at 52.5-75.0°C. From differential scanning calorimetry (DSC) peak temperature, the activation energy of gelatinization was determined to be 226± Kcal/mol, being in good agreement with the value of 254 Kcal/mol determined by the DSC method. The gelatinization temperature as measured from the half-transition was found to be 61.1°C which is slightly higher than the value of 59.1°C determined by the glucoseamylase method. From the transition curve, the van’t Hoff enthalpy was evaluated to be 117.7 Kcal/mol.


Proteins were isolated from citrus acid and sodium hydroxide extracts of navy beans (Phaseolus vulgaris), white kidney beans (P. vulgaris), and baby lima beans (P. lunatus). Urea extraction of the proteins from the different types of particulate microstructures were present in the proteins obtained by citrus acid extraction of the beans followed by precipitation by cold ethanol. It was observed that proteins obtained by extraction with NaOH solution and isolectric precipitation revealed the presence of only one type of non-particulate microstructure.


The kiwi (Chinese gooseberry, Actinidia chinensis Planch.) fruit starch consisted of small round granules with an average size of 5.5 μm and was composed of 14.4% moisture, 0.17% crude protein, 0.11% lipid, and 0.14% ash. The phosphorous content was 25.5 mg/100 g, which was the highest concentration of the minerals. The amylase content was 10.6% and the X-ray diffraction pattern was B-type. The gelatinization temperature, obtained by differential scanning calorimetry, was 72°C. The properties of the fully gelatinized form and the low degree of retrogradation were observed by 13C-NMR analysis. Free fatty acids detected in the lipid component of the starch were oleic, palmitic, linolenic, linoleic, and stearic acids.


Intract or disrupted cell walls from tomato pericarp cannot be suspended in the juice, even at higher densities (up to 1.2 g/ml) or viscosities (up to 70 centistokes) than those of natural juice serum. Hence, the viewpoint of swelling precipitate rather than suspension in tomato juice. Degradation of insoluble pectin by a relatively high concentration of exogenous pectinases led to breaking of the wall and partial dispersion of the microfibrillar system in the wall. Cellulase activity led to partial or complete degradation of the microfibrillar system, with increase of the precipitate. Expansion of the microfibrillar system and retention of its ability to withstand collapse under gravity stress, make possible the swelled precipitate and homogeneous appearance of insoluble particles along the juice column during shelf life.


The genus Panchum has been shown to have species exhibiting a range of leaf digestibility. Leaf anatomy as well as differences in degradation of specific tissues have been implicated as factors influencing the digestibility. The objective of the present report was to examine microscopically specific cell wall types in leaves of upper and lower nodes for the pattern of attack and the microorganisms involved in cell wall degradation. Examination of the digestive tract, the rate of digestion, the activation energy of gelatinization was determined to be 226± Kcal/mol, being in good agreement with the value of 254 Kcal/mol determined by the DSC method. The gelatinization temperature as measured from the half-transition was found to be 61.1°C which is slightly higher than the value of 59.1°C determined by the glucoseamylase method. From the transition curve, the van’t Hoff enthalpy was evaluated to be 117.7 Kcal/mol.

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CULTIVATION AND MICROSCOPICAL ORGANIZATION OF HARASAKI

Harasakas samples collected from different processing stages were obtained from one commercial crushing plant for the present investigation. The samples included (a) whole seeds, (b) flaked seeds, (c) press cakes, (d) solvent-extracted meal, (e) desolventized meal and (f) cooled, defatted flakers. Frozen and/or lyophilized mass and/or embedded sections of the samples were examined using the techniques of fluorescence microscopy. Mechanical crushing tended to fragment the cells. After separating and expelling pres- sure, individual protein bodies fused to form large masses excreting phytin-containing globoids. Storage lipids also congealed into larger droplets. Most of the oil was removed after solvent extraction and was absent inside cotyledon cells after desolventization. The final meal product con- tained primarily hull and cotyledon fragments. The cotyledon fragments consisted of an amorphous protein matrix embedded with phytin globoids and supported by a network of broken cell walls. The structural and microchemical organization of the hull were not much affected by the processing. Phenolic compounds, oxidation, cell wall polysaccharides, chlorophyll, storage proteins, and lipids could be detected by various fluorescence microscopic techniques.

[ENZYME SUSCEPTIBILITY OF STARCH SEPARATED FROM RED BEAN ANN] [In Japanese]


Ann is a traditional Japanese cake base made from red beans or other legumes. Amylase paste consists of a mass of sepa- rated cells of cooked beans. Starch granules in beans are a source of red bean paste. It was possible to hydrolyze starch isolated from the beans using beta-amylase-polysialamase, but this enzyme did not hydrolyze starch granules in the ann. Storage of beans extremely retarded regranulation of the starch. The microstructure of ann and starch granules was studied by LN.

LITERATURE REFERENCES

ULTRASTRUCTURAL LOCALIZATION OF SOYBEAN AGGLUTININ ON THIN SECTIONS OF GELATINASE (SOYBEAN) VAR. ALTONA BY THE GOLD METHOD


The average diameter of the gold granules (Au12) used was 10-16 nm. Electron micrographs revealed presence of deep surface fissures. Gelatinization temperature range was 64-66°C and amyllose content was 28%. Gardner color values were L=92.5, a=0, b=5.6. Starch showed low swelling power and solubility in water. Brabender viscosity patterns showed neither peaks nor breakdown of hot paste. Starch formed stable gels at 5°C and higher concentrations. Gel strength was similar to that of potato starch. Retropgradation was greater in corn or wheat starch gels.

INFERENCE OF ORIENTAL MUSTARD BY NEWMATOPORA: A FLUORESCENCE AND MICROSCOPICAL ORGANIZATION OF HAPRASKI


Fluorescence LM and SEM were used to study penetration by the yeast hematospora corlyli through the seed coat and into the embryonic tissue of oriental mustard seed (Brassica juncea). Infection of the seed was associated with leaf physical injury; however, it was evident that the yeast was capable of successfully invading healthy plant cells. The histological patterns were followed in parallel using both the above types of microscopy. FoCi of yeast infection on the seed coat outer surface were characterized by swelling of the infected lateral internal cells. The inflected cells in the outer layers became evident as zones of localized erosion. Ascii and spores were visible, embedded in disorganized and disinte-grating plant tissue.

THE EFFECTS OF COMMERCIAL PROCESSING ON THE STRUCTURE AND MICROCHEMICAL ORGANIZATION OF HARASAKI


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LITERATURE ABSTRACTS

CEREALS

DEVELOPMENT OF PROTEIN BODIES IN OAT STARCH ENDOSPERM

The subaleurone layer was extracted by LM and TEM at various stages of maturation of oats (Avena sativa L.). In caryopses of 11 mg fresh mass, cells were highly vacuolated with no visible evidence of protein deposition. By 11 m.s., the vacuoles were smaller, rough endoplasmic reticulum (RER) was evident, and some vacuoles contd. protein deposits. As the caryopsis matured, protein bodies filled a greater proportion of the vacuolar space. Small protein deposits were observed within the cisternae of the RER, and direct connections between the RER and the tonoplasts of vacuoles were evident. Sclerostyes were infrequently observed at any stage. IM micrographs are consistent with the hypothesis that protein synthesized on the membrane-bound ribosomes is transported within the ER cisternae to the vacuoles, where it condenses, forming protein bodies.

FRACTURE OF CORN ENDOSPERM IN BENDING. I. FRACTURE ANALYSIS BY FRACTOGRAPHY AND OPTICAL MICROSCOPY
Balaschweiler L.H., Herum F.L., Blaisdell J.L. 1982, Transactions of the American Society of Agricultural Engineers 25(4), 1062-1068. [Escola Sup. de Agric. Luiz de queiro, Piracica, SP, Brazil.]

Fracture is corn endosperm, studied by both LM and SEM, occurs as an intergranular brittle fracture at lower temp., as an intragranular ductile fracture at higher temps (50C). The model of crack propagation is not affected by temp. and rate of loading since there were no apparent differences between ruptured surfaces due to heated-air drying. The fracture is corn endosperm was found to initiate in the centre of the kernel, apparently due to internal flaws, and propagate toward the outside through the cell walls and granule SAR.

NITROGEN FERTILIZATION EFFECTS AND ANATOMICAL, PROTEIN, AND AMINO ACID CHARACTERISTICS OF YELLOW BERRY IN TRITICALE

Yellow berry in triticale is described as an endosperm maldevelopment that gives kernels a yellowish color and is caused by a lower protein content; the reduced protein level in YB kernels in both bread wheat (Triticum aestivum L.) and durum wheat (T. durum L.) adversely affects milling and baking quality. Increased N fertilization signif. reduced YB incidence. Triticale lines differed signif. in YB incidence, implicating genetic factors in YB expression. SEM revealed that normal kernels had a more dense endosperm protein matrix with more vacuoles, and aleurone cells generally fractured around the starch granules and cytoplasm, resp., in YB kernels. The total protein content of YB kernels was typically 1 to 1.5% lower than in normal kernels. The YB kernels contained signif. more lysine as percentage of protein, but less aspartic and glutamic acids than normal kernels.

TEXTURAL AND MICROSTRUCTURAL CHANGES IN CORN STARCH AS A FUNCTION OF EXTENSION VARIABLES
Owusu-Assiam J., van de Voort FB, Stanley DW. 1984. Canadian Institute of Food Science and Technology 17(2), 65-70. [Dept. Food Sci., Univ. of Guelph, Guelph, Ont., Canada N1G 2W1.]

The effect of extrusion variables (temperature, feed moisture, and screw speed) on the expansion, breaking strength, and microstructure (SEM) of starch extrudates was studied using response surface methodology. For expansion, the most significant variable was found to be feed moisture which bore an inverse relation to the expansion response. All the extrusion variables studied were significantly related to expansion and accounted for 95.7% of the total variation. Screw speed and feed moisture variables for breaking strength, all three variables accounting for 89.7% of the total break strength variation. Microstructure of the extrudates could be related to the extrusion variables, the expansion and breaking strength responses. The porosity of the extrudates increased with decreasing moisture, with no significant change and a decrease in breaking strength. Regression equations generated from composite rotatable response surface design experiments could be used to accurately predict the responses studied.
BREAD: STRUCTURE, COMPOSITION, AND UTILIZATION


A review illustrated with 14 SEM micrographs; 206 references.

SORGHUM PROTEIN BODY COMPOSITION AND ULTRASTRUCTURE


The protein composition and ultrastructure of sorghum protein bodies isolated from mature starch endosperm material were studied. TEM showed that the protein bodies were largely circular in section and varied between 0.4 and 2.6μm across. The isolated protein bodies were subjected to an Osborne-type protein-fractionation procedure, which revealed that they are composed mainly of prolamin protein. Protein body prolamin had a virtually identical amino acid composition and gave the same electrophoretic pattern as the protein, and the matrix may function as a source of certain hydrolytic enzymes involved in the mobilization of endosperm reserves during germination.

WHEAT, WHEAT-RYE, AND RYE DOUGH AND BREAD STUDIED BY SCANNING ELECTRON MICROSCOPY


Three types of bread (100% white wheat flour, 60% wheat-40% rye flour, and 90% rye meal-10% rye flour) were examined by SEM along with appropriate samples drawn during dough mixing, various stages of breadmaking, and fermentation, and baking. In white wheat flour doughs, structure was based primarily on formation of a protein matrix. Large starch granules and especially 'stringing' of small starch granules contribute to dough structure in white wheat flour systems. In the bread, interaction occurs between protein and swollen starch (mainly large granules). Much of the starch is modified, but in some 'protected' crumb and crust areas (inside vacuoles), little modification, especially of small starch granules, was observed. In the mixed wheat-rye system, some contribution is from gum-like substances and from modifications of gluten and starch by organic acids. In rye meal systems, gum materials facilitate interactions among proteins and their solution to large granules. Whereas in white wheat or mixed wheat-rye systems, pericarp-alumcule particles are part of the problem area, in rye they are part of the solution of providing a coherent and continuous dough or bread structure. The major contributor to rye meal bread structure is modified starch; rye meal starches, unlike the flour of wheat and mixed wheat-rye flour bread, is coarse and affected by the presence of particles rich in aleurone-pericarp.

DISTRIBUTION OF STERIGMATOCYSTIN AND FUNGAL MYELIUM IN INDIVIDUAL BROWN RICE KERNELS NATURALLY INFECTED BY ASPERGILLUS VERSCOLOR


The distribution of fungal mycelium and the mycotoxin sterigmatocystin in individual brown rice kernels naturally infected by A. versicolor was studied with SEM and fluorescence microscopy. Mycotoxin content in the milled rice or rice bran at various milling stages was determined by fluorodensitometry. SEM showed that invading mycelia were often in the adjacent germ, aleurone layer, or starch endosperm. The mycotoxin usually occurred around germ as an entangled mycelial mass, but it was rarely found in the major part of the endosperm unless the grain was cracked. Characteristic yellow fluorescence caused by the mycotoxin was seen in milled rice, aleurone layer, and sometimes completely adjacent to them, and conspicuously around the germ. Concentrations of mycotoxin in brown rice were 3.8-4.3 ppm. Mycotoxin in milled rice decreased gradually from 71.6 to 7.7%, depending on the milling yields from 97.7 to 56.4%. The major portion of mycotoxin produced in brown rice could be removed at the minimum milling yield (56.5%).

LITERATURE ABSTRACTS

THE DEVELOPMENT OF KNIVES USED FOR GERMINATION AND SEDDING GROWTH OF WHEAT SYNTHETIC POLYMERS


The levels of enzymes responsible for the enzymic modification of sorghum endosperm have been followed during germination and seedling growth. A sorghum-B-glucanase was shown to be inactive towards barley α-glucan. Gibberellin acid does not appear to control the levels of a-amylase. In contrast to barley, the synthesis of this enzyme occurs in the embryo but it subsequently acts on the starch granules in the endosperm. Limit dextrinase, on the other hand, appears to be present in the endosperm as a synonym. Proteases were also examined during germination and seedling growth. Amino acid-releasing enzymes develop in the embryo and are absent from the endosperm, whereas endopeptidases can be detected in the embryos and to a greater extent in the endosperm. Amylolytic attack on endosperm starch is very extensive during the early stages of grain growth. The significance of these results to the malting properties of sorghum is discussed.

GELATINIZATION OF LOW WATER CONTENT WHEAT STARCH-WATER MIXTURES: A COMBINED STUDY BY DIFFERENTIAL SCANNING CALORIMETRY AND LIGHT MICROSCOPY


The effect of heating starch-water mixtures of low water content (between 5% and 1% water) has been observed by the techniques of both birefringence measurement and differential scanning calorimetry (DSC). The temperature at which complete gelatinization occurred, the complete loss of birefringence occurred, increased as the water content was decreased. Complete loss of birefringence occurred over almost the whole of the range of water content. A correlation was established between the loss of birefringence and passage through the DSC endotherm, assigned to the melting of starch crystallites.

A QUANTITATIVE STUDY OF BISCUIT MICROSTRUCTURE


A simple stereological analysis system was constructed in order to obtain quantitative information on the distribution of the major constituents of a biscuit at different locations within the structure. The biscuits studied were of the semi-sweet type which were baked using mainly radiation heat and were then humidified, fixed, embedded, and sectioned for microscopical examination. The constituents studied (protein, fat, native starch, gelatinized starch, and space) were identified by staining and polarized light techniques. Variations in the portion of the pattern of heat penetration and moisture loss.

CHEMISCHE MODIFIZIERUNG DER STÄRKEKOHLENFLÄCHEN, KINNBAU VON STÄRKE IN KOVALENTEN UND THERMOREVERSIBLEN NETZWERKEN ALS SYNTHERHISCHEN POLYMEREN

Chemical modification of the surface of the starch granule. Incorporation of starch into covalent and thermoreversible networks of synthetic polymers.


In order to find a new field of application for starch, methods have been investigated to improve the compatibility of starch with synthetic polymers. Preparative steps are described which allow a dense layering of synthetic chains via chain anchoring at the surface of the starch granule without any loss of its structure. The properties of products coated with poly(oxyethylene) of different chain lengths were studied. Some of the constituents are probably due to the pattern of heat penetration and moisture loss.

STREEPING — THE CRUCIAL FACTOR IN DETERMINING MALT QUALITY


A review with 20 references illustrated with SEM and LM micrographs.
LITERATURE ABSTRACTS

MACROSTRUCTURE OF SELECTED RAW STARCHES AND SELECTED HEATED STARCH DISPERSIONS


Raw starches (corn, wheat, rice, and potato starches, wheat flour, Thermox, Purity W, Nesco gel starch, amylopectin, and potato amylopectin) and potato starch dispersions (3% corn starch-water dispersions at 30, 50, 60, 65, 70, 75, 80, and 85°C, and 3% wheat starch-water dispersions at 30, 55, 60, 65, 70, and 75°C) were studied by SEM. The dispersions ranged from small and round to large and ellipsoidal in shape, with variation in uniformity and smoothness of the surface. Non-uniform corn and wheat starches in water dispersions caused swelling of the starch granules, some exudate, and eventual loss of original granule integrity.

GRAIN ENDOSPERM STRUCTURE AND END-USE PROPERTIES


A review with 59 references. Microscopical methods including photocoustic microscopy are dealt with.

FUTURE TRENDS IN THE INVESTIGATION OF WHEAT PROTEINS AND THEIR ROLE IN THE WHEAT PROCESSING TECHNOLOGY


The discussion of the following topics: (a) The role of protein-lipid and protein-carbohydrate complexes in the formation of dough and its changes during processing; (b) the correlations between protein distribution and quality and the baking value; (c) Biosynthesis of wheat proteins and its genetic background; (d) Investigation of metabolically active protein-lipid complexes; (e) Wheat protein preparations from wheat. Models of complexes formed by protein-lipid interactions are proposed.

IN VITRO PLANT REGENERATION FROM CALLUS TISSUE OF CEREALES (BARLEY, WHEAT AND TRITICALE)


Factors affecting the formation of totipotent callus cultures of barley, wheat, and triticale were studied. Plant regeneration was observed in cultures derived from immature embryos only. The standard procedure of callus induction and plant regeneration were established. Differences in chromosomal analysis was made in barley plants regenerated in vitro. No phenotypic deviations were observed in the seed progeny of diploid regenerants. Histological studies were carried out using sections stained with Fuchsin and Picroindigo-carmine.

SUSCEPTIBILITY OF RYU VARIETIES TO FUSARIUM DISEASES OF SPIKES


The results show high pathogenicity of Fusarium culmorum (W.G.S.) Sacc. Infected spikes contained a smaller number of grains than intact spikes. The weight and germination power of grains were lower than in healthy spikes. Micrographs of hyphae in the grains stained with Fast Green and Safranin are presented.

TEXTURE STUDIES ON BREAD LOAF AND BREAD

ANALYTICAL EVALUATION OF THERMALLY TREATED GRAINS FROM THE VIEWPOINT OF THEIR DIGESTIBILITY


Protein and starch digestibility of barley and maize grain was evaluated using an enzymic method with pancreatin containing both amylase and trypsin. Changes in starch accessibility to amylolytic enzymes can also be assayed by microscopic techniques (micrographs obtained by transmitted and polarized LM are presented) but the enzymic method is more convenient. The best improvement in starch and protein digestibility was achieved with the barley and maize moistened before the thermal treatment: 36-38 sec for barley and 60 sec for maize were found optimal.

PENTOSANS OF RYE GRAIN AS THE FACTOR OBSTRACTING THE EVALUATION OF ITS NUTRITIONAL VALUE FOR POULTRY


Earlier feeding experiments with Japanese quail showed that the nutritional value of ground rye grains could be greatly increased by incubating the flour with added water for 24 h. The authors concluded tentatively that this was due to the degradation of rye pentosans by endogenous pentosanases. SEM showed that after the addition of water, the cell walls appeared swollen and there was some glue-like material covering the cell constituents. After the incubation, the cell walls retained their original dimensions; the glue-like gel was no more visible. It is assumed that pentosans are probably the main factor causing the low nutritional value of untreated rye for poultry.

SCANNING ELECTRON MICROSCOPE VIEWS OF MATERIAL FROM VARIOUS STAGES IN THE MILLING OF HARD RED WINTER, SOFT RED WINTER, AND DURUM WHEAT


SEM provides an interesting perspective in the examination of wheat milling. Initial fracture patterns following passage of mill stock through the break rolls show which portions of the endosperm will become middling stocks. Continued attrition in the milling flow results in the removal of most of the endosperm until the final layer of endosperm cell walls adheres to the aleurone layer and the outer part of the bran. Major differences in the attrition patterns of hard red winter, soft red winter, and durum wheats are verified by this method of observation.

EVALUATION OF SELECTED PROPERTIES OF CHLORINATED WHEAT FLOURS IN A LEIN CAKE FORMULATION


Cake flours treated with different levels of chlorine were evaluated by use of a Kissell cake formulation. Flour particle size distributions varied with chlorination level, but pH and specific gravity of the batter did not differ. Batter viscosity differences were observed at specific temperatures during heating of total batters. Temperature profiles differed positionally in the cakes, but these patterns of heat penetration were not related to level of chlorine treatment. However, water loss rates differed depending on the level of chlorine treatment indicating a more pronounced effect of chlorination level on the water loss characteristics of the cakes during the baking process than on the temperature profiles. The largest cake volume, contour deviations, and least shrinkage from the pan sides occurred with a 0.83 % Cl2/Kg flour treatment (commercial level). SEM micrographs of crumbs showed larger starch particles with more continuous and extensive matrix development between starch particles as the chlorine level was increased. The ash analysis mentioned for sorghum and maize after baking were related to factors contributing to optimal cake structure.

S-GLUCANS IN THE CARTOPYSIS OF SORGHUM BICOLOR (L.) MUNROE


Fluorescence microscopy was used to determine the location of s-glucans in sorghum. Sections from 3 genetically different sorghum cultivars were stained with Calcofluor or Congo Red, fluorochromes which have been reported to react with s-glucans. Autofluorescence, indicative of ferulic acid in other cereals, was observed in unstained sections. When stained sections were treated with endo-s-glucanase, fluorescence was reduced or totally eliminated in pericarp, aleurone, and endosperm cell walls. s-Glucans were isolated from the endosperm of 3 sorghum cultivars when reacted with the 2 dyes, Calcofluor or Congo Red, precipitates formed immediately, a reaction which is similar to that produced by mixed linkage S-glucans from other cereals.

OIL-BINDING CAPACITY OF PRIME STARCH FROM CHLORINATED WHEAT FLOUR


Prime starch recovered by wet-fractioning serially chlorinated flour demonstrated that oil binding progressed with the rate of chlorination. This capacity was decreased when chlorinated starch was treated with 0.1% proteolytic or lipolytic enzymes or with a solution of dilute acid. Chlorinated starch did not respond well to the iodine test, until it was treated with a proteolytic enzyme. The oil-binding capacity of chlorinated starch was attributed to the starch’s having a surface coating of proteins. Treatment of such starch with certain lipid solvents had little effect on oil-binding capacity. LM was used.

COMPARISON OF OIL-BINDING ABILITY OF DIFFERENT CHLORINATED STARCHES


Direct chlorination of isolated wheat prime starch imparted lipopolypic properties to the starch. These properties nearly disappeared after treatment with 7% HCl, a-amyrase, or pepsin, but were affected very little by water-saturated butanol, chloroform-methanol (2:1), or sodium dodecyl sulfate solution, rice, potato, and corn starches also became lipophilic after chlorination. Chlorinated corn starch showed the highest oil-binding capacity. However, except for pepsin treatment, response of these starches to treatments differed only slightly from that of chlorinated wheat prime starch. Differences between chlorinated and non-chlorinated starches are illustrated by LM.

OIL-BINDING ABILITY OF HEAT-TREATED WHEAT STARCH


Prime starch fractionated from heat-treated wheat flour was found to possess high oil-binding ability. Prime starch heat-treated at 100-160°C for 1 h also possessed this ability. Treatment of heat-treated prime starch with 7% HCl, 0.84 % N4N, a-amyrase, or pepsin led to the loss of the oil-binding ability, as revealed by LM.

[EFFECT OF MATURATION ON MICROSTRUCTURE AND RHOLLOGICAL PROPERTIES OF CHINESE NOODLES] [In Japanese]


In Chinese noodles, maturation is one of the most important steps to high quality. Microstructural and rheological changes in matured Chinese noodles were studied by SEM and the Do-Corder. Maturation always promoted the production of inner vacuoles in the structure of cooked noodles but the dimensions of the vacuoles after maturation were larger when the dough was matured in the form of sheets and not as strips. The Do-Corder curve of matured noodles showed two peaks, one at 70°C and the other at 80°C.
EFFECT OF WAXY BARLEY STARCH AND REHEATING ON FIRMNESS OF BREAD CRUMB


A review with numerous references.

POLARIZATION MICROSCOPY: APPLICATIONS IN CEREAL SCIENCE


A review of fundamentals of polarisation microscopy, sample preparation techniques and applications, and integration of microscopic data.

IDENTIFICATION OF CEREAL CARBOHYDRATES BY FLUORESCENCE MICROSCOPY


A review illustrated with 12 micrographs in colour.

PHYSICO-CHEMICAL CHARACTERISTICS OF STARCHES FROM CHICK PEA, COW PEA AND HORSE GRAM


The starches were isolated in 36, 37, and 28% yields from chick pea, cow pea, and horse gram, respectively. Study of their properties revealed mixed granule population: single stage swelling with high solubility in water for cow pea and horse gram starches in comparison to chick pea starch; slightly higher solubility in dimethyl sulfoxide for chick pea starch followed by horse gram starch, a relatively high viscosity of cow pea starch in alkaline solution; and brabender amylograms indicating a low slurry viscosity and low setback in the case of chick pea starch compared to the other two starches which exhibited considerable peak viscosity as well as retrogradation. All the starches contained amylose in the range of 32-34%. X-ray diffraction patterns showed chick pea and horse gram starches to be of the B-type, whereas cow pea starch was of the A-type.

SCANNING ELECTRON MICROSCOPY OF CEREAL GRAINS


A review on specimen preparation techniques, kernel microstructure, endosperm of wheat, rye, barley, rice, sorghum, maize, and pearl millet, embryo, selection of high lysine cereal cultivars, custa and pericarp structure, digestibility of sorghum, assessment of kernel deterioration, and application of SEM to cereal research.

STRUCTURAL AND TEXTURAL CHARACTERISTICS OF BAKED GOODS


A review illustrated with numerous LM, SEM, and TEM micrographs.

FROM THE FARM TO THE TABLE - A TRANSMISSION ELECTRON MICROSCOPE ACCOUNT OF CEREAL STRUCTURE AND ITS RELATIONSHIP TO CEREAL MANUFACTURING


A review of TEM of cereals at various stages: from the farm to the elevator, on the table, and in the digestive tract.
and alkali-soluble fractions having their persistence, however, the cell walls are subject to chemical and physical modification. This study was concerned with such changes. A procedure involving ball milling and wet sieving isolated the cell walls. They were isolated from sorghum as well as from kernels after a germination period. The cell walls from both of these isolations were examined by both TEM and SEM. They were also fractionated into water- and alkali-soluble fractions as well as an insoluble fraction. Large amounts of protein were associated with the cell walls isolated from grain, but germination reduced this from 45 to 10%. During this period, the amount of cell wall was reduced, with the water- and alkali-soluble fractions having the largest reductions. EM revealed extensive pitting during germination. Interzonal and fenicular acids were the only polyphenols detected in the isolated cell walls. They were found associated with the alkali fraction, and germination did not cause any reduction in their content.

MEAT

SCANNING ELECTRON MICROSCOPY STUDIES ON CHICKEN GIZZARD STRUCTURE AS AFFECTED BY COOKING


The microstructure of raw gizzard is sponge-like and no fibrous-like structure. The treatment drastically modifies the microstructure. Prolonged cooking (auto-claving at 15 psi steam pressure for 15 min) coagulates myofibrillar and sarcoplasmic proteins of gizzard and yields a fiber-like structure. The interfibrillar meltings between individual gizzard myofibers is more pronounced after auto-claving treatment than from the water boiling process (10, 20, and 30 min). SEM micrographs have revealed that the chicken gizzard fibers are shaped like flattened nodules. Prolonged cooking tenderizes the texture of gizzard, the differences in shear force perpendicular to and parallel with the main orientations increase as the length of cooking time is increased.

PRAEFARATIONSMETHODE ZUR BEOBACHTUNG VON MYOFIBRILLEN IM RAUER-ELEKTROHENGISSEKOP UND DARSTELLUNG VON HINDERMUSKEL: K)).E.


The myofibril structure was visualized in a buffer consisting of 0.1 M KCl, 5 mM ethylenediaminetetraacetic acid, and 30 mM borax (pH 8.3). SEM revealed intact as well as partially disrupted sarcomere, the inner structure of the latter being clearly recognizable. Treatment of the myofibrillar suspension with Triton X-100 induced greater changes in the myofibrils, particularly in the region of the 1-bands.

SENSORY AND INSTRUMENTAL TEXTURE PROPERTIES OF FLAKED AND PORKED BEEF


Four experts were conducted to assess the sensory and textural properties, consumer acceptability, and instrumental-sensory correlates of flaked and formed beef steaks. In Exp. 1, the effects of additions of NaCl, sodium tripolyphosphate (TPP), and any isolates on the texture of steaks were examined using a trained texture profile panel, and the texture of these steaks was compared to that of intact steaks (ribeye) steaks. Results texture most like whole-muscle steak. Simple and multiple linear regression equations were established between sensory and shear stress measures on these steaks and these data were used to predict that the variation in texture of these steaks due to these factors was attributable to the mechanical disruption of the tissue and not to an enzymatic process.

In Exp. 4, a consumer test was conducted to assess the effect of flake size on the acceptability of flaked and formed steaks, and the instrumental and sensory perception of the products. Although few significant differences in the acceptability ratings were found for the intermediate flake sizes, it was found that consumers do not associate the texture of flaked and formed steaks with any one of a variety of traditional beef products.

NOMOGRAPHY OF MEAT BY SCANNING LIGHT MICROSCOPY


Norphometric data can be collected from meat by using a scanning stage and a photometer, both controled by a microcomputer. The passing of the measuring of the essay error in the scannings is given as an example to show that enumerative data may be biased by the expression of the width of the subject of meat, as well as being related to the size of the observing aperture. In a second example, the scanning stage is active directed by the observer and it is used to map the radial distribution of sensory texture analysis in different histochises. Types of muscle fibers. This is accomplished by the arbitrary fitting by the microcomputer of photometric curve of sensory texture during exposure to a muscle fiber diameter. Concentric zones of the resulting data matrix are unpacked to calculate radial gradients of SDV activity within muscle fibers.

DETERIORATION OF ANTARCTIC KRILL MUSCLE DURING FREEZE STORAGE


The effects of freezing on the heat-induced gelation, Ca²⁺-ATPase activity, and myofibrillar structure of Antarctic krill muscle were investigated. Muscle from freshly caught krill was immediately stored at -20°C in the presence (to prevent freezing) and absence of glycerol ('glycerol krill' and 'frozen krill', respectively). Several protease inhibitors, monosodium glutamate, and Ca²⁺ were individually added to glycerol krill to inhibit endogenous proteolysis. The examinations were carried out after 3-month storage at -20°C in glycerol krill. The calorimetric parameters of the heat-induced gels of Krill-ATPase activities of all the krill samples were similar to those of 'unfrozen' (raw fish samples) which gave the gel of good quality, although the microstructure (2-lines) of myofibrils was different among the glycerol krill samples. In frozen krill, however, the parameters of the gel were different from those of 'unfrozen': the ATPase activity was completely lost and disruption of the myofibrillar structure occurred. Refreezing (-20°C of glycerol krill after removal of glycerol resulted in a marked decrease in the gelation ability. These results suggest that freezing of krill muscle causes deterioration of the gelation ability.

COMPARISON OF SHELF LIFE AND QUALITY OF MULLET STORED AT ZERO AND SUBZERO TEMPERATURE


The shelf life and quality of mullet (Mugil spp.) caught off the Southeast Atlantic coast were evaluated during storage at subzero temperature (-2°C) either unpackaged or packaged in aluminum foil. The fish was obtained to be frozen in either 2% NaCl- 7% propylene glycol ice. Shelf life of fish stored in -2°C ice was 10 days, compared to 2 days in frozen fish. The preservation of shelf life was attributed to delayed microbial growth and slow biochemical changes. Structural changes due to ice crystals at subzero temperatures were detected by SEM and compared to those which occurred during freezing at -20°C as revealed by TEM. The minor structural changes along with insignificant biochemical changes resulted in products having acceptable eating quality for 10 days.
ULTRASTRUCTURAL CONSTRUCTION OF COLLAGEN FIBRES AS REVEALED BY THE FREEZE-FRACTURE TECHNIQUE


Schematic model of a three-dimensional constitution of the collagen fibril is proposed. The fibril is assumed to be 300 nm in length, 7 nm in width, and 3 and 4%, respectively, of the latter aspect relates to conformational changes. A similar change although the onset of the turbidity in vivo was less than that of the turbidity in vitro.

The results suggest that in pigs in some cases the reasons of meat quality defects may be of myopathic origin.

HEAT-INDUCED GELATION OF MYOSIN: RULES OF HEAD AND TAIL REGIONS OF A MYOSIN MOLECULE


The investigation was carried out on the longissimus dorsi muscle of 150 crossbred pigs (Polish Landrace x Polish Large White) kept in the industrial farm type G-0-1. The pigs were divided into 3 groups: normal, PSE, and DFD on the basis of chemical and sensory properties of the meat. Significant differences for PHm, pH50, water-binding ability, water-holding capacity, colour parameters, and activity of different enzymes were found. Significant differences were observed in carcass traits. Different intensity of the degenerative changes of the muscle matrix was observed in the different groups. The changes in the connective tissue and the incidence of giant fibres. The frequency of these changes was greater in pigs which showed the highest values. Extensive degeneration and incidence of giant fibres were found in 11.8% and 82.4% of PSE muscle, in 15.4% and 76.9% of DFD, and in 1.5% and 58.3% of the normal muscles, respectively.

The results suggest that in pigs in some cases the reasons of meat quality defects may be of myopathic origin.

ULTRASTRUCTURAL CHANGES IN PORK FROM CAMBOURGH HYBRIDS TRANSPORTED AT SHORT DISTANCES


Studies were made with the title pigs transported in the freezing during the summer at a distance of 5 or 6 km and slaughtered immediately on arrival in the slaughterhouse (test group), and after 24 h of rest (control group). In the meat the pH values were 6.4 (measured 46 min after slaughter), EM demonstrated fragmentation of Z-lines and the release of myofibrils from the sarcomeres. No sarcomeres were found in the sarcomeres of the control group (pH 6.5), the sarcomeres were unchanged in the anisotropic and ischemic groups and the sarcomeres were found in the sarcoplasm. The increased activity of the control group (pH 6.8-7.0) in the sarcomeres of the control group (pH 6.5-6.4), the anisotropic and ischemic groups were changed and there were no changes in the L- and H-zones and the W and Z-lines. Glycogen granules were found in the sarcoplasm and between the protofibrils.


Collagen was prepared from bones defatted at elevated temperatures under pressure (0.50-0.19 MPa) for 30-45 min. The results were measured in the normal, the control, and the defatted groups. No significant differences were found in the fibril stability in the defatted and the control groups. The application of the defatted condition did not cause any severe consequential changes in the protein structure since the thermal shrinkage was a two-stage process. The above defatting conditions were favourable to produce material suitable for the preparation of gelatin and glue.
IDENTIFICATION OF THE PROTEINASES RESPONSIBLE FOR THE POST-MORTEM DEGRADATION OF MYOFIBRILLAR PROTEINS


Cathepins D, B, and L were isolated from rabbit longissimus dorsi muscle and were purified. The biochemical actions toward myofibrils and various isolated myofibrillar proteins were studied using SDS-polyacrylamide gel electrophoresis, phase-contrast LM, and EM. Cathepin B almost did not degrade myofibrillar proteins at pH 5. Cathepin D hydrolyzed (optimum at pH 3) the myosin heavy chain, tropo­
in, α-actinin, and tropomyosin. A 30,000-dalton component was released from tropomyosin but not from troponin. Actin was not hydrolyzed by this enzyme. Cathepin L hydrolyzed (optimum at pH 4-5) the myosin heavy chain, actin, troposmin, and α-actinin. 30,000-dalton components were released from actin and troponin T. Tropomyosin was not hydrolyzed. The fragmentation of myofibrils was brought about by cathepin B but not by cathepin D at pH 5-6. Cathepin L degraded the Z- and M-lines of the myofibrils on the incubation with glycinated muscle fibers at pH 5.5. The results indicate that almost all the changes in myofibrils observed during post-mortem aging of muscles can be explained by the action of cathepin L. Thus, it is suggested that the action of cathepin L is dominant in the proteolysis of myofibrillar proteins in the muscles with a normal ultimate pH (pH 5.5). Cathepin D and CF at least partly contribute to the proteo­
lysis in muscles with an extremely low ultimate pH (low pH) and in the muscles with a high ultimate pH (pH 5.5), respectively.

FLEISCHAHMmCHE, STRUKTURKERTIEFISCHE EINWIRKUNG [Meat-like, structured animal proteins]


The quantity of fat-containing raw material and the relative lack of protein in the processing of meat means that there is a need to add other proteins with a high nutritive value and good emulsifying and water-binding capacities. The proteins of skim milk, whey, and blood plasma have such properties. The objective of this study was to develop procedures suitable for a low-cost production of an anisotropic 'structurates' based on the gelling ability of the proteins mentioned above. By varying the protein composi­
tion and conditions of the procedure, three types of laminar structure were obtained, which had the macroscopic form of fine pores, coarse pores, and layers. The proteins were incorporated into a gelled raw material in a production scale. Of the sausages, which contained 5-20% of added comminuted, salted, and spiced protein 'structurates', only those with the 'structurates' content higher than 10% had slightly lower sensory attributes.

IZLOSOVANIE FUNKTSIONAL'NYKH SVYAZEY BEL'KOVYX VOKOKHI, POLUCHENNYKH METODOM BEZPIL'NEGO PIYADENIA [The use of structured non-die spin proteins in the manufacture of cooked meats]


Functional properties of protein fibres, prepared by means of non-die spinning, determine their possible use in meat analogs and combination meat products, e.g. gelled meat with protein diluters added. Swelling degree, solubility, water-holding capacity, critical shear stress at various pH values and temperatures, microstructure, and biological value of vegetable spun protein fibers were studied. As the pH of the media increased (up to pH 8) the emulsion quality was improved and water absorption, water-holding capacity, and solubility were increased. Functional properties of the finished products (meat analogs, composite materials) were investigated.

The incorpora­
tion of meat tender­
er was studied. The number of muscle fibers and surface and in the interior changes contributed to the improvement of meat tender­
ness and water-holding capacity and increased the yield of the finished products. The use of such meat tender­
ners makes it possible to accelerate the curing process and to obtain a product of good sensory attributes. The use of microstructural characteristics to evaluate the extent of mechanical effects upon the muscle tissue is discussed.

GISTOLOGICHESKIE IZLOSOVANIIA STABIL'NOI FARBHEVYKH EMULSIYX [Histological studies of the stability of sausage emulsions]


The effect of chemical composition of the initial raw meat on some indices of emulsions and finished products which would characterize emulsion stability, was studied using a 4-factor experiment with 5 levels of factor varia­
tions by a full simplex-adding scheme. In addition to deter­
mine the water-holding capacity of meat emulsions, thermal shrinkage, and sensory attributes, samples of the finished products were histometrically studied by means of measuring (vol%) structural elements of the emulsions. The number of fat-filled vacuoles and the dimensions of the fat globules were increased with the increased fat content of the formul­
aton; at the same time, the amount of small fat particles distributed evenly throughout the compact water-protein mass of the emulsion was practically constant. Mathematical in­
tegration of histological data allowed the extent of fluid particles to express quantitatively the proportion of strongly bound fat to the total fat content and to establish the proportion of fat in emulsified sausages.

KORRELACII MESTE IZLOSOVAUX MATERIALNYX I MIIUKSTURKSH CHEMIE MIIUKOGO TYPYa


Rheological parameters of the meat emulsions were determined before and after preparation, using a Haake-type rotational viscometer. The Casson equation was used to charac­
terize the parameters. Histological studies were carried out on sections (3 μm) stained with Hematoxylin-Eosin, Mallory stain, and Oil Red O. The dimensions of the fat globules were the directivity factor on the Casson straight (k) and axial sections (kax) on the one hand and the microstructure of the final product on the other hand. Every distributed struc­
ture (with vacuoles in the 10- and 10-50 μm in diameter) had higher k values.
Papers published on all Food Microstructure-related topics have been included in this Index. From 1979 to 1981, such papers were published in the quarterly international journal "Scanning Electron Microscopy" (ISBN: 0-931288-22-3; $49.00 U.S. delivery, $52.00 elsewhere). From 1982 to 1984, papers on these topics were published in the new semi-annual international journal "Food Microstructure" (ISBN: 0-931288-33-9; $32.00 U.S. delivery, $35.00 elsewhere.)

Several other papers of interest to those working on Food Microstructure have been published in other issues of "Scanning Electron Microscopy" (1978 to 1984). Tables of Contents of these issues are available at $1.00 per part.

In addition, the following two books should also be of interest to readers of this issue: (1) The Science of Biological Specimen Preparation for Microscopy and Microanalysis (The proceedings of the 2nd Pfefferkorn Conference). This 246-page hardbound book contains 28 original papers presented at a conference in April, 1983 on "the rationale behind the approaches that are being used" for the preparation of biological specimens for all types of microscopy and microanalysis methods. (ISBN: 0-931288-32-0; $40.00 U.S. delivery, $43.00 elsewhere.) (2) Preparation of Biological Specimens for Scanning Electron Microscopy. This 352-page softbound compilation contains 22 papers originally published in "Scanning Electron Microscopy" from 1978 to early 1984. (ISBN: 0-931288-33-9; $32.00 U.S. delivery, $35.00 elsewhere.)

Please contact the SEM Inc. office for further details and ordering instructions.

**GENERAL--TECHNIQUE**

*REVIEW: Some Examples of Scanning Electron Microscopy in Food Science; R.J. Carroll (253/79/III)

*REVIEW: Preparation of Food Science Samples for SEM; J.F. Chabot (279/79/III)


*REVIEW: The Use of Microscopy to Explain the Behaviour of Foodstuffs--A Review of Work Carried Out at the Leatherhead Food Research Association; D.F. Lewis (391/81/III)

*TUTORIAL: Preparation of Muscle Samples for Electron Microscopy; H.D. Geissinger (415/81/III)

*REVIEW: Electron Microscopy of Milk Products: A Review of Techniques; M. Kalab (453/81/III)

*A Comparison of the Microstructure of Dried Milk Products by Freeze-Fracturing Powder Suspensions in Non-Aqueous Media; W. Buchheim (493/81/III)

**GENERAL--MISCELLANEOUS**

*Strand Structure Development in Thermally Produced Whey Protein Concentrate Gel; T. Beveridge (161/83)

*SEM Investigation of the Effect of Lactose Crystallization on the Storage Properties of Spray Dried Whey; M. Saltmarch (659/80/III)

*REVIEW: The Use of Microscopy to Explain the Behaviour of Foodstuffs--A Review of Work Carried Out at the Leatherhead Food Research Association; D.F. Lewis (391/81/III)

*Effect of Acidulants and Temperature on Microstructure, Firmness and Susceptibility to Syneresis of Skim Milk Gels; V.R. Harwalkar (503/81/III)
*Structures of Various Types of Gels as Revealed by Scanning Electron Microscopy;  
V.E. Colombo (515/81/III)

*Microstructure of Mayonnaise and Salad Dressing;  
M.A. Tung (523/81/III)

*Identification of Foreign Matter in Foods;  
J.T. Stasny (599/81/III)

**MEAT**

*REVIEW: Food Microstructure: An Integrative Approach;  
E.A. Davis (1/82)

*Detection of Buttermilk Solids in Meat Binders by Electron Microscopy;  
M. Kalab (1/82)

*Freeze-Induced Fibre Formation in Protein Extracts from Residues of Mechanically Separated Poultry;  
R.A. Lawrence (91/82)

*Instrumental and Sensory Analysis of the Action of Catheptic Enzymes on Flaked and Formed Beef;  
S.H. Cohen (99/82)

*An Alternative to Critical Point Drying for Preparing Meat Emulsions for Scanning Electron Microscopy;  
E.J. Basgall (23/83)

*REVIEW: Image Analysis of Morphological Changes in Wiener Batters During Chopping and Cooking;  
A.G. Kempton (27/83)

*Effect of Prerigor Pressurization on Bovine Lysosomal Enzyme Activity;  
E.A. Elgasim (91/83)

*REVIEW: A Review of the Muscle Cell Cytoskeleton and its Possible Relation to Meat Texture and Sarcolemma Emptying;  
D.W. Stanley (99/83)

*Myofibrillar Characteristics of Porcine Stress Syndrome;  
P.K. Basrur (111/83)

*Sensory and Instrumental Texture Properties of Flaked and Formed Beef;  
A.V. Cardello (119/83)

*Morphometry of Meat by Scanning Light Microscopy;  
H.J. Swatland (135/83)

*REVIEW: An Analysis of Microstructural Factors Which Influence the Use of Muscle as Food;  
R.G. Cassens (1/84)

*Studies on the Microdistribution of Aerobic Enzymes and Myoglobin in Pork;  
H.J. Swatland (9/84)

*REVIEW: The Role of Gap Filaments in Muscle and in Meat;  
R.H. Locker (17/84)

*REVIEW: Processing Effects on Meat Product Microstructure;  
G.R. Schmidt (33/84)

*The Effect of Salt and Pyrophosphate on the Structure of Meat;  
C.A. Voyle (113/84)

**MILK/DAIRY**

*REVIEW: Structure and Properties of the Particulate Constituents of Human Milk: A Review;  
M. Ruegg (25/82)

*Detection of Buttermilk Solids in Meat Binders by Electron Microscopy;  
M. Kalab (49/82)

*REVIEW: Ultrastructural Studies of Milk Digestion in the Suckling Rat;  
P.B. Berendsen (83/82)

*REVIEW: Electron Microscopy of Milk and Milk Products: Problems and Possibilities;  
D.G. Schmidt (151/82)

*REVIEW: Aspects of Sample Preparation for Freeze-Fracture/Freeze-Etch Studies of Proteins and Lipids in Food Systems;  
W. Buchheim (189/82)

*Morphological and Textural Comparisons of Soybean Mozzarella Cheese Analogs Prepared with Different Hydrocolloids;  
C.S. Yang (223/82)

*Electron Microscopic Localization of Solvent-Extractable Fat in Agglomerated Spray-Dried Whole Milk Powder Particles;  
W. Buchheim (233/82)
*Composition and Microstructure of Soft Brine Cheese Made From Instant Whole Milk Powder; M.M. Omar (43/83)

*REVIEW: Development of Microstructure in Set-Style Nonfat Yoghurt--A Review; M. Kaláb (51/83)

*Stranded Structure Development in Thermally Produced Whey Protein Concentrate Gel; T. Beveridge (161/83)

*REVIEW: Microstructure of Set-Style Yoghurt Manufactured from Cow's Milk Fortified by Various Methods; A.Y. Tamime (83/84)

Transportation of Fragile Food Specimens such as Milk Gels Destined for Electron Microscopy; P. Allan-Wojtas (93/84)

*Artefacts in Conventional Scanning Electron Microscopy of Some Milk Products; M. Kaláb (95/84)

A Simple Procedure for the Preparation of Stirred Yoghurt for Scanning Electron Microscopy; P. Allan-Wojtas (197/84)


*Morphological, Ultrastructural and Rheological Characterization of Cheddar and Mozzarella Cheese; M.V. Taranto (273/79/I)

*Microstructure and Rheology of Process Cheese; A.A. Rayan (635/80/I)

*Possibilities of an Electron-Microscopic Detection of Buttermilk Made From Sweet Cream in Adulterated Skim Milk; M. Kaláb (645/80/I)

*A Scanning Electron Microscopical Investigation of the Whipping of Cream; D.G. Schmidt (653/80/I)

*SEM Investigation of the Effect of Lactose Crystallization on the Storage Properties of Spray Dried Whey; M. Saltmarch (659/80/I)

*REVIEW: Electron Microscopy of Milk Products: A Review of Techniques; M. Kaláb (453/81/I)

*Electron Microscopy and Sensory Evaluation of Commercial Cream Cheese; M. Kaláb (473/81/I)

*Morphological and Textural Characterization of Soybean Mozzarella Cheese Analogs; M.V. Taranto (483/81/I)

*A Comparison of the Microstructure of Dried Milk Products by Freeze-Fracturing Powder Suspensions in Non-Aqueous Media; W. Buchheim (493/81/I)

*Effect of Acidulants and Temperature on Microstructure, Firmness and Susceptibility to Syneresis of Skim Milk Gels; V.R. Harwalkar (503/81/I)

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**PLANT FOODS**

*REVIEW: Food Microstructure: An Integrative Approach; E.A. Davis (1/82)

*Correlation of Microscopic Structure of Corn Starch Granules with Rheological Properties of Cooked Pastes; D.D. Christianson (13/82)

*Protein Bodies in Dormant, Imbibed and Germinated Sunflower Cotyledons; R.D. Allen (63/82)

*REVIEW: Grain Structure and End-Use Properties; Y. Pomeranz (107/82)

*Scanning Electron Microscopy of the Pericarp and Testa of Several Sorghum Varieties; C.F. Earp (125/82)

*The Microscopic Structure and Chemistry of Rape-seed and its Products; S.H. Yiu (135/82)

*Light Microscopy Preparation Techniques for Starch and Lipid Containing Snack Foods; F.O. Flint (145/82)

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*Microstructure of Mayonnaise and Salad Dressing; M.A. Tung (523/81/111)
REVIEWING PROCEDURE
AND
DISCUSSION WITH REVIEWERS

Each paper in this volume contains a Discussion with Reviewers. This discussion follows the text and should be read with the paper. Each paper submitted to SEM, Inc. for publication is reviewed by at least three, up to an average of five, reviewers. The reviewers are asked to separate their comments from their questions. The comments are useful in determining the acceptability of the papers as submitted. Although the comments require no written response, in several cases, the authors have included responses to comments, or to questions phrased from or based on, comments (either as a result of editorial suggestions or on the author's own initiative). Based on these comments approximately 15% of the submitted papers were not accepted for publication; while almost all of the others were asked to make changes involving from minor to major revisions.

The questions, for the most part, originate as a result of statements included in our cover letter accompanying each paper sent to the reviewers. The reviewers are asked to suppose they are attendees at a conference where this paper, as written, is being presented, and then ask relevant questions which would occur to them resulting from the presentation. From the questions so asked, some are not included with the published paper because the authors attended to them by text revisions. In some cases, editorial and/or space considerations may exclude inclusion of all questions asked by reviewers. The authors are asked to prepare their Discussion with Reviewers section in a camera-ready format. In some instances the authors edit the questions and/or combine several similar questions from different reviewers to provide one answer. While all efforts are made to check that the questions in the printed version faithfully follow the views of the specific reviewer, the editors apologize, if in some instances, the actual meaning and/or emphasis may have been changed by the author.

The cover letter to the reviewers states:

"1. Your name will be conveyed to the author with your review UNLESS YOU ASK US NOT TO.

2. The questions published in the Journal will be identified as originating from you UNLESS YOU ADVISE OTHERWISE."

In all cases sincere efforts are made to respect the reviewer's wishes to remain anonymous; however, in nearly 95% of the cases, the reviewers have given permission to be identified; so their names are conveyed to the authors and are included with the questions printed with each paper. An overall list of reviewers is provided in the opening pages of each SEM part. We apologize for any error/omissions which may occur.

Finally, readers are urged to be cautious regarding the weight they attach to the authors' replies, since the answers to the questions represent the authors' unchallenged views--except for minor editorial changes--the authors generally have the last word. Also, please consider that the questions were, in most cases, relevant to the originally submitted paper, and they may not have the same significance for the revised paper published in this volume.

If you disagree with the results, conclusions or approaches in a paper, please send your comments, as a Letter to Editor, typed in a column format (each column is 4-1/8 inches wide and 11-1/2 inches long; i.e., 10.5 by 29.3 cm.). Your comments along with author's response will be published in a subsequent issue.

The editors gratefully thank the authors and reviewers (see p. i & ix) for their contributions, invite your comments on ways to improve this procedure and seek qualified volunteers to assist with reviewing papers in the future.

ERRATA: Despite the best efforts of authors, reviewers and editors, errors may remain. Please help by pointing out errors that you notice. Please provide enough information to locate each error (volume, page, column, line, etc.) and indicate suitable correction.

The Editors
Food Microstructure
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The help of the following reviewers for papers in this issue of FOOD MICROSTRUCTURE is gratefully acknowledged. The names of the people who helped us with reviewing of the papers published in FOOD MICROSTRUCTURE Vol. 3(1) are listed in that issue on p. i.

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Important Note: The time restrictions we work under require that each reviewer returns his/her review (along with the manuscript sent) within a set time from its receipt. Please do not commit yourself if you feel that you cannot respond within this time frame; while we are grateful for your desire and efforts to help us, the reviewers who do not respond in time, in fact, seriously hamper our efforts to publish these scientific papers in time.
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Papers for Food Microstructure (FM) may be offered at any time. papers can be for publication only, or intended for oral presentation at an Annual Food Microstructure meeting in early spring. The latter papers are due two months prior to the start of the meeting; only papers acceptable for publication are allowed oral presentation. Oral presentation of a paper at some other meeting or publication as unreviewed abstract (e.g., in proceedings, etc.) does not preclude consideration of a paper by FM. The letter accompanying the paper should contain names and complete addresses of at least four persons competent to review the paper. Suggested reviewers: a. must neither be from author's current or recent affiliations, nor workers; b. should preferably be active researchers in the field (e.g., whose work is being extensively referred to); and c. need not be personally known to the authors. The editors will select the most suitable reviewers respective of their location. Each paper will be intensely reviewed by at least three reviewers. The initial paper (hereafter referred to as "paper") should conform to these Instructions. However, to be published after reviewing, the final manuscript (hereafter referred to as "manuscript") should be either a. submitted on the model sheets conforming to the Manuscript Preparation guidelines (mailed along with the reviewers' comments), or b. sent to FM Inc. for preparation at a nominal cost (per details mailed with the manuscript). In addition to all the text, the manuscript may have to contain author's publishable responses to questions raised by the paper's reviewers (the Discussion with Reviewers in papers published in FM).

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