Food Structure

Volume 5 | Number 1 Article 2

1986

Features of Food Microscopy

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FOOD MICROSTRUCTURE, Vol. 5 (1986), pp. 1-18
SEM, Inc., AMF O'Hare (Chicago), IL 60666-0507 U.S.A.

0730-5419/86$1.00+.05

FEATURES OF FOOD MICROSCOPY

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Abstract

The value of food microscopy is judged by its practical application to food handling. Hence microscopists must explain the relevance of their findings to food technologists. The food microscopist has to deal with materials that are particularly difficult to prepare for microscopy because they often contain high levels of fat, air, sugar, salt, starch or acid. Sometimes the methods used are unorthodox and could be regarded as questionable by microscopists in more traditional disciplines. This paper considers the relationship between food microscopists, food technologists and other microscopists.

An approach to interpretation of images is based on the following features.

1) All interpretations should consider preparation processes, however simple these may be.
2) Interpretations should be based, where possible, on differences between treated and control samples processed in the same way.
3) Microscopical observations should be linked to technological, chemical or physical observations.
4) Key observations should be checked by more than one microscopical technique.

This paper uses past and recent work at the Leatherhead Food R.A. to demonstrate how these criteria have been applied to fats, vegetables, meats, proteins, confectionery, surface fouling and foreign bodies.

The future of food microscopy is an exciting prospect, applying recent microscopical techniques to novel manufacturing problems.

Initial paper received February 20 1986
Manuscript received May 4 1986
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KEY WORDS: Foreign bodies, Heat exchanger fouling, Microscopy of proteins, Microscopy of fats, Microscopy of fruit and vegetables, Microscopy of meat products, Microscopy of sugar confectionery, Artefacts, Microscopy of glass fragments, Role of microscopy in food science.

Prologue

"Any fool can look down a microscope." The attitude to this statement has a profound influence on the operation of a microscopy department. Is the role of microscopists to maintain 'foolproof' techniques for others to apply, or should microscopists involve themselves with all aspects of research programmes?

Introduction

Food microscopy is the application of microscopy to technology. In this sense the microscopist has to act as the bridge between food science and technology, and the classical branches of microscopy. This role requires a mastery of the skills of microscopy and sufficient understanding of the relevant processes to be able to recognise and explain the significance of microscopical observations to food technologists. These sentiments may seem obvious but they establish the relationships between food microscopy, technology and other branches of microscopy. In this paper I should like to consider these relationships and demonstrate the contributions of microscopy to food science.

Dealing with Food Technologists

In some establishments the microscopy staff is regarded as 'just a service section', who provide pictures of relevant samples on request. Indeed, in some cases the sample is accompanied by a note of the observation required. In other places the microscopists appear to operate in isolation, with little attempt being made to integrate their observations into applied research. In my view, using a microscopy department in these ways is a quite inefficient use of a most valuable asset. The most important feature that a microscopist can add to any project is that of considering the problem from a different perspective. The food industry embraces a wide range of disciplines but most food scientists tend to view food from a background in chemistry, physics, microbiology or engineering. The result of this is that problems are tackled at two levels: either as a consideration of molecular interactions or in terms of bulk properties. At both levels the approach is normally numerical. Microscopists tend to view foods in concepts rather than numbers and at a level of organisation which is intermediate between molecular interaction and bulk behaviour. Hence the microscopist's viewpoint should add an extra dimension to the consideration of any problem. However, it is often difficult for the non-microscopist

to deal with pictures rather than numbers and to appreciate intermediate levels of structure. It is therefore essential for the microscopist to be able to relate his findings to bulk behaviour or molecular interactions in order to communicate effectively. This requires that the microscopist is fully involved with the planning and background of any project and provides opinions as well as photographs. This approach can lead to problems; in particular, 'demarcation disputes' can occur and the practical applications of microscopy often depend as much on the personalities involved as on technical considerations.

One of the problems that a microscopist faces is the question of artefacts. This fear of artefacts is often advanced as a reason for not incorporating microscopy into a research programme. In my experience a technical defence explaining preparation techniques and precautions has only limited effect. A positive approach with more impact is to compare microscopical procedures with other scientific approaches. In this way a microscopist's function is to interpret images which are produced by a controlled process in the same way as a biochemist may interpret a reading from an oxygen electrode placed in a slurry of tissue, or a chemist may interpret a burette reading as relating to a particular level of a component. In the case of the microscopist, the thought process is more obvious since a picture is, in general, not immediately interpreted numerically. With the advent of many 'black box' instruments, even in microscopy, all scientists need to be aware that their 'results' are in fact interpretations, even if the interpretations are carried out electronically. Microscopists should take a lead here.

In dealing with food technologists, therefore, microscopists need to show that they can contribute to the development of ideas and should make every effort to integrate their findings into the overall research programme.

Dealing with Other Microscopists

As I have previously mentioned, food microscopy is very much an applied branch of microscopy. Because of this, food microscopy is heavily dependent on methodology and technology developed by microscopists in other disciplines. However, food microscopy has special problems associated with specimen preparation compared with conventional biological methods. Almost invariably, processed foods are 'dead' and so the conventional view of fixation as preserving the living structure of cells does not apply. Many foods contain high levels of fat, sugar, air, salt, starch or acid, and conventional biological procedures may be inappropriate. The methods adopted to cope with particular food problems would probably horrify microscopists used to dealing with more conventional tissues. Examples of processes adopted to meet specific problems include fixation with hot uranyl acetate to follow the setting process in jam, long-term (several weeks) fixation in osmium tetroxide to preserve fat in chocolate and periodic acid/thiocarbohydrazide/osmium tetroxide fixation to preserve and stain starch gels. Where conventional fixatives are used it is sometimes necessary to use them in the presence of quite high levels of salt, sugar or acid to allow fixation before extraction. Sometimes fixation and dehydration need to be combined, e.g. using glutaraldehyde in ethanol, in order to restrict extraction of components.

In the case of an applied microscopist trying to help with a particular problem, I think that the approach 'at all costs prepare the sample for microscopy' is valid since microscopy is often the only way to obtain some structural information on the sample. This approach needs to be tempered by two concepts – respect for the microscope and respect for the sample. The first of these requires that the sample should be produced in a suitable form to allow reasonable interpretation and the second requires safeguards to be applied in considering the validity of the techniques used. The type of techniques sometimes needed will, quite naturally, present a fear of artefact production in most biological microscopists and many food microscopists. In the same way as a positive approach to artefacts is useful in dealing with food technologists, so a positive approach to artefacts should be adopted in dealing with other microscopists: 'having prepared your sample for microscopy, remember what you have done to it'. In my view, if the sample has been prepared for microscopy, the image obtained will invariably reflect the preparation technique as well as the intrinsic structure of the specimen. This view is probably acceptable to most microscopists concerning electron microscopy but it also applies to light microscopy. At a simple level the refractive index and dispersivity of a mounting medium affect the image produced in the light microscope. Even as I write this paper I see that the Proceedings of the Royal Microscopical Society contains an article by Robinson (1986), making a similar point and using the appearance of soft margarine by different illumination techniques as an example. So *every* image should be interpreted with the preparation procedures in mind, even where those procedures are regarded as the best available.

A second safeguard in dealing with artefacts is to base interpretations wherever possible on differences between control and treated samples processed for microscopy in identical ways. In this manner differences in structure can be related back to the treatment.

Thirdly, all microscopical observations should be linked to parallel technological, chemical and/or physical observations on the samples. This is the 'integrative approach' recommended by Davis and Gordon (1982).

Finally, key observations should be checked where possible by alternative preparation techniques. For example, where the method of choice is thin sectioning, some samples could be prepared by freeze-etching and vice-versa. Where possible, light and electron microscopy should be used to complement each other.

I hope that by now I have made my views clear on the role and philosophy of microscopy in food science. A particular problem arises where certain microscopy facilities are not directly available and the services of an essentially medical, biological or materials science department have to be used. In this case, persuading the microscopist operating the instruments of this general philosophy may be particularly difficult, but in my view it is also vital.

I should now like to illustrate the contribution of microscopy to food studies using examples from work at the Leatherhead Food R.A. before presenting my opinions on the future of food microscopy.

Some Past Work at the Leatherhead Food R.A.

Creaming Power of Fats in Cakes

One of the early applications of electron microscopy at the Food R.A. was a study of the creaming power of fats (Meara *et al.*, 1974). This is a good example of the integrative approach to microscopy as the work combined light and electron microscopy with X-ray diffraction, dilatometry, nuclear magnetic resonance and creaming power measurements. Several different fats were examined, thereby meeting the control

against treated criterion. The methods used were innovative, examining replicas of frozen samples and in some cases using a detergent wash prior to freezing in order to remove the liquid phase and reveal the crystal structures. An awareness of the effects of specimen preparation was needed since if the detergent was too concentrated the whole sample was dispersed, whilst if the detergent was not concentrated enough the liquid phase was not removed.

The first observation was based mainly on light microscopy but was confirmed by electron microscopy. Small individual crystals (~ 1 μm), mostly in the form of small uniformly sized clusters (3–4 μm), were found to be beneficial in producing a good creaming power fat. Polymorphic form of the crystals seemed less important although, on the whole, B′ crystals were smaller than B crystals and so produced fats with higher creaming power. The overall level of crystallinity was also important as the development of a good batter involved a liquid fat film around the air cells stabilised by a network of crystals. Smaller crystals could pack into the network better than large crystals; hence lard with very large crystals creamed very poorly whilst commercial shortening with smaller crystals creamed well. An anomaly was observed with rearranged lard which had large crystals but creamed quite adequately. This anomaly was resolved by electron microscopy, which showed that the crystals in rearranged lard had wavy edges (Fig. 1) and readily broke down during creaming to give small crystal fragments (Fig. 2). This work led to a much clearer understanding of the role of fats in creaming and to a better definition for the specification and monitoring of fats for use in cakes.

At about the same time as this work, studies on chocolate fats were underway. This has already been reported in some detail (Jewell, 1974; Berger *et al.*, 1979; Lewis, 1981).

Fruit and Vegetable Products

Unlike the work on the creaming power of fats, which studied a precise and well-defined phenomenon, the work on vegetable products has been more diverse and has been spread over a longer period. Early work on pickles related changes in microscopical appearance to Instron measurements during various stages of pickle manufacture (Saxton and Jewell, 1969; Jewell, 1972). Again, an innovative approach to methodology was used, adapting the periodic acid/thiocarbohydrazide/silver proteinate technique to study cell wall structure (Jewell and Saxton, 1970). The development of methodology indicated that hemicelluloses were most readily stained by this technique.

The work demonstrated that initial changes in texture on brining could be attributed to plasmolysis, but after several days in brine the protoplasts were completely disrupted and further textural changes during freshening and acidification were due to changes in the plant cell wall constituents. Even when the protoplast had not been degraded by brining, acidification produced breakdown of the membrane, thereby destroying any turgor pressure effect on texture. Pectin staining decreased with storage in brine and on acidification, whilst 1,2-glycol staining (presumed to be mainly hemicelluloses) decreased on storage in brine but not on acidification.

Rantsios and Jewell (1971) and Jewell *et al.* (1973) considered factors responsible for the breakdown of strawberries in jam making. From a study of the structure of strawberries they considered that strawberries with more vascular tissue would be more resistant to breakdown. The make-up of a strawberry is such that each achene (or seed) on the surface of the strawberry is linked to the centre of the strawberry with a vascular strand; hence the relative density of achenes on the surface of the strawberry should give a measure of its likely

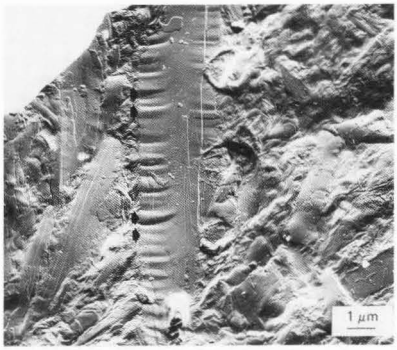

Fig. 1. TEM of rearranged lard. Replica of frozen, detergent-washed sample showing large crystals (arrow) with wavy edges.

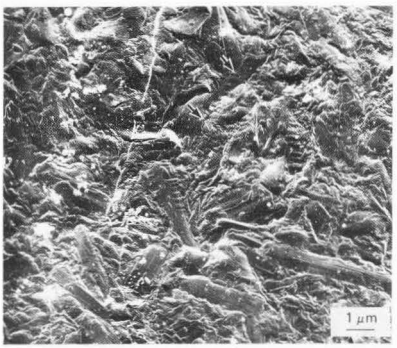

Fig. 2. TEM of cake batter prepared with rearranged lard showing many small crystal fragments (arrow). Preparation as Fig. 1.

breakdown on processing. Therefore, small strawberries with many seeds should be more resistant than large ones with few seeds. Experimental results tended to support this hypothesis although other factors such as berry ripeness and general firmness of the tissue are also active in controlling tissue breakdown.

Work on processing plant tissues continued with a study of different blanching regimes and their effects on carrots (Mirza and Jewell, 1976). This work confirmed the role of cell walls in control of texture; blanching regimes, such as microwave blanching, produced much cell wall damage, producing soft carrots, whilst those causing less cell wall damage, such as direct steam blanching, produced firmer carrots. More recently, a study of the effect of various enzymes on cell wall structure and consequent texture change has been carried out (Holgate, personal communication). These observations showed that pectinase treatment had a major effect on cell walls, causing layering of cell walls and separation of cells in potato (Figs 3–6);

these changes were accompanied by excessive softening. Cellulase had a minor effect on both structure and texture and promoted fracture of cell walls rather than cell separation. Hemicellulases, pectinesterase and proteases had little effect on texture or cell wall structure. General observations on softening processes in plant tissue produced by ripening, spoilage, heating and other processes have shown changes in the cell wall similar to those seen in pectinase-treated tissue. This tends to confirm the view that pectin behaviour is the most important feature in controlling texture in processed plant foods. The use of calcium and calcium chelating agents to produce firmer or softer products or to shorten processing times has been shown to be feasible.

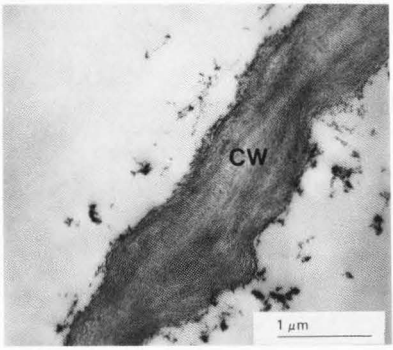

Fig. 3. TEM of thin-sectioned raw potato showing compact cell wall (CW).

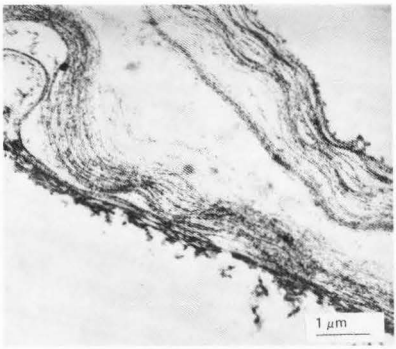

Fig. 4. TEM of thin-sectioned potato which had been treated with pectinase enzyme showing diffuse cell wall splitting into layers.

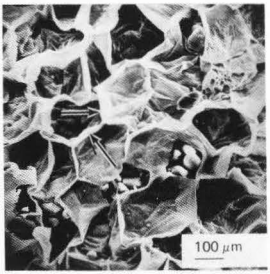

Fig. 5. SEM of cut surface of raw potato showing cell walls fractured across (arrow). Prepared by solvent dehydration followed by vacuum drying from amyl acetate.

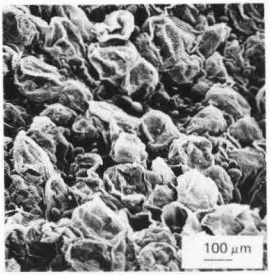

Fig. 6. SEM of cut surface of pectinase-treated potato showing cell walls separating along middle lamella. Preparation as Fig. 5.

Proteins

Light microscopy observations on pre-formed 'emulsions' prepared with a soya isolate were related to cooking losses by Parker and Lewis (1976). Salt had a large effect on the cooking losses of these systems and in particular the time of addition of salt during the chopping process was important.

Dissolving salt in the water before adding the soya isolate and fat produced high cooking losses. When salt was added at the end of the chopping process the cooking losses were lower than when no salt was present. Microscopically, stabilisation of the fat was seen to be due to the formation of a protein network entrapping clumps of fat cells and free fat. When salt was present in the water before the soya isolate then less protein was dispersed from spray-dried particles and so a coherent network was not formed. When salt was added at the end of the mixing process the soya isolate had already dispersed and the salt appeared to improve the heat stability of the network.

These observations have been followed by studies on gelation of soya isolates, in this case using mainly electron microscopy and gel strength measurements (Groves, personal communication). Early observations indicated that the fixation step was not critical in handling cooked and cooled gels although

fixation with hot glutaraldehyde was useful in following changes during cooling of the gels. Working with soya isolate at a level of 16% m/V in water, it was found that a substantial amount of the protein remained in spray-dried particles even after prolonged mixing and standing overnight. During cooking the particles swelled and more protein was extracted to form a network. The soya isolate gel was a composite structure of swollen spray-dried particles held together by an extracted protein cement (Figs 7 and 8). In this sense soya isolate gels resembled starch gels reported earlier (Lewis, 1981). The formation of a network in the extracted protein was partly a non-reversible aggregation during heating and partly a reversible aggregation on cooling. Heating to retort temperatures (110°C and 120°C) produced much greater aggregation during heating to both the extracted protein and that remaining in the spray-dried particles (Figs 9 and 10).

Salt had a marked effect on the gelation of soya isolate, particularly if the salt was dissolved in the water before the soya isolate was added. In this case the gel strength was lower than with no salt at temperatures up to 80°C but was increased at temperatures above 80°C. The gel at higher temperatures was found microscopically to be made up of swollen spray-dried particles which had very little cementing network but which interlocked together – rather like a three-dimensional jigsaw (Figs 11 and 12).

Different soya isolates were examined and some differences were observed in the extent of dispersion from spray-dried particles and the effects of salt. However, in all cases the behaviour of the spray-dried particles was a significant feature in the gels.

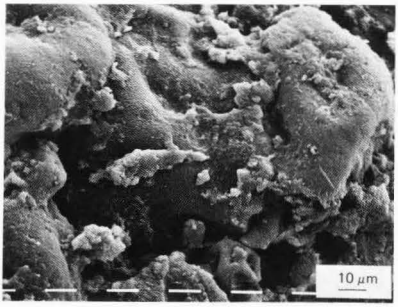

Fig. 8. SEM of soya isolate gel heated to 80°C. Prepared by solvent dehydration followed by vacuum drying from amyl acetate.

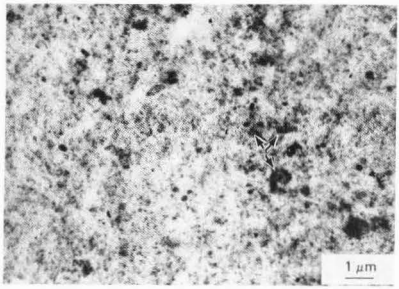

Fig. 9. TEM of thin-sectioned soya isolate gel heated to 120°C. Note aggregation of protein (arrows).

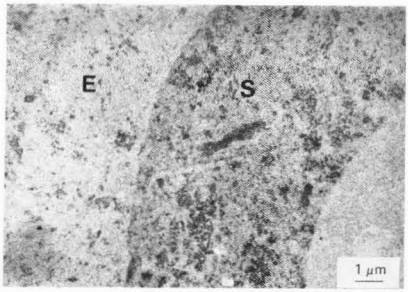

Fig. 7. TEM of thin-sectioned soya isolate gel heated to 60°C. Note spray-dried particles (S) and extracted protein (E).

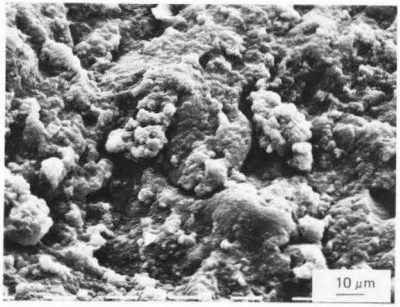

Fig. 10. SEM of soya isolate gel heated to 110°C.

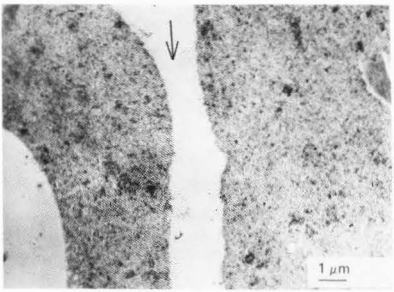

Fig. 11. TEM of thin-sectioned soya isolate gel with salt, heated to 98°C. Note very little extracted protein (arrow) between spray-dried particles.

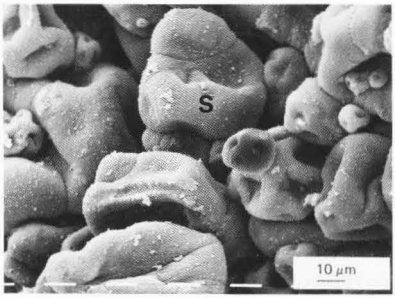

Fig. 12. SEM of soya isolate gel with salt, heated to 98°C. Note swollen spray-dried particles (S).

Meat Products

Some observations on the effects of salt and polyphosphate on pork meat have already been reported (Lewis, 1981) and further investigations are being reported elsewhere. (Lewis *et al.*, 1986).

Various meat cuts have been surveyed to assess their fat-binding ability. In the methodology used, cryostat sections were stained with acid fuchsin to show protein and osmium tetroxide to show fat. Osmium staining was found to produce less 'smearing' than the more usual stains such as Sudan Black B. This work showed that meats behaved in one of four ways.

Type I meats broke down into small pieces during chopping but did not form a network. These meats did not retain fat well. Type II meats formed a coarse network after a short period of comminution but this broke down on longer chopping to give isolated meat fragments surrounded by fat; again these meats did not retain fat well. Type III meats formed a coarse network after a short period of comminution and this developed into a finer, more extensive network on prolonged chopping; these meats generally retained fat quite well. Type IV meats formed a fine network after a short period of comminution and this network was retained on further comminution; these meats were best at retaining fat.

All samples characterised as types I and II had a pH of 6.0 or less, whilst the pH of type III and IV meats ranged from 5.75 to 6.35.

A meat which showed type II behaviour was comminuted with polyphosphate present in addition to salt and water, and in this case the behaviour became that of type III and fat retention was considerably improved.

This work indicated that the performance of some meats could be improved by the addition of polyphosphates and suggested that meats with lower pH values might be more amenable to polyphosphate action. This idea is developed further by Lewis *et al.* (1986).

Toffee and Sugar Confectionery

Campbell *et al.* (1931) studied the keeping qualities of boiled goods. They placed spots of boilings between coverslips and observed the change at the edge of these spots when the coverslips were stored in a moist atmosphere and the edge was examined daily. They showed that crystallisation started as the viscosity was reduced near to the surface by absorption of water and that the zone of reduced viscosity spread through the mass of the boiling as further crystallisation released more moisture. The effectiveness of various dusting agents in preventing moisture uptake by boiled sweets was also studied, as was the effect of porosity of the structure of sugar goods on moisture uptake. Finer dusting powders and less porous structures were found to reduce the moisture uptake.

This work established guidelines for composition and storage conditions of boiled goods that form the basis of present-day commercial practice. In these studies microscopy was used mainly to illustrate the changes occurring in boiled goods so that Grover (1941) and Lees (1965) use micrographs to illustrate features of crystallisation.

The use of microscopy as a truly explanative tool was not revived until the advent of electron microscopy at the R.A. in the late 1960s. Stansell and Jewell (1975) studied crystallising systems with microscopy and proposed the following hypothesis of sugar crystal growth.

> "Nucleation in sucrose solutions is related to the availability of free hydrogen bonding sites to permit sucrose/sucrose interaction and this occurs above 73% sucrose (s = 1.20 supersaturation). Nucleation is followed by the formation of aggregates of particles of dimensions of 25–30 nm which undergo further association to produce crystallites and thence crystals. If the mother liquor concentration falls below a supersaturation of 1.20 then growth proceeds as layer growth."

An example of aggregates packing into larger structures is shown in Fig. 13.

This hypothesis helped to explain the rapid growth of crystals during the early stages of sugar crystallisation and led to the development of a procedure for growing small crystals to make an icing sugar rather than grinding large crystals (Stansell, 1977). The technique involved shearing sugar syrups at controlled concentrations and temperatures to induce regions of crystallisation within the mass of syrup. Icing sugar produced by this technique, known as microcrystalline sugar, consisted of clean-faced crystals, as revealed by microscopical examination (Fig. 14). The properties of icing sugar were related to the microscopical characteristics of the sugar, and a large number of very fine particles (less than 0.2 μm) were found in milled icing sugars (Fig. 15). It was considered that these very fine particles could lead to the formation of a compacted cake and thus account for some of the poor handling properties of icing sugar. Microcrystalline sugar which had very few fine particles had

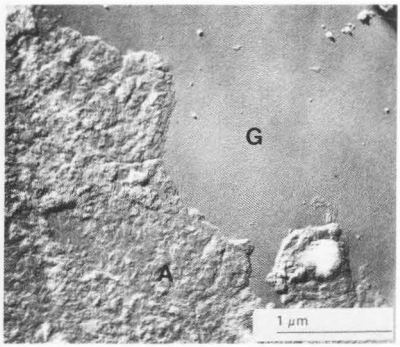

Fig. 13. TEM of freeze-etched preparation of 80% sugar syrup. Note aggregates of microcrystals (A) and glass regions (G). Shadowing direction approximately top to bottom, printed through intermediate.

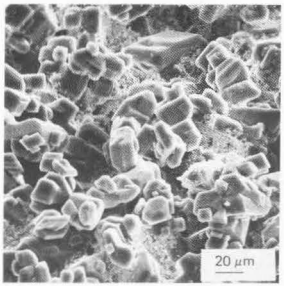

Fig. 14. SEM of microcrystalline sugar. Note clean crystal surfaces.

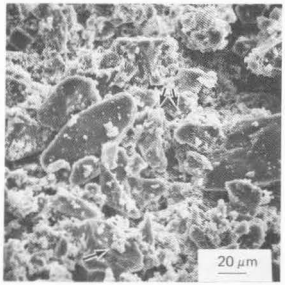

Fig. 15. SEM of icing sugar. Note many fine particles (arrows) on surface of crystals.

rather better handling and dispersibility properties than conventional icing sugar. In this case particle sizing was carried out by image analysis techniques and by Coulter Counter. It is interesting to note that the 'fines' in icing sugar represented an insignificant proportion by weight of the sugar although they represented a large proportion of the number of particles present in the samples. This indicates the importance of specifying whether a size distribution is based on weight (or volume) or on numbers of particles.

Toffee has also been studied microscopically. Grover (1937) used microscopy to study emulsification of oil in toffee making. Mineral oil incorporating a red dye was used to prepare toffees and the size of droplets was assessed at the pre-mix stage and in the finished toffee. Droplet size in finished toffee was assessed by dispersing the toffee in warm water, mixing with a little gelatin and allowing a drop of the dispersion to set under a coverslip on a microscope slide. This procedure is similar to the one currently in use at the R.A. except that agar is used instead of gelatin as this tends to produce less coalescence with some of the modified milk proteins now available. In Grover's study, three different skimmed condensed milk samples of differing consistency were used to produce toffee and the thinnest consistency milk was not quite so good as the others in producing an emulsion at the pre-mix stage. However, the emulsion that was formed by thin-consistency condensed milk was more stable on cooking to produce toffee. Later work on toffee involved electron microscopy and again the effects of different condensed milks on the structure and flow properties of the toffee were examined. This work indicated that toffee made from condensed milk with small casein micelles tended to have a small fat droplet size in the finished toffee; this led to a high-yield stress and produced toffee that was difficult to deposit. Hence it was considered that some breakdown of the emulsion during toffee manufacture was desirable. Recently, a wide range of modified milk proteins has been studied as ingredients in toffee making. In this work the light microscopy technique was adapted for electron microscopy and this has allowed the interactions between proteins occurring at the fat-droplet membrane to be studied in more detail. The toffee dispersion in agar was fixed, dehydrated and embedded for thin sectioning. Care must be exercised in interpreting results from dispersed toffees. In this case, freeze-fracture studies were carried out in parallel to the thin sectioning and light microscopy was also used to assess the toffees. This approach indicated that whey proteins tend to form a flexible membrane at the surface of the fat globule membrane and in toffees made only with whey this results in a fairly stable emulsion during toffee making. When casein is present it associates with whey at the interface and this makes the membrane more brittle, allowing some breakdown of the emulsion during cooking (Figs 16 and 17). The size of casein micelle is also important: in calcium-reduced milks the casein micelles are very much smaller than those in standard skimmed milk powders (Fig. 18). The smaller micelles do not appear to produce brittleness in the membrane and the emulsion remains stable during cooking. These findings were linked to properties of the toffees, in particular, viscosity, colour, storage behaviour and texture. The state of the emulsion and fat-droplet membranes related to the viscosity, those toffees with small droplets and flexible membranes producing toffees with lower viscosities.

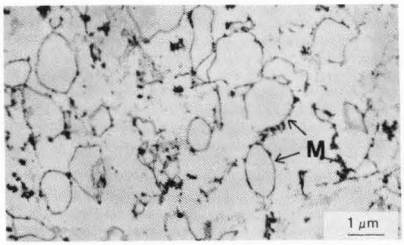

Fig. 16. TEM of thin-sectioned toffee (caramel) made with whey protein. Prepared by gently dispersing toffee in agar solution and embedding blocks of agar containing toffee dispersion. Note membrane (arrows) around fat droplets (M).

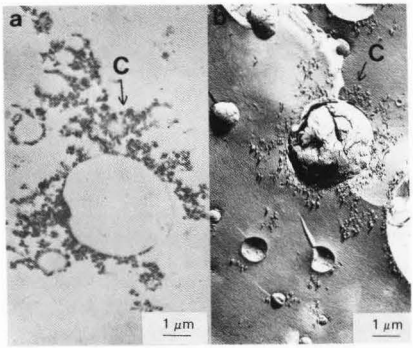

Fig. 17(a). TEM of thin-sectioned toffee made with skimmed milk powder. Prepared as Fig. 16. Note casein aggregates (C) on membrane. (b). TEM of freeze-etched toffee made with skimmed milk powder. Shadowing direction approximately top to bottom, printed through intermediate.

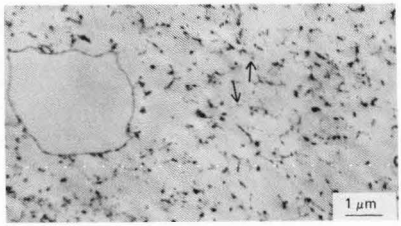

Fig. 18. TEM of thin-sectioned toffee made with calcium-reduced skimmed milk powder. Prepared as Fig. 16. Note small size of casein micelles (arrows).

Some Recent Work at the Leatherhead Food R.A.

Fouling of Heat Exchangers by Foods

The build-up of deposit on heat exchangers is a matter of considerable economic consequence to the food industry. The deposit results in poor heat transfer, which in turn means that higher temperatures and more energy are required for a given heat process. The deposit also increases the amount of cleaning time required and may break off to contaminate the product.

Most of the previously reported work on this topic has been carried out on dairy products and in these studies very little attention has been given to a study of the microstructure of the deposits.

The work at the Food R.A. combined microscopy with food engineering. A pilot-scale fouling rig was designed and built and consisted of a hot water or steam-heated pipe placed inside an insulated tube through which the product was circulated. The steam pressure or water temperature in the heating pipe could be controlled and monitored and the product inlet and outlet temperatures were recorded throughout each experiment to determine the change in heat transfer efficiency as a deposit builds up on the pipe. Blood plasma was used as the protein for most of the experimental work but critical observations were checked with soya isolate, whey protein isolate, egg albumen and gelatin solutions.

Microscopical methods. Samples were examined by light microscopy and transmission electron microscopy. Several different methods were used depending on the nature of the deposit.

1) Direct observation of deposit on heated pipe by SEM. Three-inch lengths were unscrewed from the length of the pipe and the deposit was allowed to air dry. The pipe section was carbon coated and examined in a Cambridge S250 scanning electron microscope.

2) Removal of dried deposit. When the pipe was removed from the rig whilst still hot the deposit dried on the pipe within a few minutes. Generally, the deposit could be removed from the pipe with a scalpel. The removed deposit could be examined directly in the SEM after sputter-coating with platinum.

3) Removal of wet deposit. In most cases the pipe was cooled before removal from the rig and the deposit took over an hour to dry on the pipe. In order to allow a rapid 'turn round' time of experimental runs the deposit was removed whilst still wet. The removed deposit was floated in a petri-dish containing distilled water, collected on cellulose acetate sheet and allowed to air dry for SEM examination. Samples for TEM and light microscopy were transferred to a fixative; either 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, or Mirsky's fixative (a commercial fixative available from National Diagnostics Ltd) was used. It was found that samples could be stored in the fixatives for long periods at room temperature without noticeable deterioration. After fixation, the samples were dehydrated through a graded alcohol series, embedded in LR White resin and sectioned at 2 μm thick for light microscopy and less than 100 nm thick for electron microscopy. Some samples were transferred from absolute alcohol to amyl acetate and vacuum dried, or to Freon 113 and air dried for SEM. The structures seen were essentially similar for both fixatives used and for direct air drying and solvent vacuum or air drying. Sections for light microscopy were stained with acid fuchsin or mounted and stained in 0.2% toluidine blue, 30% glycerol/0.66% phenol mountant. For electron microscopy, sections were stained in 4% aqueous uranyl acetate followed by 0.2% lead citrate

or 7% methanolic uranyl acetate without counterstain or 2% alcoholic phosphotungstic acid.

4) Replica technique. In some cases the deposit was very difficult to remove from the pipe in pieces large enough to examine by SEM. Sometimes a segmented pipe was used (method 1) but a replica technique was also developed. This technique was essentially as described by Scott (1982). A primary replica of the dried surface was prepared with Xantopren Blue (Bayer Ltd) – silicon rubber dental impression material. A secondary replica was prepared from the primary replica using Stycast 1266 (Emerson and Cumming Ltd). The directions given by the manufacturers of these products were followed. The secondary replica was sputter-coated and examined by SEM.

The replica technique was compared with some direct observations and found to give good correlations for 'low-profile' deposits, which were generally the most difficult to remove from the pipe. Thicker deposits with larger undulations gave some problems with air bubbles in the replicas; however, these could be easily recognised by SEM.

Electron microscopy was carried out on a JEOL 1200 EX electron microscope operating at 80 kV in the TEM mode and 40 kV in the SEM mode. Larger samples and samples for X-ray microanalysis were examined at ERA Technology, Leatherhead, using a Cambridge S250 SEM equipped with a Link 860 analysis system; this microscope was operated at 20 kV.

Observations. The initial conditions for operating the fouling rig used steam at low pressure as the heating medium and a heating time of 45 minutes using 1% blood plasma in tap water constantly recycled through the plant to build up a deposit. The essential structure of the deposit is shown in Figs 19–21. Figure 19 shows a stereo pair of the surface of the deposit as seen by SEM and reveals a deposit with many cavities. The nature of these voids is revealed by examining cross-sections; Fig. 20 shows the light microscopy view of the deposit and the montage presented in Fig. 21 shows the TEM view. X-Ray microanalysis spectra from the top and bottom surfaces of the deposit are shown in Figs 22 and 23. Our interpretation of these results is as follows. The deposit consists of at least two layers. A primary layer is formed close to the heated surface as a compact layer of protein, which is also associated with calcium either in the form of protein complexes or as 'scale' deposited independently of the protein. A secondary layer (or layers) is formed on top of the primary and this layer is much less compact, containing protein flocculates. The overall structure of the deposit is highly aerated, suggesting that air or steam bubbles forming at the pipe surface are acting as nuclei for the denaturation of the protein film.

This interpretation, once accepted by all those involved with the project, led to two main avenues of investigation: (i) the influence of calcium ions in the water on the deposit; and (ii) the influence of air/steam bubbles on the deposit.

The influence of calcium ions was studied using double-deionised water with calcium chloride added at various levels. In general terms the deposit was thicker at higher calcium levels and at low calcium concentrations the deposit was confined to a primary layer structure (Fig. 24). Increasing calcium levels increased the amount of secondary layer produced. Runs with tap water and no protein produced a scale deposit on the pipe (Fig. 25). The effects of steam/air bubbles was pursued in two ways. By using lower heating temperatures (e.g. water at 80°C and 90°C) it was possible to obtain similar deposit structures to those obtained with low-pressure steam, indicating that air bubbles rather than steam were the more likely cause of the deposit build-up. The sequence of events seems to be that air comes out of solution at the heated pipe surface and protein denatures at the hot air surface, trapping the air in place and building up the deposit layer. Air is a good insulator and so heat transfer efficiency falls off quite rapidly as the deposit builds up. The second approach was to apply a back pressure to the product side of the fouling rig. As the applied back pressure was increased so the amount of deposit decreased until at about 10 KPa over pressure the deposit was hardly present (Fig. 26).

Conclusion. The work on fouling is a good illustration of the practical application of microscopy. The methods used, especially allowing samples to air dry, may be considered crude but they are effective in dealing with a type of material which is probably not widely encountered outside the food industry. Apart from the technical problems of preparing samples, the presentation of the results in a convincing way was also important: in this respect the stereo pairs, which give excellent 3-D effect, were a major asset. Once the interpretation had been accepted, the follow-up experimentation to confirm the findings could be planned. Throughout the work microscopy was undertaken alongside heat transfer and thickness measurements with generally good agreement. The work presented here is an extract from a more comprehensive study of the factors affecting protein fouling of heat exchangers.

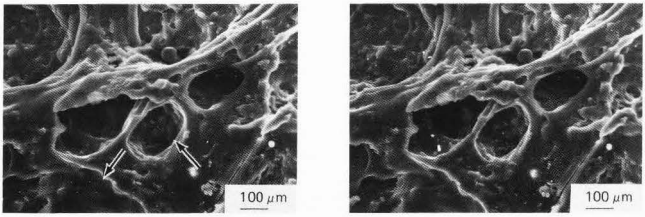

Fig. 19. SEM of deposit of blood plasma on heated surface. Stereo pair. Note large air inclusions (arrows).

Fig. 20. (above) LM of section through blood plasma deposit from tap water. Note air inclusions (arrows) in layer.

Fig. 21. (left) TEM montage of thin-section through blood plasma deposit from tap water. Note dense primary layer (P), flocculant secondary deposit (F), outer membrane (O) and air inclusions (A).

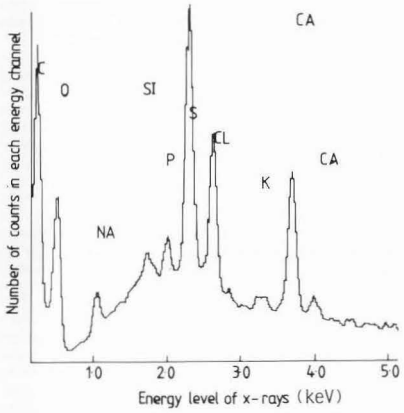

Fig. 22. Top surface of pipe deposit.

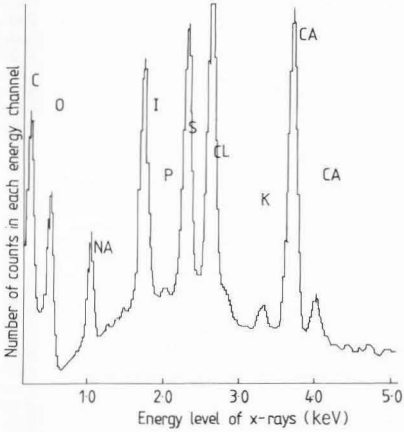

Fig. 23. Bottom surface of pipe deposit.

Fig. 24. TEM of thin-section through blood plasma deposit from deionised water. Note thin compact primary layer (arrow), same magnification as Fig. 21.

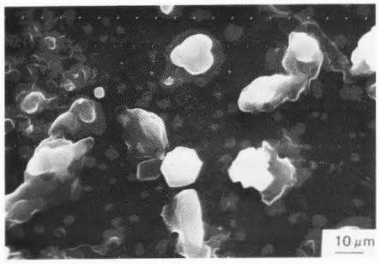

Fig. 25. SEM of scale from tap water. Prepared by replica technique.

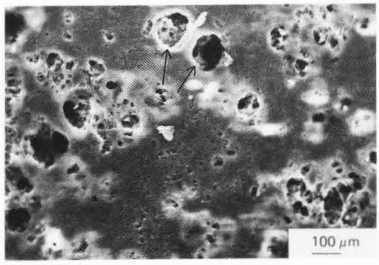

Fig. 26. SEM of deposit of blood plasma from water at 22 psi back pressure and 10 psi steam pressure. Note fewer air inclusions (arrows) in deposit.

Foreign Bodies in Foods

Foreign matter is a constant cause of concern to food manufacturers, and people who use microscopes in the food industry are often expected to assist in the investigation of foreign bodies. Consequently, the first contact between a food technologist and a microscopist often relates to a foreign body investigation, and so the technologist's first impression of food microscopy will often depend on how well a foreign body is identified. It is well worth while for the food microscopist to develop skills in handling foreign body problems since these will establish his or her reputation in the eyes of many food technologists. One of the main problems in foreign body identification is the difficulty in specifying which test or tests should be carried out since each occurrence requires an individual approach.

The literature of general approaches to foreign bodies in food is fairly limited although Gorham (1981) covers a wide range of contaminants; Wallis (1965) and "The Particle Atlas" (McCrone and Delly, 1973) are also extremely useful and cover a range of materials likely to be found as foreign matter in foods. Specific types of foreign matter, such as insects, hairs and wood, have a more extensive literature, and a recent bibliography (White and Shenton, 1984) gives a good introduction to this literature.

Two recent areas of study at the R.A. have related to foreign bodies in foods. The first of these was prepared by Smith (1983) and is presented as a scheme for examining foreign material contaminants in foods. The scheme gives a good approach to foreign bodies and suggests the following stages in identification. A preliminary examination should be carried out to obtain as much information of the sample as possible with as little disturbance as possible. This generally involves the use of a low power stereomicroscope and checking any observations against the recorded history of the sample. During the preliminary examination an attempt should be made to classify the sample, and the following categories are suggested, along with some comments to aid classification: recognisable objects; metal; metal/non-metal composite; animal/vegetable (biological); crystalline; fibrous; laminar; amorphous (hard); amorphous (soft); composite.

Once the sample has been placed in a category, a more specific identification can be attempted. This can be usefully based on checklists of possible foreign bodies for each category and confirmatory tests can be applied for each suspected item. If identification is not possible, an attempt should be made to reclassify the sample in the light of the tests carried out. A simple example may help to clarify this scheme. Some black cigar-shaped objects about 5 mm long and 2 mm wide were submitted. At first sight these were considered to be mouse droppings. However, when they were cut open they were found to be quite firm and to have white centres. These were sectioned and found to consist of masses of filaments. The bodies were now classified as fungal in origin and this was checked by staining in cotton blue in lactophenol. Finally, our mycologist confirmed that they were indeed mould sclerotia.

Checklists and suggestions for confirmatory tests are included in Smith (1983) and both the initial classification and confirmatory tests make extensive use of microscopical techniques. Many of the microscopical techniques are given in a Manual of Microscopical Methods (Lewis, 1978).

Glass fragments. Glass fragments are among the more common foreign bodies found in foodstuffs. In most years about 10% of prosecutions for foreign bodies in food relate to glass (Martin, 1984). There is a wide range of possible sources for glass contamination and it is in the food manufacturer's interest to identify the origin so that action can be taken to prevent further occurrences.

The first stage in the analysis of glass is to examine the sample in a stereoscopic light microscope at magnifications of 10 x to 40 x, asking the following questions: Are any original surfaces present? Are the surfaces moulded, polished or drawn? Are there chips or scratches which are likely to have been caused before breakage or during a food-processing operation? Is any debris present on the surfaces; is it present on all surfaces? What can be deduced about the shape of the original items; in particular can a diameter of curvature or thickness be estimated?

Typical features seen by low-power microscopy are shown in Figs 27–35. The initial examination of the glass will often give a strong indication of the glass origin and confirmatory tests can be applied. Debris from the surfaces can be removed and identified using a compound microscope; this will often reveal the nature of the foodstuffs with which the glass has been in contact. The diameter of curvature may be estimated; larger pieces can be estimated by fitting to standard curves whilst examining at low magnification, whilst smaller pieces can be derived by interference fringes. Locke (1984) describes equipment for obtaining such fringes; a less elaborate method using the interference fit between the sample surface and a glass slide is described in Lewis (1978). Some interference patterns are shown in Figs 36 and 37.

Refractive index and density are used widely in forensic science, particularly to compare samples. Hot-stage methods and dispersion-staining techniques are often used for determining refractive index, and extremely good discrimination between samples is possible (McCrone, 1974; Locke, 1985). However, in tracing the origin of an unknown glass sample, refractive index and density are of less value because of the overlap of values for different glass types. Borosilicate glass (e.g. Pyrex) can generally be identified by its low refractive index and density, but window glass and container glass tend to overlap each other. A list of refractive index and density ranges for glass types is given in Table 1.

Table 1
Refractive index and density ranges for glass types

	RI	Density
Borosilicate	1.47–1.48	2.2–2.3
Container glass	1.51–1.52	2.5–2.7
Window glass	1.51–1.54	2.4–2.6

Energy-dispersive X-ray microanalysis was investigated as a possible means of examining glass with a view to determining its origin. A total of 280 samples was examined, including food-container glass, domestic table and kitchenware glass, lighting glass, 'Pyrex' glass and some specialised glasses.

Methods. Small fragments broken from the glass were mounted, fracture side upwards, in Tempfix on a piece of microscope slide. Care is taken to align the fracture surface parallel to the slide; this is done by manipulating the sample under a dissecting microscope whilst the Tempfix is still warm. The fragments were carbon coated by evaporation from four carbon fibre sources at a pressure of $\sim 1.10^{-4}$ Torr. The glass samples were examined in a Cambridge S250 scanning electron microscope operated at 20 kV and equipped with a Link

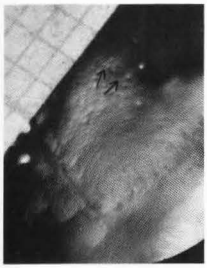

Fig. 27. Irregularities (arrows) in moulded surface.

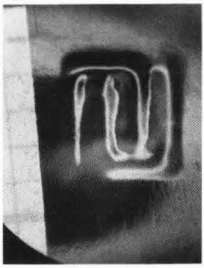

Fig. 28. Container glass sample showing Rockware logo.

Fig. 29. Groove (arrow) produced by wire in reinforced window glass.

Fig. 30. Scratches (arrows) on rim of 'Pyrex' basin.

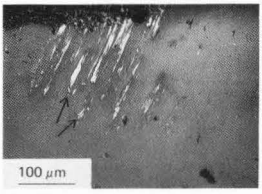

Fig. 31. Backscattered electron image of white metallic flecks (arrow) on 'Pyrex' glass. X-Ray microanalysis showed the flecks to be iron and chromium.

Fig. 32. Chips caused by machinery.

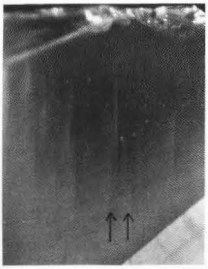

Fig. 33. 'Stretch marks' (arrows) which run around the side of drinking glasses.

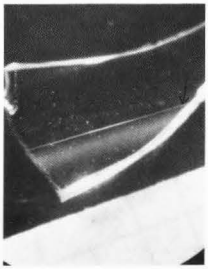

Fig. 34. Fine capillary (arrow) in drawn tubing such as fluorescent light tubes.

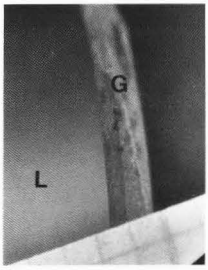

Fig. 35. Optically polished lens (L) and ground edge (G).

Fig. 36. (left). Interference pattern from drawn cylinder. Note regularity of pattern of approximately parallel fringes.

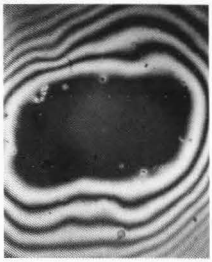

Fig. 37. (right). Interference pattern from moulded container. Note irregular pattern of fringes showing irregularity of moulded surface.

Systems 860-500 series 2 X-ray analysis system, using standardised conditions for analysis.

The system was adjusted to accept energy levels appropriate for sodium, magnesium, aluminium, silicon, sulphur, chlorine, potassium, calcium, barium, chromium, and iron. The peak area for each energy band was expressed as a percentage of the peak areas for all the elements analysed. (Figure 38 shows a typical printout of relative peak areas.)

Spectrum = Container glass sample manufactured by Rockware (sample C15)

Gain = 20 eV/Chan
Spectrum length = 1024 chans
Pre-set live time = 125 s
Actual live time = 250 s
Pre-set integral = 65535 cnts

Window label	Window centre	First channel	Last channel	Net integral	Percentage total
Na	1000	880	1140	12224	8.96
Mg	1240	1180	1320	1368	1.00
Al	1460	1400	1540	626	0.46
Si	1760	1580	1960	106789	78.26
S	2300	2240	2380	40	0.03
Cl	2600	2540	2680	0	0.00
K	3300	3180	3440	879	0.64
Ca	3660	3500	3840	14392	10.55
Ba	4480	4340	4620	17	0.01
Cr	5420	5320	5520	2	0.00
Mn	5880	5800	5980	0	0.00
Fe	6400	6200	6620	110	0.09

Fig. 38. Printout of glass analysis.

All glasses were examined under the standard conditions with an ultra-thin carbon window, and additionally some glasses were examined at 40 kV accelerating voltage and/or with a berylium window. This made it easier to discern low levels of barium, chromium and iron. With some glasses (e.g. light bulbs), inside and outside surfaces were examined in addition to fracture faces.

Currently, glass samples are analysed routinely using a berylium X-ray window and efficiency factors used to relate the counts to those obtained with a carbon window. Two standard glasses are included with each batch of analyses to check the efficiency factors.

Estimates of variation were made by repeatedly examining the same area, analysing different pieces of the same item, analysing pieces from different items in the same manufacturing batch and analysing pieces from different manufacturing batches. These suggested that within-item and within-batch variations were likely to be as great as between-batch variations and indicated that, where possible, several different pieces of glass from each sample should be analysed.

Examination of the analyses of glasses in the different categories, e.g. Pyrex, container, domestic, etc., suggested that the calcium and sodium peaks formed a basis for some discrimination. Lead-containing glasses could also be separated as specialised glass. With regard to non-lead-containing glasses, Pyrex and heat-resisting glasses had low sodium contents (3–6% of total peak area) and very low calcium levels (< 0.2%). Non-Pyrex glasses had higher sodium levels (> 7%). U.K. container glass had fairly high calcium levels (mostly 8.5–12%), whilst domestic glass was slightly lower (mostly 7–9%) and lighting glass was lower still (mostly 4–6.5%). Window glass had a variable calcium distribution, although a number of samples (including Pilkington and most car windscreens) had calcium levels around 7–8% (of total peak area) and a smaller group of window samples had levels around 11–14%. Modern window glass, produced by the float process, could often be recognised by a small tin peak on one surface. Some further differentiation seemed possible based on potassium levels and magnesium levels, and a scheme was drawn up to give a guide to the likely origins of glass samples. (Figures 39–43 show typical spectra for selected glass types.)

The scheme allowed glasses to be categorised into twelve categories. Some of the categories were quite distinctive. For example, forty-seven of the fifty-one glasses in one category were container glass; other categories contained mixtures of glass types. On the whole, the scheme seems to give a reasonable separation for the samples examined. However, the classification should not be applied too rigidly since some variation has been found between different pieces of the same glass. Also, only fairly limited numbers of samples in some categories have been examined, and it is not always possible to deduce the likely distribution of glasses as foreign bodies. However, the scheme seems to provide a guide to the likely origins of glass samples. In some cases, carrying out several analyses may help to decide between container or domestic glass but, in other cases, the overlap has to be accepted. Where possible, it is preferable to include possible sources of the glass for comparative analysis with the unknown sample, and in this way it may be possible to obtain evidence linking a particular container or other glass object with a foreign body. This can be particularly useful where a piece of glass is found within a glass container.

X-Ray analysis is a useful technique for helping to identify the origin of glass but it should always be combined with as many other observations as possible.

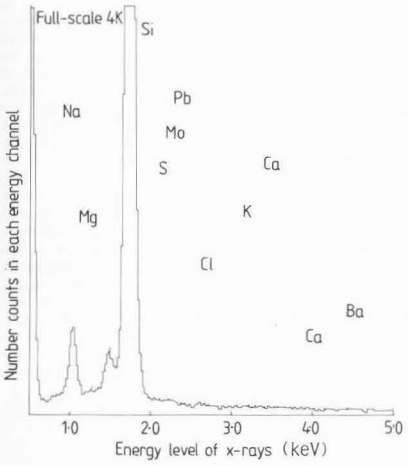

Fig. 39. Spectrum from laboratory 'Pyrex' glass.

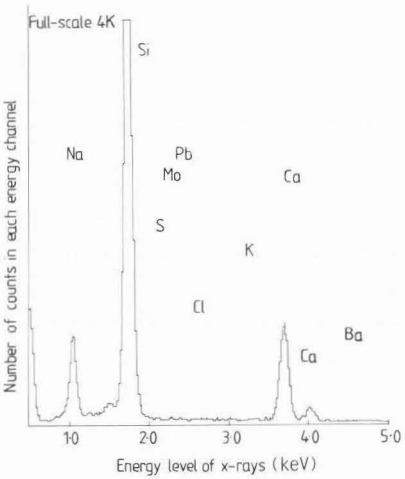

Fig. 41. Spectrum from window glass.

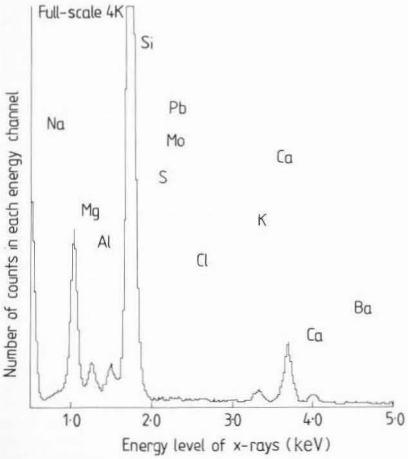

Fig. 40. Spectrum from light-bulb envelope.

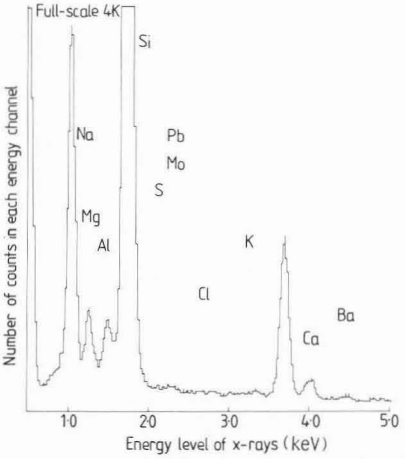

Fig. 42. Spectrum from glass mug.

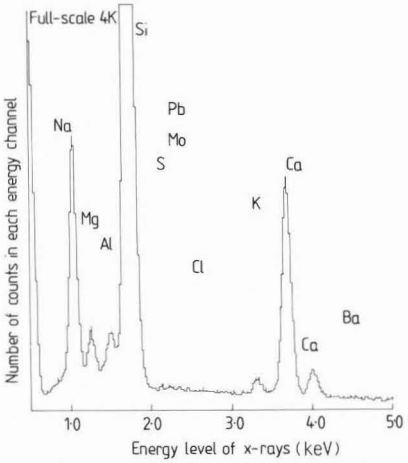

Fig. 43. Spectrum from container glass manufactured by Rockware.

The scheme has been used in practice for two years and the majority of glasses received have been recognised based on analysis and examination of surface features. In cases where known samples have been supplied (for comparison with unknowns), the scheme has accurately categorised the glasses.

Conclusion. Foreign bodies cover a very diverse range of materials and consequently no hard and fast schedule can be drawn up. Some general guidelines can be applied: firstly, gather as much information as possible whilst disturbing the sample as little as possible. From this information try to categorise the item and make a selection of confirmatory tests. If the confirmatory tests prove negative, re-assess all the available information, recategorise the sample and select some more confirmatory tests; repeat this process until the sample is identified – or no more material remains for analysis. In the identification of foreign bodies, microscopy and microscopic analytical techniques are invaluable as they allow information to be gained from a limited amount of material.

Future Prospects for Food Microscopy

There are many areas of 'conventional' food technology which are incompletely understood and where microscopy will undoubtedly be involved in providing explanations; the sources of variation in meats, protein functionality, behaviour of polysaccharide gelling agents and gums, textural consequences of low-fat and low-salt products and crystal properties of sugars, fats and water are examples of such areas. So, for a while yet, the microscopical approach which I have illustrated earlier will continue to be useful.

Taking a longer-term view, however, I see the food microscopist being asked rather more difficult questions. I think that these questions will arise from two sources: firstly, novel methods and ingredients will require understanding to allow optimisation of food processes and, secondly, microscopical techniques will allow more precise localisation of components. Some examples of the new technologies are irradiation, extrusion cooking, microwave heating, intensive processes and ingredients made by biotechnology and genetic engineering. The main difference in approach for the microscopist compared with dealing with more conventional operations will be that both the technology and the microscopy will be exploring new ground. In applying microscopy to present-day food science much of the technological behaviour of foods is well known, and hence microscopical observations can be related to well-known facts. In exploring new technologies it may well be that the microscopist will be able to guide the progress of technology rather than just aid process optimisation. As an example, Cassens *et al.* (1984) present the possibilities of producing custom-made meats with, say, a predominance of one type of fibre. The role of the food microscopist in this case could be to demonstrate the performance of each fibre type on processing and so advise on how to produce meat custom-made for particular products. This should provide an exciting role for those food microscopists bold enough to accept the challenge.

I see an equally exciting and challenging prospect when I look at the advances being made in microscopical techniques. X-Ray microprobe and other microanalysis methods, various cryotechniques, gold and fluorescent labelling, autoradiography and chemical location reactions are obvious candidates for applying to food systems and should allow the microscopist to approach more difficult problems involving the precise role of individual components in complex food mixtures. Other techniques such as scanning ion microscopy, scanning tunnelling microscopy, acoustic microscopy, scanning light microscopy, NMR microscopy, and X-ray microscopy, as yet in the early stages of development, may open up entirely new prospects for the food microscopist.

However, the food microscopist will need to adapt most of these methods in order to apply them to food systems with their high levels of salt, air, fat and sugar. Adaptation will not always be easy and will involve first mastering the techniques on conventional samples before attempting to apply them to foods. An example of one of our current problems may help to illustrate this point. An area of concern is the distribution of curing salts in bacon. At a fairly crude level the distribution can be determined by energy-dispersive X-ray microanalysis of 10 μm thick cryostat sections. This indicates a crucial role of meat connective tissue in controlling distribution, especially in injected meat samples. In order to obtain a more precise distribution of salt, particularly differences in concentration within and between different cell types, a chemical localisation technique was attempted. Pyroantimonate is widely reported as a precipitant for many cations including sodium (Simpson and Spicer, 1975). Small pieces of bacon were processed according to some of the recommended methods for sodium, in the anticipation that the likely problem would be that the precipitation would be too great throughout the tissue to allow differences in composition to be seen. In fact, the problem was that no precipitation at all was observed within the samples since all the pyroanimonate was precipitated outside the tissue by salt diffusing from the meat. As yet the problem remains unsolved although possibly cryoultramicrotomy could help if it were a more routine technique.

The explosive development of computer techniques in recent years has made it possible for image analysis to be used

routinely. This has advantages and disadvantages. The advantages are that digital imaging allows many microscopical features to be presented in a more convincing way – for example using computer-generated colours or producing electronically 'enhanced' images – and also allows data from micrographs to be presented numerically. There is a danger, however, that these computer interpretations will be accepted as absolute fact. As with all aspects of food microscopy, the microscopist must use new techniques but must use them thoughtfully.

Conclusions

Microscopy has played a vital role in the explanation of food behaviour and will continue to do so. To be more effective, microscopy must be fully integrated into all stages of research projects. The future for food microscopy is exciting for those who are prepared to accept the challenge of guiding food technologists and are prepared to learn and apply advances in other branches of microscopy. Every food microscopist should have a clear philosophy to guide his approach. The current state of food microscopy is largely exploring the effects of major structural features on food behaviour, and this suggests to me that a bias towards the application of results, as opposed to seeking ultimate structural detail, is justified. The maturation of food microscopy as an innovative science may lead to a need for more subtle variations to be understood and the emphasis may need to change. However, the need for a clear philosophy will remain.

Epilogue

The trouble with allowing fools to look down microscopes is that they are likely to come to foolish conclusions. The food microscopist has a duty to ensure that conclusions produced from images are sensible in terms of the microscopical techniques used and relevant in terms of food processing.

Acknowledgements

All figures are reproduced by courtesy of the Leatherhead Food R.A. and I am grateful to the Director, Dr A.W. Holmes, for permission to present this paper. I am also grateful to the past and present members of the Microscopy Section whose efforts have made this paper possible. Much of the work included was funded by the Ministry of Agriculture, Fisheries and Food.

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Discussion with Reviewers

R.J. Carrol: Could you comment on the future role of energy-dispersive X-ray (EDX) analysis and electron energy loss spectroscopy (EELS) in food microscopy research?

Author: I think there are two aspects to this question. Firstly, EDX is a technique which impresses chemists and food technologists and is useful in helping to overcome reservations about the use of microscopy. The image processing packages with modern systems also enhance the presentation of microscopy and encourage application of results. Although this is not strictly scientific, I think food microscopists would be well advised to take advantage of this aspect of modern instrumentation.

The second aspect is the more scientific application of these techniques. Currently, I think EDX has more applications because of its versatility, being useful in the SEM, STEM and TEM modes. I can see further developments in windowless detectors and combined use of EDX and backscattered electron detectors as leading to great improvements in the usefulness of EDX in the future.

EELS has slightly more restricted applications as it can only be used with thin specimens. However, the recently announced Zeiss microscope with integrated EELS and offering quite high resolution elemental mapping may indicate the way TEMs will develop.

Both techniques have the potential to locate elements in foodstuffs and this could lead to technological advances in food handling. For example, a knowledge of the distribution of salts in meat could lead to better microbiological control; monitoring the distribution of calcium or potassium ions in gels and plant tissues could lead to improved texture control of foods, and localising the elements of emulsifiers could lead to optimisation of recipes and processes.

The use of both EDX and EELS systems for elemental mapping in foods will, of course, require the solution of many specimen preparation problems.

D.P. Dylewski: Are only whey proteins incorporated into the membrane surrounding emulsified lipid in toffee? Or might other proteins be involved?

Author: Toffees made with whey protein as the only protein source show a membrane round fat droplets. I believe that casein, when present, associates with the whey protein in the membrane; where the casein micelles are large this results in the membrane becoming more brittle. With small casein micelles, such as in calcium-reduced milks, I suspect that the casein may be more directly involved at the fat/syrup interface. Of course, all the above is my interpretation of what I see and how the toffee performs; other explanations may be possible.

D.N. Holcomb: Are there any 'Food Microscopists' organisations? Would such a group be of value if they do not already exist?

Author: The Food Microstructure journal and meetings are the only regularly organised events for food microscopists that I know about. I believe that the American Association of Feed Microscopists and 'Sektionen Futtermittelmikroskopie und Futtermittelmikrobiologie der internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung' exist to promote feed microscopy. I think that an organisation for food microscopists would be most useful and the renewed interest in food microscopy in recent years suggests that it should be possible to start such an organisation. Possibly the Royal Microscopical Society could be persuaded to foster such a group.