

Food Structure

Volume 12 | Number 3

Article 1

1993

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Lewis, D. F. (1993) "Development of the Food Microscopist," *Food Structure*: Vol. 12 : No. 3 , Article 1.
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DEVELOPMENT OF THE FOOD MICROSCOPIST

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Abstract

This paper describes the processes through which the food microscopist develops his/her skills. It is based around a framework of Shakespeare's seven ages of man. The first stage is learning about equipment: how it works and how not to abuse it. Next, attention turns to how to prepare samples for examination whilst maintaining the validity of the observations and avoiding damage to the instrument. The "Lover" is the time at which an understanding of the basic structures of food products is gained and the "Soldier" starts to interpret these structures in terms of the performance of the food-stuff. Next, the interpretation must be negotiated with food technologists in order to influence the processes of manufacture. The sixth age sees a more managerial overview of food microscopy. The final challenge is to avoid the near oblivion of Shakespeare's last scene of all!

Key Words: Microscopy techniques, Interpretation of food structure, ice cream, meat, confectionary.

Prologue

All the world's a stage,
And all the men and women merely players:
They have their exits, and their entrances:
And one man in his time plays many parts,
His acts being seven ages.....

Jacques in "As you like it"
by William Shakespeare

There is a considerable amount to be learned as a food microscopist. It is not simple book learning either, but is rather an apprenticeship, and the learning does not stop. People develop new skills and visions as their appreciation expands. A key issue is how to assist food microscopists to achieve their full potential. One of the prime aims of this paper is to describe a view of how food microscopists develop. The intention is that this can act as a guide for microscopists and their managers, who, by recognising some of the stages of development, may be able to assist their progression. The view is based on observation of food and other microscopists over a number of years and loosely follows Shakespeare's seven ages of man although not all of his descriptions are quite appropriate. It is also necessary to realise that none of the stages is ever completed; there are always new things to learn about instruments, specimens and relationships. Hence, the seven ages represent the times at which new, additional philosophical approaches are added to the microscopist's armoury.

At First, The Infant

Most microscopists cut their teeth by appreciating instrumentation. There is much to learn. Whether the field is light or electron microscopy, the infant microscopists must learn to respect the instrument and discover its limits and limitations. As with a child, much of this discovery comes through play and it is wise to allow some time for this aspect of learning. "Playing" with the microscope is a vital exploration of its potential although, of course, the "play" needs to be guided and linked to theoretical considerations. In this respect,

Paper received December 21, 1992
Manuscript received July 6, 1993
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modern microscopes offer some advantages over their predecessors, in that, there are generally rather more safety devices to prevent damage when the operating limits are exceeded. As with all benefits, there is a price to pay; computer control is efficient and often extends the capabilities of the instrument, but it makes the operator one step removed from the microscope and some understanding of its working is lost.

Also, protecting the user from some of the more dire consequences of his/her actions can lead to a less careful approach to the instrument. It can be frustrating to realise that a trainee microscopist will never learn some of the fundamental principles because a computer automatically makes the necessary corrections, but this frustration should be countered by the knowledge that instrumental development will allow today's microscopists to achieve much more.

The process of learning about instruments is one of getting a "feel" for the microscope and so it is difficult to define precisely what will be learnt. Some examples, however, may serve to illustrate the point.

For all microscopists, one of the key early developments is to gain a sense of the most appropriate magnification. I was told that the first set of electron micrographs I took would be at too high a magnification, and that the second set would be too low! This is an important lesson to learn. The trade-off of increased detail against the benefits of seeing features in a wider context is not always easy to resolve, and does not always appear to be appreciated even by some experienced microscopists. At some stage, the microscopist also needs to appreciate the relative values of a range of instruments, and knowing, for example, when to use a polarised light microscope rather than a scanning electron microscope (SEM) is a vital learning step. Some general guidelines can be given. Magnifications in excess of $\times 1,000$ will normally dictate electron microscopy in some form, where three-dimensional information is needed then either SEM or confocal light microscopy is indicated, and where the specimen is especially susceptible to dehydration then either simple light microscopy or cryo-SEM is likely to be the method of choice. Beyond this basic guidance the microscopist's intuition, based on experience is the best guide.

Methods of increasing contrast and maintaining detail are important to the light microscopist. An appreciation of the use of the condenser diaphragm and understanding the importance of front focal plane of the condenser in obtaining contrast are key features, which should be appreciated at an early stage. Figure 1 illustrates some of these effects.

In the electron microscope, the choice of accelerating voltage on the final image is important both in transmission electron microscope (TEM) and SEM.

Figure 2 illustrates the effect of changing accelerating voltage on the appearance of bacteria on polymer-based beads. The effect of using too high an accelerating voltage is to increase the depth from which the signal is derived and hence make it more difficult to see the surface detail, including the bacteria.

Books help the microscopist to appreciate the microscope and a short list of useful books is given in the bibliography.

The process of learning about instruments never stops; for example, the Leatherhead Food Research Association (LFRA) recently bought a confocal microscope and this has required a fair amount of "play" time to explore its exciting potential. The instrumental learning phase is common to all strands of microscopy and some microscopists do not progress beyond this stage. The food microscopist, however, has a functional role and can only regard this stage as a stepping stone.

And Then The Whining Schoolboy

With some reluctance, we have to accept that the novelty of the instruments will wear off and having achieved some mastery of the instrument, the would-be microscopist now has to grapple with specimen preparation. This is especially challenging for food microscopists since probably no other discipline has to cope with such a wide range of samples and preparation techniques. At the LFRA, for example, preparation may involve polishing glass samples for X-ray microanalysis, cryostat sectioning at -45°C coupled with cold-shielded transfer to a cold stage, resin sectioning high-fat or high-sugar systems, or preparation of samples for specific labelling. It is rare indeed for a single technique to give all the necessary information and the food microscopist needs to develop an instinct for how particular samples will react to various methods of preparation and for recognising the less useful structures resulting from specimen preparation.

Many food microscopists will start with basic histology of essentially biological specimens and so sectioning will be the first technique to be tackled. Here the pupil needs to measure the merits of different approaches. Cryostat sections are quick and are useful in, say, meat products, where retention of fat or other soluble structures is important; however, resin or wax sections of fixed material often show greater detail. The value of different stains and stain combinations is also a vital point to be learned. As with learning about instruments, a certain amount of trial and error is needed to develop the reference biological methods for food use.

The transmission electron microscopist has a rather harder time in trying to prepare samples; the high fat, air, sugar, salt and acid levels encountered in some

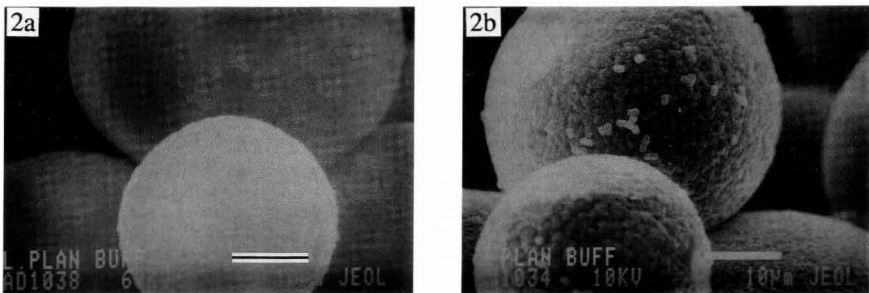
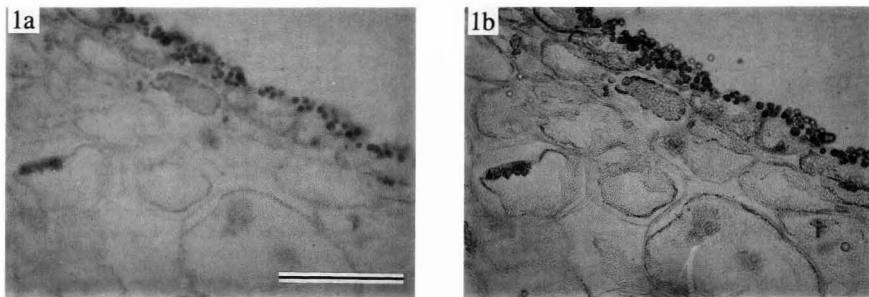


Figure 1. Effect of condenser diaphragm on image contrast and quality. Bar = 10 μ m. a) Fully open; b) two-thirds open. Sample is edge of strawberry fruit, wax section stained with saffranin light green.

Figure 2. Effect of accelerating voltage on image in SEM. Bar = 10 μ m. a) 60 kV; b) 10 kV. Sample is resin beads with adhering bacteria.

foods mean that extensive deviation from established biological methodology is often required. A key realisation has to be made: all specimen preparation techniques will modify the appearance of the final image. In other words, every image is an artifact of the specimen preparation process. Superficially, this can seem very depressing but, in fact, it frees the microscopist to tailor specimen processing to suit structural elements under consideration. An example may help to clarify this point. Long-term (several days) fixation in osmium tetroxide will allow fat to be retained for electron microscopy and will show the distribution of fat in a meat product, and reveal fat crystals within the liquid fat matrix. However, the proteins in the product will largely be extracted and in order to understand the structure

more fully, some specimens should be prepared using only glutaraldehyde fixation, which will show where the proteins are even though the fat will be removed during dehydration and embedding. A third option would be to use short osmium fixation to preserve membranes and similar structures. An understanding of the value of the replica techniques, negative staining and thin sectioning, and the way in which they can complement each other must also be achieved. In essence, this is only possible by examining samples prepared by all these different techniques. Some guidance may be found by consulting methodology based journals and looking for publications on similar specimens. Sectioning is often the easiest to understand but does involve extensive chemical treatment of the sample, replicas of frozen samples are

often much more difficult to interpret but provide an essential check on the results obtained by sectioning. Figure 3 shows meat (muscle) structure as revealed by thin sectioning and freeze etching to illustrate this point.

New instrumental techniques bring with them new challenges and opportunities. X-ray microanalysis and image processing techniques have implications for specimen preparation. A recent development has been the confocal scanning laser microscope. In this case, it is generally very easy to present the specimen to the microscope and much of the methodology development is based on finding stains to bring out key features. However, the three-dimensional capabilities of this instrument also allow some experimentation with preparation techniques. Freeze substitution has been used in biological microscopy for some time but has not been applied much to food systems. Studying ice crystals within a frozen food is of importance in terms of the eating quality of the product. It is possible to use cryostat sectioning coupled with cold-stage light microscopy to see ice crystals (Figure 4) or to use cryo-SEM (Figure 5); however, the three-dimensional nature of these approaches is rather restricted. Freeze substitution offers the possibility of retaining the outline of ice crystals within a bulk preparation of a product. Groves and Lewis (unpublished observations) have made a preliminary assessment of the application of this technique to ice cream. Figure 6 gives an indication of the results. This work is not fully developed and undoubtedly there is much to be learned but it illustrates the possibilities for method development in this area.

And Then, The Lover

Indeed, this is the stage at which a love of microscopy sets in! Now the microscopist can prepare and examine samples; he (she) learns to recognise and appreciate the key structures and to see their many variations and subtleties.

At university, one of my flat mates was studying botany and one day he described his understanding of plant structure like being able to walk round inside the tissues. I did not appreciate what he was saying until I started my doctorate work on meat structure, when suddenly I had the same experience. Although the work was mainly TEM of thin sections, and hence very two-dimensional, everything was understandable as part of a three-dimensional world. Recently one of my (younger) colleagues expressed, quite spontaneously and independently, exactly the same sentiments. Of course, microscopy is much more three-dimensional now. This change started with stereo pairs and SEM. It has developed with computer reconstructions. The wider use of confocal microscopy will extend this three-dimensional world and make it more accessible.

Figure 3. TEM of pork shoulder meat prepared by thin-sectioning (a) and freeze-etching (b). Bar = 1 μ m

Figure 4. Cold-stage light microscopy of ice-crystals in commercial ice cream. Bar = 100 μ m.

Figure 5. Cold-stage SEM of commercial ice cream. Bar = 100 μ m.

It is very difficult to teach this aspect of food microscopy; the only real way to learn is to spend time with the prepared specimens in the microscope. Books, papers and experienced colleagues can help. Often, however, the most useful work will be in unexplored areas and established sources can only be used to provide fixed points of reference to prevent the ardent microscopist from becoming completely lost. The manager's role in this process is two-fold: firstly, to allow the time for exploration of samples in sufficient detail, and secondly, to make sure that the microscopist is aware of the many traps and pitfalls that can be encountered.

Then a Soldier

The battle now is interpretation. Simply feeling at home inside the structure is no longer enough; there has to be a purpose to the exercise. The purpose of course is to understand how the structure relates to the behaviour of the food system. The strategy is to recognise the most important features and to discover to which aspects of functionality they relate. Changes may be all around and at every level of magnification or may be camouflaged within the finest levels. The campaign is to discover the key items in the relationship and then exploit and develop this intelligence.

Clearly, a link has to be made between the structure and other properties of the product. The other characteristics of the product may be physical (such as viscosity or hardness), compositional (such as variety of vegetable, protein type or sugar balance), processing performance (such as cooking loss), or sensory (such as texture or flavour release).

An example of combining sensory results with microscopy can be seen in some microscopy work carried out largely by Kathleen Groves in collaboration with the Sensory team at LFRA led by David Kilcast. Three commercially available fruit pastilles were subjected to sensory analysis and amongst other things showed differences in initial bite hardness and toughness on chewing. The textural "star diagram" (Fig. 7) illustrates the sensory attributes of these three types of pastille. Sample A had a fairly hard initial bite but softened fairly quickly on chewing; sample B had a hard initial bite and remained fairly tough through the chewing process; and sample C was fairly soft initially and remained soft

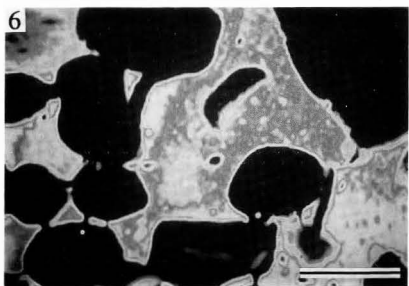
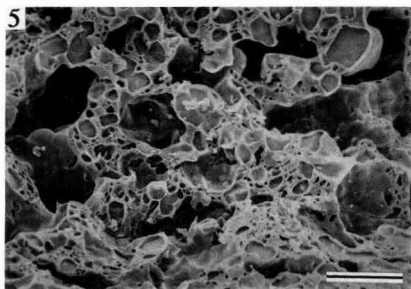
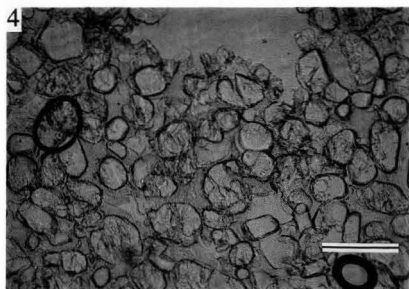
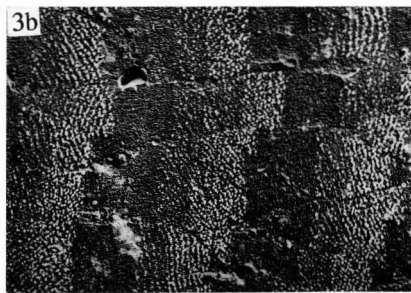
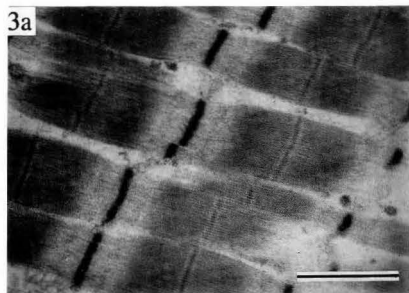
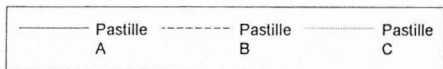
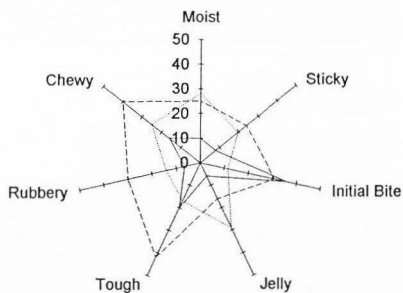


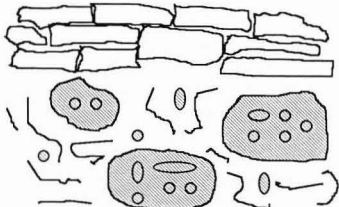
Figure 6 (above). Confocal light microscopy of freeze-substituted ice-cream. Bar = 50 μ m.

Figure 7 (at right). "Star" diagram showing textural attributes of pastille sweets.

Figure 7 - Texture Star Diagram for Pastilles

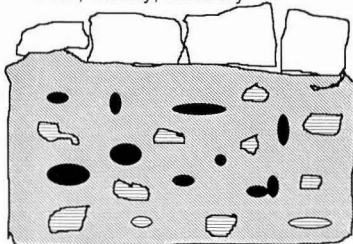


Pastille A
High Initial Bite
Soft, Moist, Breaks Down.



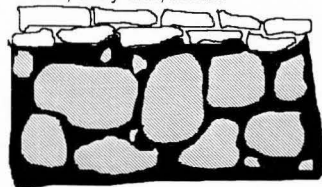
Thick outer shell of sugar crystals
Continuous syrup phase
Dispersed protein and starch

Pastille B
High Initial Bite
Firm, Chewy, Rubbery



Continuous protein phase
Dispersed starch and syrup

Pastille C
Low Initial Bite
Soft, Jelly-like, Moist



Continuous starch & syrup phase
Dispersed protein and syrup

Figure 8. Diagrammatic representation of structures in pastille sweets.

during chewing. At a macroscopic level the initial hardness in sample A could be related to a well-developed crystallised sugar layer at the outside of the sweet, samples B and C showed less extensive crystal layers. Light microscopy showed differences in the general structure. Sample B had a well-developed protein (gelatin) network with starch inclusions and this produced both the hard initial bite and the toughness on chewing. Sample C showed a starch-continuous matrix with protein inclusions and this, coupled with the restricted crystalline layer at the surface, produced a soft initial bite and a soft chewing sweet. The internal structure of sample A showed the protein as discrete areas and the starch was largely dispersed in a syrupy matrix, which accounted for the softness once the crystalline layer had been broken. Figure 8 shows a diagrammatic representation of these differences.

Using colloidal gold linked to lectins and electron microscopy, it was possible to show that the discrete areas in sample A were an association of protein with gum arabic. Interestingly, on ageing, sample A became much tougher on chewing; this was partly due to the outer crystalline layer growing but also, as this happened, the discrete protein areas fused to give a continuous matrix.

Of course, not all links between structure and behaviour are as clearly defined as in this illustration and no doubt there are many subtleties that are not explained. However, microscopy is often the key link between a number of apparently disconnected observations and the microscopist must be given and take the time to explore the connection.

And Then, The Justice

The verdict must be publicised, but the microscopist cannot expect food technologists to treat his pronouncements with the respect afforded to judges. This is perhaps the most difficult task facing the microscopist. The training and development so far have given great insight into the structure and behaviour of foods; many complex features are instantly understood. However, show the pictures to a non-microscopist and it seems that the most basic understandings are missing. People readily accept that everyday objects are recognised by their shape (or morphology) but apply the same logic to an electron micrograph and all sorts of doubts arise. Everyone recognises a banana on sight, but tell someone that a feature on Figure 9 is a casein micelle and all sorts of sophisticated chemical evidence will be requested. Even when the strict microscopical interpretation is accepted as the microscopist's domain it is difficult to gain the confidence of the technologist in allowing the microscopist to apply his (her) judgement in the area of explaining the

relevance of the structures. This is perhaps the food microscopist's most difficult task and there is no universal solution. We are now outside the area of scientific laws and into the field of human relationships. Each combination of microscopist and technologist will need to find their own way of working and, from the microscopist's point of view, different approaches should be tried until a satisfactory relationship is established.

For some people a clear diagrammatic representation of the structure and highlighting key structural changes with respect to changes in the product will be sufficient. Other people will only feel happy with numbers and the microscopist will have to find a way of converting his (her) subjective judgement into a numerical form. Image analysis systems may help here but the microscopist should retain control and ensure that the numerical output is consistent with a conventional interpretation of the images. In some cases, a heated debate, based on mutual respect, can be a most effective way of progressing.

Often the problem lies in a failure to convey the three-dimensional aspects of the structure. For example, when we were working on the fouling of heat exchanger surfaces, it was quite clear to us that an important factor was air coming out of solution at the hot surface and forming a heat-set foam, which cut down heat exchange dramatically (Lewis, 1986). However, it was only when stereo pairs were prepared that the engineers involved accepted the possibility and suggested changes to the procedure to confirm the hypothesis. In the other cases, making models of the structures may be effective. One of my early memories as a young microscopist was to see my then boss, Gerry Jewell, with the then head of Confectionery at the LFRA, Derek Stansell, on their hands and knees, building models with small white cardboard boxes. They were devising mechanisms for sugar crystal nucleation and the early stages of sugar crystal growth and this led them to propose a crystal growth mechanism. In turn, their thoughts on crystal growth allowed the demonstration of a novel process for producing icing sugar, which produced cleaner crystals and was less liable to cake. This phase is without doubt the most difficult and there are no universally applicable solutions. It is, however, the most important stage in applying microscopy findings and is one to which microscopists and their managers need to give considerable thought.

The Sixth Age

This age views microscopy in a wider field and takes the focus from the short-term interpretation of the pictures and products in order to see the overall relevance and connections between structure and performance.

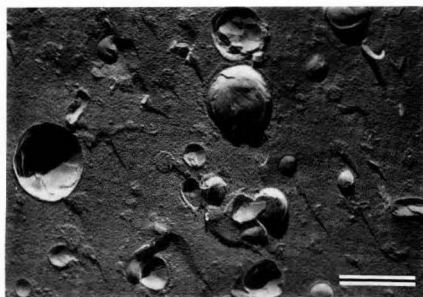


Figure 9. TEM of toffee pre-mix by freeze-etching. Bar = 1 μ m.

It is a time when the advocacy is for inclusion of structural examination as a matter of course, even in those cases where, at the outset, there is no obvious or tangible expectation of its value. The argument is based on past successes. When microscopy was included in the studies on icing sugar (see "And Then The Justice") there was no plan for its application, simply a faith that it would be justified. A role at this stage is to promote that faith with the decision makers.

Of course, it is not enough simply to include some microscopy in the project, the microscopists must be given freedom to contribute fully to the development of the project. Making the resources available for some playing with the problem is part of the role. Depending on the culture of the organisation this may be a recognised feature of the project or may need to be a bootleg activity. In Peters and Austin's book "A Passion for Excellence" the description "skunk groups" is used to describe groups of people who operate in unorthodox ways; in some cases the microscopists are well placed to take on this role which is probably vital to the well being of research establishments.

There is also a question of equipment and part of the role will be to try to keep the right level of equipment for the organisation. Philosophically, this can be a difficult balance. The good practical microscopist will always want more, better equipment; managements will generally resist, such is the nature of business life. In reality, there are two critical levels. The first will be easily appreciated as the point at which it is impossible to carry out the sort of work expected by the organisation, for example, it is no use expecting valid observations on the association of protein molecules within a gel structure and only providing a simple compound light

microscope. There is, however, an upper limit where a laboratory can be too well equipped, thought processes are inhibited, and there is a tendency to lose the insight that comes from relatively simple equipment. So often one sees papers and presentations on SEM of problems that could be better tackled by light microscopy. The "sin" is often compounded by describing the studies as "structural" or "microstructural"; in my view these terms should not be used unless several techniques are applied, each giving distinctive information. The role of the "sixth age microscopist" is to challenge their younger colleagues to ensure that the approaches are being properly considered.

Direct contact with the microscope may now be quite limited and an element of remote control microscopy is introduced. A key learning element at this stage is how to influence more junior colleagues, whilst allowing them to develop and discover their own insights into the problems. The role is one of coach and counsellor. For those, who like to see others develop, this can be a most rewarding age even if the fingers itch to try the latest innovations.

The publication of bibliographies is often a feature of this stage in a microscopist's life. I have fond memories of the late George White of Lyons Central Laboratories. Towards the end of his career, he directed the production of a most comprehensive bibliography of food microscopy. These can be found in a series of articles published in the *Journal of the Association of Public Analysts*. In earlier years, Kate and Andrew Winton had produced a compendium of food structure that has not been surpassed in over fifty years. John Vaughan in the late 1970's produced the book *Food Microscopy* that showed the way in which emerging microscopy techniques were being applied to a wide range of foods. It was around this time that the SEM meetings started to concentrate on foods and Sam Cohen, Eugenia Davis, David Holcomb and Milos Kalab were pioneers in initiating the series of meetings that has developed to be the main international meeting of food microscopists. These meetings, also led to the formation of this journal, *Food Structure*. In more recent years, David Holcomb has produced a bibliography of papers published in *Food Structure* and its predecessors.

This is the age of experience, but there is a need to realise that good advice is not always heeded; sometimes people can only learn by making their own mistakes and coping with the consequences. This thought may help to avoid the risk of bitterness. There are frustrations too, especially in organisations where there is a reasonably high turnover of personnel. Often the same arguments have to be replayed and sometimes, it is necessary to see the same mistakes being made over and over again. This, of course, applies to the technologists just as much

as to the microscopists. Sometimes it seems that no sooner has a technologist been "trained" to appreciate the value of microscopy than they are replaced and the process has to start all over again. Somehow it is easier to accept having to train younger microscopists than having to train technologists but both are essential for the most effective application of microscopy for the benefit of the food industry.

Last Scene of All

That ends this strange, eventful history;
Is second childishness, and mere oblivion;
Sans teeth, sans eyes, sans taste, sans everything.

Best not to dwell too long here! On a tangible note, many elderly microscopists seem to have rather thick glasses but hopefully this is related to monocular microscopes, and the more widespread use of binocular microscopes may preclude this defect. Some microscopists do not seem to reach this stage but remain enthusiastic and productive to a great age. An example of this was Harry Powers, once of Tate and Lyle, who was writing to us about sugar crystallisation when he was well into his nineties.

If we do reach this second childishness, at least there is the consolation of having passed through six exciting and challenging ages to get there.

Helpful Sources of Information for Each Stage

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Last age of all

Daily newspaper, gardening books, novels, radio and television.