The Beauty of Milk at High Magnification

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The task of designing milk protein-based frankfurters compelled me to use electron microscopy to understand the formation of microstructure in the product. Later, I extended microscopic studies to dairy products. Their microstructures reflect the variety of interactions which the milk constituents (caseins and whey proteins, milkfat and lactose) undergo during manufacturing, often with the participation of microorganisms (bacteria or fungi). Electron microscopy reveals the details of such interactions to manufacturers and may also be used to protect the consumer.

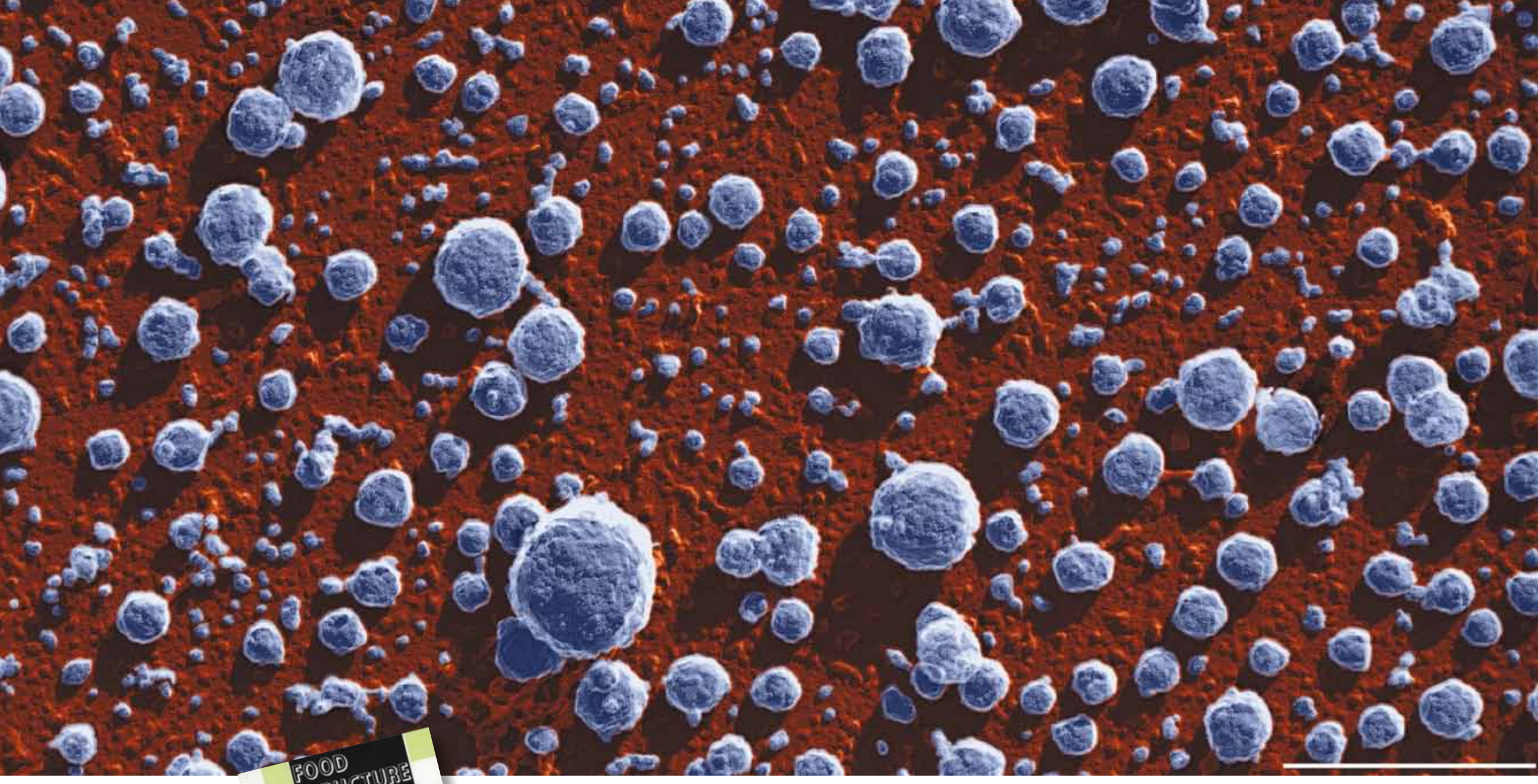

Fig. 2. *Casein micelles in cow milk by metal shadowing with platinum and carbon. False colour TEM. Bar: 0.5 µm.*

What a treat - a refreshing strawberry yogurt, fizzing kefir, forty ice cream flavours, cheese and wine, cheesy pizza. These products are all made from milk and they all have excellent nutritional value. The transformation of milk into milk products can take a short time, such as with yogurt, or it can take much longer, such as with cheese.

Fig. 1. *Food Structure – journal cover. The micrograph on issue 4, vol. 12, 1993 shows a high-resolution SEM image of cryofractured one-day old Mozzarella cheese from which fat and the liquid phase had been removed (McManus et al., 1993).*

Milk is a unique food – it comes in liquid form and contains all the nutritionally important components, such as proteins (corpuscular casein and dissolved whey proteins), fat (fat globules) and carbohydrates (milk sugar, i.e., lactose), which are required for a newborn mammal. Human cultures whose ancestors raised cattle, goats, buffaloes, sheep, camels, yaks, or horses, and used their milk as food, have retained the enzyme lactase in their gastrointestinal tract in adulthood and are thus able to digest lactose, in contrast to lactose-intolerant people who lack this enzyme. Microorganisms (bacteria, yeasts and other fungi) participate in the production of the majority of milk products and many lactic acid bacteria called "probiotic bacteria" are beneficial to human health.

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Examining milk with an optical microscope brings great disappointment since there is nothing to see except fat globules. Milk protein particles (casein micelles) are only a fraction of a micrometre in diameter. Electron microscopy is needed to see

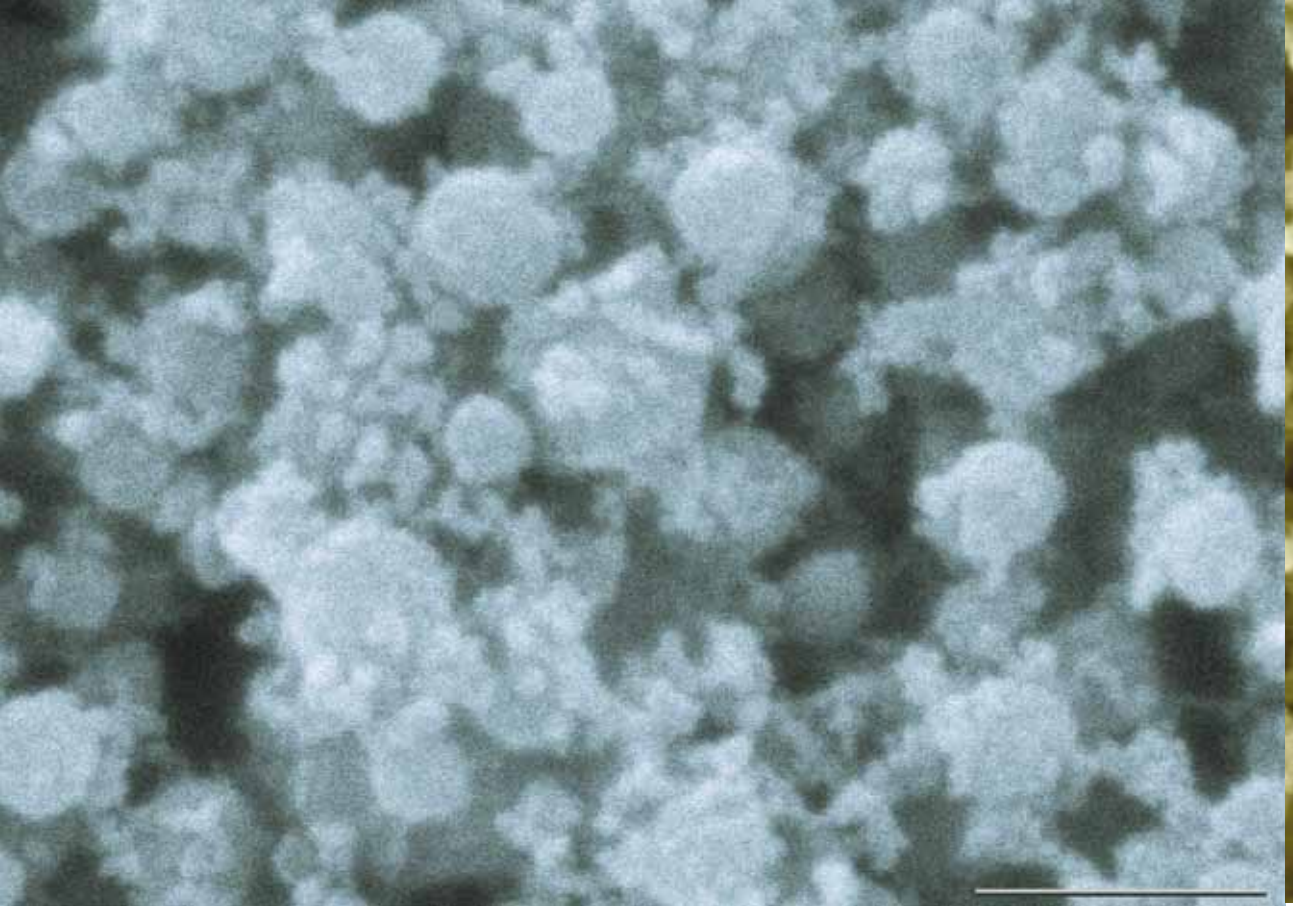


Fig. 3. *Casein micelles in a heat-induced skimmed milk gel. Reconstituted (40% total solids) skimmed milk powder in water was heated for 10 min in a boiling water bath and then cooled at 6°C for 2 hours. The resulting gel was trimmed, fixed in a buffered 2.5% glutaraldehyde solution, dehydrated in ethanol, freeze-fractured and gold-coated for SEM. Bar: 0.5 µm.*

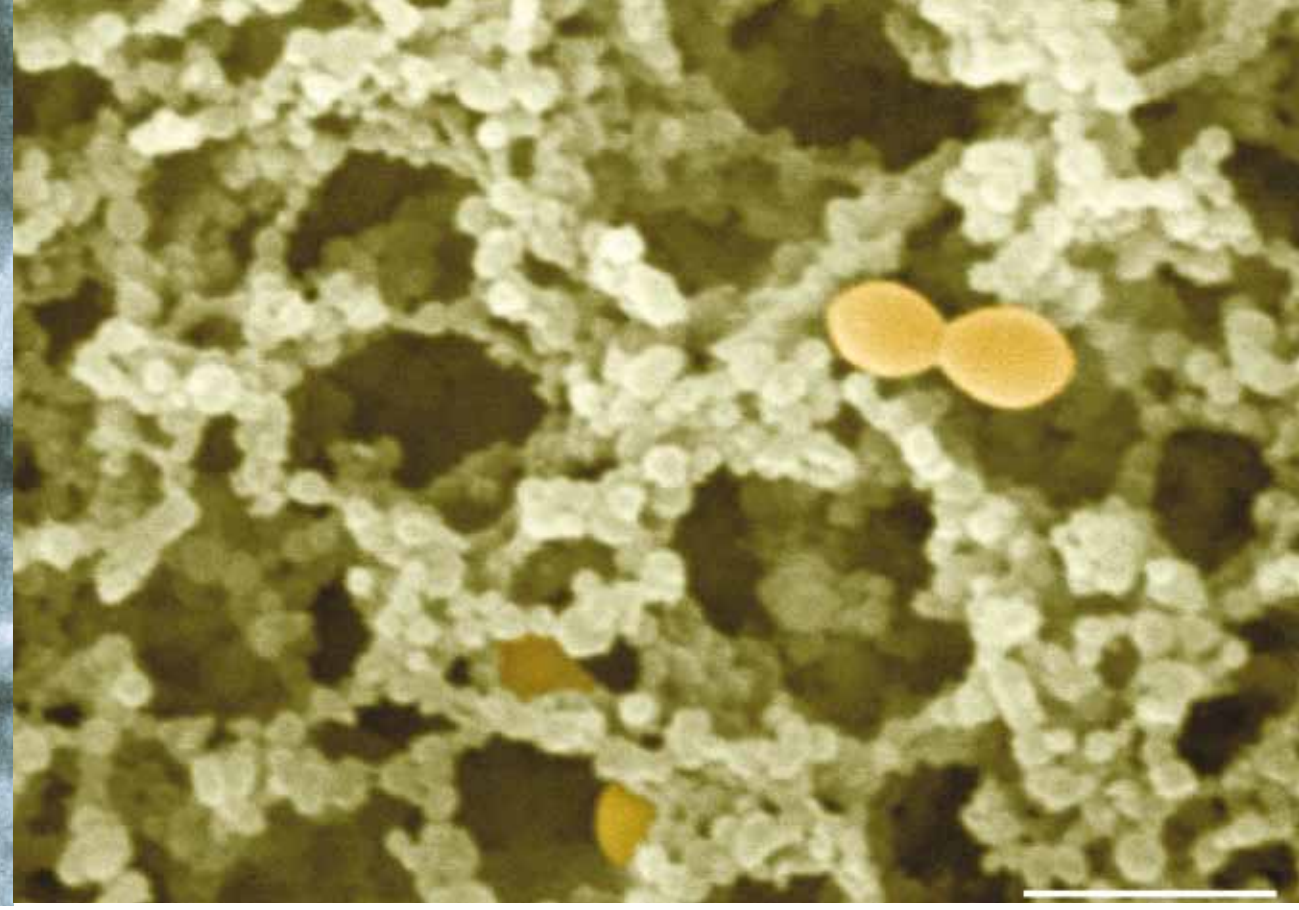


Fig. 4. *Yogurt protein matrix is composed of casein particles and attached κ-casein-ß-lactoglobulin complexes in the form of branching chains which hold the liquid phase initially present in the milk. Two streptococci are also shown. Bar: 2 µm.*

them as they are converted into various dairy foods. Their structures depend on pH, temperature, and the effects of microorganisms. Structural studies, particularly at the microscopical scale, are well justified because microstructure is closely related to physical and sensory properties of the foods such as firmness, cohesiveness, elasticity, spreadability, mouthfeel, etc. This knowledge is important in industrial production of dairy and other foods. Standard structures of traditional foods have been established and correlated with their properties. This knowledge (Aguilera & Stanley, 1999) serves food manufacturers and developers of novelty foods using non-traditional food sources or procedures.

The human tongue was found to perceive particles 1-5 µm in diameter as "fat", based on human experience with milkfat over a period of thousands of years. Any particles of such dimensions feel like fat irrespective of their composition. This finding is the principle of modern fat replacers made from microparticulated milk proteins (Singer & Dunn, 1990). Professional tasters did not distinguish any sensory differences between a control ice cream made with 16% cream and ice cream made with an equivalent amount of "microparticulated" milk protein instead of cream. Electron microscopy of the fat-free ice cream showed protein particles at the sizes of fat globules present in a control sample, i.e. 1 to 3 µm in diameter.

Early stages of electron microscopy research of milk products

The beginnings of electron microscopy research in dairy science go back to the 1970's. This is much later than its use in medicine, biology and material science. Interest gradually developed in food microstructure in general (Vaughan, 1979), including milk (Knoop, 1972). Both academic and industrial research establishments started to acquire electron microscopes and annual international meetings of food microscopists organised in the USA by Scanning Microscopy International, Inc., attracted increasing numbers of researchers on the global scale. In the eighties, a dedicated scientific journal, Food Microstructure, was established and later it was renamed Food Structure (Figure 1). Yet, interest in "what's in the foods" was not shared by the mass media. I offered an article about the microstructure of milk products such as yogurt and cheese to a large American magazine specialising in scientific discoveries and to a Canadian television station. The magazine editor reasoned that black-and-white images would not appeal to readers and suggested that illustrations in colour be provided. Six months later, when images in false colours reached his desk, the editor decided against showing any micrographs of foods in order "not to scare the readers". The television station was blunt: "We would not show that there are bacteria in yogurt – if consumers stopped buying yogurt because you show bugs in it, we could be sued by yogurt manufacturers. Take your pictures back, Sir, and have a nice day". Regrettably the publisher of Food Structure, SEM International, Inc., stopped publication in 1994, and thus deprived food microscopists of their unique journal. Yet it is common knowledge nowadays that yogurt (Tamime & Robinson, 1999), cheeses (Fox, 1987), kefir (Marshall *et al.*, 1984), and many other foods are made by the action of microorganisms. Popular articles now emphasise that "probiotic bacteria" in foods are beneficial to human health.

All kinds of foods are very interesting (Vaughan, 1979, Kaláb, 1982, Aguilera & Stanley, 1999) to observe using a microscope as their constituents and ingredients interact and other factors such as heat, pH value and moisture contribute to

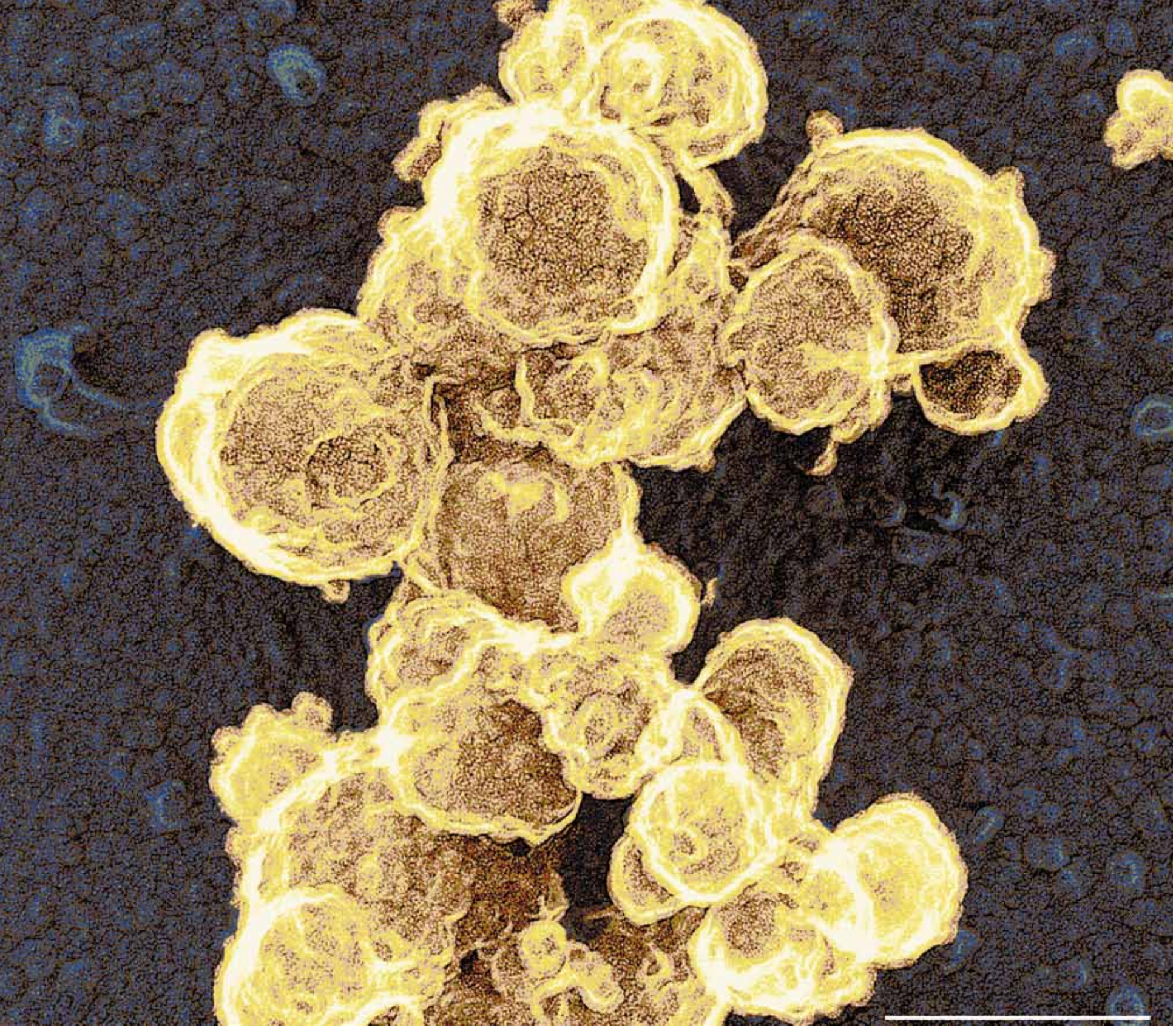


Fig. 5. *Detail of the onset of casein micelle coagulation in milk. The fresh coagulum was fixed, dispersed on a freshly split mica crystal, fixed in a glutaraldehyde solution, dehydrated in ethanol, critical-point dried, and rotary shadowed with platinum and carbon. The resulting replica was cleaned, transferred onto a carbon-coated Formvar film on a 3 mm copper grid and examined by TEM. Bar: 0.2 µm.*

their structures. I became interested in electron microscopy when I was assigned to develop frankfurters ("hot dogs") from skim milk powder by my employer. In the late sixties, there was a surplus of skim milk caused by a high demand for cream to produce ice cream and butter. I was advised to keep the technology very simple, preferably by applying heat-induced gelation. After many experiments, the product had the shape, colour and smell of the real hot dog but it had the mouthfeel of a very bad cheese. Would microscopy explain what was wrong? Let's take a look...

The major milk proteins – caseins – are in the form of minute globules 0.1 to 0.2 µm in diameter called casein micelles (Figure 2). Milkfat (globules between 1 and 10 µm in diameter) may be seen even using a light microscope but they are not present in "skimmed milk" and were not, thus, present in the milk-based "hot dogs". An electron microscopist offered assistance and the images showed the "hot dog" mass in the form of tightly packed globular casein micelles (Figure 3) (Kaláb & Harwalkar, 1974). Naturally, such structure cannot be elastic. The heat-induced milk gels were not even juicy when heated – all water, even when added in the form of gelatin droplets, which, with free fat, provide juiciness in traditional meat-based frankfurters, was tightly bound by the milk proteins. Roasting the new hot dogs produced a very unpleasant odour of burnt milk.

In the 1960's, the federal Department of Agriculture in Canada established an electron microscopy laboratory with an excellent economical and scientific concept in mind. Headed by an electron microscopist with an established scientific reputation in biology, the laboratory provided equipment and guidance to other scientists and technicians from various agricultural disciplines such as soil science, entomology, plant science and food science. These researchers were taught to carry out SEM and transmission electron microscopy (TEM) by the laboratory staff and they received additional

support. The electron microscopy laboratory was thus a place where ideas were generated and shared among various experts. This concept made it also possible to use financial support very efficiently.

Although I was focussed on the milk-based "hot dogs", my interest shifted to traditional milk products such as yogurt and cheeses. These foods are based on the ability of milk proteins to form gels. A review of electron microscopy techniques used to study milk products was published in 1981 (Kaláb,1981).

Using an electron microscope, individual casein micelles are seen to form branched chains when yogurt is made (Figure 4) or they are in the form of clusters if cheese is being manufactured. Heating milk above 85°C causes ß-lactoglobulin – one of the two major whey proteins (the other being α-lactalbumin) – to react with κ-casein on the casein micelle surface. This interaction restricts the number of places where the micelles may attach to each other. This is the principal difference between the manufacture of cheese and yogurt. Casein micelles in unheated milk coagulate without restriction into large clusters which are easily separated from the liquid phase called whey. The caseins thus form the base for cheese (Figure 5). Yogurt, in contrast, immobilises the liquid phase and its water-binding capacity is augmented with thickening agents such as corn starch, plant gums, and/or gelatin. Yogurt develops as the concentration of lactic acid produced by bacteria such as *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (Tamime & Robinson, 1999) and, more recently, *Lactococcus lactis* gradually increases and coagulates the milk.

Freeze-fracturing revealed that bacteria in yogurt were surrounded by void spaces (Fig. 7, Kaláb *et al*., 2008 – where *Streptococcus thermophilus* and not *Lactobacillus bulgaricus* is shown). It was assumed that bacterial capsules (Brooker, 1979) consisting of polysaccharide gel on the bacterial walls prevented close contact between the bacteria and the casein micelles.

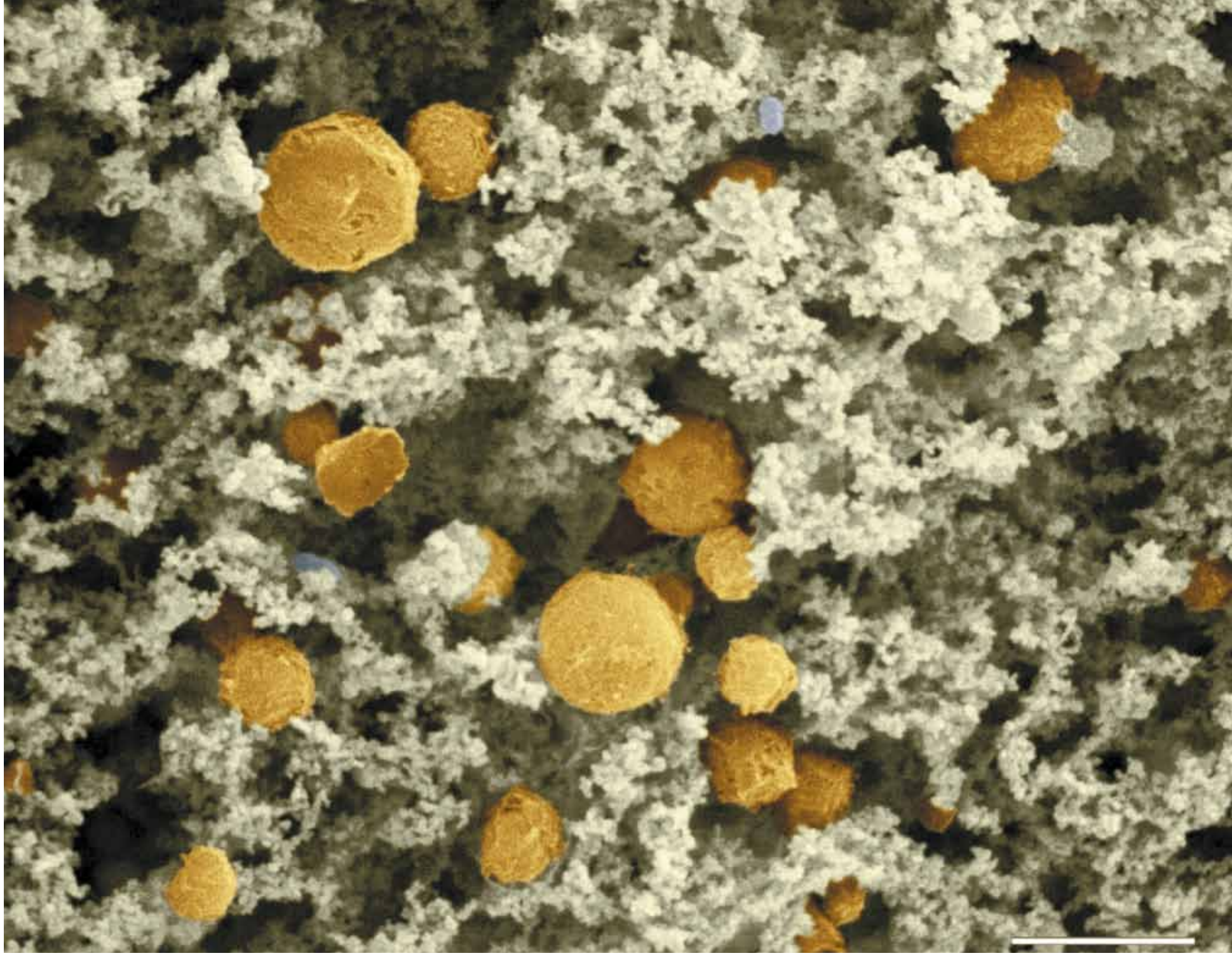


Fig. 6. *Whole milk coagulated for cheese production. Fat globules (yellow) were retained in the coagulum for SEM by fixing the curdled milk using imidazole-buffered osmium tetroxide. Two streptococci (blue) from the starter bacterial culture may also be seen. Bar: 5 µm.*

The capsules were dissolved during preparation of yogurt samples for electron microscopy and appeared as void spaces in the micrographs. If the polysaccharide gel had higher solids content, the bacteria imparted a higher viscosity on the yogurt. The polysaccharides aggregate into thin filaments around the bacteria while the sample is being prepared for electron microscopy (Fig. 6, Kaláb *et al*., 2008). This led some cheese experts in the past century to erroneously believe that such bacteria in a cheese curd "hold onto the protein matrix not to be washed away with the whey".

In contrast to yogurt, cheese is made from whole milk that has not been heated. The resulting gel

consists of casein micelle clusters and large void spaces. It collapses on heating, which makes it easy to separate the liquid phase and to concentrate the proteins. To retain the fat globules (Angermüller & Fahimi, 1982) in the gel for microscopy (Gavarić *et al*., 1989), the samples are postfixed with imidazole-buffered osmium tetroxide (Figure 6). TEM reveals the internal structure of the fat globules (Figure 7); crystals of saturated fatty acids are seen in a sample that had been cooled at +6°C for several hours before fixation.

Milk curdled by proteolytic enzymes such as chymosin (rennet) is cut with wire knives into cubes, heated, and slowly stirred. A compact protein matrix forms rapidly (Figure 8) as the cubes turn into small white granules. These are pressed and ripened to form cheese. Bacteria which had been added to the milk in the form of a starter culture ripen the cheese (Fox, 1987) and give it the characteristic flavour. Different structures may be imparted by stretching (Mozzarella cheese) or cheddaring (Cheddar cheese). Structural relationships in cheese are the subject of a book by Malin & Tunick, 1999.

Milk may also be coagulated using acidulants such as citric, lactic or hydrochloric acid. The curd produced at pH 5.5 is characteristic by a core-and-shell ultrastructure (Figure 9) which differs from curd obtained by traditional procedures. The ultrastructure is visible only by TEM but not by SEM (Gastaldi *et al*., 1996), because the free annular space between the core and the shell is narrow (50 to 80 nm) and would be partly obliterated by the regular 20 nm thick gold coating used in SEM (Kaláb, 1980). Harwalkar & Kaláb, 1981, used various acidulants to coagulate milk and reported that high temperature and pH 5.5 were conducive to the formation of the particular ultrastructure through the involvement

Fig. 7. *Fat globules and protein in curdled whole milk. Saturated fatty acids crystallised inside fat globules in yogurt kept in a refrigerator and the crystals were revealed by TEM of thin sections as light structures because they did not react with osmium tetroxide unlike unsaturated fatty acids. Bar: 2.5 µm.*

of a complex between ß-lactoglobulin and κ-casein. In practice, this kind of curd is used to make unripened cheeses such as American White cheese, Latin American Queso Blanco (Kaláb *et al*., 1991a), Ricotta cheese and Indian Paneer cheese (Figure 10, Kaláb *et al*., 1988).

There are various procedures to make cream cheese (Kaláb *et al*., 1981). In the traditional way of manufacturing, cream is coagulated and ripened similar to other cheeses but the product is different from cheeses made from milk. It consists of fat globule aggregates with milk proteins concentrated near their surfaces (Figure 11). This product is soft, moist and spreadable.

All foods, including milk products, are more or less severely altered biological tissues or fluids. Microscopic procedures designed for native biological structures cannot be applied without scrutiny. Few natural structures are as dense as processed cheese. For SEM, specimens are as small as possible, for example prisms 1.5 mm X 1.5 mm in cross section, 10 to 15 mm long. Fixation, dehydration in absolute ethanol, and freezing in liquid nitrogen thus proceeds more rapidly than with larger samples. The length of the samples makes it easy to freeze-fracture them into several fragments. Yogurt samples are considerably more porous than cheese whereas hard cheese, processed cheese and low-fat cheese are very dense and postfixation, particularly with OsO_4 to retain fat, may take more than 24 hours. Freeze-fracturing is superior to dry-fracturing because it produces smooth fracture planes. In contrast, the coarse topography of a dry-fractured specimen does not make it possible to establish the true shapes and dimensions of the individual structural components such as protein particle clusters, protein-fat interactions, pore width, etc.

Fig. 8. *Cheese curd. Casein micelles have formed a compact protein mass with embedded fat globules. Bacteria are present in the aqueous phase, which is residual whey. Thin section TEM. Bar: 1 µm.*

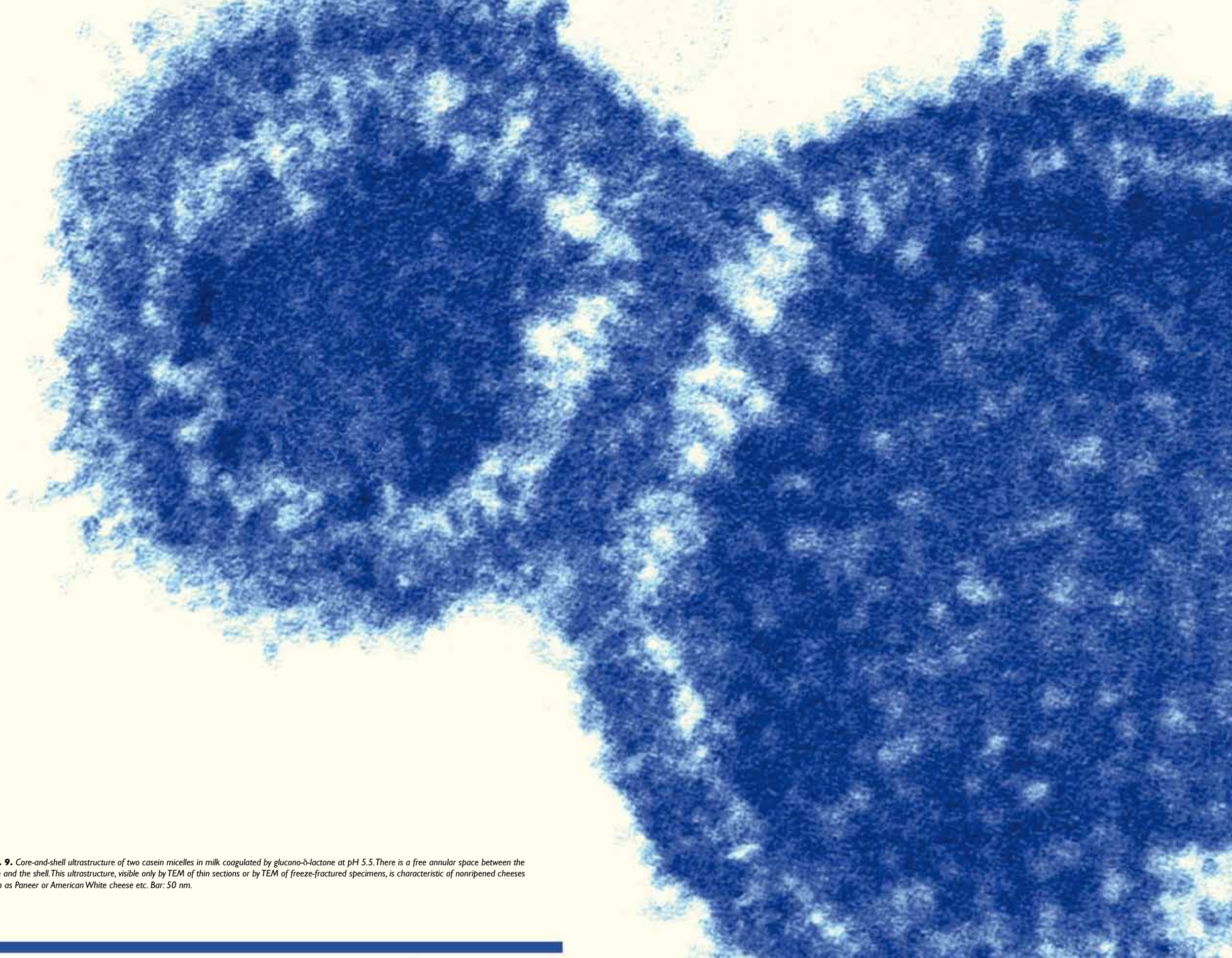


Fig. 9. *Core-and-shell ultrastructure of two casein micelles in milk coagulated by glucono-δ-lactone at pH 5.5. There is a free annular space between the core and the shell. This ultrastructure, visible only by TEM of thin sections or by TEM of freeze-fractured specimens, is characteristic of nonripened cheeses such as Paneer or American White cheese etc. Bar: 50 nm.*

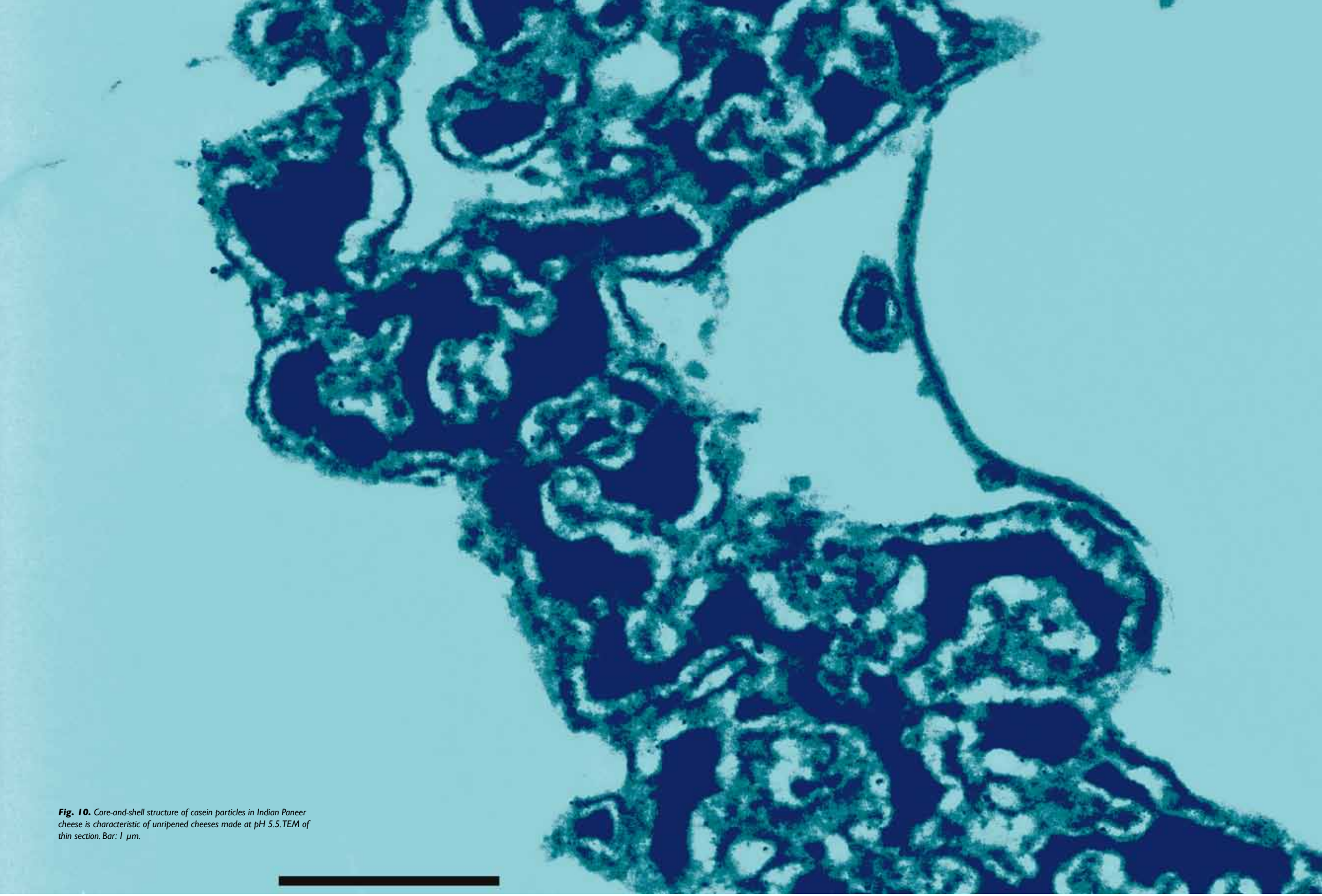


Fig. 10. *Core-and-shell structure of casein particles in Indian Paneer cheese is characteristic of unripened cheeses made at pH 5.5. TEM of thin section. Bar: 1 µm.*

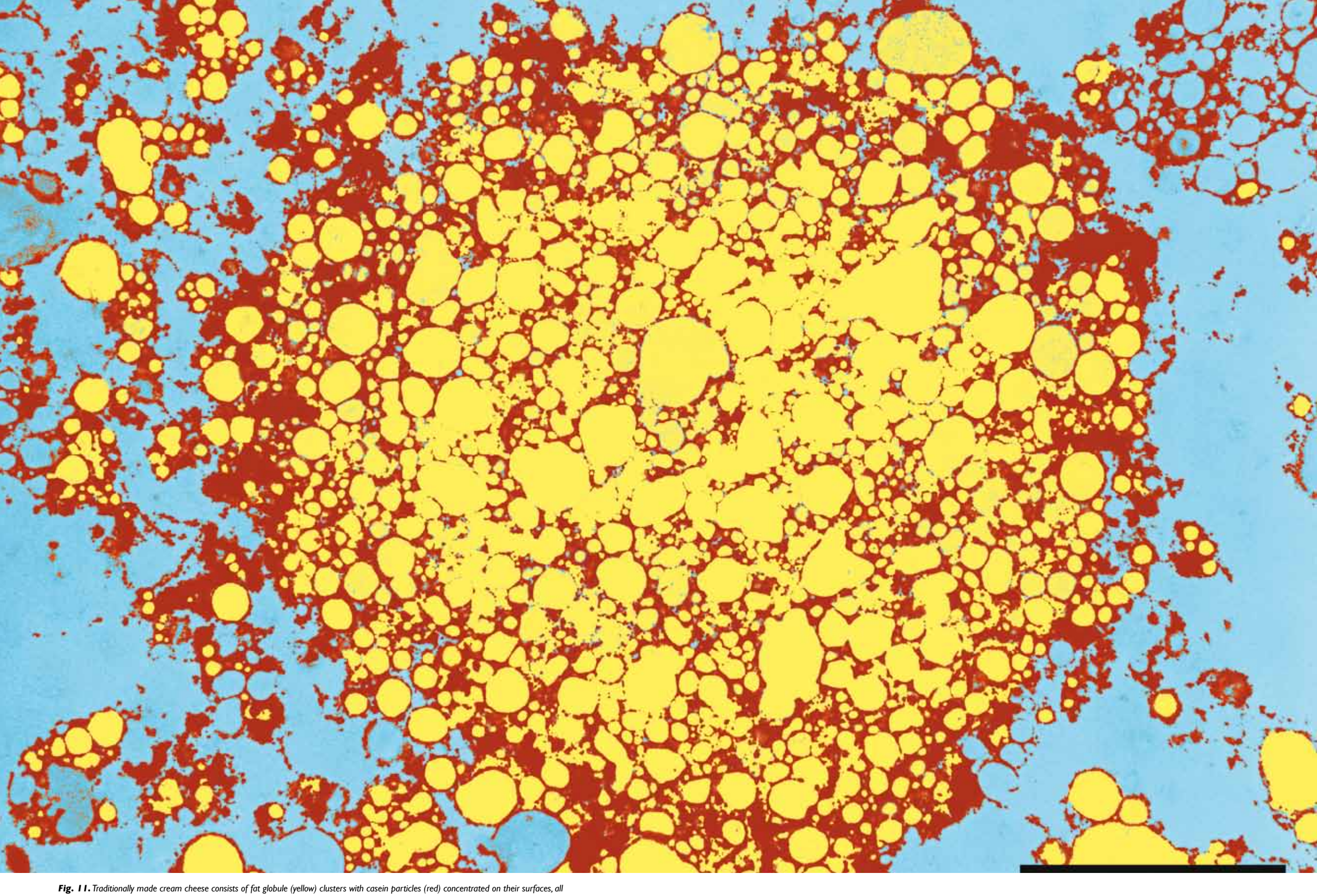


Fig. 11. *Traditionally made cream cheese consists of fat globule (yellow) clusters with casein particles (red) concentrated on their surfaces, all dispersed in an aqueous (blue) medium. False colour TEM of a thin section. Bar: 5 µm.*

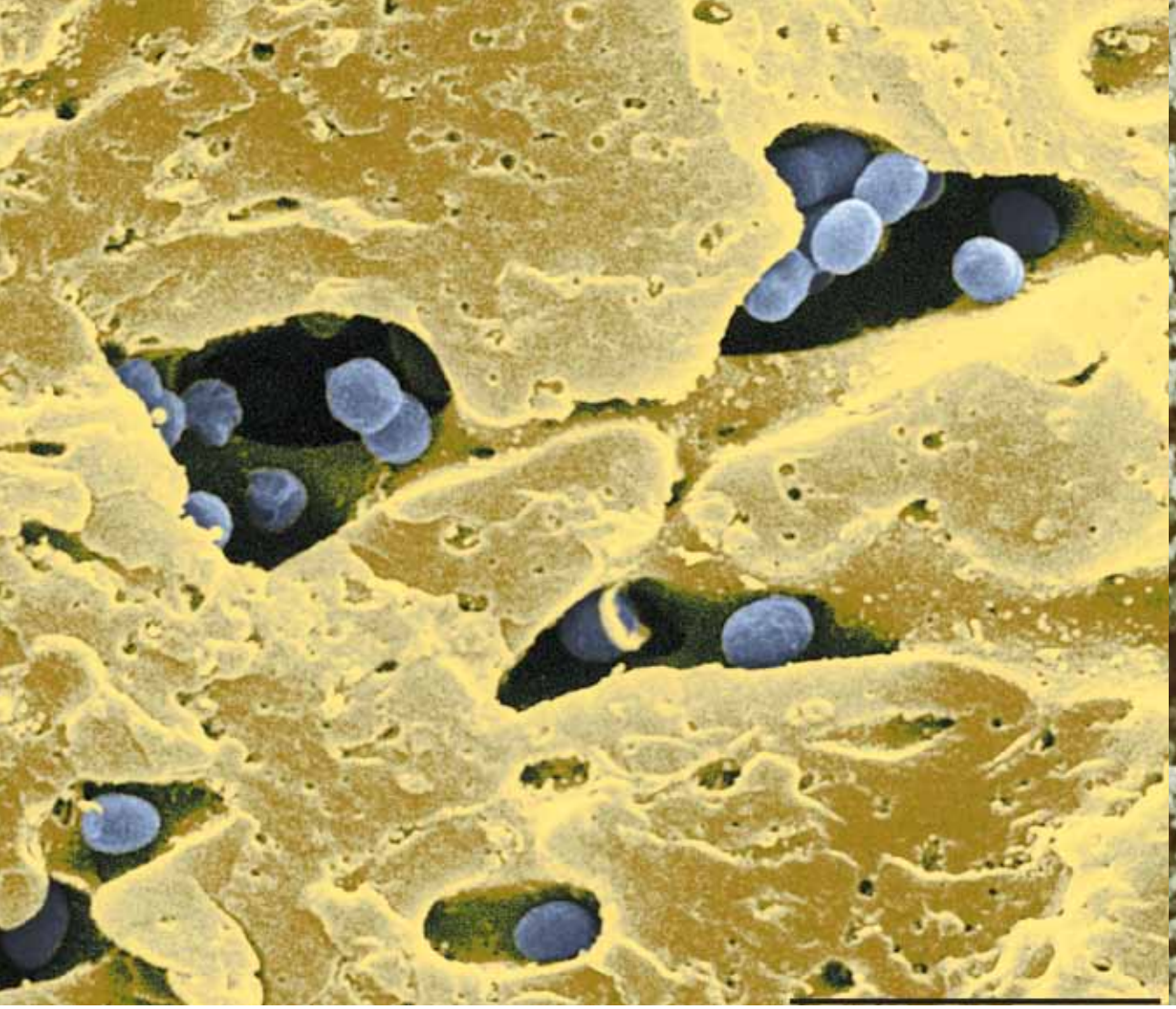


Fig. 12. *Grittiness causing particle in a cheese spread. Insufficient heating of the milk used to produce the spread resulted in the formation of compact cheese-like particles rather than a fluffy matrix similar to that of yogurt. Streptococci (globular bacteria) may also be seen. The interior of the particle was opened for viewing by freeze-fracturing. Bar: 5 µm.*

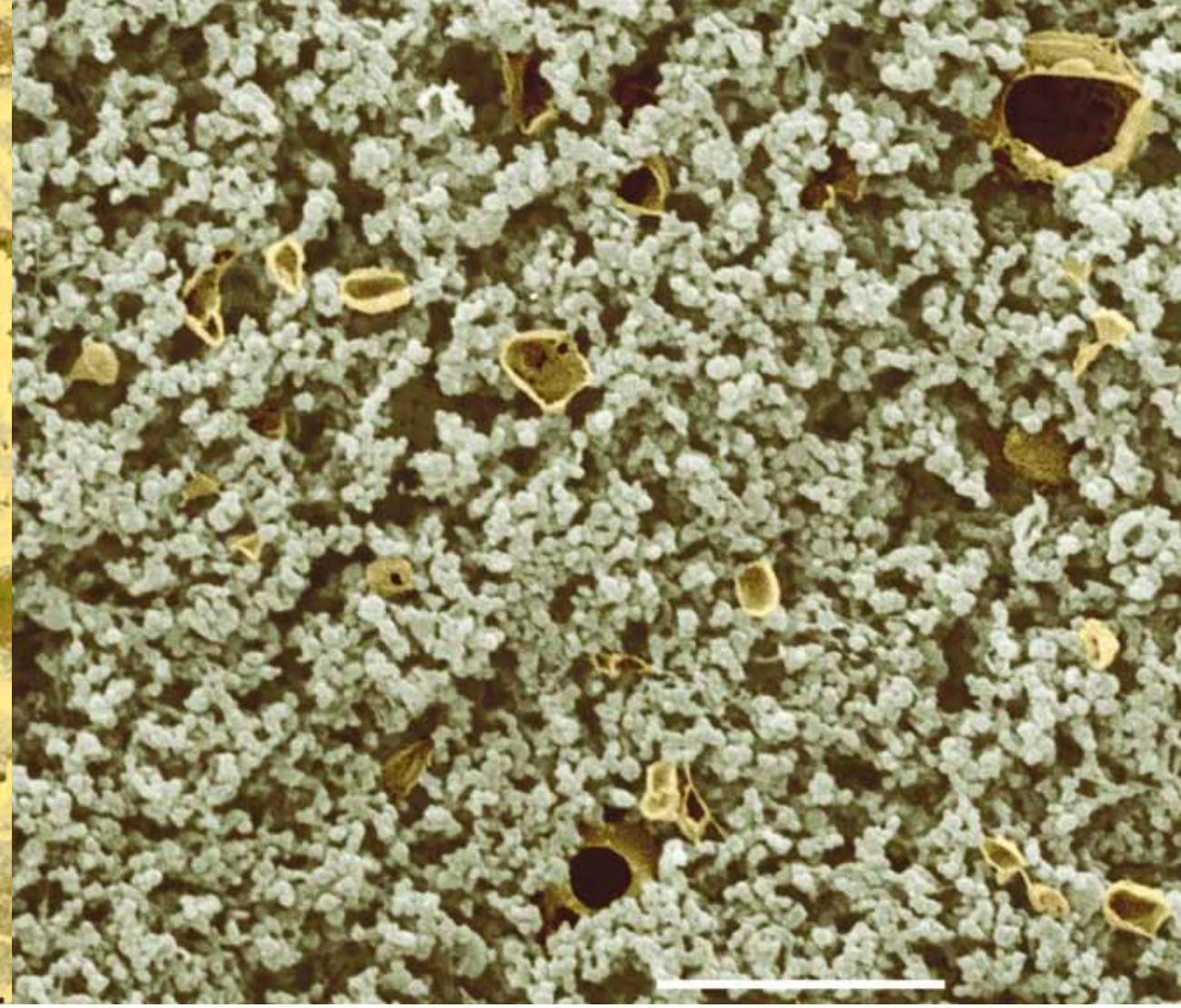


Fig. 13. *A smooth cheese spread consisted of fine particles which the tongue would not distinguish. Smoothness was achieved by heating the milk at 90°C for 10 min. Bar: 5 µm.*

Findings obtained by electron microscopy have made useful contributions to a better understanding of many milk products as an earlier scientific review has shown (Kaláb, 1993). Several of the most interesting examples are presented below.

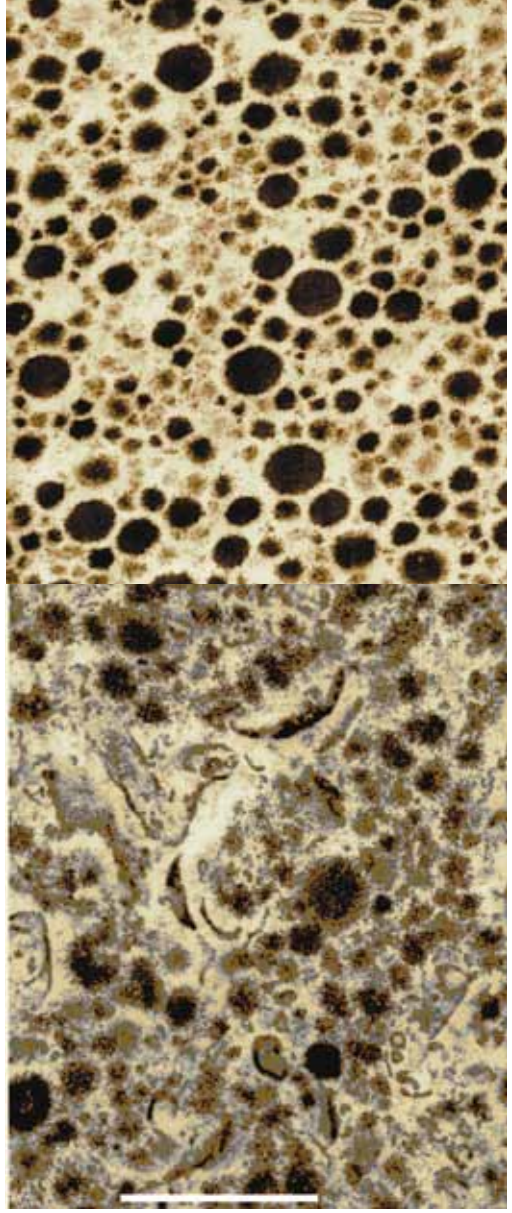
What caused a cheese spread to be gritty?

Electron microscopic assistance was sought by technologists who were developing a new cheese spread made from ~85% curd, ~10% high-fat cream, 5% sucrose and 0.2% sodium alginate. The curd was made from milk that had been pasteurised at 63°C for 30 minutes, cooled and inoculated with lactic acid bacteria. When the milk curdled, the whey was drained off and the curd was used. The blend was pasteurised again at 63°C for 30 minutes, finely dispersed in a homogeniser, packaged in plastic containers and refrigerated. When tasted, the spread was severely gritty – white particles were visible to a naked eye and their hardness was noticeable by touch. The product was a failure and all subsequent experiments were failures as well, irrespective of changing pH, sequestering calcium, using various stabilisers and extending homogenisation. Optical microscopy revealed large particles of compact protein. For SEM, the spread was encapsulated in agar gel tubes, fixed, dehydrated in ethanol, freeze-fractured, thawed and critical-point dried. The microstructure of the gritty particles was compact – similar to that of cheese (Figure 12) and different from yogurt, since it was obtained from milk that had not been heated above 85°C. In the next experiment, the source milk had been heated at 90°C for 10 minutes. The resulting cheese spread (Figure 13) was smooth – the problem was solved (Modler *et al*., 1989).

Were buttermilk solids present in sausage binders illegally?

The role of nonmeat ingredients, called sausage or meat binders, in sausages such as frankfurters (wieners, hot dogs) is to compensate for reduced functionality of lower-cost meats (Lauck, 1975). The binders may consist of a variety of ingredients including cereal flour, milk solids and spices to improve yield, sliceability and texture, to increase moisture retention and to reduce syneresis (i.e. the separation of the liquid phase) in the end product.

Although milk solids have been legally permitted to be present in the binders, buttermilk solids were not because of concern that their presence would impart a rancid flavour to the comminuted meat products such as sausages, frankfurters, bratwurst, etc. Chemical analysis would not detect buttermilk solids in the presence of other milk solids. Since the legislature did not permit buttermilk solids, there had to be a method to establish that there would



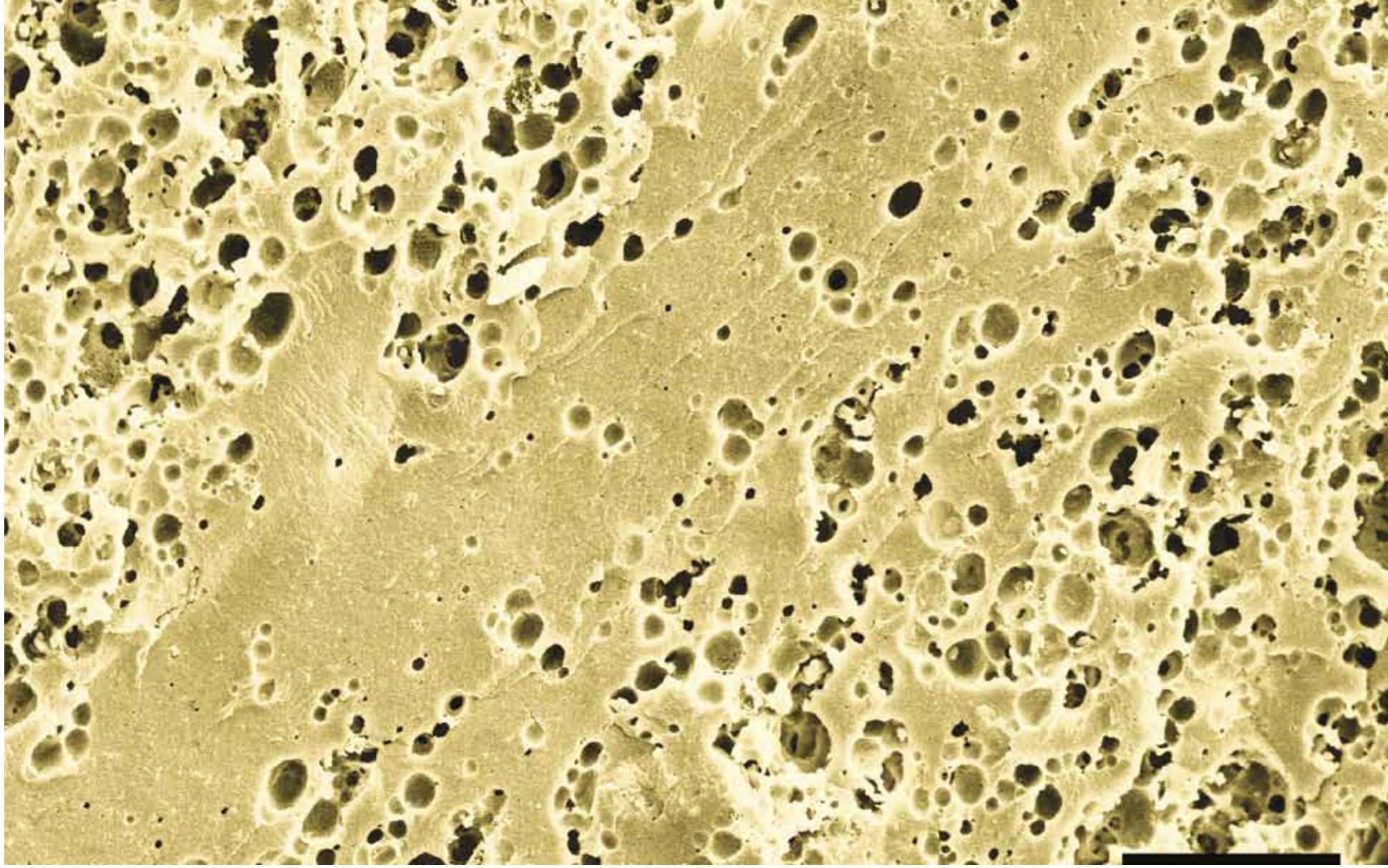


Fig. 14. *Milk solids (above) and buttermilk solids (below) isolated from meat binders and examined by TEM (thin sections). Milk solids consist almost exclusively of casein micelles varying in dimensions but buttermilk solids contain a large proportion of fat globule membrane fragments which originated from the churning of cream during butter production. Bar: 1 µm.*

Fig. 15. *The curd granule junction in cheese is the compact area with a low fat globule incidence. SEM. Bar: 20 µm.*

be no law infringement. This task was assigned to my laboratory. TEM proof is based on the fact that buttermilk solids, which are by-products of butter production, contain a high proportion of fat globule membrane fragments (Kaláb & Comer, 1982). They develop when cream is churned and the fat globules disintegrate to release milkfat (Figure 14). No milk solids other than buttermilk contain such structures. Low centrifugal force was used first to separate coarse ingredients such as flour and spices from the binders. Ultracentrifugation sedimented the milk solids and the pellet was embedded for thin-section TEM. By examining the micrographs, buttermilk solids may be detected at a concentration as low as 8% of the total milk solids.

Is it Cheddar or a stirred curd cheese?

Some SEM micrographs of ripened cheeses occasionally show areas very low in the fat globule content (Figure 15). Defatting of the cheese samples removes the fat globules and leaves globular void spaces in the protein matrix. Dissecting microscopy revealed that these areas coincided with so-called curd granule junctions. These are the areas where individual curd granules were pressed together, so they fused. A simple procedure makes it possible to visualise the junction patterns: slice the cheese 2 mm thick and trim it to 40 X 20 mm rectangles. Fix the slices in 2.5% glutaraldehyde solution for 24 hours and then dehydrate in 3 changes of 95% ethanol followed by defatting in 3 changes of n-hexane. Dry the slices between two weighed pieces of filter paper to prevent the cheese from warping, then carefully sandpaper it to make it smooth. With the dust removed, characteristic curd granule junction patterns will appear (Figure 16 top).

Cheddar cheese has yet another kind of junction – the so-called milled curd junctions. During cheddaring, the curd in the cheese vat, in the form of large slabs, is placed on top of one another. As the curd slowly flows down, the curd granules in the slabs become elongated. Then the slabs are milled into "finger-like" pieces. They are salted and pressed. The surfaces of the larger pieces fuse together and form a stronger version of new junctions (Figure 16 bottom).

Traditional cheddaring is a relatively labour-intensive and thus an expensive process. To reduce

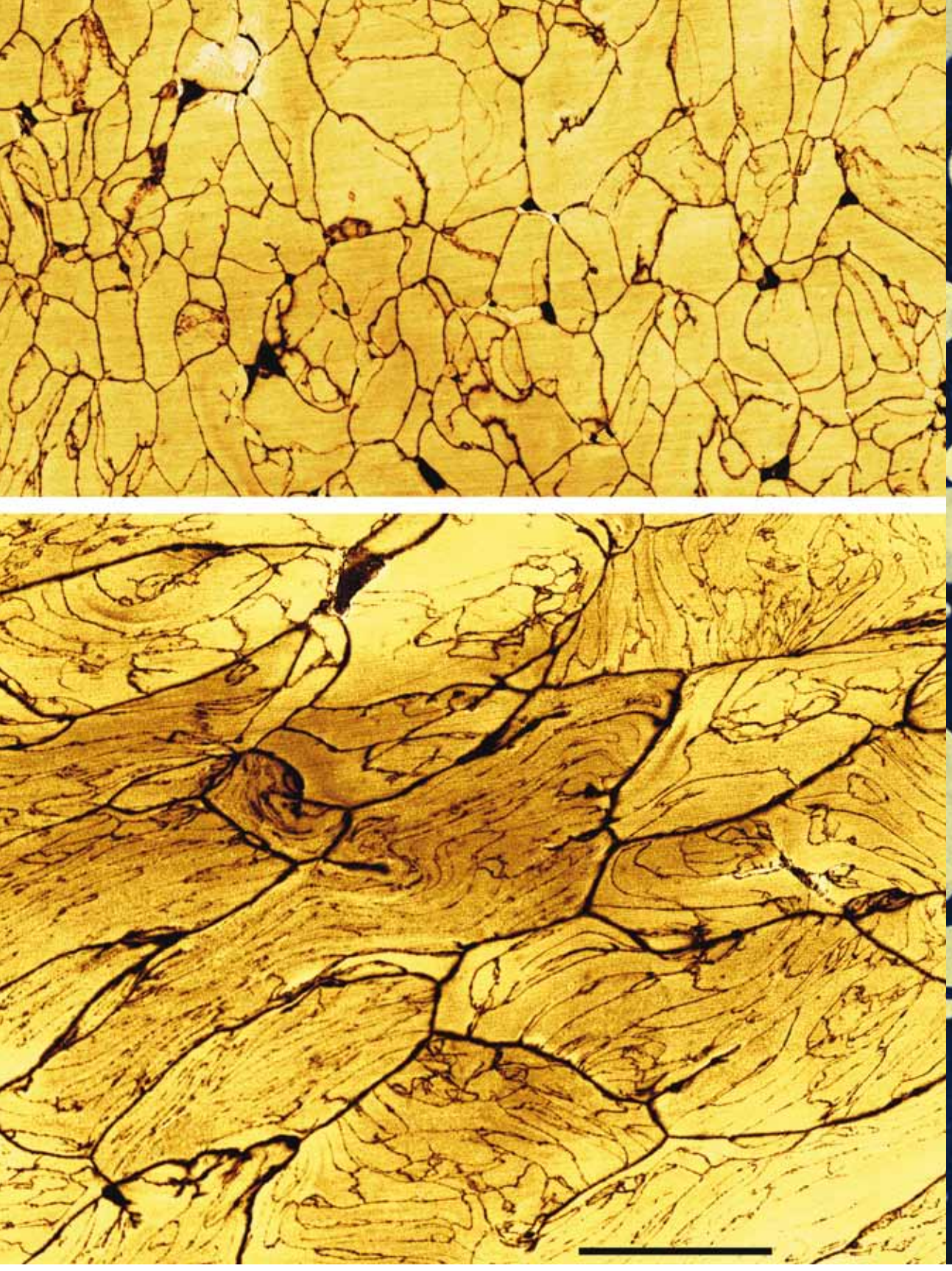


Fig. 16. *Curd granule junction patterns in stirred-curd cheese (top). Milled curd junction patterns in traditionally made Cheddar cheese (bottom). Bar: 10 mm.*

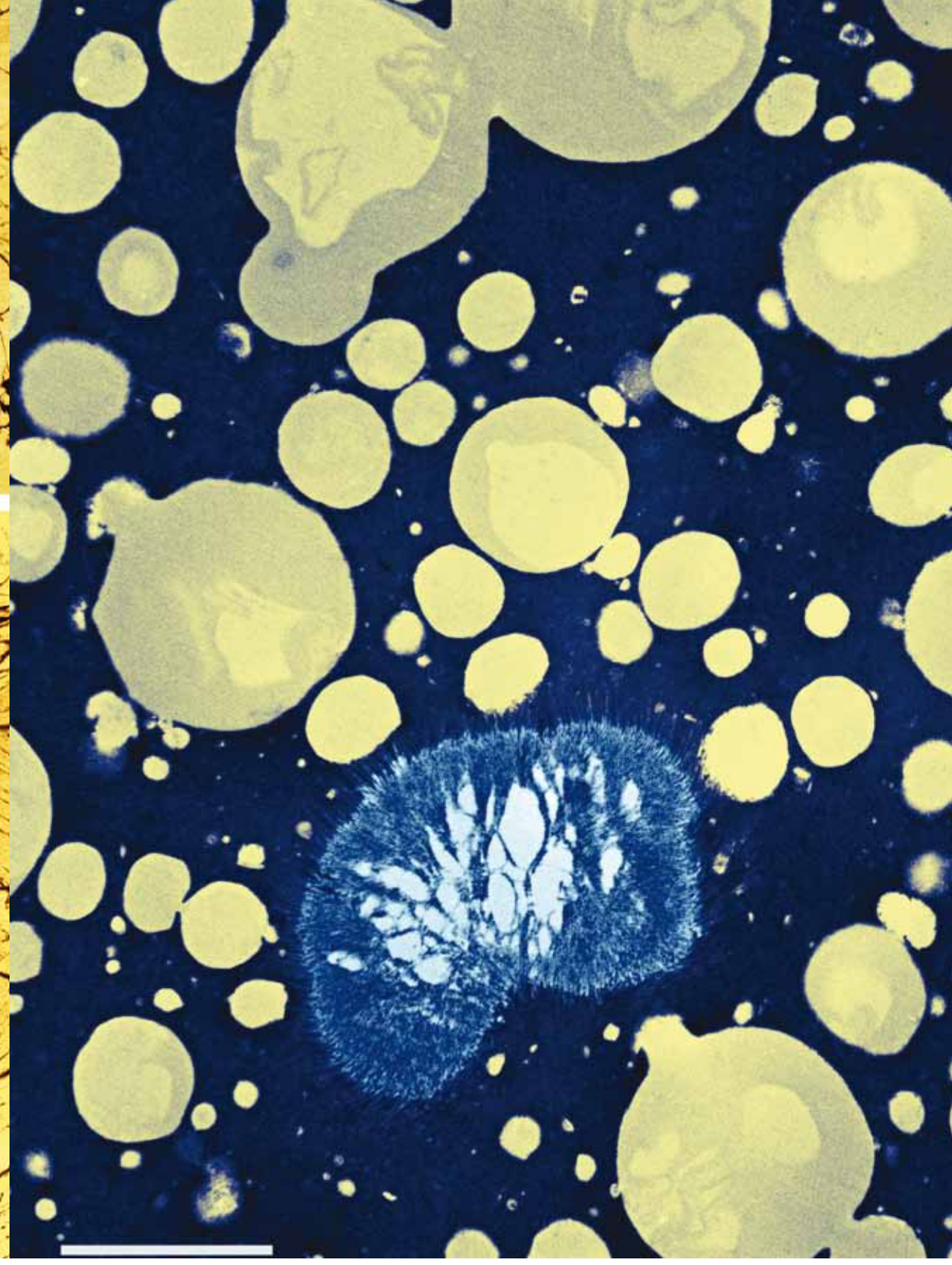


Fig. 17. *Processed cheese. Fat globules separating into smaller globules (top) are characteristic of milkfat emulsification taking place during cheese processing. A freshly developed calcium phosphate crystal is shown in blue. Bar: 5 µm.*

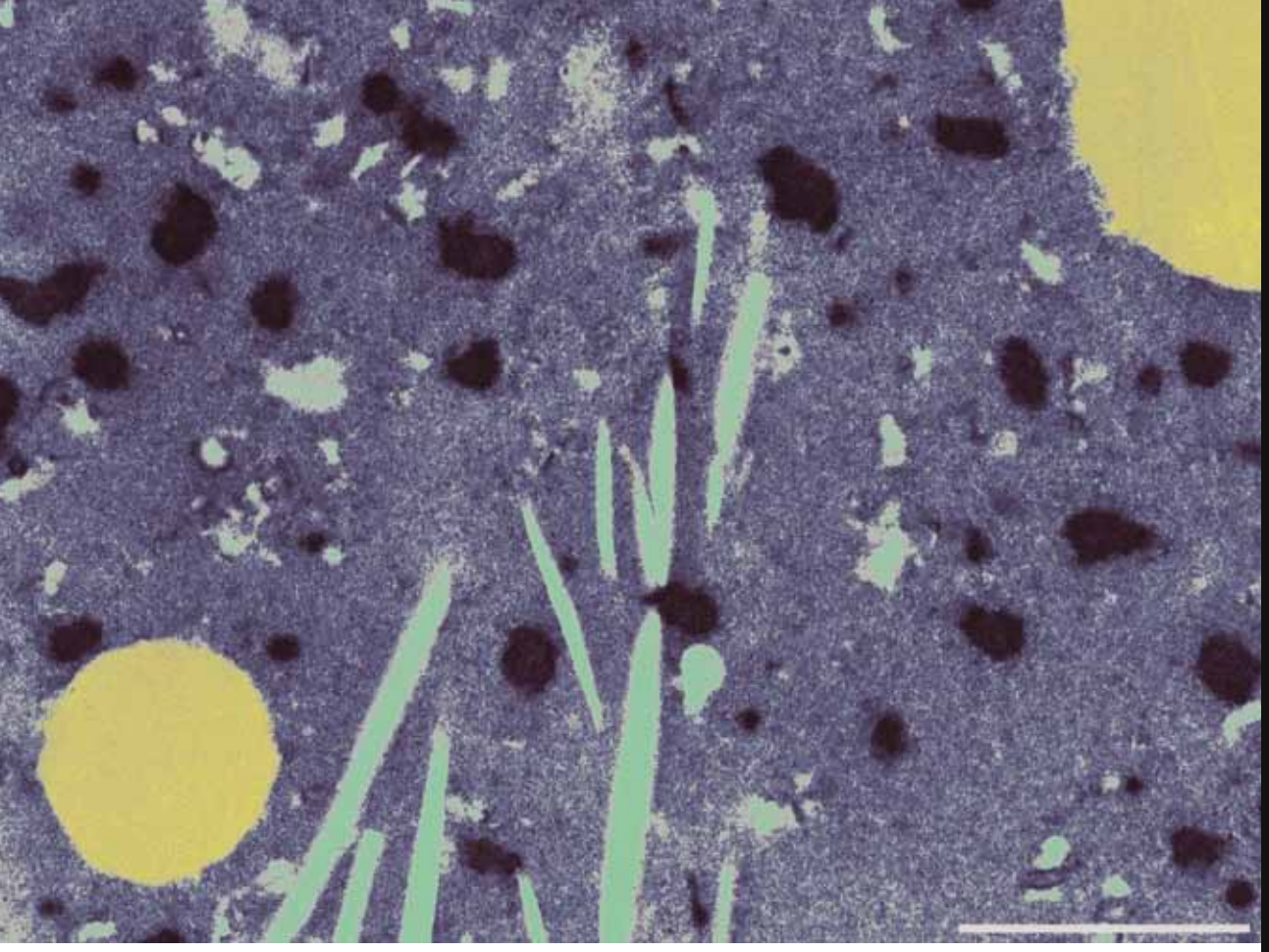


Fig. 18. *Overheated processed cheese. Dark spots were found by TEM in processed cheese that was overheated and lost its ability to melt. It is used as "rework" in freshly made processed cheese and its presence there may be detected by TEM. Bar: 2.5 µm.*

cost and to improve productivity, mechanised or even fully automated processes are nowadays in use. They all produce the milled curd junctions (Lowrie *et al.*, 1982) characteristic of the cheddaring equipment used, although they look different from the traditional ones. Cheese inspectors once intercepted some retail Cheddar cheese samples with the characteristic milled-curd junctions missing. This indicated that the cheddaring step had been omitted during the manufacture. The producers claimed innocence but the junction patterns documented their "omission" and, consequently, the proper cheddaring procedure was restored. During that study, tens of Cheddar cheese samples were received from cheese manufacturers, who used traditional, mechanised or automated cheddaring procedures. Specific characteristics were attributable to the equipment used. There was, however, an exception. One processed cheese specimen sent by a cheese inspector to the laboratory from a small Canadian town had milled curd junction patterns which raised Dr. Lowrie's doubt that it had been produced in the local Cheddar cheese plant. He was convinced that the cheese had been made in another town, about 200 km farther north, where a different procedure was used, which would correspond with the kind of the curd granule junctions observed. Dr. Lowrie was right – the cheese inspector who acquired that sample, had sent it together with other samples from his next destination, as the cheesemaker's registration number on the sample had proved. This shows that even mechanised and automated processes leave an equivalent of "finger prints" in the form of milled curd junctions.

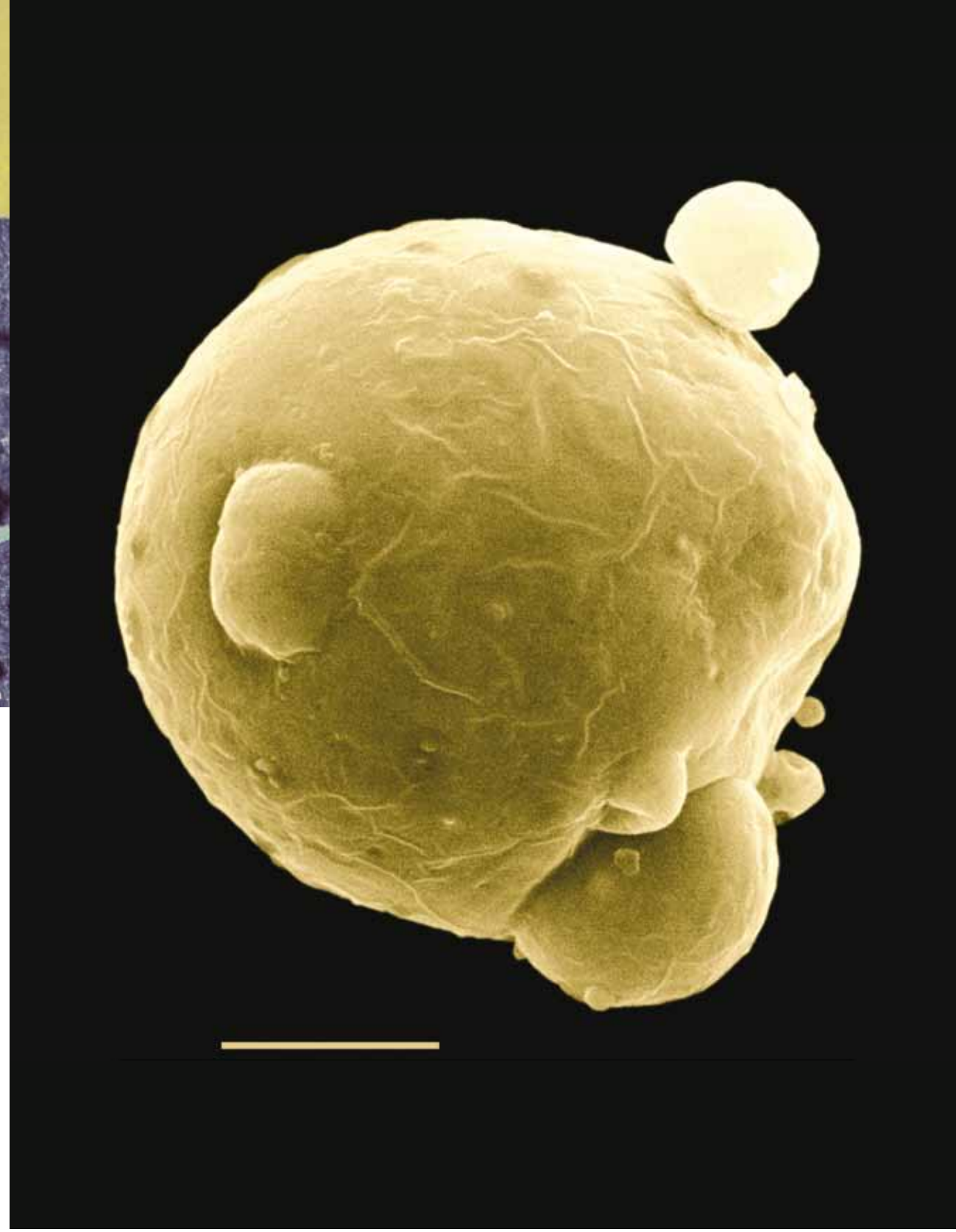


Fig. 19. *Spray-dried milk particle by SEM. A smaller particle was captured by the larger milk powder particle. Bar: 20 µm.*

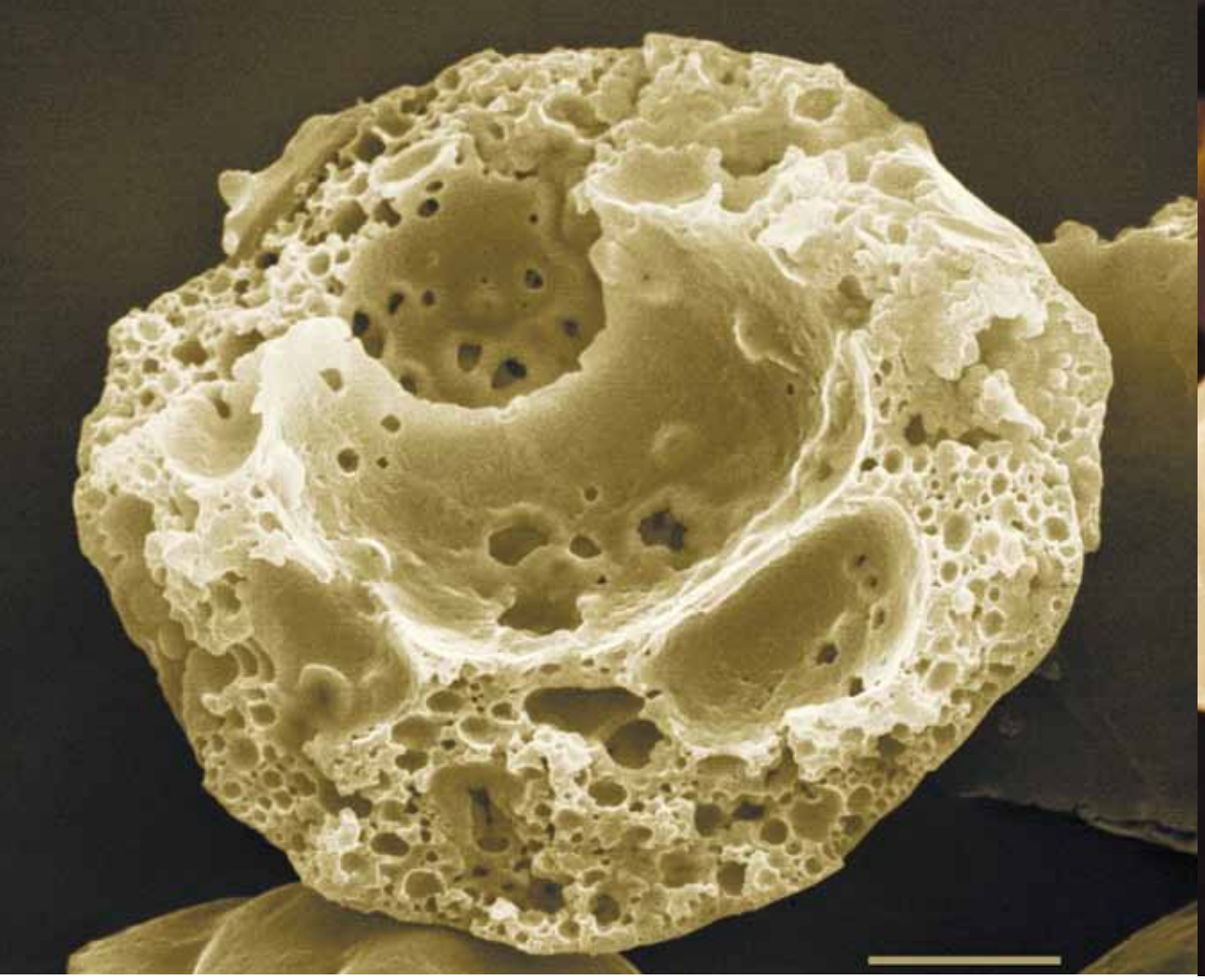


Fig. 20. *There are vacuoles inside each spray-dried milk particle. Bar: 3 µm.*

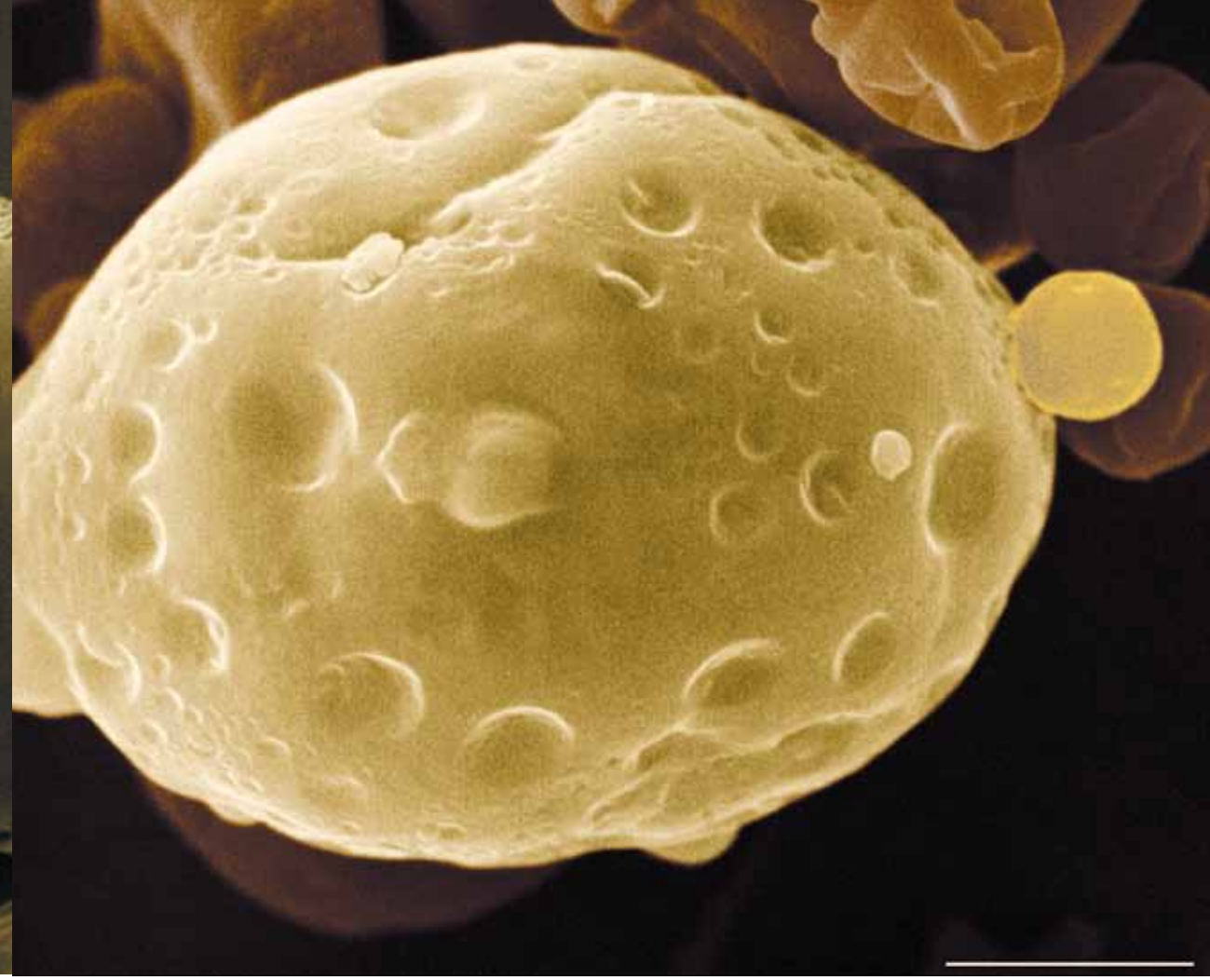


Fig. 21. *Some spray-dried milk particles have dimples on their surfaces. Bar: 10 µm.*

Can defective cheese be salvaged as food?

When butter turned slightly defective more than a hundred years ago, it was rendered and the fat was saved. Cheese makers wondered if this would also be possible with deformed cheese and its trimmings. Recycling cheese was not as easy as recycling butter. Merely heating cheese separated its fat from protein and made the product inedible. Finally, a solution was found. Sodium citrate or sodium phosphates, added to shredded natural cheese at about 2% concentration and stirring the mixture at a high temperature, restored the emulsifying ability of cheese proteins to bind fat. The resulting product is called "processed cheese" (Carić & Kaláb, 1987) and the salts are called "melting salts". Processing, of course, alters the microstructure of natural cheese. Milkfat globule membranes disintegrate and the globules are emulsified into many smaller fat globules (Figure 17). There is a correlation between the dimensions of the emulsified fat globules and the firmness of the processed cheese: the smaller the fat globules, the harder the cheese. It is possible, however, that highly efficient melting salts may also affect the properties of proteins. Newly formed calcium phosphate crystals (Figure 17) and also undissolved melting salt crystals (Figure 18) may be seen in most micrographs of processed cheese.

Minute dark areas were sometimes found in TEM micrographs of processed cheese (Figure 18). Their origin was puzzling until a problem developed in a processed cheese plant. The molten cheese could not flow through the pipes and eventually solidified and had to be retrieved. This product could not be marketed and would be comminuted (ground) as "rework" to be added at a small concentration to a fresh batch of cheese to be processed. The dark areas in the micrographs of the commercial processed cheese were characteristic of the experimentally "overprocessed" (overheated) cheese (Kaláb *et al.*, 1987). The origin of the dark areas is not fully understood: either they have a higher affinity for the heavy metal stains (osmium tetroxide, uranyl acetate, lead citrate) used to stain thin sections or the proteins are more compacted. The findings by Taneya *et al.*, 1980, by freeze-fracturing and replication favour the latter assumption. The presence of relatively evenly distributed dark spots in TEM micrographs of processed cheese indicate that rework was used as an ingredient. A large North-American processed cheese manufacturer was surprised to learn what electron microscopy could tell them about their product.

TEM of processed cheese may reveal even whether non-ripened cheese was part of the cheese blend to be processed. The core-and-shell structure of casein particles in American White cheese or Indian Paneer and similar cheeses is so stable that it may be observed in processed cheese.

Milk powder

There are many reasons to dry milk and other liquid milk products such as buttermilk, cream and whey, and to store them in the form of powders for future use (Carić & Kaláb, 1987b). In fact,

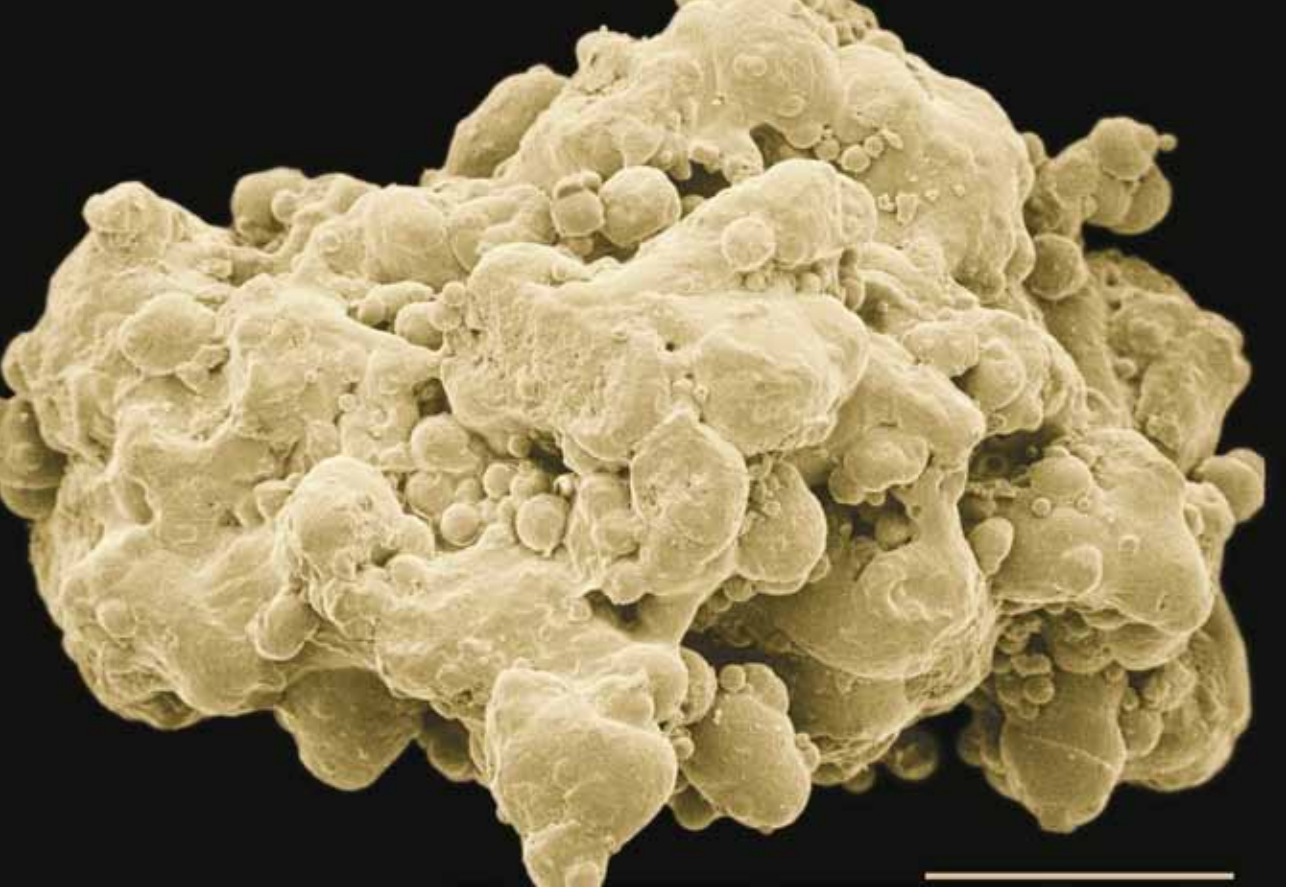


Fig. 22. *Instant milk powder consists of agglomerated primary spray-dried particles. Bar: 100 µm.*

dried milk products also include yogurt, various milk-based beverages and even cheeses (Carić, 1994), ultrafiltration permeate (Kaláb *et al*., 1991b), casein and caseinates. Drying considerably reduces the volume of the milk and increases the shelf life of the product. Nowadays, most milk powder is produced by spray-drying. Evaporated milk is dispersed ("atomised") into a stream of hot air in a spray-drying chamber. Spray-dried milk particles are globules 10 to 250 µm in diameter (Figure 19) with one or several large central vacuoles and many small vacuoles distributed throughout the globule body (Figure 20). The external appearance may vary.

In some powders, the globular particles are marked with depressions (Figure 21) whereas other powders consist of particles which appear as if smaller globular particles are emerging from their interior (Figure 19). The surface of the globules may be wrinkled. According to Dr. Norman Singer (private communication), the processes inside the drying chamber determine the appearance of the powders. It is known that smaller particles dry more quickly than large particles. If such small, dry particles collide with large particles which are still wet, they become captured on the surface of the large particles. If, however, the large particles are no more sticky but still soft, the colliding small, dry and hard particles ricochet after having caused dimples (depressions) in the large particles.

A large part of the milk sugar (lactose) in the primary spray-dried milk particles is in an anhydrous glassy form. In humid air, it slowly recrystallises into α-monohydrate and this leads to caking of the powder (Ibach & Kind, 2007). Controlled recrystallisation associated with agglomeration of the powder (Figure 22) is the principle of instant milk powder production. Its objective is to improve the reconstitution properties of the primary powder (Carić, 1994), i.e. the rate at which the powder particles dissolve in water without sticking together.

Conclusions

Electron microscopy has markedly contributed to the understanding of interactions among the milk constituents (caseins, whey proteins, milkfat, lactose) and other ingredients during the manufacture of milk products (Kaláb, 1991). There are many researchers who have contributed other important EM findings. It is impossible to name them all. Dalgleish *et al*., 2004, and McMahon and Oommen, 2008, suggested a new model of the casein micelle based on their EM studies. Heertje *et al*., 1985, followed acid-induced coagulation of casein micelles by freeze-fracturing and Brooker used electron microscopy extensively to study a variety of milk products including the dextrans produced by lactic acid bacteria (Brooker, 1979). He also described microscopic crystalline inclusions in Cheddar cheese (Brooker *et al*., 1975), explained the origin of peculiar structures in fresh milk as the residues of air bubbles in milk foam, to the surfaces of which casein micelles first became attached and then dissociated from them leaving the membranes floating in the milk (Brooker, 1985). Buchheim and Dejmek, 1990, studied the microstructure of milkfat globules and dairy emulsions including the microstructure of cream liqueurs. Goff has used low-temperature SEM and TEM to study the recrystallisation of ice crystals, partial-coalescence of fat globules and stabilization of air bubbles in ice cream (Caldwell *et al*., 1992, Goff *et al*., 1999).

This list is incomplete, of course. Additional information may be found in other books (McClements, 2007) and in journals such as the Journal of Dairy Science, International Dairy Journal, Food Science and Technology (LWT) and other food science journals, and also in the now defunct Food Microstructure and Food Structure journals.

Acknowledgments

I thank Dr. Adrian Burden for his invitation to write this review and Dr. Barbara Blackwell and Dr. H. D. Goff for useful suggestions and reviewing the manuscript. Since electron microscopy produces grayscale micrographs, Adobe Photoshop was used to present all illustrations in false colours. A great part of information on this subject may be found on the Internet, e.g. the list of scientific papers published in Food Microstructure and Food Structure (www.foodsci.uoguelph.ca/dairyedu/journal2.htm), pages on Dairy Science and Technology at the University of Guelph, and several websites dedicated to the microscopy of food.

References

Aguilera J. M., & Stanley D. W. (1999) Microstructural Principles of Food Processing and Engineering. 2nd ed., Aspen Publishers, Inc., Gaithersburg, MD, USA, 432 pp.

Angermüller, S. & Fahimi, H. D. 1982. Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy. Histochemical Journal 14, 823-835.

Brooker, B. E. (1979) Electron microscopy of the dextrans produced by lactic acid bacteria. p. 85-115. In: Microbial Polysaccharides and Polysaccharases. R. C. W. Berkeley, G. W. Gooday & D. C. Ellwood (eds.), Academic Press, New York.

Brooker, B. E., Hobbs, D. G., Turvey, A. (1975) Observations on the microscopic crystalline inclusions in Cheddar cheese. Journal of Dairy Research 42, 341-348.

Brooker, B. E. (1985) Observations on the air-serum interface of milk foams. Food Microstructure 4(2), 289-296.

Buchheim, W. & Dejmek, P. (1990) Milk and dairy-type emulsions. In: K. Larsson & S. E. Friberg (eds.) Food Emulsions, 2nd ed., Marcel Dekker, Inc., New York & Basel, 203-246.

Caldwell, K. B., Goff, H. D., Stanley, D. W. (1992) A low-temperature scanning electron microscopy study of ice cream. I. and II. Food Structure 11(1), 1-10 and 11-24, resp.

Carić, M. (1994) Concentrated and Dried Dairy Products. VCH Publishers (UK) Ltd., Cambridge, UK, 249 pp.

Carić, M. & Kaláb, M. (1987a) Processed cheese products. In: P. F. Fox (ed.) Cheese: Chemistry, Physics and Microbiology. Vol. 2. Major Cheese Groups. Elsevier Applied Science, London & New York, 339-383.

Carić, M. & Kaláb, M. (1987b) Effects of drying techniques on milk powders quality and microstructure: A review. Food Microstructure 6, 171-180.

Dalgleish, D. G., Spagnuolo, P., Goff, H. D. (2004) A possible structure of the casein micelle based on high-resolution field emission scanning electron microscopy. International Dairy Journal 14, 1025-1031.

Fox, P. F. (1987) Cheese: Chemistry, Physics and Microbiology. Vol. 2. Major Cheese Groups. Elsevier Applied Science,

London & New York, 393 pp.
Gastaldi, E., Lagaude, A., Tarodo de la Fuente, B. (1996) Micellar transition state in casein between pH 5.5 and 5.0. Journal of Food Science 61(1), 59-64, 68.
Gavarić, D. D., Carić, M., Kaláb, M. (1989) Effects of protein concentration in ultrafiltration milk retentates and the type of protease used for coagulation on the microstructure of resulting gels. Food Microstructure 8(1) 53-66.
Goff, H. D., Verespej, E., Smith, A. K. (1999). A study of fat and air structures in ice cream. International Dairy Journal 9, 817-829.
Harwalkar, V. R., & Kaláb, M. (1981) Effect of acidulants and temperature on microstructure, firmness and susceptibility to syneresis of skim milk gels. Scanning Electron Microscopy/1981/III, 503-513 and Studies of Food Microstructure, D. N. Holcomb & M. Kalab (eds.), Scanning Electron Microscopy Inc., O'Hare 1981, USA, pp. 211-221.
Heertje, I., Visser, J., Smits, P. (1985) Structure formation in acid milk gels. Food Microstructure 4(2), 267-278.
Ibach, A. & Kind, M. (2007) Crystallization kinetics of amorphous lactose, whey-permeate and whey powders. Carbohydrate Research 342(10), 1357-1365.
Kaláb, M. (1980) Milk gel structure. XII. Replication of freeze-fractured and dried specimens for electron microscopy. Milchwissenschaft 35(11) 657-662.
Kaláb, M. (1981) Electron microscopy of milk products: A review of techniques. Scanning Electron Microscopy/1981/III: 453-472 and Studies of Food Microstructure, D. N. Holcomb & M. Kalab (eds.), Scanning Electron Microscopy Inc., O'Hare 1981, USA, pp. 123-142.
Kaláb, M. (1982). Electron microscopy of foods. In: M. Peleg & E.B. Bagley (eds.) Physical Properties of Foods, AVI Publishing Co., Inc., Westport, Connecticut, USA, 43-104.
Kaláb, M. (1991) Food structure and milk products. In: Y. H. Hui (ed.) Encyclopedia of Food Science and Technology, John Wiley & Sons, Inc. New York, USA, 1170-1196.
Kaláb, M. (1993) Practical aspects of electron microscopy in dairy research. Food Structure 12(1), 95-114.
Kaláb, M. & Comer, F. (1982) Detection of buttermilk in meat binders by electron microscopy. Food Microstructure 1(1), 49-54.
Kaláb M. & Harwalkar V. R. (1974) Milk gel structure. II. Relation between firmness and ultrastructure of heat-induced skim-milk gels containing 40-60% total solids. Journal of Dairy Research 41, 131-135.
Kaláb, M. , Sargant, A. G., Froehlich D. A. (1981) Electron microscopy and sensory evaluation of commercial cream cheese. Scanning Electron Microscopy/1981/III:473-482, 514 and Studies of Food Microstructure, D. N. Holcomb & M. Kalab (eds.), Scanning Electron Microscopy Inc., O'Hare 1981, USA, pp. 153-162.
Kaláb, M., Yun, J., Yiu, S. H. (1987) Textural properties and microstructure of process cheese food rework. Food Microstructure 6(2), 181-192.
Kaláb, M., Gupta, S. K., Desai, H. K., Patil, G. R. (1988) Development of microstructure in raw, fried, and fried and cooked Paneer made from buffalo, cow, and mixed milks. Food Microstructure 7, 83-91.
Kaláb, M., Modler, W., Carić, M., Milanović, S. (1991a) Structure, meltability, and firmness of process cheese containing White cheese. Food Structure 10, 193-201.
Kaláb, M., Carić, M., Milanović S. (1991b) Composition and structure od demineralized spray-dried milk permeate powder. Food Structure 10(4), 327-332.
Kaláb, M., Yang, A.-F., Chabot, D. (2008) Conventional scanning electron microscopy of bacteria. infocus, Issue 10, June 2008, 42-61.
Knoop, E. (1972) Electron microscopic studies on the structure of milkfat and protein. Milchwissenschaft 27, 364-373 (in German).
Lauck, R. M. (1975) The functionality of binders in meat emulsions. Journal of Food Science 40(4), 736 – 740.
Lowrie, R. J., Kaláb, M., Nichols, D. (1982) Curd granule and milled curd junction patterns in Cheddar cheese made by traditional and mechanized processes. Journal of Dairy Science 65, 1122-1129.
McClements, D. J. (ed.) (2007) Understanding and Controlling the Microstructure of Complex Foods. 772 pages. Woodhead Publishing, Cambridge, UK.
McMahon, D. J. & Oommen, B.S. (2008) Supramolecular structure of the casein micelle. Journal of Dairy Science 91, 1709-1721.
McManus, W. R., McMahon, D. J., Oberg, C. J. (1993) High-resolution scanning electron microscopy of milk products: A new sample preparation procedure. Food Structure 12(4), 475-482.
Malin, E. L. & Tunick M. H. (eds.) (1999). Chemistry of Structure-Function Relationships in Cheese. Plenum Press, New York, 396 pp.
Marshall, V. M., Cole, W. M., Brooker, B. E. (1984) Observations on the structure of kefir grains and the distribution of the microflora. Journal of Applied Bacteriology 57, 491-497.
Modler, H. W., Yiu, S. H., Böllinger, U. K., Kaláb, M. (1989) Grittiness in a pasteurized cheese spread: A microscopic study. Food Microstructure 8(2), 201-210.
Singer, N. S. & Dunn, J. M. (1990) Protein microparticulation: The principle and the process. Journal of the American College of Nutrition 9(4), 388-397.
Tamime, A. Y. & Robinson, R. K. (1999) Yoghurt Science and Technology. Second edition, CRC Press LLC, Boca Raton, 619 pp.
Taneya, S., Kimura, T., Izutsu, T., Buchheim, W. (1980) The submicroscopic structure of processed cheese with different melting properties. Milchwissenschaft 35, 479-481.
Vaughan, J. G. (ed.) (1979) Food Microscopy. Academic Press, New York, 651 pp.

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As a chemical engineer (M.Eng., 1952), Miloš worked in beet sugar manufacture, vegetable canning, meat refrigeration and in food research in his native Czechoslovakia. He obtained his Ph.D. degree in chemistry (pectic substances) from the Slovak Academy of Sciences in Bratislava. In 1966, he was Associate Professor of Biochemistry at Palacký University (Olomouc) when he was accepted as a postdoctoral fellow at the National Research Council of Canada in Ottawa to work on blood serum lipoproteins.

In 1968, Miloš was hired by then Agriculture Canada to develop wieners from skimmed milk powder. To better understand the transformation of milk proteins, he learned to carry out most electron microscopy techniques thanks to a unique Electron Microscopy Lab system where scientists and technicians of various disciplines interacted and learned one from each other with the assistance from Dr. G. H. Haggis.

Since 1979, Miloš has helped Dr. Om Johari (Scanning Microscopy International, USA) organise international meetings of food microscopists and in 1982 to establish a new scientific journal "Food Microstructure" (later renamed "Food Structure"). He served as the Editor-in-Chief for 12 years.

The American Dairy Science Association conferred the Pfizer Award on him in 1982 for his microstructural research of cultured milk products. Miloš served as a United Nations FAO consultant at the National Dairy Research Institute in Karnal (India) and shared his expertise with food scientists in Japan thanks to a grant from the Government of Japan. Miloš has published over 140 scientific and technical papers. After his retirement in 1995, Miloš has volunteered as an Honorary Research Associate doing part-time electron microscopy of microorganisms, foods and other subjects.

Websites... worth a look

www.edwardhfd.info
www.pageant.org.uk
www.robkesseler.co.uk
www.millennium-microscope.org
www.microworldofgems.com
www.sciencetolife.org

http://home.att.net/~seberhard/
www.deckart.de
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