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Preparation of Milk Products for SEM Examination

Rosemary Walsh, Penn State University

This laboratory has compared several techniques in our effort to produce artifact-free SEM images in order to compare the effect of different stabilizers on microstructure of soft serve frozen yogurt and stirred yogurt. Such samples are a porous matrix of casein micelles fused to form chains which immobilize the liquid phase. Two sample preparation protocols were used: cryo-SEM for the product sampled at lower temperature, and encapsulation of those semi-liquid or soft-set yogurts within an agar gel tube or in drilled aluminum stubs / molds coated with low melting point 3% agar.

Cryo-SEM

If the product's temperature is close to freezing, samples may be taken using a small copper rivet which is topped with a second inverted rivet and placed onto dry ice or into the sample holder cooled to -20°C . The sample is quickly transferred to the -196°C stage in the sample prep chamber (Oxford C1500 Cryotrans system) and the top rivet is removed by fracturing the sample once the chamber is evacuated. The freshly fractured sample is transferred to the cryo-stage in the SEM, and the etch period is started by raising the stage temperature from -196°C to -100°C . After a brief 4 to 5 minute sublimation of the surface contamination, the sample is removed to the stage in the prep chamber and coated with 30 seconds of gold at -196°C . The frozen, fractured sample is returned to the SEM for image recording. It is possible to image the sample during the etching period at 2.5 kV but an accelerating voltage of 5 to 10 kV is possible once the sample is coated.

If the sample has no stabilizers and little fat content, ice crystal formation can distort the distribution of protein matrix and the air vacuoles in the foam. One remedy to ice crystal formation is to keep the sample size as

small as is possible. Kalab et al. (1988) describe sample holders for cryo-SEM of viscous foods.

Agar gel tube

If the product's microstructure is so fragile that it disintegrates when placed into an aqueous fixative, Misolav Kalab (1988) devised a method to encapsulate the semi-liquid sample with an agar gel tube. The sample is aspirated into a glass pipette which is then placed into 3% agar sol, coating the sample and sealing it with a thin layer of agar. The encapsulated sample is then treated as a

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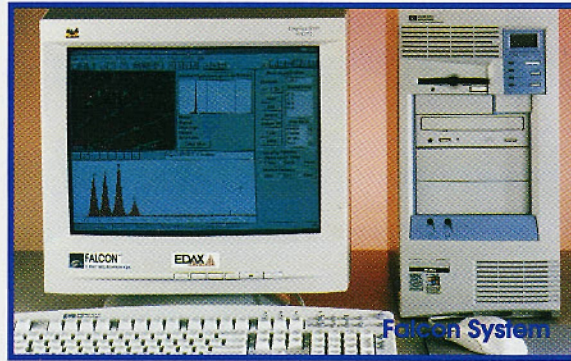
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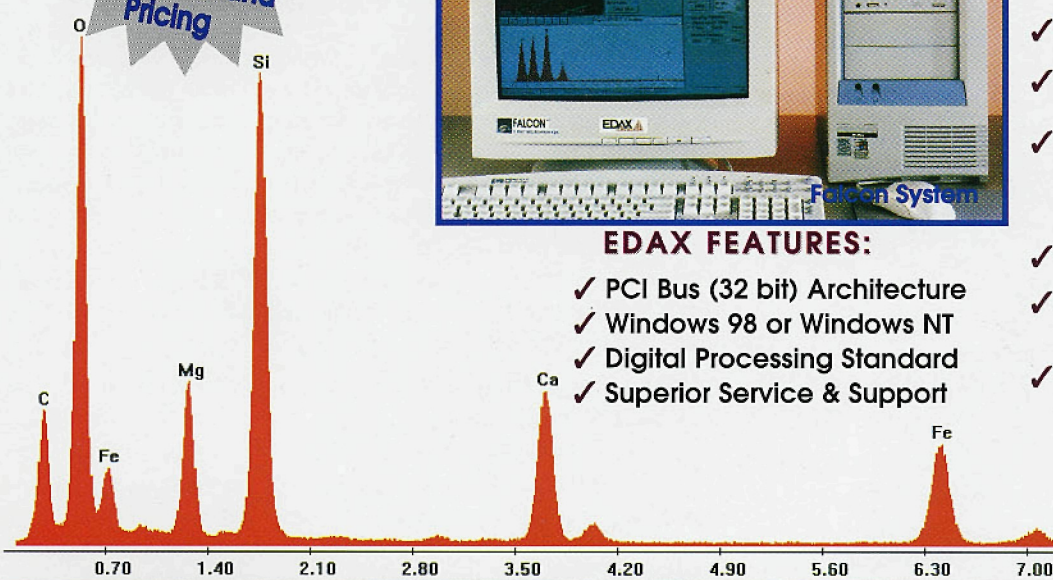


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conventional biological sample which can be placed into a glutaraldehyde fixative followed by osmium fixation, dehydration, critical point drying. The samples were placed into specimen carriers lined with fine nylon mesh for the critical point drying step. Once dry, the samples were mounted onto an aluminum stub, a fracture was initiated with a double edge razor and the complimentary piece glued in place. Colloidal silver was carefully added to the edges of the agar tube and the sample coated with 20 nm of gold/palladium.

A variation of this procedure was developed by Teggatz and Morris (1990) who used aluminum stubs with small 3 mm diameter holes drilled 1 mm into the surface as a mold to hold samples of sheared yogurt. Drawn pipettes were used to pipette the yogurt into the holes and the entire stub was dipped into 3% agar sol and allowed to solidify. Following fixation, dehydration, and critical point drying, the top layer of agar was lifted off and mounted onto a second stub. In some cases the fracture was initiated with a double sided razor and the second stub, covered with sticky tape, was inverted onto the mold to obtain complimentary surfaces. Again care must be taken when applying colloidal silver to the edges of the agar since the sponge-like sample rapidly wicks up any excess solvent in the conductive paste. ■

References:

- Kalab, M., P. Allan-Wojtas and A.F. Yang. 1988. Sample Holders for solid and viscous foods compatible with the Hexland Cryotrans CT 1000 assembly. *Food Microstructure* 7:115-120
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