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TECHNICAL NOTE:

**A SIMPLE CARRIER FOR FREEZING DIFFICULT FOOD SAMPLES
IN PREPARATION FOR SCANNING ELECTRON MICROSCOPY**

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Abstract

A thin aluminum foil carrier allows several pre-shaped samples to be plunge-frozen simultaneously. The disposable, inexpensive, simple-to-make carrier allows effective freeze-fracture electron microscopy of troublesome samples.

Introduction

Successful scanning electron microscopy (SEM) of freeze-fractured samples having a low total solids content such as yoghurt or renneted milk curd occasionally requires extraordinary treatment (1, 3); these samples remain to be very soft even after prolonged glutaraldehyde fixation. Treatments usually involve mixing the samples with agar sol (4) or encapsulation in agar gel tubes (2); both disrupt sample structure and are suitable only to study spreadable viscous foods. Agar becomes integrated into the sample structure in the former case, whereas encapsulation requires aspiration and extrusion of the sample into tubes, which disturbs the initial structure. The best preparation for difficult samples is the one that minimizes handling and disruption of structure.

Cracking and breaking of soft samples during handling with forceps is particularly troublesome during plunge-freezing; submerging the sample in the cryogen and holding it there is difficult, but was made possible by the use of a simple, disposable aluminum foil carrier.

Materials and Methods

The carrier, made from thin (0.01 mm thick) aluminum foil (Fisher Scientific, Nepean, Ontario) allowed the maximum sample cooling rate during plunge-freezing. Three selected folds of a 10 mm x 25 mm foil piece formed a pocket to contain the samples (Fig. 1a-c); the dull side faced inwards to reduce sample adhesion to the carrier.

Samples, fixed in a glutaraldehyde solution and dehydrated in ethanol were loaded into the carrier and a few drops of ethanol were added to keep the samples wet (Fig. 1d). The carrier was closed (Fig. 1e) and the assembly was gripped using forceps (Fig. 1f), plunged into a melted pool of Freon 12 at its freezing temperature, and submerged for 15-20 s. Then the carrier was transferred into liquid nitrogen and the sample was taken out (Fig. 1g). The samples were fractured as usual (Fig. 1h). Standard transferral of fractured particles to the critical point drying basket and subsequent particle handling followed (Fig. 1i).

Results and Discussion

Advantages of using the aluminum foil carrier over direct specimen handling using forceps include: increased efficiency when carriers are made ahead of time and are used to prepare multiple samples simultaneously (Fig. 1h) and reduction of damage when preparing difficult samples. Pre-shaping of samples further increases the efficiency and success of the technique. If samples are pre-shaped (cut

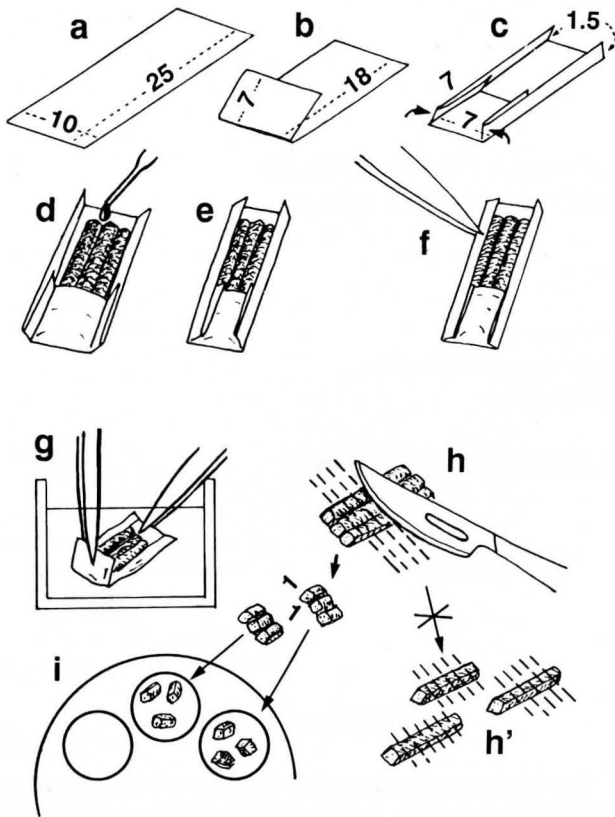


Fig. 1. Formation and loading of carrier

a: The piece of aluminum foil before folding (dimensions are in millimetres); **b:** First, the dull side of the foil is folded to the inside. **c:** The 2nd folds (arrows) are made on the sides to complete the pocket. **d:** The carrier is opened, the samples (~15 mm long prisms, 1x1 mm in cross section) are loaded, and a few drops of ethanol are added to keep the samples wet; **e:** The loaded carrier is closed, ready for freezing; **f:** Using a pair of tweezers, the loaded and closed carrier is plunged into a pool of melted Freon 12 at -150°C ; **g:** The carrier, with the frozen samples inside, is transferred to liquid nitrogen and the samples are taken out; **h:** The block of cemented samples is fractured under liquid nitrogen. The fractures extend across all the samples at once. Only a few passes across the block with the chilled blade are required as compared with many passes which would be necessary if the samples were frozen and fractured separately (**h'**); **i:** The fractured samples separate into pieces upon thawing in absolute ethanol when loaded into the critical point-drying basket.

into sticks 15 mm long with a square face of 1 mm per side) before glutaraldehyde fixation and processing for SEM, the only further manipulation of the sample required is at the freeze-fracture step. After critical point-drying, the dried samples may be mounted on stubs without any further trimming. Trials were carried out where samples including yoghurts, milk gels, and cheeses were trimmed later in the processing schedule. Some samples were found to be more difficult to handle; after glutaraldehyde fixation and/or dehydration in ethanol, cheeses became very tough and brittle, and, in some cases, shattered when cut. Cheese samples were tougher and more brittle when trimming was delayed until after critical point drying. Yoghurts and milk gel samples became firmer and were slightly easier to handle without damage if cut later in the processing schedule. When trimmed after critical point-drying, they sometimes broke into small, useless fragments. It is therefore recommended that all samples be pre-shaped before processing. The benefits of adopting this practice include better penetration of chemicals into the samples, and reduction of the exposure of the microscopist to chemicals compared to the situation when large samples are trimmed in the later stages of the processing schedule.

Pre-shaping of the samples allows precise control of fracture location; this is important when samples contain some structural orientation (e.g., cheddared curd or stretched Mozzarella cheese) and fracturing in more than one

orientation is desired. Careful alignment of the samples during carrier loading allows more efficient fracturing by having each blade stroke fall across all aligned pieces simultaneously. Straight fractures are effected, saving more time during mounting and electron microscopy of fracture faces.

The carriers have been used in the author's laboratory for a wide range of dairy food samples, and have been found to be very versatile. They are also useful in the preparation of routine samples, where they allow for rapid preparation for the SEM.

Acknowledgment

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