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TECHNICAL TIP

**CRITICAL POINT DRIER AS A SOURCE OF CONTAMINATION IN
FOOD SAMPLES PREPARED FOR SCANNING ELECTRON MICROSCOPY**

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Abstract

A valve in a critical-point drier regulating the flow of liquid carbon dioxide was found to be the source of minute metal particles which contaminated the fragments resulting from freeze-fracturing of food samples.

Introduction

Various artifacts may develop in food samples during preparation for scanning electron microscopy (SEM). Some of the artifacts have already been identified and described [3]. In conventional SEM, hydrated food samples containing protein such as most milk and meat products, are fixed in a glutaraldehyde solution, dehydrated in a graded ethanol series, impregnated with absolute ethanol, rapidly frozen, and freeze-fractured. The fragments are thawed in absolute ethanol and subsequently critical-point dried [1]. The dried fragments are mounted on SEM stubs, coated with gold, and examined in the scanning electron microscope.

Fragments selected for mounting were consistently found for some time to be contaminated with minute particles of unknown origin. Examination of all individual steps during sample preparation resulted in the identification of the critical-point drier as the source of contamination.

Materials and Methods

Commercial set-style yoghurt samples were cut into prisms of dimensions approx. 1 x 1 x 10 mm. The prisms were fixed in a 2.8% aqueous glutaraldehyde solution for 24 h, washed with distilled water, dehydrated in a graded ethanol series (20, 40, 60, 80, 95, and 100% ethanol), frozen in liquid Freon 12 at -150°C, and transferred into liquid nitrogen. The frozen samples were freeze-fractured as usual [2] in a brass dish (45 x 45 x 45 mm) immersed in liquid nitrogen. Fracturing was accomplished using a cooled steel scalpel. The fragments were returned into 20°C absolute ethanol and subsequently dried in a Samdri PVT-3 critical point drier. This commercially produced device had been modified by adding a liquid carbon dioxide line wrapping around the pressurized cell so that it is possible to independently maintain the temperature at about 10°C. This modification has extended the period for liquid carbon dioxide to replace ethanol in compact food samples such as cheese.

The dried fragments were placed in glass vials (15 mm in diameter, 42 mm high) with screw caps and were later mounted on double sticky dry mount disks attached to aluminum SEM stubs. The edges of the fracture planes and the sides of the fragments were rendered electrically conductive using a silver-based cement (Ladd Research Industries, Inc., Burlington, VT).

Until the contaminating source was identified and the problem was corrected, the contaminating particles were blown off the food fragments using a gentle stream of compressed air in order to obtain artifact-free micrographs.

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Key Words: Artifacts, contamination, critical-point drier, freeze-fracturing, scanning electron microscopy, SEM.

Results and Discussion

Minute shiny particles were observed for some time to contaminate the fracture planes of freshly obtained fragments (Fig. 1). Several sources of this contamination were considered: (a) liquid carbon dioxide, (b) the glass vials with the screw caps, (c) the brass dish used to freeze-fracture the samples, and (d) the critical-point drier.

A new filter was installed on the carbon dioxide cylinder to ensure that most impurities would be eliminated. The clean filter on the carbon dioxide line did not resolve the problem. The glass vials and the screw caps were carefully cleaned with cotton-wool swabs to eliminate them as a source of contamination, but the problem persisted. The brass dish was a more likely source of contamination because the scalpel used to fracture the samples could chip the brass dish as it came in contact with it. The contamination was too evenly distributed on all surfaces of the samples for the brass to be the primary cause. The brass dish was nevertheless replaced by a Pyrex glass dish to definitely remove the brass dish as a possible source of contamination. The last probable source of contamination was the critical-point drier. An observation had been made that sample fragments allowed to dry in air were not contaminated; this suggested that the contamination occurred before critical-point drying. All the valves in the drier were cleaned and the lines were flushed. The problem was temporarily resolved. When the contamination reappeared within a month, the inlet valve, which is a brass valve, was replaced with a new one. The old valve showed wear and tear and metal dust (Fig. 2) was found around its stem. The problem has been solved and contamination has not occurred again.

Acknowledgments

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Discussion with Reviewers

L.J. Veto: The authors have mentioned that the old valve showed wear and tear and metal dust (Fig. 2) was found around its stem. The origin of the "minute metal particles" (Fig. 1) on the contaminated yoghurt fragment is unknown. (a) What evidence to the authors have that the contamination was of metallic nature? Could it be organic? (b) Figs. 1 and 2 are secondary electron images. Have the authors examined the minute unknown particles by backscatter electron imaging? (c) Another suggestion is to examine and compare the minute particles by energy dispersive spectrometry (EDX).

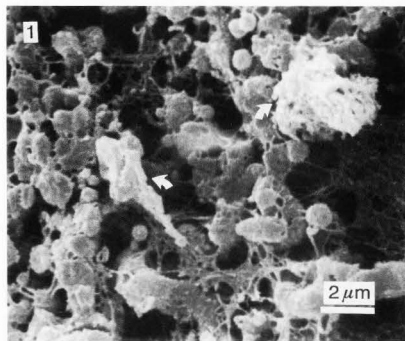


Fig. 1. Scanning electron microscopy of a contaminated yoghurt fragment. Arrows point to minute metal particles.

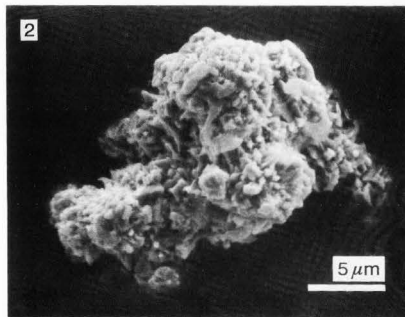


Fig. 2. Scanning electron microscopy of a metal dust particle isolated from a faulty valve in the critical-point drier.

Authors: The minute particles had a metallic appearance under a dissecting microscope and differed markedly from minute yoghurt particles which may develop during freeze-fracturing. Dust of this nature, unlike the metallic particles, is very easy to remove from the fragments by a stream of compressed air. It is true that backscattered electron imaging or EDX could be used to determine the origin of the contaminant. However, the correlation between the incidence of metal dust around the worn valve and the minute shiny particles contaminating all surfaces of the yoghurt particles was obvious. Since the objective of the search for the contaminant was to identify its source rather than its composition, this objective has been accomplished. It may serve as a warning and a suggestion to select high-quality parts when modifying instruments used in sample preparation for electron microscopy.

Introduction
to the Food Microstructure Symposium
at the 8th World Congress of Food Science and Technology
Toronto, September 29 - October 4, 1991.

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Physical relationships of individual food components to each other in multiphase or structurally complex natural food systems are as important as or more important than the gross composition of the food. This fact is recognized by the growing body of work dedicated to revealing these relationships through application, usually, of the microscope in all its various forms.

Canadian food scientists have contributed significantly to the microscopical examination of food, particularly over the past 20 to 25 years, to which the following illustrative (and certainly not exhaustive) examples will attest. Early in this period, William Powrie, newly appointed head of the Food Science Department at the University of British Columbia collaborated with Daryl Schaller on the application of the scanning electron microscope to muscle foods (Schaller and Powrie, 1972), while Bernard Dronzek and Walter Bushuk studied cereals and cereal products at the University of Manitoba (Dronzek et al., 1974). Also beginning in the early 1970's at Guelph University, David Stanley published studies of the microstructure of muscle, milk, vegetable, and extruded foods (Stanley et al., 1972; Stanley and Geissinger, 1972). In a prolific series of studies extending to the present day (Aguilera and Stanley, 1990), Stanley's influence on food microstructure research is as pervasive as the number and variety of his co-workers and students who have worked with him in the subject area. About the same time, Miloslav Kalab, working at Agriculture Canada's Food Research Institute in Ottawa, began extensive studies related to the microstructure of milk and milk products (Kalab and Harwarwar, 1973). These ongoing studies have brought world recognition to Kalab for his pioneering work (Aguilera and Stanley, 1990, Chapter 6).

Throughout Canada, a host of food scientists have made use of microscopical examination of food, usually in support of studies using other techniques and often building on the leadership of Stanley, Kalab, and others. Williard Mohr (1972) studied sogginess in french fries using TEM. Marvin Tung and his students at the University of British Columbia (Tung and Jones, 1981; Gill and Tung, 1978;

Cumming and Tung, 1975) applied microscopy to thermally gelled protein systems and mayonnaise. Shai Barbut (1988) of Guelph University has examined microstructure in meat products and Thomas Beveridge and co-workers at McGill University and Agriculture Canada (Beveridge et al., 1980; Beveridge and Ko, 1984; McKenzie and Beveridge, 1988) have examined gelled protein products, egg products, and opalescent apple juice particulate. The new generation of food scientists in the field include Rick Yada and Douglas Goff of Guelph University (Yada et al., 1990, Caldwell et al., 1992, Xu et al., 1992) and Paula Allan-Wojtas and Linda Poste of Agriculture Canada's Centre for Food and Animal Research (Allan-Wojtas and Poste, 1991).

With such a rich background in food microstructure, it is no surprise that an international meeting organized and held in Canada should have a symposium program featuring food microstructure. In the following pages, four internationally recognized experts discuss food structure each from their own perspective. Suk Yiu, currently seconded to the Ontario Ministry of Agriculture and Food from Agriculture Canada discusses the application of microstructural techniques, particularly those based on optical microscopy, to the study of the nutritional quality of cereal foods. She has worked extensively to delineate the ways in which the physical structure of cereals influence the nutritional availability of carbohydrates and minerals. Brian Brooker, of the AFRC Institute of Food Research in Reading, U.K., widely known for his expertise in dairy food microstructure and the application of microstructural techniques to food quality questions, discusses the formation of air emulsions (foams) in diverse products such as whipped cream and cake batter. The stabilization of these materials by proteins and fats is of special interest, especially the role of fat-protein interactions in foam stabilization. Isaac Heertje of the Unilever Research Laboratories in the Netherlands brings insight to the study of the relationship of structure to function in fatty emulsion systems such as butter, margarines, and low-fat spreads. Recent

techniques such as confocal scanning laser microscopy are described which allow direct observation of emulsifiers at oil-water interfaces and study of the competitive processes which shape the nature of these interfaces. Miloslav Kalab of Agriculture Canada's Centre for Food and Animal Research has worked extensively on the electron microscopic examination of dairy products with a view to explaining the role of processing in the generation of structure and texture. The detection of defects in food products by microstructural means and their explanation in chemical or technological terms has been an additional, ongoing interest. Here, Kalab brings these two interests together reviewing the electron microscopy of dairy products particularly as the microstructure relates to the quality (or lack thereof) in dairy foods.

Four world-known food microscopists met in Toronto in October, 1991, during the 8th World Congress of Food Science and Technology. It was our pleasure to receive these visitors and co-chair their respective presentations. We extend to our speakers our thanks for their patience throughout the organizational process. Their cooperativeness, helpfulness, and further patience throughout the publishing process for the microstructure symposium was especially appreciated.

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Managing Editor's Note: The organizers of Food Microstructure Symposium (see above) held as part of the Food Science and Technology Congress in Toronto in 1991 approached us in early 1993 suggesting that Food Structure publish the manuscripts by I. Heertje, M. Kalab, B.E. Brooker, and S.H. Yiu, because of specific requirements related to high quality reproduction of micrographs. The manuscripts were provided to Food Structure in January and were reviewed and edited to Food Structure standards.

We are pleased to be able to present these important papers to our readers in this issue of Food Structure (pages 77-133).

Om Johari