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# OBSERVATIONS OF REAL-TIME DYNAMIC CHANGES TO FOOD MICROSTRUCTURE USING THE ENVIRONMENTAL SCANNING ELECTRON MICROSCOPE (ESEM)



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In this report we describe the first use of the production model environmental scanning electron microscope (ESEM) to observe dynamic morphological changes in fresh tomato and celery. The use of an Environmental SEM eliminated the need for time consuming preparative methods such as fixation, freeze drying, etc. Use of this type of microscope should prove helpful in studying dynamic changes to both food and nonfood items at the high resolution and high magnification obtained by a SEM.

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## SUMMARY

An environmental scanning electron microscope (ESEM) was used for the first time to observe and videotape real-time dynamic changes to the microstructure of fresh, wet untreated tomato and celery tissues. No sample preparation methods such as dehydration, freezing, critical point or freeze-drying were necessary for charge-free visualization at from 15 to 25 kV. Since the specimen chamber allows for control of the chemical and/or physical states of the specimens, it is possible to keep each sample wet and to observe cell shrinkage and distortion as the chamber pressure is reduced. The consequence of pressure reduction to fresh vegetable cells and comparisons with freeze-dried vegetables is discussed. The experimental results demonstrate that the capability for altering a food sample's environment (temperature, pressure, gas or chemical) within the ESEM offers many possibilities in the study of food microstructure and is a significant improvement over conventional scanning electron microscopes.

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# PREFACE

This project on the first use of the environmental scanning electron microscope (ESEM) at Natick was undertaken during the period October 1986 to September 1988. The work was funded under Program Element No. 1L161102, Project No. AH52, Task No. 03, and Work Unit Accession No. DA309672.

The authors thank Dr. Charles A. Voyle for reading this paper at the Food Microstructure Symposium in Reading, U.K., in September 1988.

# OBSERVATIONS OF REAL-TIME DYNAMIC CHANGES TO FOOD MICROSTRUCTURE USING THE ENVIRONMENTAL SCANNING ELECTRON MICROSCOPE (ESEM)

## INTRODUCTION

In the study of biological microstructure, interpretation of results should be made with consideration given to preparative methods (Lewis, 1986). For example, cell volume is greatly affected by sample preparation and cell structure can be altered by processing differences (Heathcock and Chapman, 1983), such as variations in fixation and post-fixation techniques (Irving and Becker, 1985). The occurrence of artifacts, which are a consequence of most preparative techniques, must be minimized to assure proper sample evaluation (Flint, 1982).

Problems encountered by food scientists in sample preparation for microscopy are similar to those generally encountered by biologists, namely: fixation and dehydration methods produce structural changes, including distortion and shrinkage (Chabot, 1979). The best way to overcome these problems is to eliminate dehydration and retain the sample's natural state (Robinson, 1978). This has recently been accomplished with a newly developed environmental scanning electron microscope (ESEM). By use of the ESEM's vacuum gradients, scientists can examine charge-free, wet and uncoated nonconductive samples (Harniman, 1988).

Technologies upon which this microscope are based include: 1) a specimen chamber with which samples can be examined in a saturated $H_2O$ vapor environment (Danilatos and Robinson, 1979); 2) a scanning electron microscope, which permits visualization of samples at atmospheric pressure (Danilatos, 1980); and 3) a differential pumping system, which permits live and wet specimens to be examined at pressures up to 6 kPa (Danilatos, 1981, 1988).

The ESEM is a scanning electron microscope that can operate at a range of pressures from the typically low pressure of a standard SEM to pressures at which $H_2O$ can be observed (Danilatos, 1988). In other words, the ESEM has a region within the electron column where the vacuum is optimum for generating and focusing the electron beam at pressures less than $1.3 \times 10^{-4}$ Pa and another region (the specimen chamber) where the pressure can exceed 2.6 kPa. These two regions are separated by two apertures, which effectively limit pressure build-up in the column. By using pressure-limiting apertures, the desired specimen chamber pressure is maintained and specimen transfer is facilitated.

As the electron beam passes into the specimen chamber it diffuses after collision with gas molecules; however, if a detector is positioned close enough to the sample, detection of the emerging signal will occur. Parameters such as resolution and magnification are basically the same as a standard SEM (Baumgarten, 1989); however, if the sample within the ESEM is completely covered with $H_2O$, the resulting secondary electron image will be very low in contrast (Robinson, 1978). An additional significant capability of the ESEM is that dynamic surface microstructural changes can be videotaped so that these real-time changes can be studied at a later date.

In this report we describe preliminary experiments using for the first time the production model environmental scanning electron microscope to observe dynamic changes to the microstructure of fresh, wet, untreated, uncoated vegetable tissues.

## MATERIALS AND METHODS

For comparative purposes, both fresh and freeze-dried vegetables (celery and tomato) were evaluated.

Fresh vegetables obtained from a local market were cut into 1 $cm^3$ cubes and placed into the cylindrical well (2.5 cm in diameter and 2.5 cm deep) of a specially fabricated SEM stub. In addition to saturating the specimen chamber of the ESEM with $H_2O$ vapor to 100% relative humidity via a reservoir chamber, the well contained $H_2O$, which helped keep the sample moist.

The initial pressure within the ESEM specimen chamber of 2.66 kPa (19.9 torr) $H_2O$ vapor was gradually reduced over a period of time via a computerized differential pumping system to 10.6 Pa (1 h 33 min) for celery, and from 2.85 kPa to 2.66 X $10^2$ Pa (2 h 3 min) for tomato. The dynamic sequence of events occurring to the microstructure, especially the cell walls, of each of the vegetables was recorded on videotape and still photographs were made from TV screen images.

Freeze-dried celery and tomato were cut into 1 $cm^3$ cubes, affixed to the surface of SEM stubs with silver paste, sputter coated with 15 nm of AuPd and examined with a Zeiss CSM 950 SEM at 20 kV (the ESEM was operated at either 20 kV or 15 kV). Photographs were taken using Polaroid type 52 film.

## RESULTS AND DISCUSSION

Photographs of celery and tomato taken within 1 min of insertion into the specimen chamber of the ESEM whose vapor pressure was 2.7 kPa can be seen in Figs. 1A and 2A. As the pressure within the chamber was reduced to 8.6 X $10^2$Pa in 30 min, some shrinkage and distortion occurred to the celery cell walls (Fig. 1B) and similar changes occurred to the tomato (Fig. 2B). After 30 min at 2.0 X $10^2$ Pa pressure, the tomato cell walls collapsed (Fig. 2C); however, because of the natural rigidity of celery, collapse of the cell walls (Fig. 1C) took longer (1 h 26 min at 1.1 X $10^2$ Pa) to occur than with the tomato.

Cell walls of both the celery (Fig. 3) and tomato (Fig. 4) appear well defined and typical of freeze-dried vegetable cell walls. Since these samples have been dehydrated and coated with AuPd, to relate their microstructure to those of Figs. 1A and 2A, which contain $H_2O$, would be misleading. The gross appearance in Figs. 3 and 4, more closely resembles Figs. 1B and 2B whose cell walls have undergone some dimensional changes due to reduction in chamber pressure and subsequent shrinkage due to diminished $H_2O$ content.

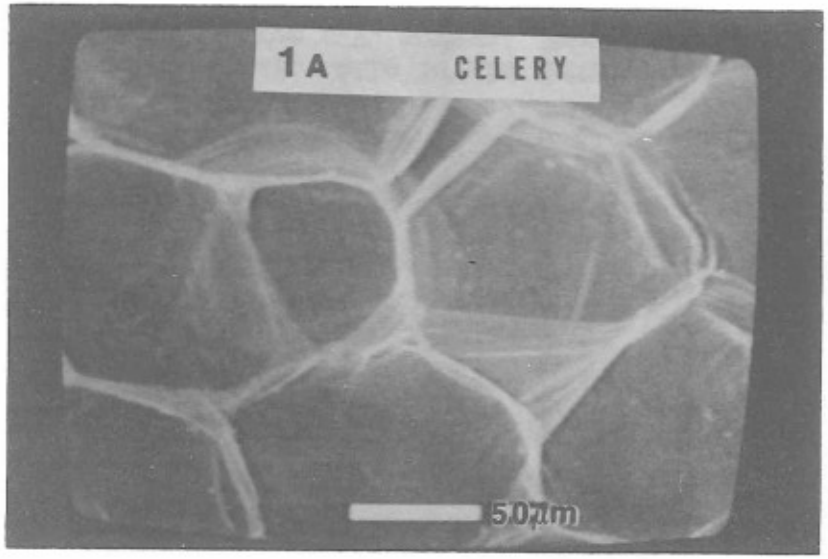


Fig. 1A. Celery cells 1 min after insertion into ESEM. Photo taken from T.V. screen. Bar = 50 µm

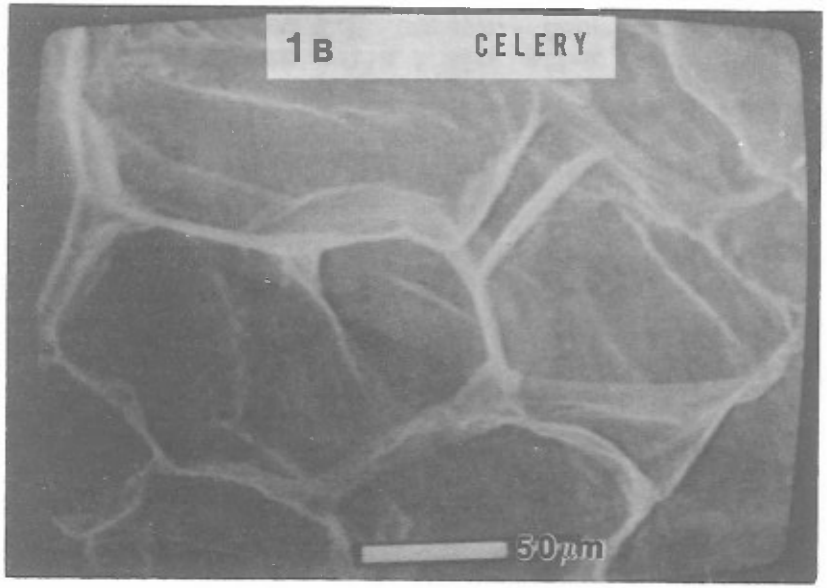


Fig. 1B. Celery cells 30 min after insertion into ESEM. Photo taken from T.V. screen. Bar = 50 µm

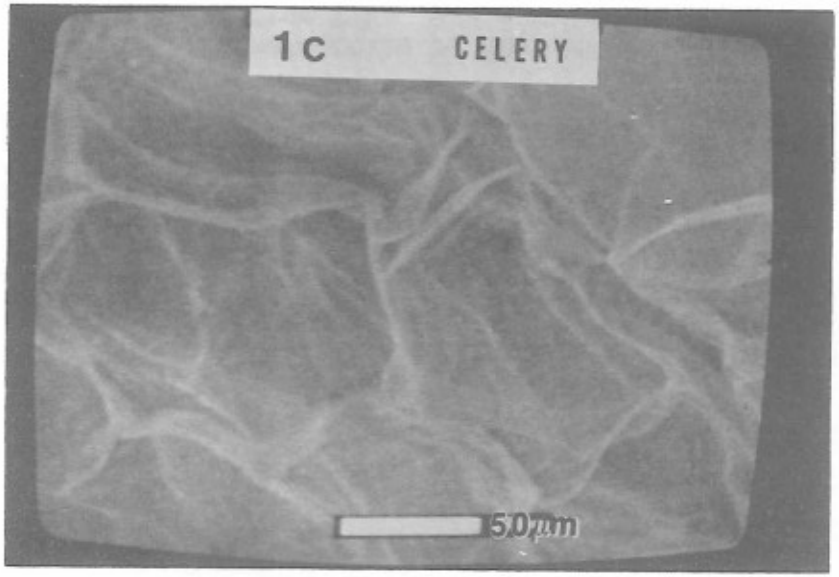


Fig. 1C. Celery cells 1 h 26 min after insertion into ESEM. Photo taken from T.V. screen. Bar = 50 µm

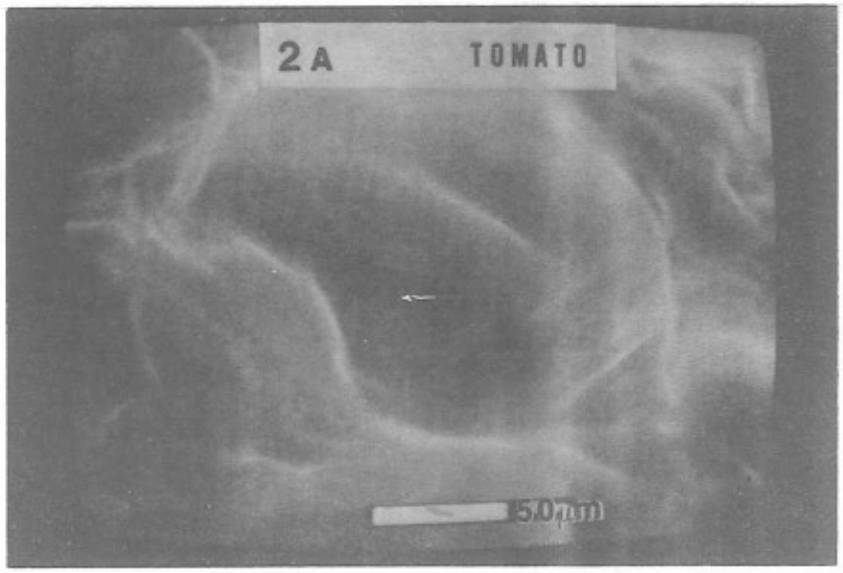


Fig. 2A. Tomato cells 1 min after insertion into ESEM. Photo taken from T.V. screen. Bar = 50 µm

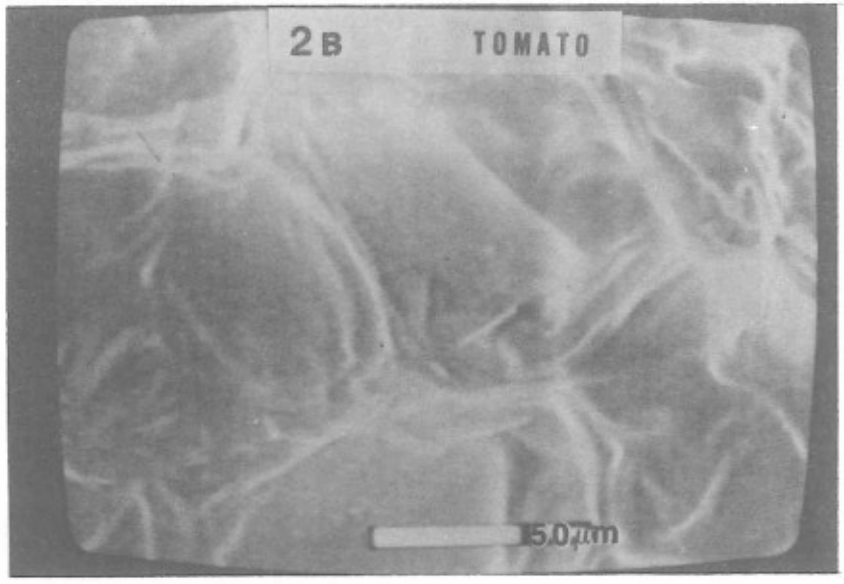


Fig. 2B. Tomato cells 10 min after insertion into ESEM. Photo taken from T.V. screen. Bar = 50 µm

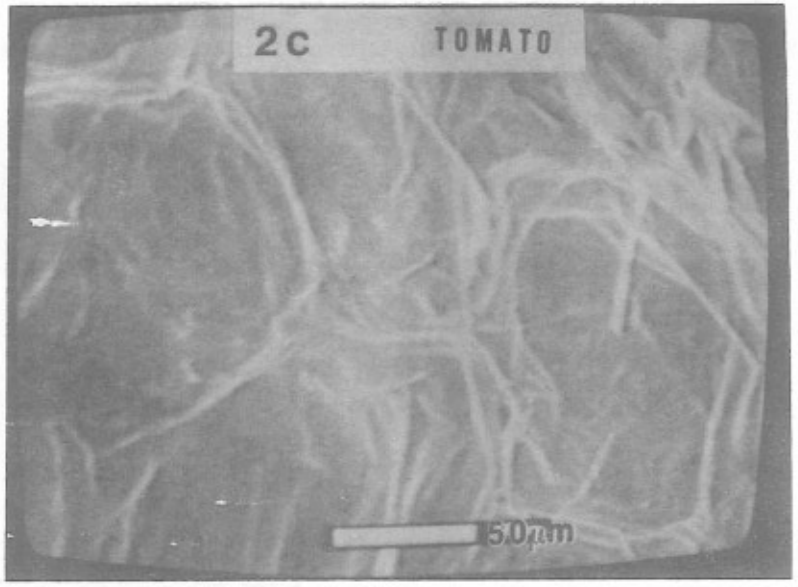


Fig. 2C. Tomato cells 20 min after insertion into ESEM. Photo taken from T.V. Bar = 50 µm

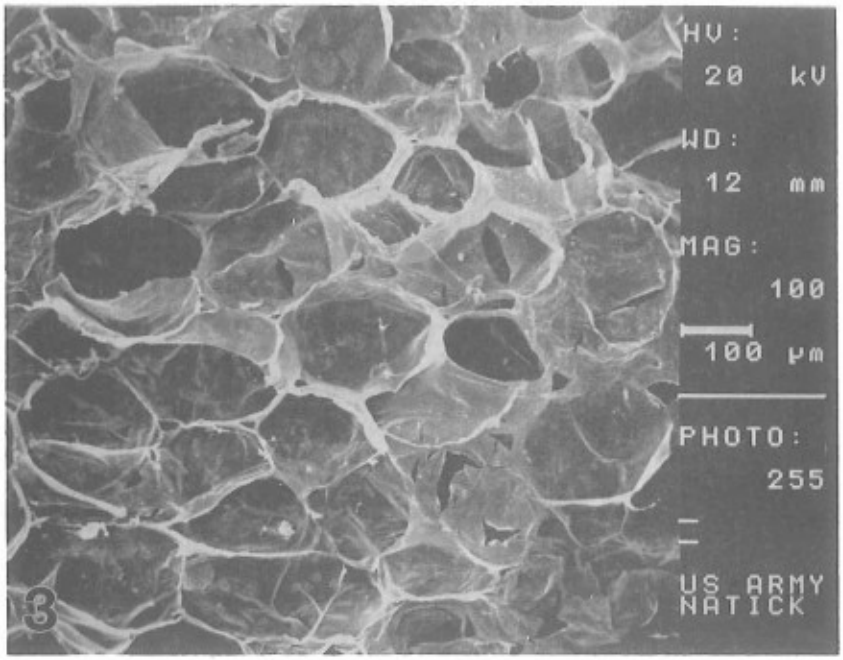


Fig. 3. SEM micrograph of freeze dried celery cells.

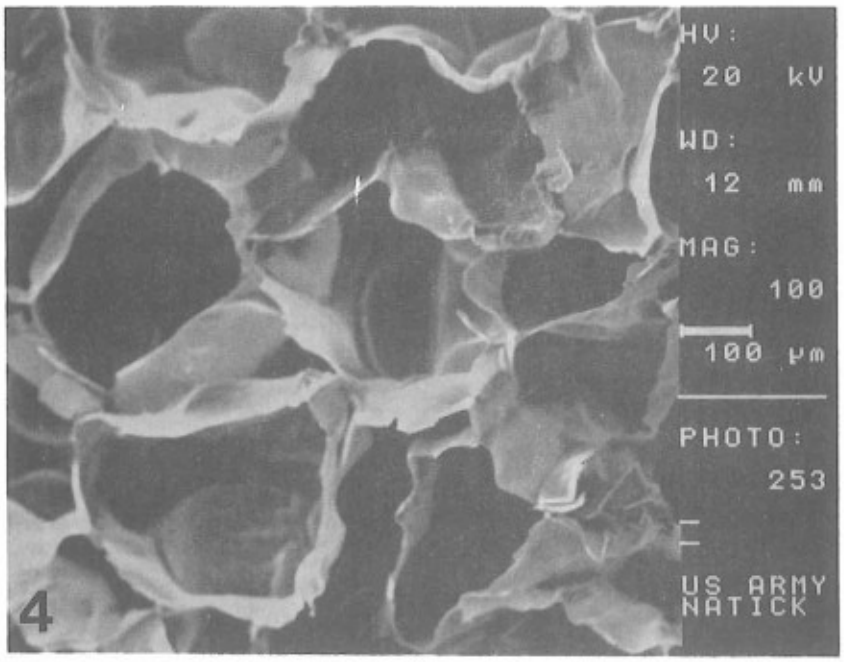


Fig. 4. SEM micrograph of freeze dried tomato cells.

## CONCLUSIONS

The environmental scanning electron microscope is a very useful tool in the study of food microstructure because no sample preparation is needed and microstructural changes can be observed in real-time. The preliminary findings presented here demonstrate that the capability for altering a sample's environment within the ESEM is a significant improvement over conventional scanning electron microscopes.

The potential uses of the ESEM should benefit not only food ration research, but research in such areas as chemistry and materials science. Work is currently in progress to study the diffusion of metal ions in polymeric films.

This document reports research undertaken at the US Army Natick Research, Development and Engineering Center and has been assigned No. NATICK/TR-90/022 in the series of reports approved for publication.

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