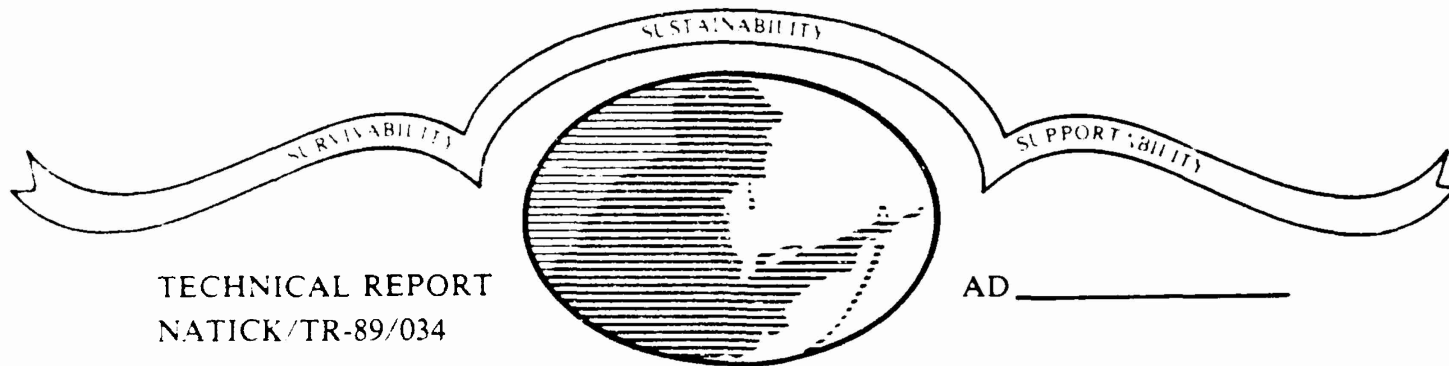




MICROSTRUCTURAL EVALUATION
OF POROUS NUTRITIONAL
SUSTAINMENT MODULE
EXTRUDATES AND INFUSATES

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JULY 1989
FINAL REPORT OCTOBER 1986 - SEPTEMBER 1988

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY SEP 07 1989			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release. Distribution unlimited.		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NATICK/TR-89/034			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Natick RD&E Center		6b. OFFICE SYMBOL (if applicable) STRNC-YSP		7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) Kansas Street, Natick, Ma 01760		7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION		8b. OFFICE SYMBOL (if applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 611102	PROJECT NO. 1L1 611102AH52	TASK NO. 03	WORK UNIT ACCESSION NO. DA309672
11. TITLE (Include Security Classification) (U) Microstructural Evaluation of Porous Nutritional Sustainment Module Extrudates and Infusates					
12. PERSONAL AUTHOR(S) Samuel Cohen, Charles Voyle, Richard Harniman, Robyn Rufner, Ann Barrett, and Carolyn Hintlian					
13a. TYPE OF REPORT FINAL		13b. TIME COVERED FROM Oct 86 TO Sept 88		14. DATE OF REPORT (Year, Month, Day) July 1989	
15. PAGE COUNT 25					
16. SUPPLEMENTARY NOTATION Authors addresses: Charles Voyle, AFRC, Institute of Food Research, Langford, Bristol, BS187DY, U.K.; Richard Harniman, Electroscan Corp., 100 Rosewood Drive, Danvers, MA 01923; Robyn Rufner, 503 Foxwood Circle, W. Peabody, MA 01960					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	MICROSTRUCTURE; EXTRUDATES; PYCNOMETRY; MICROSCOPY;		
			INFUSATES; SCANNING ELECTRON MICROSCOPY (SEM);		
			NUTRITIONAL SUSTAINING MODULE (NSM); POROSIMETRY INFUSION;		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
A variety of microscopies (light, scanning electron, transmission electron, fluorescence, and environmental scanning electron) were used to demonstrate morphological characteristics of nutritional sustainment module (NSM) extrudates and infusates. The microscopic data proves that internal cell wall pores measuring less than 1.0 μm exist. Also, uniform infusion takes place when there is communication between cell wall pores and the much larger external pores. Some of the methods used should prove helpful in selecting optimum extrudate/infusate formulations.					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL Samuel Cohen			22b. TELEPHONE (Include Area Code) 508-651-4578		22c. OFFICE SYMBOL STRNC-YSP

SUMMARY

NSM extrudates contain many external pores which permit infusion to take place quite easily, depending of course on the number and size of pores and the size of particles within the infusate. In addition to the external pores there exist many internal pores within the cell walls which do not communicate with the outside. As was clearly shown using the environmental scanning electron microscope infusion is blocked after some period of time when the infusate just cannot penetrate through the cell walls. Therefore, it seems as if not only is pore size a limiting factor when the particles within the infusate block the pores, but lack of communication between adjacent cells also contributes to impeded penetration of infusate. As a result, even the liquid portion of the infusate can't get through.

The microscopic methods used to study the NSM matrix microstructure were quite valuable as a means of understanding the relationship between processing variability, extrudate microstructure and ease of infusion.

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PREFACE

This final report describes research performed during the period October 1986 to September 1988 under project ILI61102AH5203021.

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MICROSTRUCTURAL EVALUATION OF POROUS NUTRITIONAL
SUSTAINMENT MODULE EXTRUDATES AND INFUSATES

INTRODUCTION

The nutritional sustainment module (NSM) (Briggs et al., 1986), developed in the Food Engineering Directorate, is a ration whose high caloric density (up to 7.1k cal/cc) and nutritional quality is designed to permit the continuation of a soldier's performance under stressful environmental conditions. By using "ICE" (infusion, compression and extrusion) technologies three families of food components, dairy bars, infused extrudates and compressed or infused meat bars, all of which are calorie dense, have been produced.

The objective of this report is to describe the results of a three year effort (from FY86-88) whose purpose was to examine NSM extrudates and infusates microscopically and by so doing show the interrelationships between extrudate porosity and facility of infusion.

NSM extrudates consist of porous matrices made from various flours (corn, wheat, rice, etc.) whose large range of pore sizes, from a few micrometers to several millimeters in diameter, permit rapid liquid infusion. However, nonhomogeneity and high concentration of particulates in infusate suspensions, with some particles being larger than the extrudate's pores, contributes to pore blockage and often prevents complete infusion.

Among the methods currently used to evaluate extrudate characteristics are porosimetry, pycnometry, force deformation analysis and chemical analysis. However, microscopy is the only way to visually differentiate and evaluate physical changes within extrudates (Stanley, 1986a). Microscopy can also be used to study infusates, although viscometry, mechanical spectrometry and light scattering analysis can all be used to determine various infusate characteristics. Methods such as scanning electron microscopy (Gomez and Aguilera, 1983; Harper, 1986; Owusu-Ansah, et al., 1983; Stanley, 1986b; and Cohen and Voyle, 1987a), fluorescence microscopy (Fulcher, 1982) and transmission electron and light microscopy (Cohen and Voyle, 1987b) have all been used to characterize either extrudate or infusate morphologies. In addition, recent work by Cohen et al. (1988) using an environmental scanning electron microscope (ESEM) has shown how dynamic microstructural changes can be observed without sample preparation while still keeping the sample moist.

MATERIALS AND METHODS

NSM samples used in this study were obtained from the Food Engineering Directorate and produced as follows: Corn flour was cooked at 250-355°F for a couple of minutes under pressures of 500-1500 psi to gelatinize the starch and denature the protein. The cooked mass was then released to the atmosphere through the orifice of a die. The sudden release of pressure accompanied by the rapid loss of steam resulted in the formation of a porous honeycomb-like matrix. To obtain a desired cell and pore morphology the matrix can be engineered by changing processing and ingredient parameters.

Flavored and fortified melted lipids were added to the porous matrices by placing the extrudate into the chamber of a vacuum infuser where, upon evacuation, the infusate (the melted lipid containing flavors and nutrients) penetrates throughout the extrudate.

The final step in this process was to toast the sample in a hot air oven at 240°F. The approximate composition of our samples was 68-98% corn meal, 0.75% corn oil, 0.0015% BHA, 0.005% Vitamin E, 0.75% salt and 0.25% lecithin.

Light Microscopy (LM). 4 mm² fragments of extrudate were placed on a glass micro scope slide and examined using a Zeiss Ultraphot Microscope equipped with Luminar optics and a 16 mm objective lens. Photographs were taken with reflected and transmitted light using Polaroid 55 P/N film.

Scanning Electron Microscopy (SEM). 4 mm² fragments of extrudate were affixed to an SEM stub with silver paste then sputter coated with gold palladium in a Hummer X sputter coater. The samples were examined in the SEM mode of a Hitachi 600-2 scanning transmission electron microscope (STEM) at 50 kv. Photographs were taken using Polaroid 55 P/N film.

Freeze-dried samples were cut into 1 cm³ 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. Photographs were taken using Polaroid type 52 film.

Transmission Electron Microscopy (TEM). 1 mm² fragments similar to those used above were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, immersed in 1% osium tetroxide and then washed in buffer

(see Table 1). Next, the samples were dehydrated with ethyl alcohol (50% through 100%) and then infiltrated in a 1:1 epon/alcohol mixture followed by immersion in fresh epon containing DMP-30 (the composition of the embedding medium is given in Table 2). Then the fragments were removed from the epon, placed into embedding capsules and polymerized overnight at 60° in a vacuum oven.

TABLE 1
Fixation, Dehydration and Embedding Schedule

1. 2.5% Glutaraldehyde in 0.1 M Phosphate buffer pH 7.2	Overnight
2. Buffer wash	1 h
3. Buffer wash	1 h
4. Buffer wash	1 h
5. Osmium Tetroxide	1 h
6. Buffer wash as in steps 2, 3, and 4	1 h each
7. 50% Ethyl Alcohol	30 min
8. 70% Alcohol	30 min
9. 80% Ethyl Alcohol	30 min
10. 90% Ethyl Alcohol	15 min
11. 95% Ethyl Alcohol	15 min
12. Absolute Alcohol	15 min
13. Absolute Alcohol	15 min
14. Absolute Alcohol (over Silica Gel)	15 min
15. Absolute Alcohol=Epon mix (equal parts) w/out DMP-30	2 h
16. Epon mix with DMP-30	2 h
17. Polymerize at 60°C under vacuum	24 h

TABLE 2

Composition of Embedding Medium

Solution A	Epon 812	3.1 mL
	DDSA	5.0 mL
Solution B	Epon 812	5.0 mL
	NMA	4.9 mL

Final Mixture

Solution A	7.0 mL
Solution B	3.0 mL
DMP-30	0.15 mL

After polymerization had taken place the blocks were trimmed and then thin sectioned with a Sorvall MT2B ultramicrotome using a glass knife. The 100 nm sections were cut and mounted on 3 mm copper grids and then stained with uranyl acetate and lead citrate. The grids were then examined using the TEM mode of Hitachi 600-2 STEM and photographs were taken using Kodak 2415 35 mm Technical Pan film. The STEM was operated at 50 kV and the objective moveable aperture was set at 50 μ m to obtain an image with greater contrast.

Fluorescence Microscopy. The use of fluorescence in microscopy is based on the phenomenon that occurs when light of one UV wavelength strikes a substance and is changed to a longer visible wavelength. The resulting

fluorescence is either due to the object's natural fluorescence (autofluorescence) or induced fluorescence, which occurs when a substance impregnated with a dye called a fluorochrome begins to fluoresce when excited by UV radiation.

Attempts to locate the infusate within the extrudate matrix were made using fluorescence microscopy. The Zeiss microscope described above was used, only now it was equipped with a mercury vapor lamp, Neofluor objectives, an Epi-illuminator (reflected light) and a variety of UV excitation filters (65 nm, 53 nm, 50 nm, 47 nm, 44 nm and 41 nm). To confirm the area of interest within the sample, transmitted light was used first, followed by reflected light. The stain used was Nile Blue A which is a specific fluorochrome for lipids. Samples were dipped into a 1% aqueous solution for less than 10 seconds then washed in distilled H₂O for about 1 minute. Next, the sample was mounted under a coverslip on a glass slide examined with the Zeiss and photographed using either Ektachrome ASA 400 film or Polaroid type 55 P/N film.

Environmental Scanning Electron Microscopy (ESEM). The ESEM is based on a scanning electron microscope which can operate at a range of pressures from the typically low pressure of a standard SEM to pressures at which H₂O 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 1×10^{-6} torr and another region (the specimen chamber) where the pressure can exceed 20 torr. These two regions are separated by two apertures which effectively limit pressure build-up in the column. 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; however, if the sample within the ESEM is completely covered with H_2O the resulting secondary electron image will be very low in contrast. 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.

Extrudate samples 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 which was then inserted into the ESEM. In addition to saturating the specimen chamber of the ESEM with H_2O vapor via a reservoir chamber, the well contained liquid margarine (some margarine was heated until it melted and then pipetted into the well) which helped keep the sample moist.

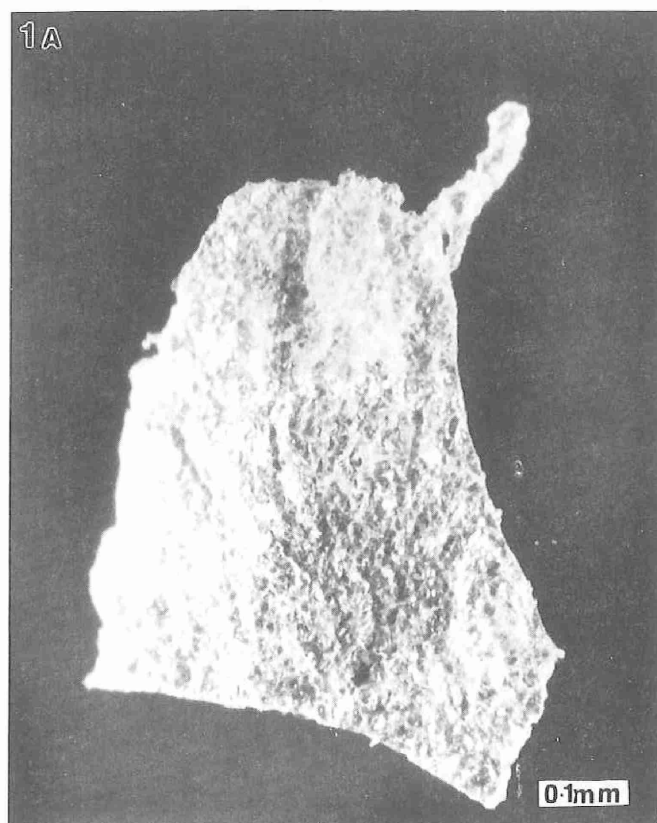
The initial H_2O vapor pressure within the ESEM specimen chamber of $2.66 \times 10^3\text{ Pa}$ (19.9 Torr) was gradually reduced over a period of time (1 h 33 min) via a computerized differential pumping system to $1.06 \times 10^2\text{ Pa}$ (0.79 Torr) while the voltage of the microscope was maintained at either 15 or 20kV. The dynamic sequence of events occurring to the microstructure, especially the cell walls, of each of the samples was recorded on videotape and still photographs were made from T.V. screen images.

RESULTS AND DISCUSSION

Reflected light microscopy (IM) of the NSM's highly porous corn-based extrudate air cell wall showed a rough surface (Fig 1a); however, when transmitted light was used (Fig 1b) air spaces or voids can be clearly seen throughout the specimen. The air spaces were separated by septa (s); however, these structures could not be readily distinguished at this relatively low magnification. Using scanning electron microscopy (SEM) a cross section through the sample (Fig. 2) reveals the extensive number of air cells. In addition, as seen in Fig 3, closer examination of the surface of an air cell wall as in Fig 1A, reveals several pores, some of which are smaller than 1mm in diameter.

When transmission electron microscopy (TEM) was used, those structures which were barely visible with transmitted IM could now be seen (Fig 4). Air spaces (A) of varying dimensions separated by septa (arrows) within the cell wall matrix (Fig. 4) are probably analogous to Pomeranz and Meyer's (1984) description of minute vacuoles inside the walls of larger vacuoles of bread crumbs.

During extrusion, expansive forces cause wall thinning (Gomez and Aguilera, 1984) and both pore size and wall thickness are related to the moisture content of the extrudate (Harper, 1986). These findings, partially based on SEM data, showed that processing conditions which affect the morphologies of large extrudate structures in the 50 to 100 micrometer range also affect those smaller internal pores within air cell walls.



1B

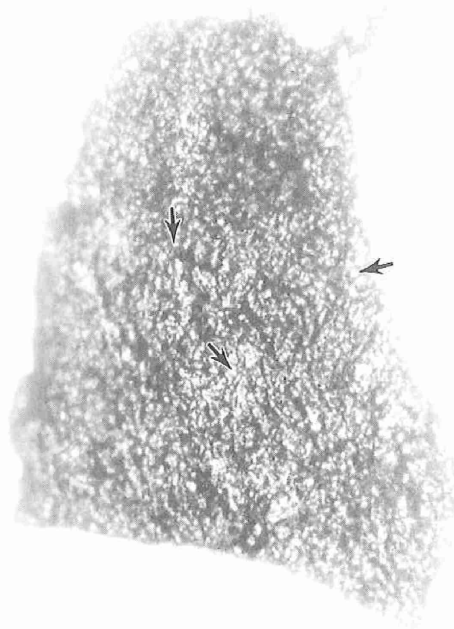
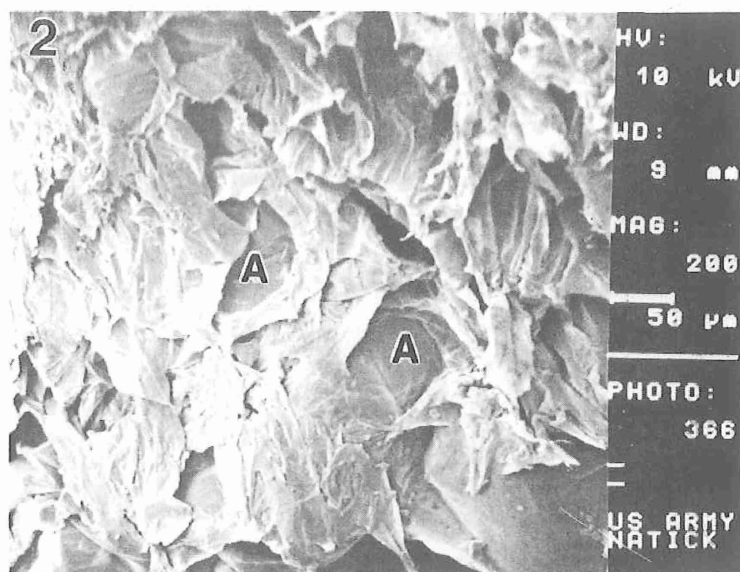


Figure 1. a. Reflected light micrograph of NSM sample showing rough surface. Bar = 0.1 mm.

b. Transmitted light micrograph showing air spaces seen as small pinpoints of light (arrows) throughout the sample. Bar = 0.1 mm.



- Figure 2. Scanning electron micrograph showing air cells. Bar = 50 μm .
3. Scanning electron micrograph of portion of sample seen in Fig 1a. Few pores (arrows) can be seen. Bar = 50 μm .

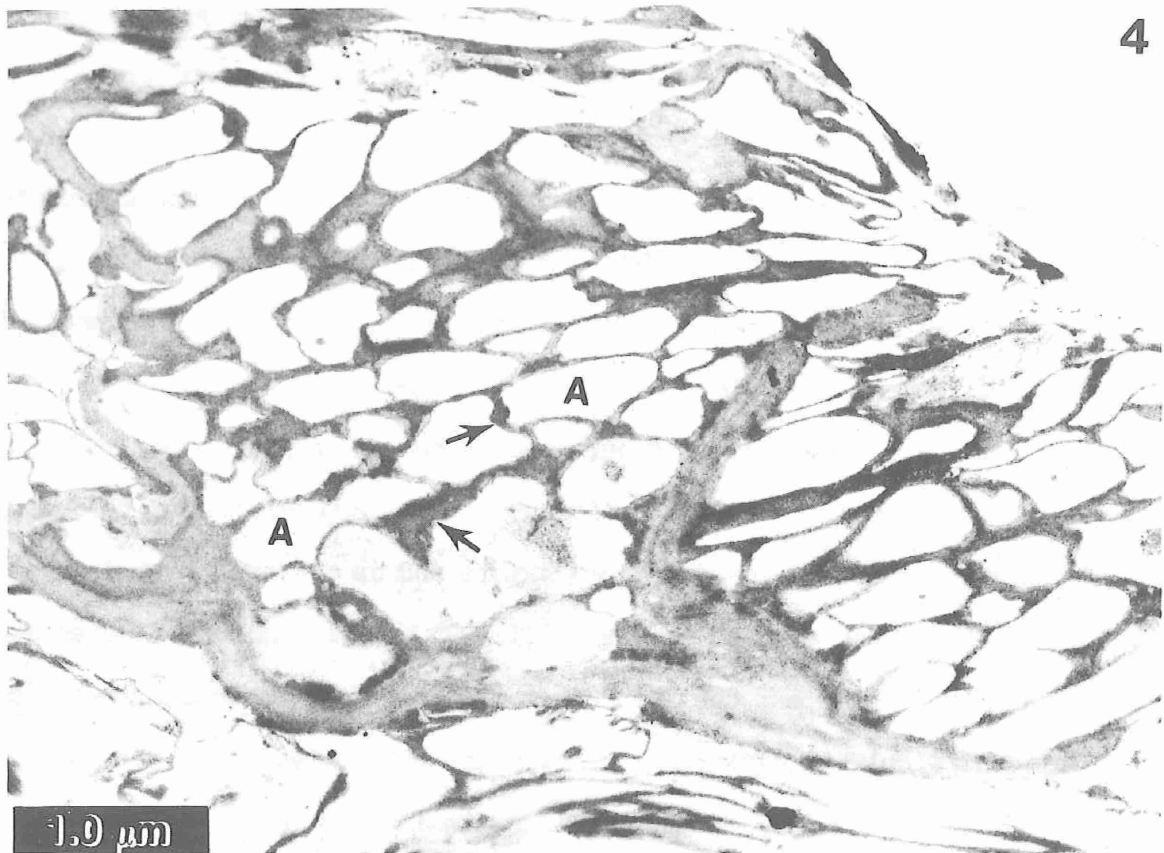


Figure 4. Transmission electron micrograph showing minute air spaces (A) and septa (arrows) of varying thicknesses. Bar = 1 μ m.

Processing variability such as differences in temperature also influence NSM microstructure (Cohen and Voyle, 1987a). For example, Figures 5 and 6 show the surfaces of two similarly extruded corn-based flatbreads which were processed at different temperatures. The sample in Fig. 5 was processed at 340°F whereas the sample in Fig. 6 was processed at 355°F. A 15°F higher temperature resulted in excess gelatinization and coagulation (arrows) which smoothed out the surface and probably blocked whatever surface pores that were present.

Elucidation by TEM of the internal microstructure of the extrudate air cell wall permits a more informed evaluation of the consequences of how changing extrusion parameters may affect infusion extrudate morphology and texture (Cohen and Voyle, 1987b).

There are two types of fluorescence: natural and induced. Natural fluorescence occurs without treatment of the sample; induced fluorescence occurs when a stain is added to a sample and is directed towards a specific area which, when exposed to a specific UV wavelength, then fluoresces. With the NSM samples, the infusate, unless it is made to fluoresce is often quite difficult to differentiate from the extrudate. When stained with Nile Blue A the lipid containing infusate fluoresced (F) as expected (Fig. 7); however, there was extensive background fluorescence (B) which we could not significantly reduce without injuring the sample.

Vegetable oil was added to those extrudates we examined to aid in solidifying all the additives and to provide some lubrication for the extruder. The induced fluorescence of the vegetable oil obscured all lipid

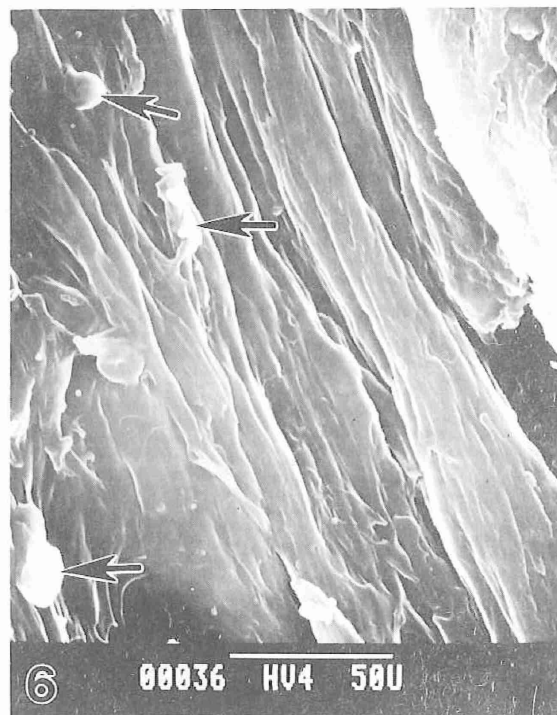
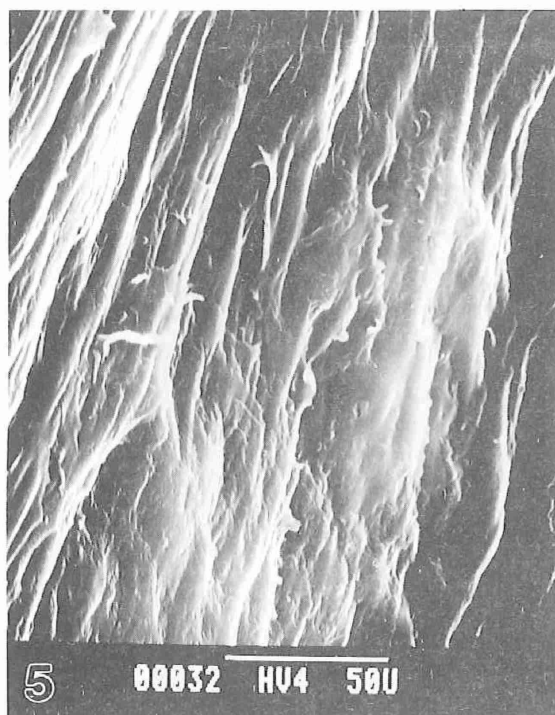


Figure 5. Scanning electron micrograph showing surface of sample extruded at 340°F. Bar = 50 μ m.

6. Scanning electron micrograph showing surface of sample extruded at 355°F. Note coagulated material (arrows) on the surface. Bar = 50 μ m.

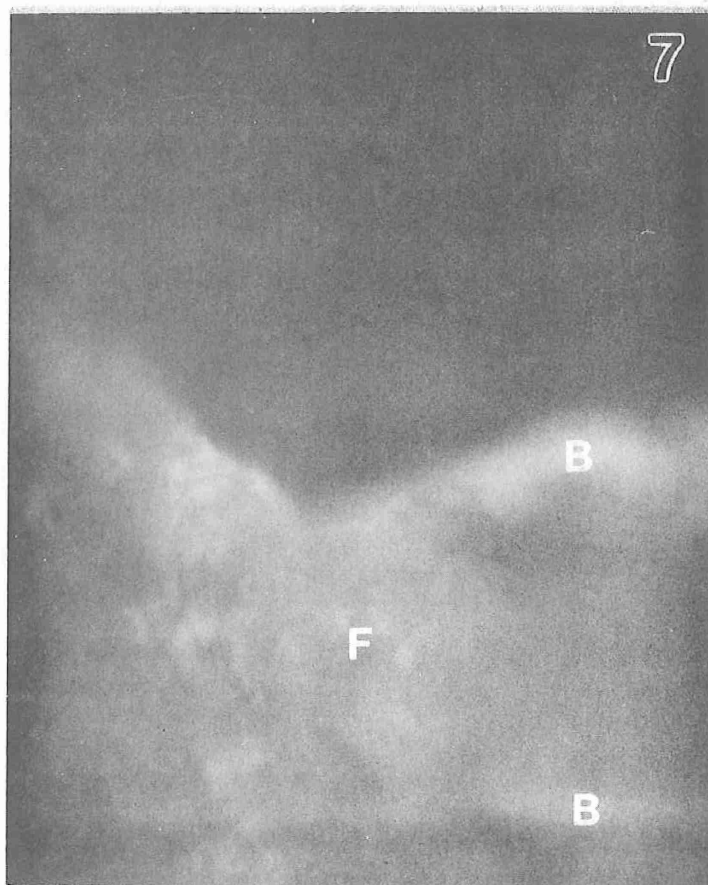


Figure 7. Surface of NSM sample with lipid specific fluorescence (F)
(caused by Nile Blue A stain) and background fluorescence (B).
Magnification = 400 X.

infusate fluorescence. This artifact of unwanted fluorescence reduced our ability to accurately localize the infusate particulates and determine whether or not they caused blockage of some pores.

In the study of microstructure, interpretation of results should be made with consideration given to preparative methods (Lewis, 1986). The example given above where vegetable oil had been added is a valid one, since the occurrence of artifacts must be minimized to assure proper sample evaluation (Flint, 1982).

Photographs of the NSM sample which had been infused with liquid margarine can be seen in Figs 8, 9 and 10. As the pressure within the specimen chamber of the ESEM was reduced, shrinkage and distortion occurred. In addition, as time passed the infusate obscured more and more of the large pores of the NSM. Although the infusate appears to fill the pores quite rapidly, even after more than 1 h within the specimen chamber some of the larger pores could still be seen. The penetration of margarine throughout the extrudate could clearly be seen with the ESEM. Prior to the development of this type of microscope wet samples of this size could only be seen by light microscopy.

Even after the infusion by margarine had taken place over an extended period of time, not all the pores were filled. In addition the margarine appeared to be concentrated around the extrudate pore cell walls, not at the bottom of the cells, a fact which supports our TEM findings that lack of communication between large and small pores is a limiting factor in the infusion process.

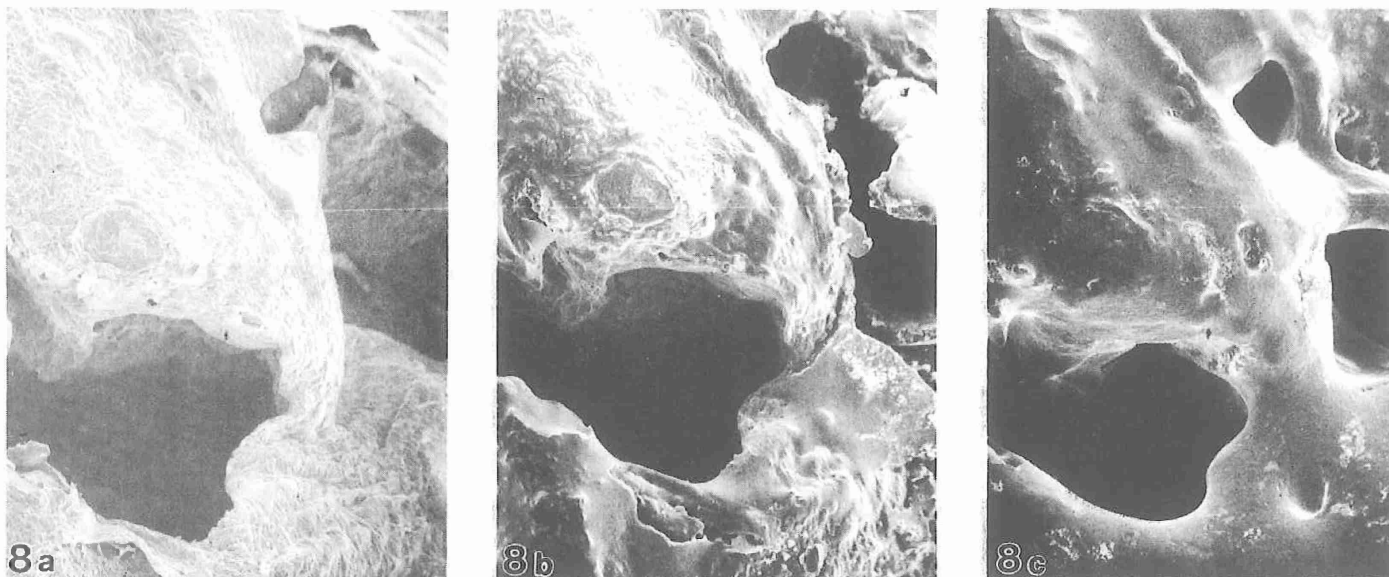


Figure 8. a. Environmental scanning electron micrographs showing infusion of liquid margarine into NSM sample 5 min after sample put into microscope.

b. Environmental scanning electron micrographs showing infusion of liquid margarine into NSM sample 30 min later.

c. Environmental scanning electron micrographs showing infusion of liquid margarine into NSM sample 1 hour 30 min later.

The infusate has gradually filled the air cells of the extrudate. Magnification = 1000 X.

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