Food Structure

Volume 2 | Number 1 Article 4

1983

An Alternive to Critical Point Drying for Preparing Meat Emulsions for Scanning Electron Microscopy

Edward J. Basgall

Peter J. Bechtel

Floyd K. McKeith

FOOD MICROSTRUCTURE, Vol. 2 (1983), pp. 23-26
SEM Inc., AMF O'Hare (Chicago), IL 60666 U.S.A.

0730-5419/83$1.00+.05

AN ALTERNATIVE TO CRITICAL POINT DRYING FOR PREPARING MEAT EMULSIONS FOR SCANNING ELECTRON MICROSCOPY

Edward J. Basgall, Peter J. Bechtel and Floyd K. McKeith

Departments of Animal Science and Food Science, Muscle Biology Laboratory, 1503 South Maryland Drive, University of Illinois, Urbana, Illinois 61801, U.S.A.

Abstract

A rapid sample drying technique is described which is useful for the simultaneous preparation of large numbers of samples as an alternative to critical point drying. The cryofractured face of meat emulsions was visualized after applying this technique. The fine structure of lipids and proteins were found to be well preserved in comparison to other reports which used critical point dried meat emulsions. Lipid was readily discerned from the protein matrix by selective fixation of the components in duplicate samples. Stereo imaging was useful in enhancing the texture of the cryofractured surface and as an aid in differentiating the protein matrix from the fat component of meat emulsions.

Initial paper received March 18, 1983.
Final manuscript received June 27, 1983.
Direct inquiries to P.J. Bechtel.
Telephone number: 217-333-3787.

KEY WORDS: Cryofracture, Meat Emulsions, Fat, Protein, Critical Point Drying, X-Ray Microanalysis

Introduction

Meat emulsion systems as well as many other food systems have been examined with the Scanning Electron Microscope (SEM) in order to evaluate the complex structural components. Some major problems faced by many investigators, especially those doing comparative studies, have been the long periods of time required for sample preparation and difficulties in scaling-up procedures in order to process a large number of samples quickly. Several techniques for cryofracturing biological tissues have been reported (Humphreys et al., 1974, Nemanic, 1972, Boyde, 1974), some of which are very useful to food systems, while others have limited application. Preparation of meat emulsion samples requires freezing and cryofracturing prior to fixation if the surface components are to be exposed to the fixative solutions. In order to minimize ice crystal formation it is important to freeze any material for SEM very rapidly. This requires the use of a small sample size and/or replacement of sample water with a low surface tension solvent such as ethanol (Humphreys et al., 1974), acetone, ether, 1, 2 epoxy propane or amyl acetate (Boyde, 1976 and 1972). Problems associated with the use of these solvents include the potential to remove the lipids if the sample has not been osmium fixed.

SEM samples must be frozen quickly utilizing an intermediate fluid which has a high boiling point and a low freezing point. Commonly used fluids having relatively high boiling points and low freezing points include: Freons 12 and 22 (Boyde, 1972), isopentane (Theno and Schmidt, 1978), and acetone (Carroll and Lee, 1981). One of the drawbacks of critical point drying is that extensive dimensional changes occur during the process. Boyde et al. (1981) showed that drying from Freon 113 produced less shrinkage artifacts than critical point drying.

One drawback of SEM is the inability to clearly distinguish between lipid and protein. SEM results have been correlated with light microscopy and specialized staining techniques used as an aid in identifying lipid and protein components in serial sections of cooked meat emulsions (Ray et al., 1979). The globular appearance of the lipid component was often used as one characteristic to distinguish it from protein,

and this was confirmed by use of selective fat stains with light microscopy (Ray et al., 1979). Carroll and Lee (1981), using a combination of light microscopy studied the relationships between structure and thermal stability in beef emulsions.

Due to the difficulties in rapidly preparing large numbers of samples for comparative SEM studies, a faster procedure was developed that could accommodate a large number of samples using conventional laboratory equipment. This technique involves an initial quick freezing of small samples followed by cleavage fracture and then fixation. After ethanol-Freon 113 substitution, a desiccator drying technique is employed (Liepins and de Harven, 1978) which produces results similar to critical point drying. A distinct advantage of this technique is that it allows for the rapid preparation of a large number of samples simultaneously. In addition, using cryofractured meat emulsions and selectively preserving either the protein matrix alone or both the protein and lipid matrix together, one can readily distinguish the two components.

Materials and Methods

Two test groups of franks were prepared: 1) control, and 2) phosphate addition during processing. From each group three franks were selected, two sample slices were taken from each frank. 1.5mm x 1.5mm cubes were cut from each sample (4°C) with a chilled razor blade. Each cube was dipped into a 1% toluidine blue solution in order to facilitate identification of the fractured surface for x-ray microanalysis. Each sample was quenched in liquid Freon 12 (BP = -29 C, FP = -158 C). Once thoroughly chilled, the samples were immersed in liquid nitrogen. As shown in Figure 1, the frozen cubes were transferred to a multiwelled metal block submerged in liquid nitrogen and cryofractured by sharply striking a precooled metal blade positioned over each cube. These samples were immediately removed from the liquid nitrogen and fixed in 3% glutaraldehyde fixative buffered with 20 mM MES [2(N-Morpholino) ethane sulfonic acid], pH 6.0 for 12 to 18 hr at 4°C. All of the samples were fixed in glutaraldehyde in order to preserve the protein matrix and half of the samples were postfixed with 1% OsO_4 (osmium tetroxide) in distilled water for 30 min to stabilize the lipid. After fixation, the samples were rinsed twice in 20 mM MES, pH 6.0 for 5 min.

Samples were dehydrated in a graded ethanol series of 25, 50, 75, 90, 100, 100, and 100% (ethanol:water, v/v) for 10 min each at 23°C, followed by a graded Freon 113 series of 25, 50, 75, 90, 100, 100, and 100% (Freon 113:ethanol, v/v) for 10 min each at 23°C. The samples were dried in a vacuum desiccator attached to a running water aspirator for 3 hr, a slight modification of the drying technique of Liepins and de Harven (1978). The dried samples were then mounted under a dissecting microscope with the fractured surface exposed, onto 13 mm Cambridge style stubs using a 1:1 (v:v) mixture of DAG (Colloidal graphite in isopropanol): DUCO cement. An SPI sputter coat was used to coat the samples with gold-palladium under a vacuum of 170 microns and an ionization current of 40 mA for 45 sec.

SEM micrographs were made using an ISI DS-130 scanning electron microscope equipped with a LaB_6 (lanthanum hexaboride) electron gun at 10 kV accelerating potential. Stereo micrographs were made with a tilt angle difference of 10 degrees.

The energy dispersive X-ray (EDX) samples were freeze-dried directly from liquid nitrogen, mounted onto stubs as described above and coated with a heavy layer of carbon in a Denton DV-502 vacuum evaporator. These samples were used to obtain the EDX spectra on a JEOL U-3 scanning electron microscope equipped with an ORTEC detector and multi-channel analyzer operated at an accelerating potential of 15 kV.

Results and Discussion

Examination of the cryofractured surface of frankfurters fixed in 3% glutaraldehyde reveals a noticeable absence of lipid material (Fig. 2a); although, the protein matrix is well preserved. The surface is pock-marked with empty voids, most of which once contained fat droplets that varied in size from 50 µm to several µm in diameter. A higher magnification of the denoted area shown in figure 2b reveals the fine structure of the protein matrix of the meat emulsion. Similar protein matrix structures have been observed by Theno and Schmidt (1978) and Ray et al. (1979). Figure 3a depicts the cryofractured surface of frankfurters fixed in 3% glutaraldehyde and post-fixed in 1% OsO_4 to preserve the lipid structure. Both the protein and lipid components are well preserved. Upon closer examination (Fig. 3b), it is evident that a large number of round fat particles are present which range in size from 50 µm for the large globules to several µm in diameter for the finely dispersed small droplets. The fat globules are evenly distributed throughout the frankfurter matrix.

Stereo imaging is a very useful and informative technique for visualizing surface texture. Stereo imaging was used to further examine the cryofractured surface of glutaraldehyde fixed, OsO_4 post-fixed frankfurters (Fig. 4). As observed in figure 3, the protein matrix appears ladened with well dispersed lipid globules. Some of the globules appear to have been drawn out from the exposed fracture face and appear as small, uniform, rounded droplets. This could be circumvented by fixing the samples initially in OsO_4 prior to glutaraldehyde fixation as described by Carroll and Lee (1981). The protein matrix is seen to be very finely textured with some larger fat droplets, and a few voids can be seen where the fat droplets have been dislodged. Lipid composed predominantly of saturated fats is difficult to preserve because osmium tetroxide may not adequately fix them due to the absence of unsaturated bonds with which to react (Dawes, 1979). Also, the voids may be the result of air pockets (Carroll and Lee, 1981). When figure 4 is compared to figures 2 or 3, it is apparent that the stereo pair results in a more informative image of the emulsion surface.

Two groups of frankfurters were

cryofractured and directly freeze dried without fixation, one set had an additional 0.5 percent phosphate added to the preblend. Energy dispersive x-ray microanalysis was performed on the fractured face of these samples. Qualitative x-ray profiles are shown in figure 5 illustrating the relative content of the major ions between the two groups of frankfurters and indicates that

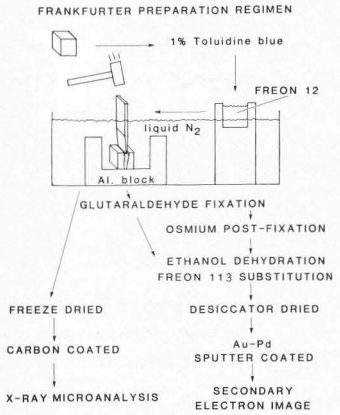


Fig. 1. Flow diagram of frankfurter preparation regimen.

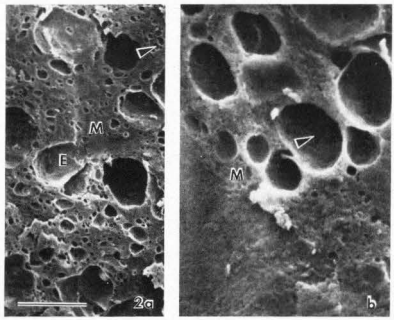


Fig. 2. (a) Cryofractured surface of glutaraldehyde fixed frankfurter. The protein matrix (M) is preserved with no noticeable lipid component. Empty vacuoles (E) remain where large fat globules have been dissolved away. Smaller vacuoles (arrow) which once contained smaller fat droplets are also present. Bar = 100 μm. (b) Higher magnification (10x) of area indicated by arrow in Fig. 2a. Note absence of lipid material in protein matrix.

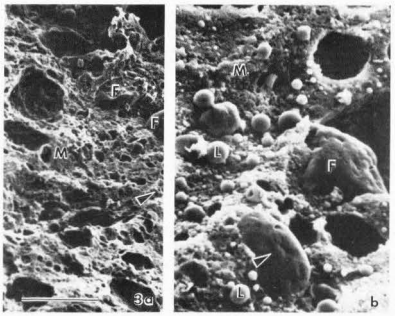
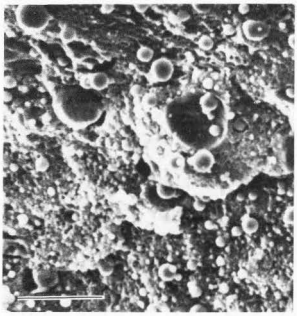


Fig. 3. (a) Cryofractured surface of glutaraldehyde fixed, osmium tetroxide post-fixed frankfurter. Large globules of fat (F) are embedded in a finely textured protein matrix (M). Arrow denotes smaller fat droplet. Bar = 100 μm. (b) Higher magnification (10x) of area indicated by arrow in Fig. 3a. Small fat droplets (F) fill vacuoles depicted in figure 2a. At this magnification, even smaller, finely dispersed fat droplets can be seen (L).

Fig. 4. Stereo pair of glutaraldehyde fixed, osmium tetroxide post-fixed, cryofractured surface of frankfurter. The fine structure of the protein matrix is well preserved. Stereo imaging provides excellent 3-D visualization of rough, lipid (L) laden fractured surface. Small fat droplets (F) readily stand out in a finely textured protein matrix (M). The arrow denotes a few empty vacuoles where fat droplets may have been dislodged. Bar= 10 μm.



the additional phosphate added can be detected by this method. State-of-the-art instrumentation exists which allows for the quantitative x-ray profile analysis of many SEM samples; unfortunately, due to specimen preparation problems, direct application to all food products has not been realized.

In terms of preservation of structural relationships in meat emulsion systems, the techniques developed in this study provide comparable SEM results to those reported by others (Carroll and Lee, 1981; Ray et al., 1979; Theno and Schmidt, 1978). In addition, the use of osmium tetroxide post-fixation provides a quick method of distinguishing protein from lipid in meat emulsion systems and may be useful for the examination of structural changes in the lipid and protein components of many food products. Major advantages of the techniques described in this paper are the relative rapidity and capacity to handle a large number of samples simultaneously as compared to critical point drying. A typical critical point drying device consists of a rather small, high pressure cylinder which restricts the number of samples that can be processed with each run. The large desiccator technique described not only allows for the simultaneous preparation of a large number of samples, but also is safer and less expensive.

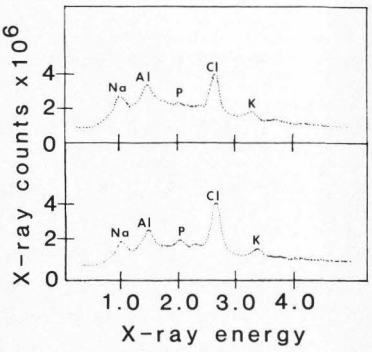


Fig. 5. Qualitative x-ray profiles of cryofractured, unfixed, freeze-dried surface of frankfurter. Relatively abundant ions are labelled. (Al peak is due to x-ray emission from mounting stub.) Upper profile is the control, bottom profile is from frankfurters treated containing additional phosphate. The higher phosphorus content can be readily observed.

References

Boyde A. (1972). Biological specimen preparation for the scanning electron microscope - An overview. Scanning Electron Microsc. 1972; 257 - 264.

Boyde A. (1974). Freezing, freeze-fracturing and freeze-drying in biological specimen preparation for the SEM. Scanning Electron Microsc. 1974; 1043-1046.

Boyde A. (1976). Do's and don'ts in biological specimen preparation for the SEM. Scanning Electron Microsc. 1976; I: 683-690.

Boyde A, Franc F and Maconnachie E. (1981). Measurements of critical point shrinkage of glutaraldehyde fixed mouse liver. Scanning. 4, 69-82.

Carroll RJ and Lee CM. (1981). Meat emulsions - Fine structure relationships and stability. Scanning Electron Microsc. 1981; III: 447-452.

Dawes CJ. (1979). Chemical Fixation for TEM. In: Techniques for Transmission and Scanning Electron Microscopy, Ladd Research Industries, Burlington, VA. 30-36.

Humphreys WJ, Spurlock BO, Johnson JS. (1974). Critical point drying of ethanol-infiltrated, cryofractured biological specimens for scanning electron microscopy. Scanning Electron Microsc. 1974; 276-282.

Liepins A and de Harven E. (1978). A rapid method for cell drying for scanning electron microscopy. Scanning Electron Microsc. 1978; II: 37-44.

Nemanic MK. (1972). Critical point drying, cryofracture, and serial sectioning. Scanning Electron Microsc. 1972; 297-304.

Ray FK, Miller BG, Van Sickle DC, Aberle ED, Forrest JC and Judge MD. (1979). Identification of fat and protein components in meat emulsions using SEM and light microscopy. Scanning Electron Microsc. 1979; III: 473-478.

Theno DM and Schmidt GR. (1978). Microstructural comparisons of three commercial frankfurters. J. Food Sci. 43; 845-848.

Discussion with Reviewers

R. J. Carroll: Have you compared this procedure which includes cryofracture followed by fixation with fixation followed by cryofracture? Do you find any differences?
S. H. Cohen: What would have happened if the samples were fixed prior to freezing? Why were the samples frozen immediately?
Authors: This study utilized only cryofracture followed by fixation and fixation followed by cryofracture was not attempted. The permeability of the fixative in this system is unknown; therefore, the degree of fixation of the internal matrix is questionable. As a consequence, fixation was performed after cryofracture to assure complete fixation of the exposed surface.

S.H. Cohen: Why was MES chosen for sample preparation?
Authors: MES buffer was used because the pKa is 5.96 and the pH of the solution was 6.00, thus it has much buffering capacity in this range.