## TECHNICAL NOTE

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# Scanning Electron Microscopy Determination of String Mozzarella Cheese in Gastric Contents

**REFERENCE:** Platek SF, Crowe JB, Ranieri N, Wolnik KA. Scanning electron microscopy determination of string mozzarella cheese in gastric contents. J Forensic Sci 2001;46(1):131–134.

**ABSTRACT:** As part of a suspected homicide investigation, a sampling of the gastric contents from the victim was forwarded to the U.S. Food and Drug Administration's Forensic Chemistry Center (FCC) for analysis of specific, selected components. The victim was known to have consumed string mozzarella cheese, as a snack, less than 24 h before his disappearance and the subsequent discovery of the body. The investigation sought to confirm or dismiss speculation the victim may have been fed a meal or eaten additional food prior to his death. Analysis of the stomach contents involved examination by stereoscopic light microscopy (SLM) and isolation, processing, and analysis of suspect materials by scanning electron microscopy (SEM). Several wax-like, off-white to cream-colored objects were noted by SLM examination and removed from the gastric contents. Through a series of fixation, sectioning, drying, and coating steps, these objects were prepared for analysis by SEM. Comparison of the suspect material with laboratory control string mozzarella cheese showed excellent correlation between the analyzed samples, confirming the suspect material from the stomach contents as string mozzarella cheese.

**KEYWORDS:** forensic science, cheese, mozzarella cheese, stomach contents, gastric contents, stereoscopic light microscopy, scanning electron microscopy, glutaraldehyde

The forensic analysis of gastric contents has multiple applications, including identification of the gastric components and determination of the relationship between the deceased and a specific location where the food item(s) were consumed or prepared, or both, possible determination of cause of death, and approximation of time of death. Review of available literature has shown some reported research which investigated the possible use of gastric contents to determine the time of death (1–3). Other authors have reported analytical methods to identify various types of food, including vegetable food items recovered from the stomach (4,5). The purpose of this forensic investigation was to identify in the stomach contents a specific component (mozzarella cheese), which was the last food item the victim was known to have consumed. The confirmation of mozzarella cheese in the stomach contents may provide valuable information in this suspected homicide investigation.

From the mouth to the stomach, ingested food items are cut, chopped, crushed, and mixed with a number of enzymes and stomach acids to accelerate the digestive process. As the mixture of liquids and solids in the stomach continues the digestion process, the contents are processed toward a more homogenous mixture, making identification of ingested food items more difficult with increased residence time. A number of contributing factors, including type of food item, particle size, volume of food product consumed, and temperature and individual metabolism may have an effect upon the digestion and residence time of ingested food items in the stomach (and throughout the entire gastrointestinal tract). It has been noted that the isolation or separation of solids from stomach contents is important for physical or morphometric analysis of partially digested food items and subsequent identification of the food (6).

### *Description of Suspect Material in Stomach Contents*

In the subject case, one of the last known items reported to have been consumed by a suspected homicide victim was a stick of mozzarella string cheese as a late afternoon snack. Examination of the gastric contents from the victim found several off-white to cream-colored masses in the nearly liquefied gastric contents, which exhibited a characteristic shape and texture. These masses had a wax-like consistency and were amorphous to globular in shape and with some elongation in a few pieces, which measured from 1 to 3 cm along the longest axis. Cross-sectional cuts perpendicular to the long axis of the elongated pieces produced an amorphous shape with both rounded and a few angled edges. Gentle probe dissection of the pieces of wax-like material revealed an orientation of fibrous material with a columnar or bundled appearance (Fig. 1*a*). Suspecting the material was cheese (specifically string mozzarella cheese per police report), additional sample preparation and analysis was performed using scanning electron microscopy (SEM) analysis for confirmation.

## Materials and Methods

The stomach contents were received frozen and were thawed at room temperature to soften them prior to examination. A representative piece of a suspect cream-colored mass (~0.5 $cm^3$) was isolated from the stomach contents and placed in a clean plastic petri dish. Using a razor blade, outer surfaces of the mass were

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cut away to expose only the inner core of the suspect mass, which had not been directly exposed to gastric fluids. The resultant "block" of the material measuring ~0.25 $cm^3$ was cut into several smaller pieces and placed in a labeled, 30 mL glass vial with a plastic screw-on cap. A similar-sized piece of a local control sample of 100% natural string mozzarella cheese from an individual serving, snack-size package was processed as above and placed in a second glass vial.

Using a modified Oberg et al. method (7), a 0.001 M buffering solution of sodium phosphate was prepared by dissolving 0.07 g of sodium phosphate (dibasic, anhydrous) in 500 mL of distilled, deionized water. The fixative solution was then prepared by adding 4.0 mL of 50% glutaraldehyde (Sigma Chemical) solution to a 200 mL volumetric flask and filling to volume with the 0.001 M sodium phosphate solution. Approximately 25 mL of the fixative was added to both the suspect and control vials and allowed to fix for a period of ~42 h. The fixative was replaced once during the fixation time after 24 h.

Following fixation, the suspect material and control mozzarella cheese sections were individually washed two times with 25 mL of distilled, deionized water with a soak time of at least 10 min per wash. In order to prepare the materials for dehydration and subsequent SEM analysis, the material in each vial was taken through a graded replacement series of ethanol and deionized water, increasing the ethanol percentage from 30, 50, 70, 90, and 100%, and to a second 100% wash. Although longer time intervals may be necessary with larger pieces of material (protein, tissue, etc.), a minimum immersion interval of 10 min per series for water removal and ethanol replacement was found to produce excellent dehydration of the material sections.

The suspect material and control mozzarella cheese blocks were further trimmed with razor blades. Cuts were made in specific directions, both parallel with and perpendicular to the arrangement of fibrous bundles in the material. All pieces were then placed in a graded replacement series of room temperature, Pel-Dri II® (substitution drying fluid, Ted Pella, Inc., Redding, CA) and ethanol, increasing the Pel-Dri II® percentage from 30, 50, 70, and 90% to 100%. Each immersion series was for a minimum of 10 min.

[AUTHORS' NOTE: The Pel-Dri II® fluid (Ted Pella, Inc.) is no longer available but the product Tetramethylsilane (TMS) (Ted Pella, Inc.) can be used instead. Critical point drying (CPD) using a commercially produced critical point dryer may also be used in place of the Pel-Dri II® steps once the material is dehydrated by replacement series to 100% ethanol.]

Once the suspect material was saturated in 100% Pel-Dri II®, the vials were quick chilled in an ice bath then placed (vial top opened) under vacuum (house vacuum pressure) in a vacuum desiccator. The preparations were left in the vacuum desiccator until all Pel-Dri II® had completely sublimed (approximately 2 h).

Pieces of the dried suspect material were mounted on labeled regions of double-sided, carbon-impregnated, conductive tape on a 25-mm-diameter carbon planchet. The pieces were oriented to view both longitudinal and cross-grain (perpendicular or transverse) exposed surfaces. The mounted preparation was coated with a conductive layer of gold-palladium in a sputter coater (pressure of ~125 to 150 mtorr at 45 mA for 60 s at ~7.0 cm anode to cathode distance). The coated preparation was placed in an SEM under vacuum and imaged by secondary electron imaging (SEI) using an accelerating voltage of 10 kV and magnifications up to ~×2000. SEM photomicrographs were prepared of both the longitudinal and cross-grain cut surfaces from the suspect material and the local control string cheese material.

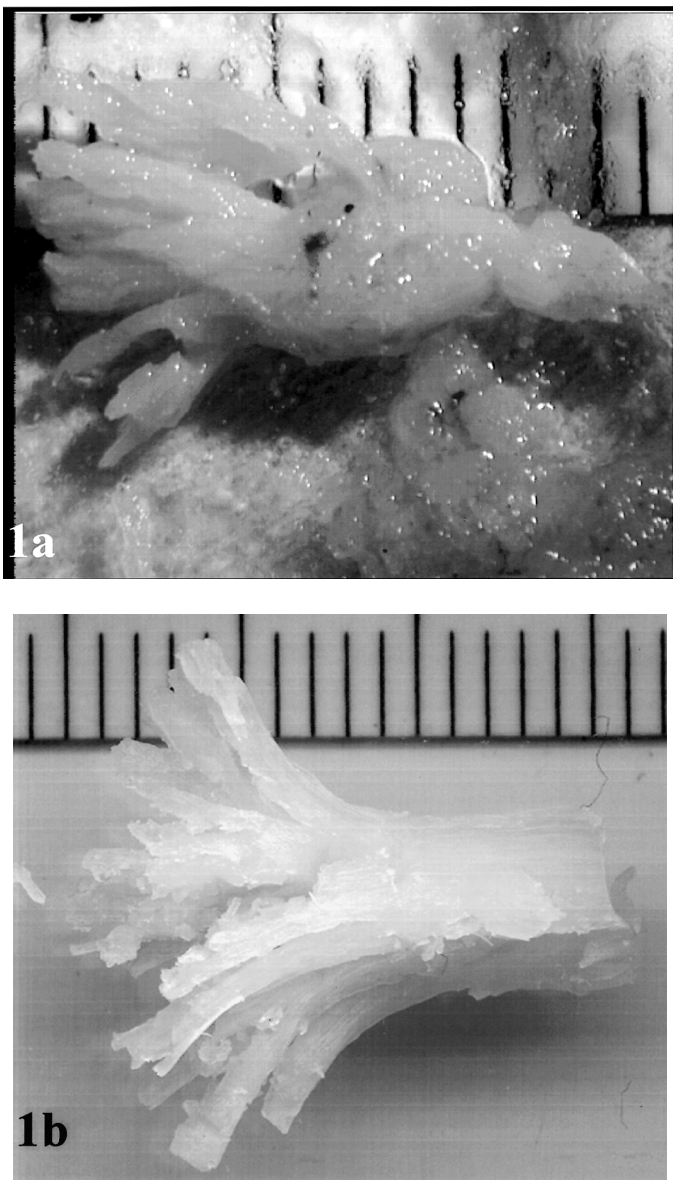


FIG. 1—(a) *Suspect cheese-like material recovered from stomach contents showing the distinct bundled appearance of longitudinal filaments slightly "fanning out" at one end. Scale intervals in mm.* (b) *Local control string mozzarella cheese showing the distinct bundled appearance of longitudinal filaments (purposely "fanned out" on one end). Scale intervals in mm.*

## Results and Discussion

Both the suspect material from the stomach contents (Fig. 1*a*) and the local control string mozzarella cheese sample (Fig. 1*b*) were off-white or cream-colored with a wax-like texture. Both were easily separated into smaller elongated bundles showing a definite parallel arrangement of these elongated bundles. Scanning electron microscopy (SEM) analysis on the longitudinal cut surfaces showed a distinct elongated arrangement of microstructure filaments on both the suspect material (Fig. 2*a*) and local control string cheese (Fig. 2*b*). The longitudinal cut surface also showed what appeared to be chains of spherical or cocci bacteria (annotated "B") (species not identified) among the filaments and into pores between filaments on both the suspect and control material. Mi-

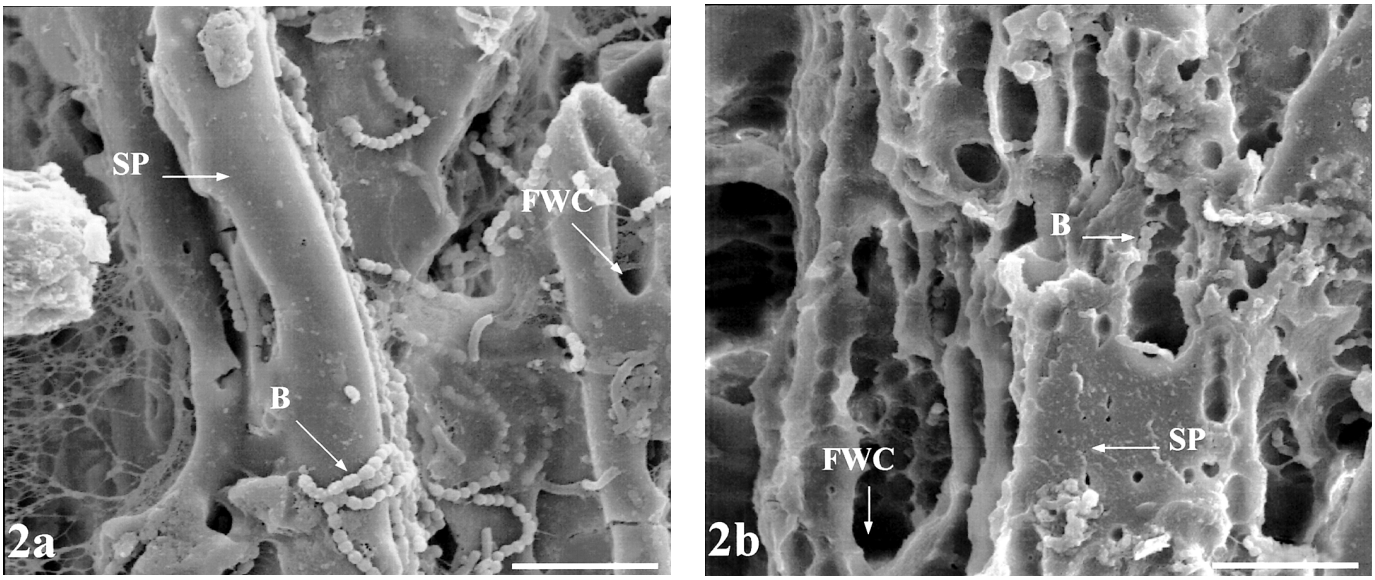


FIG. 2—(a) *Suspect cheese material (longitudinal section) showing elongated protein filaments and fat/whey columns with chains of bacteria. Bar = 10 μm.* (b) *Local control cheese material (longitudinal section) also showing elongated protein filaments and fat/whey columns with chains of bacteria. Bar = 10 μm.* B—*bacteria,* FWC—*fat/whey columns,* SP—*smooth protein.*

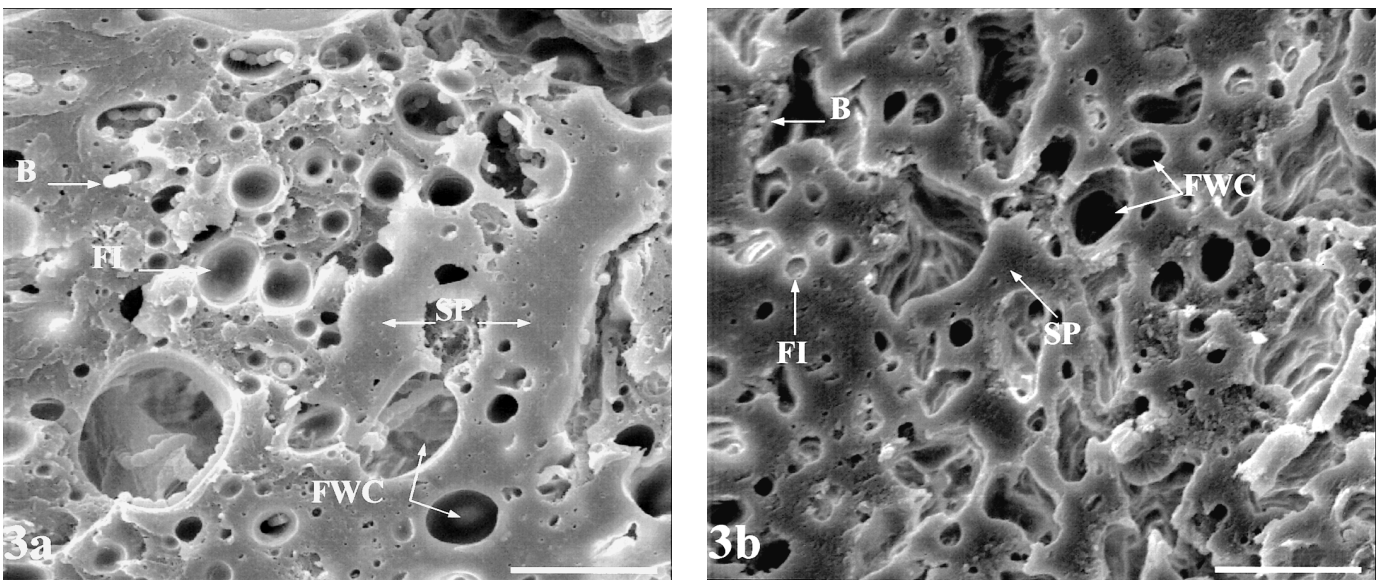


FIG. 3—(a) *Suspect cheese material (cross section) showing elongated protein filaments and fat/whey columns with chains of bacteria. Bar = 10 μm.* (b) *Local control cheese material (cross section) also showing elongated protein filaments, fat indentions, fat/whey columns and bacteria. Bar = 10 μm.* B—*bacteria,* FI—*fat indentions,* FWC—*fat/whey columns,* SP—*smooth protein.*

crostructural analysis of mozzarella cheese has shown the elongated filaments to be smooth protein (annotated "SP") and the channels to be fat/whey columns (annotated "FWC") (7). Both the suspect and control cheese material had the same type of filaments with approximately the same diameters. Both had the same appearance of the smooth protein as well as the fat/whey columns previously reported by others (7). The sizes of the microstructure features including the bacteria were consistent between the suspect material and the local control string cheese.

SEM analyses of the perpendicular or cross-grain cut preparations from the two samples were also consistent. Both the suspect material (Fig. 3*a*) and the local control string cheese (Fig. 3*b*) were comparable with visible exposed fat/whey columns (annotated "FWC") and fat globule indentions (annotated "FI") in the protein matrix. Bacterial colonies (annotated "B") were visible in the fat/whey columns, which was also consistent with observations made by other researchers (7).

A method to chemically fix particulate organic/food material (including proteins and fats) was used on suspect cheese recovered from the stomach of the decedent. Scanning electron microscopy analysis of the material demonstrated suspect off-white to cream-colored material from the stomach contents to be consistent with a local control sample of string mozzarella cheese. Consistencies between the suspect material and control cheese products included

material color, texture, fibrous bundled morphology, and microstructure. Based upon these analyses, the suspect material was shown to be consistent with local control string mozzarella cheese and mozzarella cheese analyses reported by other investigators (7).

This method demonstrated that string cheese, which is primarily fat and protein, could successfully be prepared for detailed forensic analysis by SEM, which requires placing the specimen under extreme vacuum pressures. The analyses successfully identified mozzarella string cheese in the stomach contents of the victim and provided information on the last item(s) consumed by the deceased. Additional scanning electron microscopy studies conducted to investigate other variables in the analysis of mozzarella cheese (8) may further enhance cheese analysis by SEM. The successful identification of a specific food product (such as string mozzarella cheese) that is normally difficult to image by SEM due to its composition of fat and protein suggests that it may be possible to identify other related food products by the same technique, providing valuable information in the forensic examination of stomach contents and postmortem analyses.

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## References

1. Baur C, Spann KW, Troger HD, Schuller E. Stomach contents and time of death. Beitr Gerichtl Med. 1980;38:193–7.
2. Jaffe FA. Stomach contents and the time of death. Re-examination of a persistent question. Am J Forensic Med Pathol. 1989;10(1):37–41.
3. Horowitz M, Pounder DJ. Is the stomach a useful forensic clock? Aust N Z J Med. 1985;15(2):273–6.
4. Spann KW. Identification of vegetable foodstuffs in stomach contents of corpses. Z Rechtsmed. 1978;Nov 10, 82(2):129–35.
5. Hayashiba Y, Kimura K, Kashimura S, Nagata T, Imamura T. Identification of vegetable species in gastric contents using HPLC. Int J Legal Med. 1996;108(4):206–9.
6. Rabl W, Sigrist T. Separation of stomach contents using the sieve-tower technique. Arch Kriminol 1992;May, 189(5–6):164–8.
7. Oberg CJ, McManus WR, McMahon DJ. Microstructure of mozzarella cheese during manufacture. Food Structure 1993;(12):251–8.
8. Kiely LJ, Kindstedt PS, Hendricks GM, Levis JE, Yun JJ, Barbano DM. Effect of draw pH on the development of curd structure during the manufacture of mozzarella cheese. Food Structure 1992;(11):217–24.

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