

1988

Encapsulation of Viscous Foods in Agar Gel Tubes for Electron Microscopy

Miloslav Kalab
Agriculture Canada

Follow this and additional works at: <https://digitalcommons.usu.edu/foodmicrostructure>

Recommended Citation

Kalab, Miloslav (1988) "Encapsulation of Viscous Foods in Agar Gel Tubes for Electron Microscopy," *Food Structure*: Vol. 7 : No. 2 , Article 12.

Available at: <https://digitalcommons.usu.edu/foodmicrostructure/vol7/iss2/12>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Food Structure by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



Technical Note: **ENCAPSULATION OF VISCOUS FOODS IN AGAR GEL TUBES FOR ELECTRON MICROSCOPY**

Miloslav Kaláb

Food Research Center, Agriculture Canada, Ottawa, Ontario, Canada
Telephone: (613) 995-3722 x 7707

(Received for publication Nov 26, 1988, and accepted for publication November 30, 1988)

Abstract

Viscous food is aspirated into a glass capillary tube with the inner diameter of approximately 0.5 mm if the food is to be examined by transmission electron microscopy. If the sample is destined for examination by scanning electron microscopy, it is aspirated into a Pasteur pipette having the diameter of 1.0 mm. In each case, the lower end of the glass tube is sealed with a droplet of 40°C warm 3% agar sol. After the sol solidifies, the pipette is dipped in the same agar sol and a coating, 0.2 to 0.5 mm thick, is formed around the glass tube by manipulating it while the sol is still liquid. Dipping may be repeated in order to form a uniform coating of desired thickness. The agar gel sleeve is then trimmed, and the pipette is withdrawn, whereby the sample slides into the agar gel sleeve. The free upper end of the agar gel tube is then sealed with a drop of the agar sol. The subsequent preparation of the encapsulated sample for electron microscopy is the same as that of a solid sample.

KEY WORDS: Agar gel tubes, Electron microscopy, Encapsulation, Viscous foods.

Introduction

Encapsulation of biological samples in agar gel tubes for subsequent examination by electron microscopy has been described by several authors [1, 3-7]. This technique may be used for scanning electron microscopy (SEM) as well as transmission electron microscopy (TEM). Food samples as diverse as milk [3, 4], orange juice [5], stirred yoghurt [1], and mayonnaise [2] have successfully been examined using this technique.

The techniques developed earlier consist of forming an agar gel tube around a piece of steel wire or a glass rod and using the wire or the glass rod as a piston when aspirating the sample into the agar gel sleeve. In this note, an easier and a more rapid approach is described. Its main features were published earlier [6].

Materials and Methods

Agar sol (3%) was made using distilled water and was stirred continually with a magnetic bar at 40°C.

A glass Pasteur pipette with inner diameter of 1.0 mm was used as obtained from the supplier for samples destined for SEM or was drawn out into a capillary tube to an inner diameter of approximately 0.5 mm for use with samples destined for TEM.

Commercial stirred-style yoghurt samples were aspirated into the thin capillary tubes to a length of approximately 2 mm, or were aspirated into the Pasteur pipettes to a length of 15 to 20 mm. The lower ends of the glass tubes were wiped clean with paper tissue and were sealed with droplets of the agar sol (Fig. 1).

After the sealed end had solidified, the capillary tube or the Pasteur pipette was dipped into the agar sol

and then was manipulated to form a thin layer of agar gel on the glass surface around the sample. Dipping was repeated once or twice to form a uniform agar gel layer around each sample. The agar gel sleeve was then trimmed at the upper end of the sample and removed. The capillary tube or the pipette was then withdrawn from the agar gel sleeve, whereby the sample slid from the glass tube into the gel tube. The sample column in the agar gel tube was somewhat shorter than was its initial length in the glass tube following its removal because the inner diameter of the agar gel tube was larger than that of the glass tube. The freed upper end of the agar gel tube was then trimmed with a blade approximately 0.5 mm above the sample and was sealed with a droplet of the agar sol. It was then possible to handle the encapsulated samples as solid samples during the subsequent preparatory steps for electron microscopy.

Results and Discussion

The advantages of encapsulating viscous samples for electron microscopy have already been discussed in the literature [1-5]. However, some of the techniques are quite laborious. A high degree of manual dexterity is required to properly aspirate the sample into the agar gel tube using it as a cylinder while the solid rod around which the tube had been formed is used as a piston. This procedure leads to another problem, that is the need to seal both ends of the tube after the sample is aspirated and the piston is withdrawn.

The suggested technique (Fig. 1) simplifies the encapsulation procedure and markedly increases the productivity of the technician. The manipulation required to form a uniform gel coating by dipping the glass tube

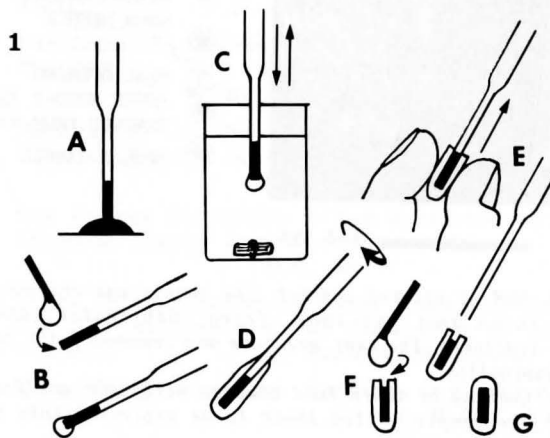


Fig. 1. Encapsulation of viscous food samples in agar gel tubes. A: Aspirate sample, B: Seal lower end of tube, C: Dip into agar sol, D: Rotate tube to form agar gel sleeve, E: Withdraw glass tube, F: Seal upper end of tube, G: Encapsulated sample.

containing the sample into agar sol can easily be learned. Should this appear difficult even after making several attempts, gels may be cast around the glass tubes using a technique that has been described elsewhere [5].

Very viscous samples such as Cream cheese can be encapsulated also provided that the above technique is slightly modified. As it is impossible to aspirate them into the tubes, the samples are placed in the tubes by repeatedly tapping the tubes into the samples which are placed on a firm support such as a microscope glass slide.

The ease with which the sample may be transferred from the glass tube into the agar gel sleeve depends on the viscosity of the sample and also on the quality of the seal at the lower end of the tube. If difficulties are encountered and the agar gel sleeve collapses, the seal should be strengthened with another agar sol droplet and a thicker agar gel sleeve should be formed.

Resin blocks containing food samples which had initially been encapsulated in agar gel tubes must be trimmed in such a way that the entire agar seal is removed along with the bordering area where the food sample and the agar gel may be mixed together.

Food samples encapsulated in agar gel tubes for subsequent SEM examination may be freeze-fractured following their fixation, dehydration, and impregnation with absolute ethanol. The agar gel coating may be left on the sample [1] or may be removed (Fig. 2).

Foods, which disintegrate in aqueous solutions and yet cannot be placed in glass capillaries to be prepared for TEM, may be coated with a thin agar gel layer in a different way. The sample is placed on the tip of a needle and touched with a droplet of warm agar sol (Fig. 3). The sol coats the food particle and immediately solidifies. The bead thus formed is removed from the needle and the exposed area of the food sample is sealed with another agar sol droplet. Again, the encapsulated sample can be treated as a conventional solid particle in any further preparatory steps.

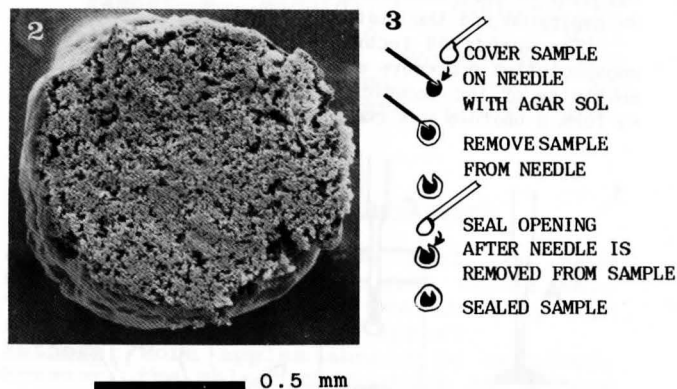


Fig. 2. SEM of stirred yoghurt. The sample was encapsulated in an agar gel tube, fixed, dehydrated, and freeze-fractured. The agar gel tube was removed prior to SEM examination.

Fig. 3. Coating of thick food samples with agar gel for TEM if the sample is too thick to be aspirated into a glass capillary tube.

Acknowledgments

Electron Microscopy Unit, Research Branch, Agriculture Canada in Ottawa provided facilities. Contribution 795 from the Food Research Centre.

References

- Allan-Wojtas P, Kaláb M. (1984). A simple procedure for the preparation of stirred yoghurt for scanning electron microscopy. *Food Microstruc.* **3**, 197-198.
- Dylewski DP, Unger RS, Martin RW. (1988). Microstructure of oil-in-water fat spreads. *Food Microstruc.* **7** (in press).
- Henstra S, Schmidt DG. (1970). Ultrathin sections of milk by means of the microcapsulation method. (In German). *Naturwissenschaften* **39**, 247.
- Henstra S, Schmidt DG. (1974). The microcapsule technique. An embedding procedure for the study of suspensions and emulsions. LKB Application Note No. 150.
- Jewell GG. (1981). The microstructure of orange juice. *Scanning Electron Microsc.* 1981;III: 593-598.
- Kaláb M. (1987). Encapsulation of viscous suspensions in agar gel tubes for electron microscopy. *Electron Microsc. Soc. Am. Bull.* **17**(1), 88-89.
- Salyaev RK. (1968). A method of fixation and embedding of liquid and fragile materials in agar microcapsulae. *Proc. 4th Europ. Reg. Conf. Electron Microsc. Rome, II*, 37-38.

Discussion with Reviewers

D. P. Dylewski: I would like to express some caution for the application of the above procedure to samples high in their lipid content. I would like to discuss the following three points:

- (1) How is a sample, especially one high in lipid content, affected morphologically when it is dipped repeatedly into molten agar at a temperature of 40° to 45°C?
- (2) When the removal of the space occupied by the glass tube is completed, how does the sample "pack" into the agar cylinder? Would mayonnaise, for example, retain its native or near natural form, that is, tight packing of lipid droplets?
- (3) Using Salyaev's method [7], the sample is drawn in one step into the agar cylinder. Using your method, the sample is handled twice: first it is drawn into the pipette, then blown out into the agar gel cylinder. What are the effects, if any?

Author: The method has been developed in order to facilitate SEM investigation of small hard particles causing grittiness in protein-based milk products such as stirred yoghurt and soft cream cheese. The effect of temperature on high-fat foods such as mayonnaise would have to be tested. If problems are encountered, the agar gel tube around the glass capillary tube may be formed by smearing the agar sol around it rather than dipping the glass tube into the agar sol. This would limit the effect of heat on the sample as the smear cools quite rapidly.

Your concern for the loss of space and the effects of handling are closely related. In my opinion, greater effects on the packing of the sample constituents may be anticipated to originate from the initial aspiration rather than from the subsequent sliding of the sample into the agar gel tube. In viscous samples, there is no continuous matrix that would be at risk of disintegration. The distribution of corpuscular components would not be affected unless air is aspirated along with the samples and a new gas-liquid interface is thus formed in them.

It is advisable, however, that the effects of the factors which you have mentioned be investigated in the case of viscous high-fat foods.