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TECHNICAL NOTE

ENCAPSULATION OF VISCOUS HIGH-FAT FOODS IN CALCIUM ALGINATE GEL TUBES AT AMBIENT TEMPERATURE

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Abstract

Viscous high-fat foods such as cream, egg yolk, or mayonnaise are co-extruded with a 3% sodium alginate solution from a syringe into a 50 mM calcium chloride solution. The food sample passes through the inner tube of a double needle assembly while the alginate solution is simultaneously extruded through a mantle surrounding the inner needle. As the sodium alginate solution forms a gel on contact with calcium ions, the food sample becomes encapsulated in the calcium alginate gel formed on the surface of the food sample. The encapsulation procedure may be carried out within a temperature range between 0°C and 25°C. Samples may be prepared for scanning electron microscopy or for transmission electron microscopy by selecting either wide or narrow bore needles, respectively.

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Introduction

The original procedure for the encapsulation of suspensions and dispersions of biological origin in agar gel tubes designed by Salyaev [7] has been modified by several authors for use with foods [3-6]. In a recent modification [6], the sample is aspirated into a glass Pasteur pipette, the lower end of the pipette is sealed with agar gel, and the pipette is repeatedly dipped into a warm (40°C) agar sol to form a sleeve around the Pasteur pipette. The sample is then transferred into the agar gel tube by pulling the Pasteur pipette out. In the *Discussion with Reviewers* in paper [6] Dylewski noted that the microstructure of some heat-sensitive high-fat foods may be affected by repeatedly dipping the food in the pipette into the warm agar sol. In order to reduce the exposure to heat, Goff (personal communication) has been using low-temperature gelling agarose for this purpose. Viscous foods may also be aspirated into agar gel tubes made around glass or metal rods [5] using the rod as a piston, and, thus, avoiding the exposure of the foods to heat, but this procedure is more difficult to perform than the former procedure [6]. In particular, it is difficult to properly seal both ends of the agar gel tubes following their contamination with fat.

A different approach is possible by using a sodium alginate solution which forms a gel on contact with calcium ions. This reaction makes it possible to instantly encapsulate viscous food samples at any temperature between 0°C and room temperature (~25°C) and is the subject of this technical note.

Materials and Methods

Sodium alginate (BDH Chemicals, Ltd., Poole, England) was dissolved in distilled water to form 2 to 4% solutions. Calcium chloride, 0.1 to 1.0 M solution, was mixed with a 2.5% glutaraldehyde solution (1:9, v/v) and the pH was adjusted to 6.5 using 0.2N NaOH.

High-fat food (egg yolk, cream, or mayonnaise) destined for examination by scanning electron microscopy (SEM)

was placed in a 5-mL syringe and a double needle assembly was attached to it. The double needle assembly consisted of an inner needle (~1 mm inner diameter) concentrically positioned inside an outer stainless steel tube (mantel) with a 0.3-mm gap between both tubes (Fig. 1). Three ribs, each 3 mm long, were soldered on the inner tube, 5 mm from the tube end, as spacers in order to maintain concentric position of both needles. Another 5-mL syringe filled with the sodium alginate solution was attached to the outer needle (Figs. 1 and 2). Both the food and the alginate solution were manually simultaneously extruded into the calcium chloride solution.

The long column of the encapsulated food (Fig. 3) was examined under a dissecting microscope and the best parts were cut into 10-15 mm sections as soon as the calcium alginate tubes were firm enough to be handled, *i.e.*, within 1-2 min. The food-containing gel tube was compressed with a pair of fine tweezers and cut at that location with a scalpel. The wet cut surface was blotted with paper tissue, covered with a droplet of the sodium alginate solution, and this droplet was gelled using a droplet of the calcium chloride solution, thus sealing the food inside the cut gel tube. These shorter sections were washed with a 50 mM calcium chloride solution, pH 6.5, and postfixed for 24 h at 6°C with a 2% osmium tetroxide solution made up in a combined imidazole and veronal-acetate buffer [1] in order to retain fat. The samples were then dehydrated in a graded ethanol series and freeze-fractured [1]. The fragments were immediately critical-point dried from carbon dioxide in a Samdri 3PVT apparatus; the heater of this apparatus was switched off at 35°C in the final step of drying. The dried sample fragments were mounted on aluminum stubs using a silver-based cement, coated with gold in a Technics Hummer II sputter coater, and examined at 20 kV in an ISI DS-130 scanning electron microscope equipped with an external oscilloscope [2]. Micrographs were taken on 35-mm 100 ASA film.

For transmission electron microscopy (TEM), the foods were prepared in two ways. Provided that fixation and dehydration sufficiently hardened the samples, they were cut into smaller (< 1 mm3) particles for embedding in a Spurr's low-viscosity medium (J.B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada). Otherwise, a similar encapsulating apparatus was used but the diameter of the inner needle was reduced to <0.5 mm and the diameter of the outer needle was adjusted accordingly. Pulsed extrusion resulted in small encapsulated beads rather than tubes. The beads were better suited for TEM and were postfixed, dehydrated, and embedded in the same way as other solid samples [1].

Results and Discussion

Co-extrusion of high-fat food and a sodium alginate solution through concentric needles into a calcium chloride solution, which contained glutaraldehyde as a fixative, resulted in the encapsulation of a long column of the food under study in a calcium alginate gel (Fig. 3). The end of the double concentric needle assembly should be about 5 mm above the calcium chloride solution; if it is too low, the gel may form at the needle outlet and clog the needle. The diameter of the food column and the thickness of the calcium alginate coating depend on the diameters of the needles and the rates at which each of the two components are extruded: rapid extrusion of the alginate solution and slow extrusion of the viscous food produce a thick gel coating on a relatively thin food column. If the extrusion rates are reversed, an uneven gel coating may develop on the food column and the column may disintegrate in the calcium chloride solution. The optimum conditions founds were a 3% sodium alginate and 0.05 M calcium chloride solutions. The rate of extrusion varies with the consistency of the food under study and should be established experimentally.

The need to seal the food under study following the division of the long column into shorter segments is easy to meet. Compared to an agar gel coating, which is brittle, the calcium alginate coating is elastic; the cut may be sealed with a droplet of the sodium alginate solution by gelation.

Freeze-fractured cream samples which had been encapsulated for SEM, postfixed with a 2% osmium tetroxide solution in a buffered imidazole solution are shown in Fig. 4. The calcium alginate gel adhered to the samples through all treatment steps. In comparison with the *dipping* procedure using agar for encapsulation [6], no difference could be found between samples encapsulated according to this procedure and samples encapsulated in calcium alginate gels. The co-extrusion method, however, has been developed to provide an alternative to the agar gel encapsulation and to prevent exposure of heat-sensitive food samples to an elevated temperature before fixation.

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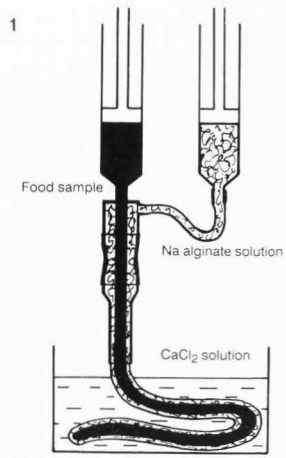

Fig. 1. Diagram of the apparatus for the encapsulation of viscous food samples in calcium alginate gel tubes by co-extruding the food samples with a 3% sodium alginate solution into a 50 mM calcium chloride solution.

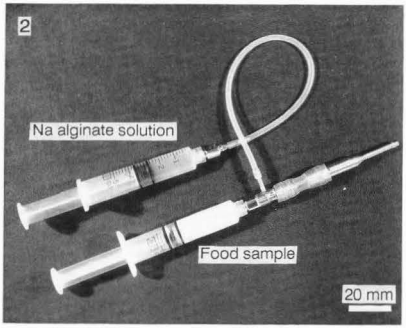

Fig. 2. A photograph of the encapsulation apparatus.

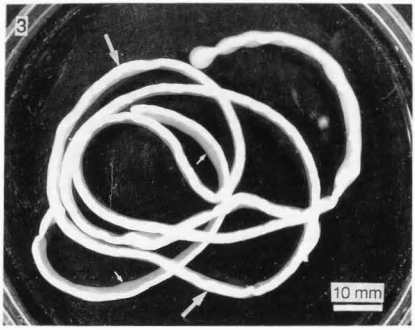

Fig. 3. Cream (large arrows) encapsulated an calcium alginate gel (small arrows). The long and curled encapsulated column will be cut into shorter sections for freeze-fracturing.

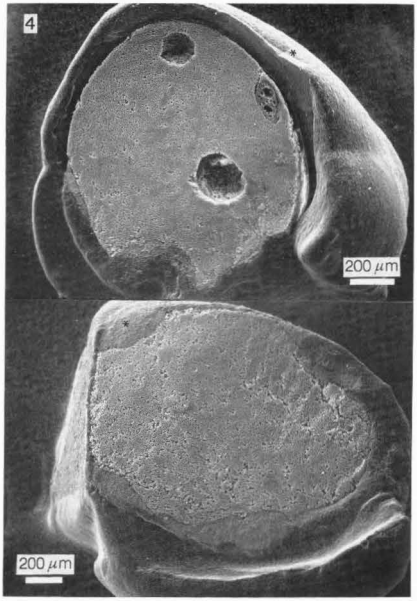

Fig. 4. SEM of 2 freeze-fractured cream samples shows smooth fracture planes and adherence of the alginate gel (*) capsules to the samples.

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Discussion with Reviewers

Reviewer 1: What effect could calcium ions have on the food under study?

Authors: With milk products, calcium has been recommended as an ingredient in buffered fixatives by several authors [9, 12, 13] in order to maintain the integrity of the casein micelles. The concentration of calcium ions penetrating the food sample may be reduced by transferring the encapsulated food column into a glutaraldehyde solution containing 5 mM $CaCl_2$.

Reviewer 1: May any liquid food sample be examined by electron microscopy using the encapsulation technique described?

Authors: There are reports in the literature that this is possible [10, 11], but other authors mentioned problems encountered with milk [8]. Although milk may be encapsulated, the distribution of the casein micelles is affected by fixation with glutaraldehyde and the removal of the soluble components of the milk serum. When the serum is replaced with ethanol during dehydration, the fixed casein micelles sediment in the capsules irrespective whether they consist of agar or calcium alginate gels.

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