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TECHNICAL NOTE

A SIMPLE PROCEDURE FOR THE PREPARATION OF STIRRED YOGHURT FOR SCANNING ELECTRON MICROSCOPY

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Abstract

Stirred yoghurt is aspirated into agar gel tubes having 1.2 mm interior diameter, fixed in glutaraldehyde, dehydrated in ethanol, freeze-fractured under liquid nitrogen, and critical-point dried. Agar gel encapsulation protects the sample and prevents it from disintegration during the preparative steps. Scanning electron microscopy of the mounted fragments reveals the corpuscular microstructure of this type of yoghurt which develops due to stirring and pumping of the product during manufacture.

Introduction

Development of microstructure in set-style yoghurt was studied by electron microscopy (3-6, 8). Scanning electron microscopy (SEM) in particular has been found to be useful to show the porosity of the protein matrix (5), distribution of lactic acid bacteria (8), and the presence of fat globules in yoghurt made from whole milk (1). Preparation of stirred yoghurt for SEM is difficult due to disruption of the rigid gel matrix during manufacture caused by mechanical agitation to produce a smooth flowing viscous product. If a sample of stirred yoghurt is placed in an aqueous fixative, the broken matrix disperses and the sample disintegrates. However, the Salyaev's (7) procedure, initially devised to encapsulate liquid samples, including milk (2), destined for embedding in a resin for sectioning for transmission electron microscopy (TEM) was found suitable to preserve the liquid yoghurt samples during preparative steps for SEM. The objective of this technical note is to describe the use of the encapsulation procedure in SEM and show micrographs of stirred yoghurt.

Materials and Methods

Agar sol (2%) was made using distilled water and was maintained at 40°C with constant stirring. A glass rod (a flame-sealed Pasteur pipet, 1.2 mm in outer diameter) was dipped repeatedly into the agar sol and rotated until the sol gelled and formed a uniform thin (0.3-0.5 mm) layer. The lower and upper parts of the gel tube were trimmed to form a 20 mm long gel sleeve on the glass rod.

Commercial stirred-style yoghurt samples varying in consistency were used in this study. A volume of approx. 3-5 mL of the yoghurt was placed on a glass plate and a small part of it was aspirated into the agar gel tube as shown in Fig. 1. Because of the high viscosity of the yoghurt and the relatively large diameter of the soft agar gel tube, aspiration of the yoghurt was done very slowly at a low angle; a 10 to 12 mm column of the yoghurt was aspirated followed by the aspiration of 1 mm of air. The end of the agar gel tube was gently blotted with tissue paper and sealed with 2 drops of warm agar sol. The gel tube was trimmed at the upper end 1 mm away from the yoghurt column and was also sealed with agar sol.

Yoghurt samples thus encapsulated were fixed in a 3.5% glutaraldehyde solution at 6°C for 24 h, dehydrated in a graded ethanol series, frozen in Freon 12 at -150°C, freeze-fractured under liquid nitrogen, melted in absolute ethanol, and critical-point dried from carbon dioxide. The fragments were mounted on aluminum SEM stubs using silver cement, sputter-coated with gold, and examined in a Cambridge Stereoscan Mark II scanning electron microscope operated at 20 kV.

Results and Discussion

Two steps are essential to this procedure: immobilization of the liquid sample in the agar gel tube and freeze-fracturing to obtain smooth fracture planes suitable for SEM examination.

The sample to be examined by SEM is considerably larger than that destined for TEM. Also, because stirred yoghurt is a dense and viscous suspension of casein micelle clusters, it is easier to aspirate it into agar gel tubes of a diameter larger than that used for TEM.

During all the preparatory steps, the agar gel tube remained to be part of the sample. There was no separation of the yoghurt sample from the agar gel (Fig. 2) and the gel appeared to be relatively dense (Fig. 3). The sample fragments remained cohesive and were easy to mount on metal stubs using silver cement. Sputter coating provided sufficient conductivity to examine the sample at 20 kV without encountering charging artefacts. Freeze-fracturing revealed the corpuscular microstructure of the sample (Fig. 4). However, a detail of casein micelle chains and clusters in Fig. 5 is in agreement with images obtained with set-style yoghurt (5, 8).

Thus, this simple procedure makes it possible to prepare stirred yoghurt for SEM and to study the effects of manufacturing conditions on the dimensions and distribution of protein particles. It is probable that other similar foods such as cultured buttermilk can be prepared for SEM using this procedure.

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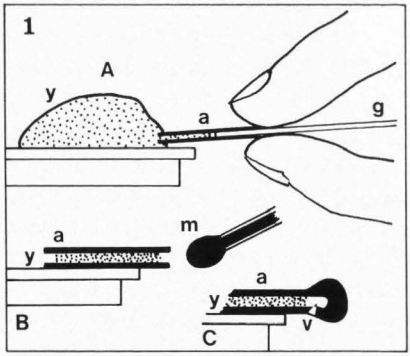
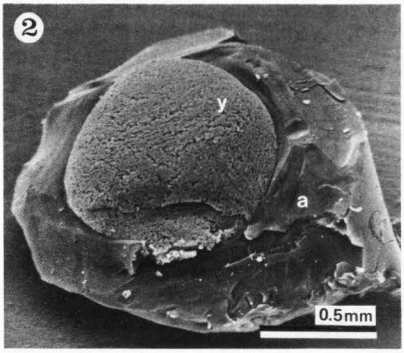
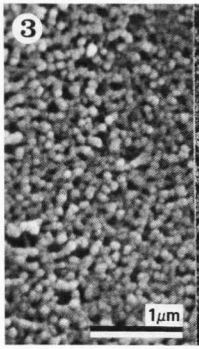
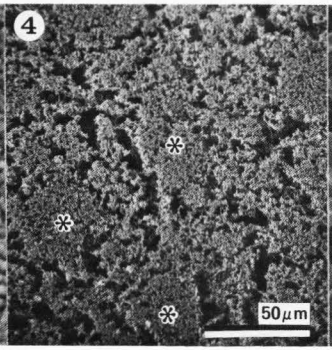
KEY WORDS: Agar gel; Encapsulation; Freeze-fracturing; Scanning electron microscopy; Stirred yoghurt.









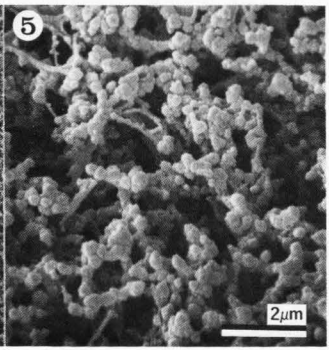


Fig. 1. Aspiration of stirred yoghurt into an agar gel tube (A) and its sealing (B and C).
a = agar gel tube; g = glass rod acting as a piston; m = molten agar; y = yoghurt sample; v = void air space.

Fig. 2. SEM micrograph of a stirred yoghurt (y) fragment encapsulated in an agar gel tube (a).

Fig. 3. Detail of the dense microstructure of the agar gel tube.

Fig. 4. Overall corpuscular microstructure of stirred yoghurt. Protein particles composed of casein micelles are marked with asterisks.

Fig. 5. Detail of the casein micelle matrix.

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