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A METHOD FOR THE EXAMINATION OF THE MICROSTRUCTURE OF STABILIZED PEANUT BUTTER

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Abstract

A method for light and scanning electron microscopy of damaged resting peanut seed tissue was adapted as a research tool for evaluating the microstructural features of commercially available stabilized peanut butter. This method was used in the present study to evaluate the degree of homogenization of stabilized peanut butter by examining the spatial relationship which exists among the microstructural features. Light and scanning electron microscopy of three commercially available stabilized peanut butters revealed varying degrees of homogenization of broken cell and tissue fragments, protein bodies, and starch grains within a matrix of stabilized oil.

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Key Words: Light microscopy, scanning electron microscopy, microstructure, fixation, peanuts, peanut butter, protein, starch, oil.

Introduction

During the grinding of peanuts into butter, various liquid additives such as stabilizers may be introduced by means of metering units complete with proportioning pumps. Woodroof (1983) claims that achieving a thorough, homogenous mix during the grinding process is extremely difficult. Improper homogenization may result in an undesirable lack of uniformity which may affect the texture and consistency of the peanut butter.

Over the years, our peanut laboratory has performed a substantial number of quality evaluations on different types of commercially available peanut butters as well as other peanut products. Recently Young and Schadel (1989) developed a method for light and scanning electron microscopy of damaged resting peanut seed tissue. The purpose of the present study is to adapt this method as a research tool for evaluating the microstructural features of commercially available stabilized peanut butter. By examining the spatial relationship which exists among the microstructural features, the degree of homogenization in smooth peanut butter can be determined.

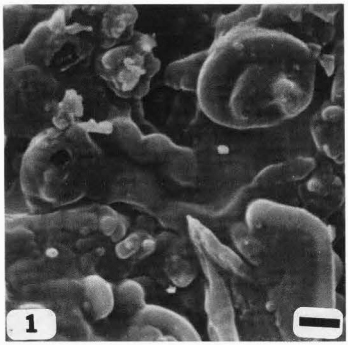
Materials and Methods

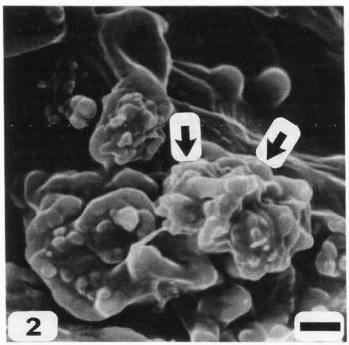
Sources of Stabilized Peanut Butter

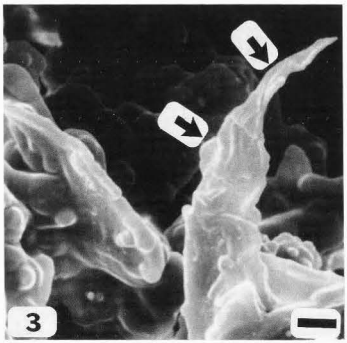
One jar of each of the three leading commercial brands of stabilized peanut butter as purchased at a local grocery store were used. In the present study, these will be referred to as Commercial Brands #1, #2, and #3.

Fixation Method for Light and Scanning Electron Microscopy

Peanut butter samples (1 mm^3) were carefully sectioned from the contents of the three jars with razor blades. These samples were dropped directly into a Karnovsky's (1965) fixative as modified by Young and Schadel (1989). Our modified fixative, as originally devised for damaged resting peanut seed tissue and adapted for peanut butter, was prepared by mixing 25 mL of 8% formaldehyde, 3.6 mL of 70% glutaraldehyde and 28.6 mL of 0.1 M sodium phosphate buffer (hereinafter referred to as buffer). The pH of the mixture was adjusted to 7.0. The peanut butter samples were fixed under vacuum for 30 minutes at room temperature and then at atmospheric pressure for 48 hours at 4°C. Following a 24 hour wash in 6 changes of 0.1 M buffer (4°C, pH 7.0), the material was post-fixed for one hour in 1% osmium tetroxide in 0.1 M buffer (4°C, pH 7.0). After







Bars = 10 micrometers on each figure.

Figures 4-6 on the color plate, facing page.

Fig. 4. Light micrographs of Commercial Brand #1: **a)** representative section of a stabilized peanut butter with a high degree of homogenization of microstructural features; and **b)** section in which broken cell wall fragments (W), protein bodies (P), and starch grains (S) are well-dispersed in a matrix of stabilized oil (O).

Fig. 5. Light micrographs of Commercial Brand #2 sample showing: **a)** incomplete homogenization of microstructural features; and **b)** cellular contents (primarily protein bodies) that remained coalesced (arrow) and incompletely dispersed.

Fig. 6. Light micrographs of Commercial Brand #3 sample revealing: **a)** incomplete homogenization of microstructural features; and **b)** comparatively large cell and tissue fragments (arrows).

post-fixation, the material was washed for 30 minutes in 0.1 M buffer (4°C ph 7.0) and dehydrated at 15 minute intervals in a graded series of aqueous ethanol (10, 25, 50, 75, and 95%) and then finally for 30 minutes in absolute ethanol producing the dehydrated samples.

Preparation for Light Microscopy

Dehydrated peanut butter samples were embedded in Spurr's resin using the methodology of Spurr (1969) for long pot-life resin. Sections, 3 micrometers in thickness, were cut using a Reichert ultramicrotome and glass knives. After mounting sections on glass slides, the sections were stained with acid fuchsin and toluidine blue using the methods of Feder and O'Brien (1968). Stained sections were photographed using a Wild light microscope fitted with a 35 mm camera.

Preparation for Scanning Electron Microscopy (SEM)

Dehydrated peanut butter samples were prepared for SEM by critical point drying in a Tousimis PVT-3B unit using liquid CO_2. Dried samples were mounted on aluminum specimen stubs using double-sided tape and silver conducting paint. Prepared stubs were coated with 30 nm gold-palladium alloy at room temperature in a Hummer V sputter coater fitted with a Technics Digital Thickness Monitor. Specimens were viewed with a Philips 505T SEM at a working distance of 15 mm and an accelerating voltage of 15 kV.

Results

Scanning Electron Microscopy (SEM)

Observations with SEM revealed differences in the degree of homogenization among the three commercially available brands of stabilized peanut butter.

Figures 1-3 (at left). Scanning electron micrographs of samples from Commercial Brands #1, #2, and #3 respectively. **Fig. 1** is representative of a stabilized peanut butter with a high degree of homogenization of microstructural features. Note the broken cell wall fragments, protein bodies, and starch grains are well dispersed in a matrix of stabilized oil.

Fig. 2 shows a sample with a variable degree of homogenization in which cellular contents remained coalesced (arrows) and incompletely dispersed.

Fig. 3 shows a sample with a variable degree of homogenization in which comparatively large cell wall fragments (arrows) protrude from the surface.

Commercial Brand #1 is representative of a stabilized peanut butter with a high degree of homogenization of microstructural features. Broken cell wall fragments were well-dispersed in a matrix of stabilized oil (Fig. 1). Commercial Brand #2 possessed a variable degree of homogenization in which cellular contents remained coalesced and incompletely dispersed (Fig. 2). Commercial Brand #3 also possessed a variable degree of homogenization in which comparatively large cell and tissue fragments were observed (Fig. 3).

Light Microscopy (LM)

Observations with LM also revealed differences in the degree of homogenization among the commercially available brands of stabilized peanut butter. Commercial Brand #1 possessed a high degree of homogenization in which broken cell wall fragments, protein bodies, and starch grains were consistently well-dispersed in a matrix of stabilized oil (Figs. 4a and 4b). Commercial Brand #2 possessed a variable degree of homogenization which ranged from high (Fig. 5a) to intermediate (Fig. 5b) in which cellular contents (primarily protein bodies) remained coalesced and incompletely dispersed. Commercial Brand #3 also possessed a variable degree of homogenization which ranged from high (Fig. 6a) to intermediate (Fig. 6b) in which comparatively large cell and tissue fragments were observed.

Discussion

The use of SEM has limited capability for evaluating the differences among stablized peanut butters. Although SEM was capable of detecting incompletely homogenized cellular contents and tissue fragments protruding from the specimens, the additional use of LM provided an excellent complement to SEM for a more thorough understanding of the spatial relationship of the microstructural features. This spatial relationship is best preserved by plastic embedding which enables thin-sectioning for LM examination without disruption of the spatial relationship. By preserving the spatial relationship, the degree of homogenization of the microstructural features of stablized peanut butter can be determined. For example, Commercial Brand #2 exhibited a coalescence of protein bodies (Figs. 2 and 5b) which indicated incomplete homogenization.

Vix et al. (1972) reported pressure as the cause of coalescence of protein bodies in partially defatted, hydraulically pressed peanuts. We believe that the observance of coalesced protein bodies in stabilized peanut butter may also be related to incomplete homogenization.

Lastly, the use of plastic embedding for LM also enables the observance of large tissue fragments that would otherwise be crushed by smearing the peanut butter on a slide for examination under the light microscope.

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Discussion with Reviewers

N. Krog: Is there any correlation between your structural observations and the rheological properties, surface shine, etc. of the different peanut butter samples?

I. Heertje: Is the difference in homogenization between the three samples in some way reflected in the organoleptic properties?

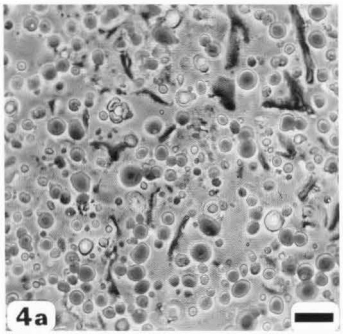
Authors: Yes, our structural observations reveal differences between the three samples in which homogenization of microstructural features varies from complete to incomplete and is correlated with the smoothness character note of peanut butter during eating, and is also related to changes in rheological properties and surface shine.

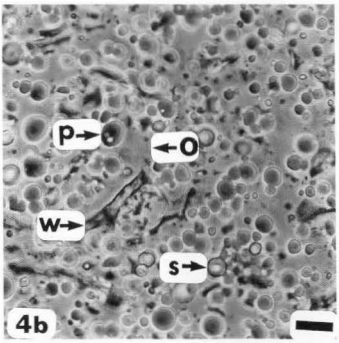
I. Heertje: Is a fixation time of 1 hour in osmium tetroxide adequate for fixing the continuous oil phase? I would fear that, under those conditions, part of the oil would be lost during the washings with ethanol. Consequently the distribution of the dispersed particles may have been affected.

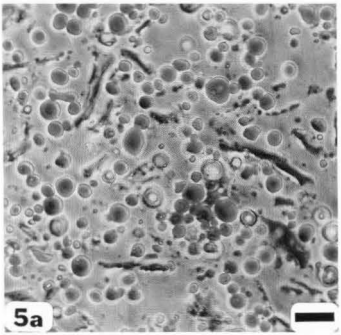
Authors: A fixation time of one hour in 1% osmium tetroxide is adequate for samples that are approximately 1 mm^3 in size. Since the osmium tetroxide completely blackens the oil in the samples, any potential oil loss should appear as a stream of blackened oil from the sample during the ethanol washings. Neither the removal of blackened oil nor sample size changes (i.e. shrinking) were observed during the ethanol washings.

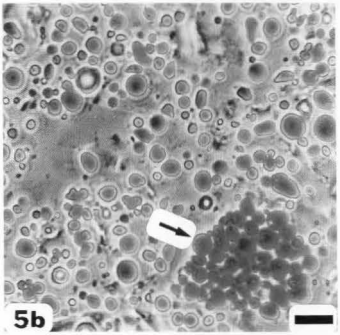
F.O. Flint: Would 5 mm diameter cryostat sections give more reliable results than plastic embedded sections that are only 1 mm in diameter?

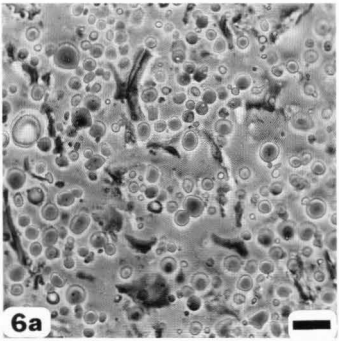
Authors: The sample size of 1 mm^3 is determined by the fixation requirements of the plastic embedding of peanut butter. Our experience with larger cryostat sections is that the peanut butter is smeared during cryostat sectioning and, therefore, is unsatisfactory since the method of preparation alters the sample.

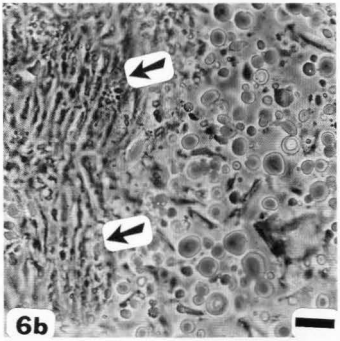












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