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Characterization and identification of surface crystals on smear-ripened cheese by polarized light microscopy

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ABSTRACT

Surface crystallization and radial demineralization of Ca, P, and Mg occur in smear-ripened cheese. Furthermore, crystals of ikaite, struvite, calcite, and brushite have been identified in cheese smears by powder x-ray diffractometry (PXRD), and ikaite and struvite exist in smears as single crystals. Polarized light microscopy (PLM) is a simple, inexpensive, and well-established method in geology to detect and identify single crystals. However, use of PLM to identify cheese crystals has not been reported previously. The specific objectives of this research were (1) to identify crystals in cheese smears using selected PLM criteria; (2) to compare identification by PLM against PXRD; and (3) to develop and evaluate a novel treatment for smear material to improve crystal analyses by both PLM and PXRD. Duplicate wheels of 4 cheeses produced by different manufacturers were obtained from retail sources. Scrapings of surface smears were prepared and analyzed by PLM and PXRD by previously described methods. Crystals were categorized by PLM based on angle of extinction (AE), birefringence behavior under crossed polarizers and quartz filters, and size and shape (circularity) by image analysis. Crystals observed by PLM fell almost exclusively into 2 readily differentiated groups based on birefringence behavior and estimated angle of extinction. Group 1 (n = 18) were highly birefringent with AE = 88–92°, whereas group 2 (n = 28) had no birefringence with AE = 13–26°. Group 2 crystals were significantly larger and more circular than group 1 crystals. Group 1 and 2 were identified as struvite and ikaite, respectively, based on known birefringence and AE characteristics. Struvite was identified in all 4 cheeses by PLM but in only 3 cheeses by PXRD. Ikaite was identified in 3 cheeses by PLM but in only 2 cheeses by PXRD. These discrepancies occurred because the smear scrapings from 1 cheese contained excessive amorphous matter that caused extreme background noise, potentially obscuring diffractogram peaks that may have been present. To minimize noise, smear scrapings were dispersed in aqueous NaOH (pH 10) before analyses, which resulted in consistent results by PXRD and PLM. The method also rendered high-quality images by PLM. Data suggest that PLM may offer a simple and inexpensive means to identify struvite, ikaite, and possibly other single crystals in cheese smears.
Key words: cheese, crystal, polarized light microscopy

INTRODUCTION

Crystallization of calcium phosphate at the surface of soft white mold surface ripened cheeses, such as Brie and Camembert, sets up pronounced outward migration of endogenous cheese minerals, such as calcium, magnesium, and phosphorus, during ripening (Le Graet et al., 1983; Karahadian and Lindsay, 1987; Le Graet and Brule, 1988; Tansman et al., 2017a). The surface crystals chelate minerals and deplete the water phase of mineral components, which in turn establishes a concentration gradient with net mineral migration outward that fuels further crystallization. This process leads to demineralization of the curd body and results in pH-dependent radial softening, significantly affecting the overall texture and body of the resulting cheese (Metche and Fanni, 1978). A similar process of radial softening occurs in soft, surface-ripened, washed-rind (smear-ripened) cheeses. However, the surface crystals that form on soft, washed-rind cheese consist mostly of ikaite (calcium carbonate hexahydrate), struvite (magnesium ammonium phosphate hexahydrate), and small amounts of calcite (calcium carbonate), with only minor and evidently transient formation of brushite (calcium phosphate dihydrate; Tansman et al., 2015, 2017b,c). Anecdotal reports from industry and informal

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observations from our laboratory suggest that these surface crystals can grow to sizes that cause a gritty or sandy texture in the rinds of soft, washed-rind cheeses.

Advances in analytical approaches have helped to fuel a growing interest in cheese crystal research, including several recent studies that employed new applications of powder x-ray diffractometry (**PXRD**; Tansman et al., 2014, 2015, 2017a,b). A limitation of this technique, however, has been the presence of high background noise due to the difficulty of removing noncrystalline cheese material from the harvested crystals, which can make interpretation of x-ray diffraction patterns difficult and result in unidentified crystal phases (Bloss, 2012). This difficulty seems especially problematical when analyzing surface scrapings from soft, washed-rind and bloomy rind cheeses (Tansman et al., 2017a,b).

Polarized light microscopy (**PLM**), a technique in optical mineralogy and crystallography, is another promising approach that has been recently applied to study cheese crystals (Tansman et al., 2017b,c). Use of PLM offers a simple and inexpensive analytical approach when crystals occur as single crystals because the anisotropic nature of single crystals lends them unique identifying features, such as angle of extinction and birefringence, when viewed using this methodology (Nesse, 2004). Angle of extinction refers to the specific position that some crystals go extinct, or dark/transparent, when viewed under cross-polarized light (Nesse, 2004). Birefringence refers to the coloring some crystals take on when viewed under cross-polarized light. The type and intensity of birefringence can help indicate crystal type (Dyar et al., 2008). Single crystals also tend to exhibit characteristic shapes and geometries that can aid in identification (Bloss, 2012). Image analysis software can be used to quantify the shape and size of crystals, allowing for robust analysis and differentiation. These crystal morphologies, along with intensity of birefringence and angle of extinction, can all be used to tentatively identify specific crystal species.

Previous studies confirmed that the principal surface crystals on soft, washed-rind cheeses, namely ikaite and struvite, occurred as single crystals that were readily observed using PLM under crossed polarizers (Tansman et al., 2017b,c). The present work applies the above criteria to identify single crystals present on the surface of soft, washed-rind cheeses. The specific objectives of this work were (1) to identify surface crystals on soft, washed-rind cheese using specific PLM criteria, (2) to compare PLM identification against PXRD, and (3) to develop and evaluate a novel treatment for cheese surface smear material to improve the analyses of cheese crystals by both PLM and PXRD.

MATERIALS AND METHODS

Cheeses Samples

Four different soft, washed-rind cheese varieties were purchased at local retailers in the Burlington, Vermont, area. Two wheels of each cheese variety with identical code date and vat identifier (if available) were obtained and served as duplicate experimental units. All analyses were performed identically on each of the 2 wheels for each of the 4 varieties. All cheese samples were stored at 3°C for up to 3 d until analyses were conducted. A flat-tip pH electrode (Thomas Scientific, Swedesboro, NJ) was used for surface pH measurements. The probe was placed on 3 separate rind locations (top, side, and bottom) and measurements were averaged. (6 total pH measurements per cheese variety) Moisture analyses were conducted in triplicate using a forced-draft oven set at 100°C. A wedge of cheese from each wheel was homogenized using a mortar and pestle. Approximately 2 g of homogenized sample were weighed into predried and preweighed aluminum pans and then allowed to dry for 24 h at 100°C, until a constant weight was reached (6 total moisture content measurements per cheese variety).

Smear Collection

Smear scrapings were collected from a 1-cm^2 section of cheese rind using a metal spatula. Care was taken to limit the fracturing of crystals. Scrapings were applied directly to microscope and diffraction slides for PLM and PXRD analyses, respectively, using a metal spatula and a dissection needle.

Alkaline Dispersion

Alkaline dispersions (pH = 10) of smears were prepared by submerging a sample of smear (collected as described above) in 15 mL of 0.0001 *M* NaOH (Fisher Scientific, Pittsburgh, PA) in a 50-mL beaker. Beakers were vortexed gently to encourage dispersion of smear matrix. Alkaline mixtures were allowed to sit at room temperature for approximately 3 h. Crystals that settled to the bottom of the beaker were used for PLM and PXRD analyses.

PXRD

A zero-background diffraction slide was used for all PXRD analyses. Cheese smear scrapings were mounted directly on the slide and overlaid with a thin film of mineral oil, as described in previous work (Tansman et

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