



J. Dairy Sci. 100:1–11

<https://doi.org/10.3168/jds.2017-13067>

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Crystallization and demineralization phenomena in washed-rind cheese

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ABSTRACT

This report documents an observational study of a high-moisture washed-rind cheese. Three batches of cheese were sampled on a weekly basis for 6 wk and again at wk 10. Center, under-rind, rind, and smear samples were tested for pH, moisture, and selected mineral elements. Powder x-ray diffractometry and petrographic microscopy were applied to identify and image the crystal phases. The pH of the rind increased by over 2 pH units by wk 10. The pH of the under-rind increased but remained below the rind pH, whereas the center pH decreased for most of aging and only began to rise after wk 5. Diffractograms of smear material revealed the presence of 4 crystal phases: brushite, calcite, ikaite, and struvite. The phases nucleated in succession over the course of aging, with calcite and ikaite appearing around the same time. A very small amount of brushite appeared sporadically in center and under-rind samples, but otherwise no other crystallization was observed beneath the rind. Micrographs revealed that crystals in the smear grew to over 250 μm in length by wk 10, and at least 2 different crystal phases, probably ikaite and struvite, could be differentiated by their different optical properties. The surface crystallization was accompanied by a mineral diffusion phenomenon that resulted, on average, in a 217, 95.7, and 149% increase in calcium, phosphorus, and magnesium, respectively, in the rind by wk 10. The diffusion phenomenon caused calcium, phosphorus, and magnesium to decrease, on average, by 55.0, 21.5, and 36.3%, respectively, in the center by wk 10. The present study represents the first observation of crystallization and demineralization phenomena in washed-rind cheese.

Key words: crystal, ikaite, demineralization, washed-rind cheese

INTRODUCTION

Washed-rind cheese is a broad classification that includes cheeses of widely varying moisture content and aging time. The qualifying characteristic of washed rind cheeses is the treatment of the rind with a brine during aging, which can be applied by hand or with an implement such as a brush. Cheeses treated in this manner tend to form a complex microbiota on the surface that contributes to the appearance and flavor of the ripe cheese. The present observational study focuses on the development of a high-moisture washed-rind cheese that is characterized by a thick bacterial smear.

There exists a striking homology between the ripening of washed-rind cheese and the ripening of white mold (bloomy rind) cheese in that both types of cheese possess a highly active surface biomass that is carefully cultivated by the cheesemaker. In white mold cheese, the surface flora's activity causes a local increase in pH at the surface which causes pH-sensitive calcium phosphate crystals to nucleate in the rind (Le Graet et al., 1983). The elevated pH reduces the solubility of calcium and phosphate in the rind and causes calcium and phosphate ions to diffuse to the rind from the center along a concentration gradient. These ions are deposited in growing crystals, which results in calcium phosphate accumulation in the rind and concurrent diminishment in the center. Elevated rind pH during aging has been reported in washed-rind cheese (Gobbetti et al., 1997), but corresponding investigation of crystallization and demineralization in washed-rind cheese appears to be absent from the literature.

Rind alkalization in washed-rind cheese is a function of the various microbial communities that colonize the cheese surface and metabolize cheese components. At the initial pH of washed-rind cheese (typically between 5.0 and 5.5), the bacterial species that characterize the mature smear of washed-rind cheese cannot grow (Gori et al., 2007) and, instead, a succession of microbes colonizes the cheese surface as the collective microbial metabolism raises the pH (Mounier et al., 2006). The first species to appear are the yeasts, such as *Debaryomyces hansenii* (Riahi et al., 2007b), *Kluyveromyces lactis*, and *Saccharomyces cerevisiae*

Received April 23, 2017.

Accepted August 2, 2017.

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(Kagkli et al., 2006), that grow on account of their high acid and salt tolerance (Larpin et al., 2006). Yeast activities, including metabolism of lactic acid (Masoud and Jakobsen, 2005) and ammonia production (Gori et al., 2007), during early aging contribute to a significant increase in surface pH. The acid sensitivity of successive bacterial colonies varies, but in general the bacterial species begin to grow around pH 6 (Bockelmann and Hoppe-Seyler, 2001; Gori et al., 2007).

The main bacterial species that tend to appear on the cheese surface after the yeasts are gram-positive bacteria including *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Micrococcus*, and *Staphylococcus* (Feurer et al., 2004). The process of washing the surface of the cheese with a brine has the effect of spreading the bacterial colonies over the rind and creating a uniform mass (Brennan et al., 2002). These bacteria form a sticky biofilm or microbial mat on the cheese surface (Leclercq-Perlat et al., 2004; Wolfe et al., 2014) that consists of the bacteria and excreted substances (Larpin et al., 2006). In contrast to the well-characterized microbial diversity, an analysis of the extracellular components of the smear appears to be absent from the literature. The bacterial smear communities continue to metabolize the cheese substrate, with further release of ammonia (Leclercq-Perlat et al., 2000b) and consumption of lactate (Leclercq-Perlat et al., 2000a).

The isolation and identification of crystals from the smears of 2 washed-rind cheeses was reported by the authors of the present manuscript (Tansman et al., 2017b). The crystals, which had never been observed in cheese, ranged in size with the largest crystals measuring several hundred microns in length. One of the isolated crystals was identified as ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$), which is a rare metastable crystal that is mostly associated with freezing marine and lacustrine environments. The other crystal was struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$), which is often associated with bacterial activity.

The primary goal of the present study was to observe the nucleation and growth of crystals in a washed-rind cheese during aging and to measure the changes in mineral element concentrations at different depths of the cheese. Novel crystallographic techniques were employed to observe the maximum size of crystals and to identify the crystal phases at different points during aging.

MATERIALS AND METHODS

Cheese Manufacture

Cheeses were obtained from The Cellars at Jasper Hill (Greensboro, VT; herein referred to as The Cellars). The cheese variety observed in the present study

corresponded to cheese A in the authors' previous publication (Tansman et al., 2017b) and is commercially marketed as Winnimere. The general recipe for this variety is as follows: cheeses are manufactured from nonstandardized raw cow milk produced at Jasper Hill Farm during the winter months using a washed-rind procedure approximately similar to that described by Sozzi and Shepherd (1972). The milk is inoculated with cultures that are proprietary and therefore the specific cultures are not included in this description. The curds are dipped at pH 6.55 and drained in molds measuring 14 cm in diameter. The curds drain at room temperature until the pH reaches approximately 6.0, at which point they are transferred to a refrigerated chamber at approximately 2°C and drained overnight. The wheels are demolded at a pH of approximately 5.45 and dry salted to a target salt-in-moisture of approximately 2.6%. This salt content is relatively low, but is within the range reported by Mounier et al. (2005) for other varieties of washed-rind cheese. Target cheese moisture content at demolding is approximately 55%, although considerable drainage continues during the first few days. The cheese is aged in a 13 to 14°C (94 to 95% humidity) room for the first week, after which it is transferred to a 10 to 11°C (96% humidity) aging vault. Immediately before being placed in the aging vault, the cheeses are wrapped with a strip of spruce bark around the outer diameter of the cheese, which is fastened with a rubber band. The cheese is scrubbed with a medium-length-bristle brush dipped in brine 3 times in the first week and then twice per week until the fourth week. From demolding, the cheese is aged on wire racks for a total of 6 wk and then wrapped in semi-permeable paper and stored at 4.5°C until shipment. At the time of wrapping, each wheel weighs approximately 570 g. The cheese described by Sozzi and Shepherd (1972) is considered ripe at 20 d, but due to the regulatory environment, the cheese in the present study is aged at a lower temperature and for a longer time before packaging and distribution.

Cheese Sampling

Cheeses were sampled at wk 1, 2, 3, 4, 5, 6, and 10, on the same day of the week. The wk-1 samples were delivered 2 d after demolding, which dictated the day of the week that the subsequent samples were collected. The cheeses were wrapped in cheese paper and delivered by overnight post to the Department of Nutrition and Food Sciences at the University of Vermont in insulated coolers with ice packs. Two wheels were collected from The Cellars at wk 1, 2, 3, 4, and 5. Four wheels were collected at wk 6, which was the last week of cave-aging and the first week of packaging and cold storage. Two

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