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Development and Application of Image Analysis to Quantify Calcium Lactate Crystals on the Surface of Smoked Cheddar Cheese*

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ABSTRACT

Calcium lactate crystals that form white specks or haze on the surface of cheese constitute a significant quality problem for producers of Cheddar cheese. Subjective methods to evaluate crystal coverage of cheese surfaces have been reported previously, but objective methods are currently lacking. The objectives of this work were to develop and evaluate an objective method to measure the area occupied by calcium lactate crystals on surfaces of naturally smoked Cheddar cheese samples using digital photography and image analysis. Coefficients of variation ranged from 1.29 to 4.68% for 5 replicate analyses of 3 different cheese surfaces that ranged from ~2 to 49% of total surface area occupied by crystals. Thus, results showed a high degree of repeatability for the 3 cheese surfaces, which ranged from very slight and geometrically simple to very heavy and geometrically complex crystal coverage. The method underestimated total area occupied by crystals on the 3 surfaces by 0.24 to 4.83% unless the fainter crystal regions that went undetected during initial thresholding were manually segmented and quantified. The wet weight of crystal substance collected per unit of surface area from 20 different cheese samples increased exponentially as the percentage of total surface area occupied by crystals increased. These data were consistent with subjective observations that crystal regions appeared to grow vertically as well as horizontally as they expanded to occupy greater surface area. Image analysis was well suited for evaluating changes in crystal coverage during cheese aging because measurements were made nondestructively and with minimal disruption to the cheese. The area occupied by crystals on 6 different surfaces from 3 different cheese samples increased linearly (R^2 = 0.94 to 0.99) during storage at 4°C for up to 33 wk. However, the rates of increase differed significantly among the 3 cheese samples. Image analysis may serve as a useful tool to quantitatively evaluate the effects of factors such as cheese composition, packaging conditions and storage temperature on rate of crystal growth and time of crystal appearance during storage.
(**Key words:** cheese, calcium lactate, crystal)

INTRODUCTION

It is well established that calcium lactate crystals are responsible for white crystalline specks and haze that sometimes occur on the surface of Cheddar cheese (Tuckey et al., 1938; McDowall and McDowell, 1939; Shock et al., 1948; Farrer and Hollberg, 1960). These crystals are believed to form when the serum phase of the cheese becomes supersaturated with calcium and lactate ions, followed by the nucleation of calcium lactate crystals that develop into larger aggregates (Dybing et al., 1988; Kubantseva et al., 2004; Swearingen et al., 2004). They are considered visual quality defects because consumers mistake them for spoilage microorganisms. Although not harmful, these crystals are a challenge to manufacturers with regard to product reputation and financial loss due to rejected cheese (Chou et al., 2003; Swearingen et al., 2004).

Several researchers have investigated various factors associated with calcium lactate crystallization on the surface of Cheddar cheese, including high lactose levels in milk (Pearce et al., 1973); milk concentrated by ultrafiltration without diafiltration (Sutherland and Jameson, 1981); selection of certain starter culture strains (Swearingen et al., 2004); biofilm formation and contamination of cheese by nonstarter lactic acid bacteria that are able to racemize L(+) lactate to D(–) lactate (Johnson et al., 1990b; Chou et al., 2003); low storage temperature and loose packaging (Johnson et al., 1990a); low salt-in-moisture levels and low cheese pH during early aging (Swearingen et al., 2004; Rajbhandari and Kindstedt, 2005); and dehydration at the cheese surface caused by natural smoking (Rajbhandari and Kindstedt, 2005).

Although much progress has been made toward understanding some of the causes of calcium lactate crys-

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tallization and methods of prevention, cheese industry researchers (Swearingen et al., 2004) and anecdotal reports from commercial cheese manufacturers indicate that problems continue to persist in the industry. Furthermore, the basic mechanism of crystallization at the cheese surface is not completely understood. Kubantseva et al. (2004) recently characterized the solubility of calcium lactate in simple aqueous solutions, which the authors stated would provide a basis for future kinetic studies of crystallization in aqueous solutions. However, the kinetics of crystal formation and growth at the surface of cheese are likely to be more complex than in simple aqueous solutions because the protein matrix of the cheese and occluded fat globules therein impede the diffusion of dissolved substances through the serum phase (Geurts et al., 1974, 1980). Furthermore, the serum phase of cheese is complex and variable with respect to dissolved solids content (Morris et al., 1988; Swearingen et al., 2004) and may contain nucleation sites (e.g., dead bacteria) that are not present in simple aqueous solutions (Kalab, 1980). Finally, the calcium content in cheese is not completely soluble but instead exists in a pH-dependent equilibrium between the casein-associated and serum-soluble states (Kindstedt et al., 2001; Ge et al., 2002; Swearingen et al., 2004). All of these factors may affect the nucleation and growth of crystals in ways that may be difficult to predict from simple aqueous model systems.

Progress toward elucidating the mechanism of crystal formation and growth in cheese has been hindered by limitations in existing methods to quantify crystal growth. Dybing et al. (1986) described a simple, nondestructive visual method to rate the extent of crystal development (0 = no crystal development, 4 = very heavy to encrusted crystal development) on the surface of colored Cheddar cheese. This approach provided an estimate of crystal coverage, which the authors subsequently used to evaluate changes in crystal coverage during cheese aging (Dybing et al., 1988). Applying a similar subjective approach, Johnson et al. (1990b) used a 10-point scale to visually evaluate the extent of crystal development on Cheddar cheese during aging. However, little progress has been reported toward measuring crystal coverage objectively, and there continues to be a need for more accurate, repeatable, and sensitive methods to quantitatively and nondestructively measure the extent of crystal coverage on cheese surfaces.

Digital imaging technology may offer a means for quantitative measurement of surface crystals. This approach has been used widely to evaluate visual attributes of various objects, including many food applications. Image analysis has been applied successfully in cheese research to measure the size and distribution of blisters on pizza (Yun et al., 1994); correlate the size and shape characteristics of cheese shreds with other functional characteristics (Apostolopoulos and Marshall, 1994); measure topping percentage and distribution on pizza (Sun, 2000); measure the melting, browning, and oiling-off properties of Cheddar and Mozzarella cheeses as a function of time, temperature, and sample dimensions; correlate meltability with empirical test results (Wang and Sun, 2001, 2003, 2004); measure the surface area occupied by gas holes in Emmental and Ragusano cheeses (Caccamo et al., 2004); and quantify slits in Cheddar cheese (Caccamo et al., 2004). Image analysis measurements have also been shown to be more sensitive and able to differentiate cheese meltabilities more precisely than the traditional Schreiber and Arnott tests (Wang and Sun, 2002). The objectives of the present study were to develop and evaluate an image analysis method to quantify calcium lactate crystal coverage on the surface of naturally smoked Cheddar cheese. Naturally smoked cheeses are particularly well suited for image analysis because smoking renders the cheese surface orange-brown in color, which contrasts sharply with white calcium lactate crystals that may be present at the surface.

MATERIALS AND METHODS

Random weight (~300 g) retail samples of naturally smoked Cheddar cheeses exhibiting white surface crystals were obtained from local supermarkets. All cheese samples were produced at the same cheese plant, and their dates of manufacture were determined from information supplied by the manufacturer. The dimensions of a typical cheese sample were approximately $47 \times 60 \times 100$ mm. Samples were stored at 4°C until analysis. Before the samples were photographed for image analysis, all labels were removed from the packaging film and any remaining traces of label or adhesive were gently etched away to render the film completely transparent without altering the appearance of the crystals beneath the film. After the samples were photographed, crystals from approximately one-third of the samples were carefully dislodged from the photographed surfaces using a sharp blade (1991 Trimming knife blade, Stanley Tools, New Britain, CT) and analyzed for D(–) and L(+) lactate contents by the enzymatic method described in the test kit (Boehringer Mannheim/R-Biopharm AG, test kit no. 11112821035, Darmstadt, Germany).

Photography and Image Analysis

Image generation. A digital still camera (Sony model no. MVC-FD87, 1.3 megapixel, Sony Corp., Tokyo, Japan) was used to photograph cheese surfaces.