Characterization of Calcium Lactate Crystals on Cheddar Cheese by Image Analysis

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ABSTRACT

Previous research demonstrated that crystal coverage on the surface of Cheddar cheese can be quantitatively and nondestructively measured using image analysis of digital photographs of the cheese surface. The objective of the present study was to extend image analysis methodology to quantify and characterize additional features of visible crystals on cheese surfaces as they grow over time. A random weight (~300 g) retail sample of naturally smoked Cheddar cheese exhibiting white surface crystals was obtained from a commercial source. The total area occupied by crystals and total number of discrete crystal regions on one of the surfaces (~55 × 120 mm) was measured at 3-wk intervals for 30 wk using image analysis. In addition, 5 small (~0.3 mm radius) individual crystals on that surface were chosen for observation over the 30-wk period. The crystals were evaluated for area, radius, and shape factor (circularity) every third week using image analysis. The total area occupied by crystals increased in a linear manner ($R^2 = 0.95$) from about 0.44 to 7.42% of the total cheese surface area over the 30-wk period. The total number of discrete crystal regions also increased but in a nonlinear manner that was best described by a quadratic relationship. Measurement of discrete crystal regions underestimated the true number of crystals present at the cheese surface due to merging of adjacent crystals as they grew and merged into a single crystal region over time. Throughout this period, the shapes of the 5 individual crystals closely approximated perfect circles, except when adjacent crystals merged to form a single irregular crystal region, and the area occupied by each of the 5 crystals increased in a near-linear manner ($R^2 = 0.95$). Image analysis approaches may be used to evaluate crystal formation and growth rates and morphology on cheese.

Key words: calcium lactate, crystal, Cheddar cheese, image analysis

INTRODUCTION

Calcium lactate (CL) crystals that sometimes form on Cheddar cheese surfaces are a significant expense to manufacturers due to trimming losses during cut-and-wrap operations (personal communication with US cheese manufacturers) and consumer rejection of retail cheese (Dybing et al., 1988; Swearingen et al., 2004). Numerous researchers have studied various factors that contribute to CL crystal defects, and much progress has been made toward identifying conditions that promote crystal formation (Johnson et al., 1990a,b; Somers et al., 2001; Chou et al., 2003; Kubantseva et al., 2004; Swearingen et al., 2004; Agarwal et al., 2005, 2006; Rajbhandari and Kindstedt, 2005a).

Crystallization in aqueous solutions occurs in 3 stages. First, the solution must become supersaturated with respect to the reactant species. Second, a stable crystal nucleus must form, after which the third stage, crystal growth, may proceed as new reactant species from the surrounding solution move to the crystal interface and orient into the lattice (Hartel, 2001). Although supersaturation of the aqueous phase is a prerequisite to crystallization, the rate of nucleation and the growth rates of individual crystals following nucleation also influence the time required for crystals to reach sufficient size and number for visual detection (Hartel, 2001).

Crystallization at the surface of cheese is subject to conditions that do not occur in simple solution because of the complex physical environments that occur within the cheese body and at the surface. It is possible that some of these environmental conditions may influence nucleation and growth rates of CL crystals at the cheese surface. For example, the movements of Ca and lactate ions through the cheese serum to the crystal interface probably occur through an impeded diffusion process, as has been observed for Na⁺ and Cl⁻ ions in cheese, and are likely influenced by similar factors such as obstructions of fat globules and globular protein particles, porosity of the protein matrix, and frictional effects of protein-bound water (Guinee and Fox, 2004). Furthermore, the complex physical conditions that occur at the interface of the cheese surface and packaging

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material could conceivably influence the number of sites that are predisposed to support nucleation and subsequent crystal growth, which may explain why factors such as packaging tightness and packaging with gas-flushing strongly influence the development of CL crystal defects at the surface of Cheddar cheese (Johnson et al., 1990b; Agarwal et al., 2005).

Little is known about the specific sites where CL crystals form on the surface of cheese. Such sites may be highly clustered rather than uniformly distributed (Rajbhandari and Kindstedt, 2005b), and we have observed patterns of crystal growth that appeared to follow defined contour lines etched into the cheese surface during cutting (P. Rajbhandari and P. S. Kindstedt, unpublished data). Kalab (1980) speculated that dead bacterial cells could serve as nucleation sites for the formation of crystalline inclusions in cheese. Other researchers have demonstrated that bacteria can serve as nucleation sites for ice crystals in various foods (Blanshard and Franks, 1987; Charoenrein et al., 1991). Crystal nuclei consist of stable clusters of the reactant species that are too small to be detected; therefore, it is difficult to study the nucleation process directly because there is a lag time between nucleation and crystal detection (Hartel, 2001). However, nuclei at the surface of cheese may eventually grow into crystals that exceed the threshold for visual detection. Thus, the crystal formation rate (i.e., number of visible discrete crystals that form per unit of cheese surface area per unit of time) may be considered an indicator of the number of surface sites at which crystal nucleation occurred. Similarly, the rates at which individual discrete crystals increase in area over time when viewed perpendicular to the cheese surface may be considered an indicator of crystal growth rate (i.e., rate at which individual crystals increase in size over time).

Presumably, the amount of time that it takes for CL crystals to become visible and attain sufficient coverage on a cheese surface to constitute a visual defect is a function of both crystal formation rate and the crystal growth rate. Few attempts have been made to measure these parameters quantitatively or to differentiate the role of crystal formation rate from that of crystal growth rate in determining the timing and severity of surface crystal defects. Such information, if available, could provide new insight into the process of crystal formation as it occurs at the surface of cheese and assist in the development of strategies to combat crystal defects.

Recently, we evaluated an objective method that uses image analysis of digital photographs to quantify the collective or total area occupied by CL crystals on Cheddar cheese surfaces (Rajbhandari and Kindstedt, 2005b). We hypothesize that image analysis methodology can be extended to quantify and characterize addi-

tional features of visible CL crystals that relate to crystal formation rate, growth rate, and morphology. The specific objectives of the present study were to demonstrate the use of image analysis to measure changes in the number of discrete crystal regions (**DCR**), which may be considered an indicator of crystal formation rate, and changes in the area and circularity of individual discrete crystal regions, which may be considered indicators of crystal growth rate and morphology, respectively, on the surface of Cheddar cheese during refrigerated storage.

MATERIALS AND METHODS

One retail-sized sample (~300 g) of naturally smoked Cheddar cheese that exhibited white surface crystals was obtained from a retail outlet. Natural smoking causes chemical changes in Cheddar cheese that favor elevated lactate and calcium levels in the serum phase (Rajbhandari et al., 2007); therefore, smoked Cheddar is a useful test model for cheese that is susceptible to CL crystallization. The dimensions of the sample were approximately 120 × 55 × 40 mm. The cheese was 25 wk old from the date of manufacture at the start of the 30-wk study. The cheese was produced by a milled curd procedure using an automated production line that included enclosed vats (22,727-kg capacity) for coagulating the milk and cooking the curd; an enclosed conveyor series for continuous curd dewheying, cheddaring, and milling; enclosed mechanical metering of dry salt and automated stirring of salted curd; and block-forming towers that produced 19.1-kg blocks. Cheese blocks were vacuum packaged and aged for about 4 mo before being cut into random-weight chunks for smoking in a commercial smoke house for approximately 6 h at 20°C. Smoked samples were then cooled, vacuum packaged, and sent to retail distribution. Conditions during retail distribution such as storage temperature were not determined.

Upon receipt of the cheese sample, labels were carefully removed from the packaging film to render the film completely transparent for photography and image analysis. Particular care was taken to avoid applying pressure that could disrupt the cheese surface or crystals thereon. The sample was then stored for 30 wk at 4°C, digital photographs of one of the large flat surfaces (~55 × 120 mm) were taken repeatedly at 3-wk intervals, and the total area occupied by crystals on this surface was measured using Metamorph imaging software (Molecular Devices, Downingtown, PA) as described previously (Rajbhandari and Kindstedt, 2005b). In addition, the number of DCR on the cheese surface was measured using the integrated morphometry analysis

Figure 1. Digital images of one surface of a retail sample of smoked Cheddar cheese that was stored for 30 wk at 4°C. Calcium lactate crystals appear as white regions against the gray cheese surface; A) wk 0; B) wk 12; C) wk 21; D) wk 30.

function of the Metamorph program and was expressed as number per square centimeter of surface area.

On the same surface, 5 small (~0.3 mm radius) specific DCR were selected to analyze over the 30-wk period. These DCR were numbered as 1, 2, 3, 4, and 5. Each DCR was individually outlined using an ellipse region tool and measured for area (mm²), equivalent radius (mm), and shape factor (circularity) using the Metamorph program. Shape factor is measured as circularity = $4\pi \times \text{area}/(\text{perimeter})^2$. Values for shape factor ranged from 0 to 1; a value of 1 indicated a perfect circle, whereas a value <1 indicated irregular shape.

Regression analyses were performed using Proc GLM in SAS (SAS Inst. Inc., Cary, NC). Differences between variables were considered to be significant at the $P < 0.05$ level. Both linear and quadratic terms were used to test the relationship of area and radius of DCR over time. To determine whether DCR grew at the same rate, the slopes of the regression equation of area measurements were compared over a period of 30 wk.

At the end of the 30-wk study, visible crystals were scraped from the cheese surface and analyzed for contents of moisture and L(+) and D(−) lactate as described previously (Rajbhandari and Kindstedt, 2005a). Moisture and L(+) and D(−) lactate contents in the cheese were also determined as described previously (Rajbhandari and Kindstedt, 2005a).

RESULTS AND DISCUSSION

The cheese sample used in this study contained 34.34% moisture and 0.92% L(+) lactic acid and 0.024%

D(−) lactic acid, which were within the range of values reported previously for smoked Cheddar cheeses produced at the same manufacturing plant (Rajbhandari and Kindstedt, 2005a,b). The crystals collected from the cheese surface contained 38.3% moisture and 26.6% L(+) lactate [negligible D(−) lactate was detected], which were also within the range of values reported previously (Rajbhandari and Kindstedt, 2005a,b).

The progression of crystal growth and proliferation on the cheese surface during 30 wk of storage at 4°C is shown in Figure 1. Surface CL crystals can be clearly distinguished as round and irregularly shaped white regions against the darker background of the smoked cheese surface. Crystal regions were not distributed uniformly across the cheese surface but occurred mostly as clusters that became more pronounced over time. This suggests that physical sites on the cheese surface that were favorable to nucleation occurred in clusters, presumably due to surface irregularities that created a heterogeneous environment for nucleation.

The total area occupied by crystals, expressed as a percentage of total cheese surface area, increased from approximately 0.44 to 7.42% in a linear manner over 30 wk of refrigerated storage (Figure 2), in agreement with earlier results (Rajbhandari and Kindstedt, 2005b). A comparison of the visual image series in Figure 1 with quantitative crystal area data in Figure 2 suggests that the measurement of total crystal area (Figure 2) may provide a quantitative index of the severity of crystal defect as perceived by consumers.

The number of DCR, expressed per square centimeter of cheese surface area (Figure 3), displayed more varia-

Figure 2. Changes in the total area occupied by calcium lactate crystals, expressed as a percentage of total cheese surface area, on one surface of a retail sample of smoked Cheddar cheese that was stored for 30 wk at 4°C.

tion over time than did total crystal area (Figure 2) and increased in a curvilinear manner (rather than linear) over the 30-wk storage period that was best described by a quadratic relationship. More variation in DCR number and the declining rate of increase in DCR with increasing time of storage were likely the outcome of 2 competing phenomena that affected DCR measurements. New crystal regions became clearly visible as storage time increased (Figure 1) and thereby caused the number of DCR to increase over time. However, as visible crystal regions increased in size over time, crystals that were spatially adjacent to one other in clusters (Figure 1) merged to form a contiguous mass

Figure 3. Number of discrete crystal regions expressed per unit of cheese surface area (cm²) on one surface of a retail sample of smoked Cheddar cheese that was stored for 30 wk at 4°C.

Table 1. Changes in shape factor measurements of 5 discrete crystal regions (DCR₁ to DCR₅) on the surface of a retail sample of smoked Cheddar cheese during 30 wk of storage at 4°C

Storage time (wk)	Shape factor ¹				
	DCR ₁	DCR ₂	DCR ₃	DCR ₄	DCR ₅
0	1	1	1	1	1
3	1	1	1	1	1
6	1	1	1	1	1
9	1	1	1	1	1
12	1	1	1	1	1
15	0.713	1	1	1	1
18	0.859	1	1	1	1
21	0.883	1	1	1	1
24	0.797	1	1	1	1
27	0.858	1	1	1	1
30	0.771	1	0.994	1	0.903

¹ $4\pi \times \text{area}/(\text{perimeter})^2$; values of 1 indicated a circular shape and values <1 indicate deviation from circularity.

that could no longer be differentiated by image analysis, as discussed below, and thus was measured as a single region from that time forward. Thus, the measurement of DCR number underestimated the true number of individual crystals and, hence, the number of sites on the cheese surface where nucleation occurred. Furthermore, the extent of underestimation became greater as clusters of adjacent crystals grew over time and progressively merged with one another.

Shape factor values for each of 5 DCR that were evaluated over the 30-wk study are presented in Table 1. A shape factor value of 1 indicates a circular shape, whereas values <1 indicate deviation from circularity. The 5 DCR were circular throughout the 30-wk study, with 2 exceptions that occurred with DCR₁ and DCR₅ at wk 15 and 30, respectively. A visual evaluation of the image at wk 15 revealed that DCR₁ merged with an adjacent circular crystal that was not present at the start of the study but that subsequently became visible. The merger of these 2 circular DCR resulted in a single crystal region that remained noncircular from that time forward (Table 1). Similarly, DCR₅ merged with a newly visible adjacent crystal at the end of the study (wk 30) to form a noncircular crystal region (Table 1). In summary, the 5 CL crystals grew in circular manner when viewed perpendicular to the cheese surface, although the eventual merger of 2 adjacent round crystals resulted in the formation of a noncircular DCR. It is possible that these crystals, which appeared round from a 2-dimensional aerial view, actually grew as spheres in 3-dimensional space, but this study did not attempt to characterize 3-dimensional morphology.

Changes in the areas of the 5 DCR over the 30-wk storage period are shown in Figure 4. Statistical evaluation of the data using linear and quadratic models revealed that DCR area data increased in a linear rela-

Figure 4. Changes in the area (mm^2) of 5 discrete crystal regions (DCR) on one surface of a retail sample of smoked Cheddar cheese that was stored for 30 wk at 4°C ; $\bullet$ = DCR₁; $\triangle$ = DCR₂; $\circ$ = DCR₃; $\blacksquare$ = DCR₄; $\blacklozenge$ = DCR₅; DCR areas followed linear growth curves.

tionship with storage time. In contrast, a quadratic relationship represented the best fit for radii data (data not shown), as would be predicted for circular crystals (Table 1) that show linear growth in area over time. Linear increases in the areas of the 5 DCR (Figure 4) were consistent with the linear increase in total crystal area presented in Figure 2. This suggests that most or all of the DCR on the cheese surface underwent linear increases in area.

The ANOVA for DCR area data (Table 2) revealed that storage time, crystal identity, and the interaction of storage time and crystal identity all significantly affected area measurements. Thus, the 5 crystals did not all increase in area at the same rate (Figure 4). Pairwise comparisons to test for differences in the slopes of the regression lines for individual DCR (Table 3) revealed that the slope for DCR₄ differed significantly from those for DCR_{1, 2, 3} and DCR₅, whereas the latter 4 DCR did not differ significantly among themselves. The reason for the slower growth rate of DCR₄ is not known but could have been related to impeded diffusion of Ca and lactate ions to this particular region of the cheese surface caused by factors such as localized differ-

Table 2. Degrees of freedom, mean squares (MS), and probabilities for area measurements of 5 discrete crystal regions (crystal) on the surface of a retail sample of smoked Cheddar cheese during 30 wk of storage time at 4°C

Source	Area		
	df	MS	P-value
Time	1	9.300	<0.001
Crystal	4	0.082	0.006
Time $\times$ crystal	4	0.139	<0.001

Table 3. Degrees of freedom, mean squares (MS), and probabilities for test of slope differences for area measurements of 5 discrete crystal regions on the surface of a retail sample of smoked Cheddar cheese during 30 wk of storage at 4°C

Contrast	Area		
	df	MS	P-value
Slope 1 vs. 2	1	0.0011	0.8110
Slope 1 vs. 3	1	0.0361	0.1817
Slope 1 vs. 4	1	0.2744*	0.0005
Slope 1 vs. 5	1	0.0048	0.6227
Slope 2 vs. 3	1	0.0501	0.1173
Slope 2 vs. 4	1	0.2402*	0.0011
Slope 2 vs. 5	1	0.0012	0.7999
Slope 3 vs. 4	1	0.5097*	<0.0001
Slope 3 vs. 5	1	0.0673	0.0706
Slope 4 vs. 5	1	0.2065*	0.0022

* $P < 0.05$.

ences in the porosity of the casein matrix or size and frequency of fat globules. Also, it has been observed that crystals of uniform size and shape growing in the same environment may grow at different rates in many food systems because of factors such as differences in surface microstructure, surface coverage by a foreign impurity or its incorporation into the crystal lattice. This results in a distribution of growth rates that is called growth rate dispersion (Hartel, 2001).

CONCLUSIONS

The image analysis approaches presented in this report may serve as tools to measure the area and circularity of DCR and the total number of DCR on the surface of Cheddar cheese. The crystal formation rate (i.e., number of visible discrete crystals that form per unit of cheese surface area per unit of time) may be considered an indicator of the number of surface sites at which crystal nucleation occurred. However, it is important to take into account that DCR number underestimates the true number of crystals, and thus crystal formation rate, when crystals form in clusters and subsequently grow and merge with one another over time. The DCR evaluated in this study grew as near-perfect circles over 30 wk of refrigerated storage, except when 2 adjacent DCR merged to form a single contiguous crystal mass that was no longer circular. Circular and noncircular crystal regions were readily distinguished by shape factor measurements, which may be useful in future work to evaluate whether CL crystals grow in circular morphology only, or whether noncircular growth also occurs. Image analysis can also be used to evaluate crystal growth rates by measuring the increase in areas of individual DCR over time.

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