Cheese

Crystals are a girl's best friend

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To the general public, crystals are most prominent in fine jewelry. To a cheese enthusiast, crystals are speckles found in the most beloved cheeses. Curiosity around cheese crystals began at the turn of the 20th century, but has recently gained traction with the increase in technology enabling crystal research (Kindstedt and Polowsky 2020). The first cheese crystals were observed in Cheddar cheeses stored at cold temperatures and presumed to be calcium lactate pentahydrate salt crystals (Babcock and Russell 1902). It wasn't until the 1920s that cheese crystals began to be studied using bright-field microscopy, followed by electron microscopy and X-ray crystallography in the 1970s for characterization (Kindstedt and Polowsky 2020). Initially, the study of cheese crystals was driven by food safety concerns, as consumers believed crystals to be a defect in the cheesemaking process. Once the field learned more about the natural formation of crystals as cheeses age, they became more appreciated. In cheesemaking, casein proteins in milk coagulate to form solid cheese curds, which get pooled together and pressed into cheese wheels. As cheese ripens, microorganisms contribute to proteolysis, or the breakdown of casein into its amino acid subunits. Amino acids are extremely small, existing on the angstrom or 10^{-10} meter scale, but their release via proteolysis is critical in the biochemical composition of the cheese matrix. Tyrosine is the amino acid most frequently described by cheesemongers when advertising hard cheeses, such as Parmigiano Reggiano or aged Cheddars. However, tyrosine is just one of the 20 possible amino acid building blocks, so it is intriguing to ask if all white crystally speckles are in fact tyrosine. More broadly, are all cheese crystals made of amino acids and if so, how do subunits on the order of 10^{-10} meters become visible to the naked eye? Here, I provide a thorough examination of how cheese crystals can be characterized, what they look like, and how they impact consumption. Similarly to diamonds having four Cs (cut, color, clarity, and carat weight), I present the four Ss of cheese crystals: subtype, structure, size, and satisfaction.

Although the term "tyrosine crystals" has increased in popularity in the artisan cheese industry, this classification describes only one type of cheese crystal structure that can form. Cheese crystals can be characterized into two major subtypes: organic and inorganic. This terminology is rooted in the chemistry of the crystal, where organic is derived from a living organism and inorganic is mineral-based. Examples of organic crystals are calcium lactate pentahydrate (CLP), calcium citrate, and amino acids (tyrosine, leucine, phenylalanine, etc.). Inorganic crystals include calcium phosphate, calcium carbonate, magnesium phosphate, or magnesium ammonium phosphate (Kindstedt and Polowsky 2020). Aside from the amino acids, all examples listed above are salts, whether they be organic or inorganic salts.

Cheese crystals can also be intuitively characterized by location: on the surface of rindless cheeses, on the surface of rinded cheeses, or embedded in the paste (Kindstedt and Polowsky 2020). CLP crystals commonly form on the surface of rindless cheeses, such as Cheddar or Colby, or on chunks of cheese that have been repackaged. Roughness of the cut surface, tightness of the seal, and storage temperature all regulate CLP crystal formation (Kindstedt and Polowsky 2020). The serum that sweats from the cheese acts as a medium for calcium lactate diffusion around the surface of the cheese, which can be trapped by the packaging. When calcium lactate concentrations get high enough, it will "crash out" of the serum and crystallize on the surface of the cheese. More recently, leucine crystals have been reported on the surface of Parmesan-style cheeses, but organic CLP crystals represent the majority of surface crystals on rindless cheeses (Kindstedt and Polowsky 2020). Surface crystals on bloomy and washed-rind cheeses are a byproduct of microorganism metabolism and are typically inorganic salts. On white mold-ripened cheeses like Brie, *Pencillium camemberti* fungi produce ammonia and increase the pH of the surface, which prompts the migration of calcium and phosphorous from the paste to the rind of the cheese (Kindstedt and Polowsky 2020). Once calcium phosphate has saturated at the surface, it will form white crystals that contribute to the white mold rind. Washed-rind cheese crystallization operates very similarly but can be even more dramatic due to the higher production of surface ammonia from *Geotrichum candidum* and can occur three weeks post-production (Kindstedt and Polowsky 2020). Perhaps the most coveted cheese crystals occur within the cheese paste. Organic salts, inorganic salts, and amino acid crystals have all been reported in the interior of aged cheeses imparted by salt trapping between curds during curd washing and pressing or from starter bacteria culture that has since died and released its cellular contents of free amino acids into the paste. Inorganic calcium phosphate salts have been observed in Parmigiano Reggiano, Grana Padano, Pecorino Sardo, Roquefort, Sbrinz, Swiss, Tilsit, Trappist, and Manchego. Organic amino acid crystals, primarily tyrosine but also leucine, isoleucine, methionine, valine, and phenylalanine, have been reported in Gouda, Norvegia, Emmental, Sbrinz, Comté, Grana Padano, and Parmigiano Reggiano (Kindstedt and Polowsky 2020). Since most of the cheese crystal diversity arises from interior crystallization, the following sections on structure and size concentrate on these crystals.

Especially since there are many types of crystals found in the paste of cheeses, determining their intricate structures has helped to identify them. Three techniques that have greatly contributed to what is known about the unique patterning of various crystals are scanning electron microscopy (SEM), transmission electron microscopy (TEM), and powder X-ray diffractometry (PXRD). In 1982, Washam and colleagues artificially induced crystal formation on Cheddar cheese with known compounds, extracted the crystals, imaged them with SEM, and deduced characteristic morphologies of sodium chloride, calcium phosphate, calcium lactate, tyrosine, and sorbic acid crystals. They found that sodium chloride appeared as aggregates of cubic crystals, calcium phosphate appeared coral-like in morphology, calcium lactate as bundles of ribbon-like fibers, tyrosine crystals as large fibrous needle-like aggregates, and sorbic acid as a more disorderly version of calcium lactate fibers (Washam, Kerr and Hurst 1982). Although images take in Figure 1 are at a much lower magnification, it is clear that a tyrosine crystal from an aged Cheddar **(Fig. 1Ai)** has a distinct morphology than what is presumably a calcium lactate crystal from a blue cheese vein **(Fig. 1Aii)**. As shown in **Fig. 1B**, the definition of the fibrous aggregates increases with magnification **(Fig. 1Bv,vi)** and reveals beak-like bundle structures of these fibers visible on the micron scale. The definition of these tyrosine crystals gets lost when embedded in the cheese matrix, which is called the “closed” morphology (Tansman, Kindstedt and Hughes 2015; Kindstedt and Polowsky 2020). Examples of “closed” morphology salt and amino acid crystals are presented in Figure 2, where the darker masses are denoted with black arrowheads in the cheese paste **(Fig. 2A-D)**. Although the Washam (1982) SEM images were of induced crystals, their work was one of the first to emphasize that different-crystal subtypes have distinct crystalline patterns. Later work from Tansman et al. (2015) and D’Incecco et al.

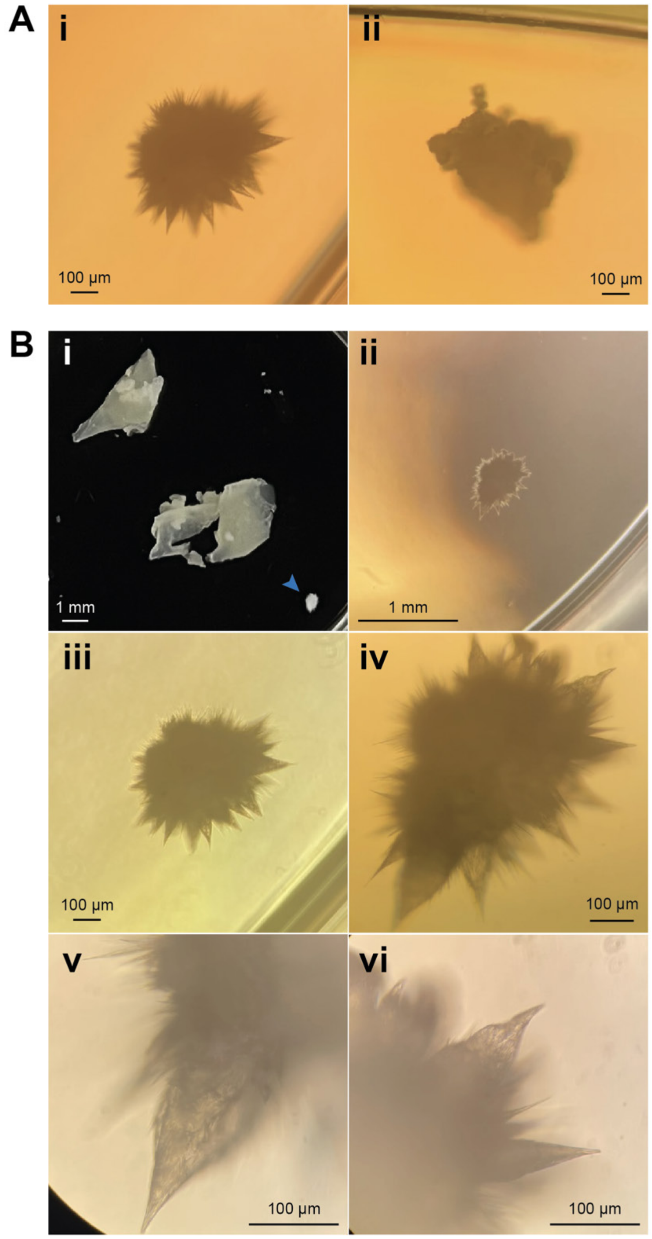

Figure 1: Brightfield microscopy images of a tyrosine crystal. A. Side by side comparison of an extracted (i) tyrosine crystal from Beecher’s Handmade Flagship Reserve and (ii) calcium lactate crystal from Point Reyes Bay Blue. **B.** Beecher’s Handmade Flagship Reserve tyrosine increasing in magnification from (i)-(vi). The blue arrowhead in (i) denotes the crystal zoomed in on in (ii)-(vi). Scale bars are approximate. Images were taken on a Leica Microsystems DM IL inverted microscope equipped with a 10x eyepiece and use of 4x, 10x, 20x, and 40x objectives for 40x, 100x, 200x, and 400x total magnification (property of the Cheeseman Laboratory).

(2016) used PXRD and TEM to analyze thin sections of cheese samples containing crystals, further supporting the idea of cheese crystal structure fingerprinting.

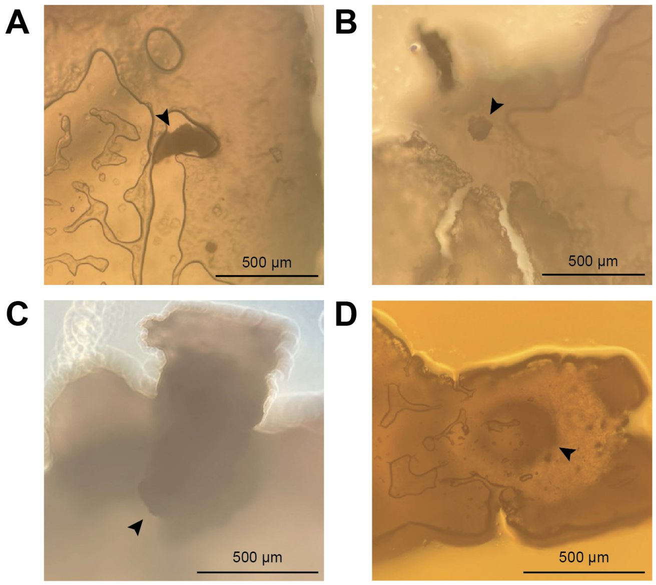

Figure 2: Brightfield microscopy images of various embedded cheese crystals. A. Sample of Sangles Sparrenschors at 100x magnification with kidney-shaped crystal indicated with a black arrowhead. The liquid blobs are sweat or serum pooling on the exterior of the slice. **B.** Sample of 18-month aged Gouda with a spherical crystal. The ridges or valleys are gaps in the curd of the sample slice. **C.** Sample of Beecher's Handmade Flagship Reserve with the black arrowhead indicating the bottom left corner of a rectangular crystal. **D.** Sample of Cougar Gold with a larger spherical crystal. There appears to be a gap or lighter density in the center of the crystal itself. Scale bars are approximate. Images were taken on a Leica Microsystems DM IL inverted microscope equipped with a 10x eyepiece and use the 10x objective for 100x total magnification (property of the Cheeseman Laboratory).

Although somewhat less informative than crystal structure in precise characterization, crystal size can approximate aging duration and is what draws consumer attention. Sizes of cheese crystals generally exist on three scales: spots, speckles, and microscopic crystals (D'Incecco et al. 2016). Spots are 4-5 mm in size appearing 10-12 months into aging, with common examples being in aged Gouda or Grana Padano, while 2-3 mm speckles start forming within 3-6 months and are commonly seen in Parmigiano Reggiano (D'Incecco et al. 2016). Microscopic crystals refers to crystals that are less than 2 mm in diameter, which encompass all crystals shown in figures 1 and 2. The extracted tyrosine and calcium lactate crystals in figure 1A were approximately 0.6 mm in diameter, while the closed morphology crystals in figure 2 span roughly 0.1-0.5 mm. In all cheese samples, there was variability between crystal sizes, but spots and speckles were more difficult to image due to crystal thickness and objective focusing. D'Incecco et al. (2016) also notes that microcrystals can appear as circles, ovals, or kidney-shaped, which are represented in figure 2. If cheeses like Cheddar, Gouda, Parmigiano Reggiano, and Grana Padano are aged longer than two years, crystals can grow to be 5-10 mm in size and are sometimes referred to as pearls (Tansman, Kindstedt and Hughes 2015). Spots, speckles, and microcrystals are somewhat arbitrary cutoffs, but they provide a general framework for referencing cheese crystals in the field.

When considering both crystal structure and size, the fact that amino acids exist on the 10^{-10} meter scale becomes much more intriguing. For example, 18-25 month aged Grana Padano was found to contain 6 mm diameter pearls primarily composed of the amino acid leucine (Tansman, Kindstedt and Hughes 2015). If the crystal was tightly packed with only leucine, seeing a 6 mm crystal by eye would require on the order of trillions of leucine molecules. The same would be true of tyrosine crystals, such as the one shown in figure 1. Even when on the highest magnification of the bright-field microscope (400x), there are billions of tyrosine molecules that form the visible beak-like structures **(Fig. 1Bv,vi)**. This means that in order to form a tyrosine or leucine crystal, free amino acids must be highly abundant at the crystal nucleation site. Material accumulates with time at these positions through casein proteolysis or the release of intracellular amino acids from dead bacteria starter culture. Mentioned previously, tyrosine and leucine are just two of 20 possible amino acids that are using as the building blocks of proteins. Isoleucine, methionine, valine, and phenylalanine have also been reported, drawing the total to six out of 20 amino acids represented (Kindstedt and Polowsky 2020). This group of amino acids is largely hydrophobic (water-hating), meaning that they would preferentially bind with other hydrophobic molecules over hydrophilic molecules like water. An additional dimension explaining the likelihood of crystal formation between amino acids within the group of six is their solubility in water. Tyrosine is two times less soluble in water than phenylalanine at room temperature, which is the next least soluble of the group, and at colder temperatures, tyrosine becomes significantly less soluble than the others (Ji et al. 2019). Lower solubility means that they are less tyrosine molecules needed at the crystal nucleation site for tyrosine to "crash out" of solution and crystallize. Hence, tyrosine is the most likely to form crystals even when tyrosine concentrations are relatively low (still billions of molecules), making it the most prominent form of amino acid crystal. Over time, additional tyrosine molecules can pack with the initial crystal, forming intricate structures like the one in figure 1Ai.

While the scientific exploration of cheese crystals is fascinating, the most important consideration for cheesemongers and consumers alike is how cheese crystals impact cheese perception. Historically, cheese crystals were considered to be undesirable and presumed to be mold or some fermentation defect, which caused consumers to return purchased cheeses and the distributors to lose sales (Washam, Kerr and Hurst 1982). The uncertainty of crystal identity lead to increased efforts to study them and determine that they were safe for consumption (Kindstedt and Polowsky 2020). Now, there is an appreciation for cheese crystals and their contribution to texture and taste, although this is not true for all consumers (D'Incecco et al. 2016).

To assess the final of the four Ss, satisfaction, I conducted anonymized online survey of family, friends, lab members, and other MIT graduate students. To begin, I provided five photos of different types of cheese crystals and asked respondents to select all crystals that they had seen or tasted **(Fig. 3A)**. Only three out of 50 respondents had never encountered a cheese crystal, and most respondents reported having seen white speckles or mixed size spots in hard cheeses, such as Parmigiano Reggiano **(Fig. 3A)**. At both the beginning and the end of the survey, respondents rated the likelihood that they would seek out a crystal-containing cheese on a scale of 1-5 (highly unlikely to highly likely). I tracked both responses from each participant to deduce trends in likelihood once they had thought more about crystals over the course of the survey. Somewhat likely was the most frequent answer and stayed relatively stable per person throughout the survey **(Fig. 3B)**. As a control, I asked participants to self-report their enjoyment of cheese on a scale of 1-7 (hate to love) and found effectively no correlation between cheese enthusiasm for and likelihood to seek out crystally cheeses **(Fig. 3C)**. These data indicate that in general, consumers are likely to try a crystal-containing cheese but not necessarily go out of their way to get. Respondents also had to indicate if they consider crystals to be a defect and if they had ever thrown away a cheese with crystals because of the crystals. Three quarters of participants noted that crystals are not a defect of cheese, but one quarter believed crystals to always or in some instances be a defect **(Fig. 3D)**. Thankfully, the majority of respondents (83%) had not yet throw a crystal-containing cheese away, but surprisingly almost a fifth of them had **(Fig. 3E)**.

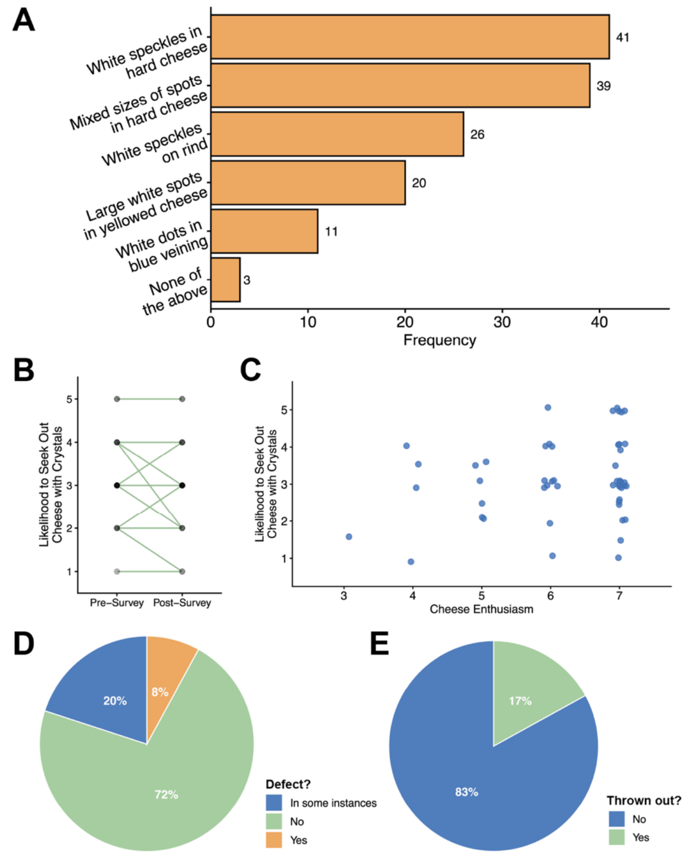

Figure 3: Results from the crystal satisfaction survey regarding consumer observation, likelihood to seek out, and perception as a defect. A. Histogram of types of cheese crystals previously observed by participants. The responses were accompanied with a representative photo and respondents could select all that apply (N = 50). **B.** Connected dot plot of likelihood to seek out a crystal-containing cheese at the beginning (pre-survey) and end (post-survey) of the survey. Likelihood was on a 1-5 scale where 1 was highly unlikely and 5 was highly likely. The darkness of the points indicates the frequency of that response. The green lines connect one participant's pre- and post-survey likelihoods, revealing trends in increases or decreases in likelihood throughout the survey. **C.** Dot plot of cheese enthusiasm compared to likelihood to seek out a crystally cheese per participant. Cheese enthusiasm was measured on a 1-7 scale, where 1 is hate all cheeses and 7 is love cheese (or at least willing to try anything). Likelihood presented here is an average of pre- and post-survey ratings. Since there were no "1" or "2" responses of cheese enthusiasm, those axis labels have been excluded. **D.** Pie chart of the perception of cheese crystals as defects. Respondents could select either yes, no, or in some instances, which are colored per the legend. **E.** Pie chart of people who had thrown away a cheese with crystals in it because of the crystals. Respondents could select either yes, no, or not applicable (not shown here), which are colored per the legend.

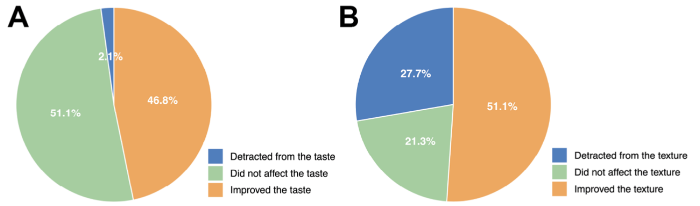

Figure 4: Results from the crystal satisfaction survey on the impact of crystals on taste and texture. A. Pie chart of the influence of crystals on cheese taste. Respondents could select either detracted, did not affect, improved, or not applicable (not shown here), which are colored per the legend. **B.** Pie chart of the influence of crystals on cheese texture. Respondents could select either detracted, did not affect, improved, or not applicable (not shown here), which are colored per the legend (N = 50).

Inquiring about the satisfaction of crystal-containing cheese sampling revealed that most people believe that crystals either had no impact or improved the taste of the cheese **(Fig. 4A)**. Texture was a bit more controversial, where half reported crystals improved the texture, a quarter said they did not affect the texture, and a quarter said that they detracted from the texture **(Fig. 4B)**. Despite the growing popularity of crystals in the artisan cheese community, some consumers are either still hesitant or simply do not prefer crystally cheeses. The hesitation is likely a product of the uncertainty of what cheese crystals actually are. To assess this, respondents had to select all that they believed could contribute to crystal formation from the following: milk fat, amino acids, sugar, salt, mold, yeast, or bacteria. While most people identified that salt could play a role, others said that crystals could be mold, yeast, or bacteria, which in theory could be harmful **(Fig. 5)**. Only a third of participants thought that amino acids could form crystals **(Fig. 5)**, which highlights a major gap in the public's knowledge of cheese crystals.

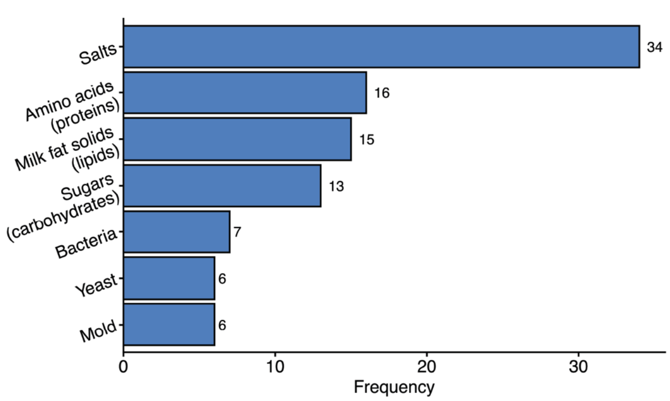

Figure 5: Results from the crystal satisfaction survey regarding the composition of cheese crystals. Histogram of what participants believed could be causing the formation of cheese crystals. Respondents could select all that apply (N = 50).

Cheese crystal culture is on the rise due to recent advances in our understanding of the four Ss: subtype, structure, size, and satisfaction. In the course of around a hundred years, the cheese industry has discovered, questioned, discarded, researched, appreciated, and enjoyed cheese crystals. They are hidden gems in the world of artisanal cheese, but are still faced with hesitation among the general public. The hope is that cheesemongers and enthusiasts can share the knowledge of the four Ss of crystals to consumers that may be interested yet skeptical of cheese crystals. After all, they are a girl's best friend.

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Supplemental Figure 1: Favorite cheeses of cheese satisfaction survey respondents. The larger, orange text cheeses were represented three times, the green text cheeses were represented twice, and the small, blue text cheeses were represented once (N = 50).