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Historical Aspects of Cheese Crystals

Historical Presence of Cheese Crystals

The first mention of cheese crystals in the American dairy literature probably occurred in Wisconsin, around the start of the 20th century. Babcock and Russell (1901), of Wisconsin's Agriculture Experiment Station, noted that cheddar cheese cured at low temperatures (<50°F) developed "white specks". Upon more thorough inspection, many cheeses kept in cold storage were found to contain these specks. They mentioned that the usual practice of the era was to avoid storage of cheese at cold temperatures (<50°F). Cheesemakers believed (erroneously) such treatment would result in a bitter product. Interestingly, the work by Babcock and Russell in implementing lower storage temperatures to improve cheese quality probably encouraged the growth of these "white specks". A year later, Babcock et al. (1902) performed a series of experiments in order to determine the factors affecting formation of these specks. They concluded that storage temperature and salt content of the finished cheese were the main factors determining speck formation. High storage temperatures (>60°F) and high salt content seemed to limit the formation of these white specks. Looking at these experiments through the lens of modern science, it's highly likely that they were observing the first wide-spread occurrence of calcium lactate crystals. Cooler temperatures can result in lower solubility and more pronounced crystallization. Higher salt content will lead to a larger salt-to-moisture ratio of the final cheese. This can affect starter culture metabolism and limit the growth of these microbes. With reduced starter culture activity, less acid is developed *via* fermentation, and the resulting cheese has a higher pH. These effects were confirmed to reduce calcium lactate formation over a century later (Agarwal et al. 2008). The first work done to determine the composition of these "white specks" was likely performed at New York State's Agricultural Experiment Station by Van Slyke and Publow (1909). Through chemical analysis, they determined calcium was present within these crystals. Based on the waxy texture, fat/fatty acids were thought to be another major substituent. The combination of these phases led Van Slyke to postulate that these crystals were "calcium soaps". To account for their occurrence at lower temperatures, Van Slyke made the supposition that fat was being broken down by "bacteria acting only at lower temperatures" (Van Slyke and Publow 1909). Up to this point, much of the knowledge of

these “white specks” were isolated in research communities. Researchers noted that the specks were innocuous, and largely not noticed by the cheese industry. In 1910, “white specks” were described in an article entitled *Cheese Defects* in the trade publication *The New York Produce Review and American Creamery* (Monrad 1910). This publication was the leading trade journal at the time, and was in large circulation among the dairy industry. Around the same time, Arthur Dox of University of Connecticut’s Storrs Agricultural Experiment Station, claimed he found the first occurrence of tyrosine crystals in cheese (Dox 1911); he noted white crystalline entities in Roquefort bleu cheese. This determination was made through exclusionary testing, and the positive chemical identification of phenol groups.

Throughout the early observations of these crystals, visual inspection and rudimentary chemical testing were the primary analysis methods. By the 1920s, microscopy techniques began to be employed to further study cheese crystals. Professor Otakar Laxa, of the University of Chemical Technology in Prague, conducted extensive microscopic studies of cheese that included many mentions and sketches of crystals (Laxa 1926). Laxa noted the presence of leucine, tyrosine, and lactate of lime (calcium lactate) across many different cheese varieties; crystals appeared to be endemic in alpine-style cheeses, grana-style cheeses, blue cheeses, and others. A year later, Laxa further studied the morphological differences of tyrosine and leucine in cheese (Laxa 1927). He indicated tyrosine, leucine, and their anhydrous forms all had unique structures that could be identified *via* microscopy. Several years later, H. H. Sommer of the University of Wisconsin, captured micrographs of calcium tartrate crystals in processed cheese (Sommer 1930). He concluded this was the cause of sandiness in processed cheese, a common defect of the period.

Rather serendipitously during this same time period, the discovery and novel use of X-rays was underway. In 1895, Wilhelm Röntgen observed the florescent effects of “invisible” cathode rays. This, ultimately, turned out to be the discovery of X-rays (i.e. Röntgen rays). Shortly thereafter, Sir William Lawrence Bragg and his father developed Bragg’s Law, describing the interaction of X-rays and crystals (Bragg and Bragg 1913).

This would lay the foundation for X-ray diffraction, and create a field that has been responsible for some the greatest scientific discoveries of the modern era.

USE OF POWDER X-RAY DIFFRACTOMETRY ON CHEESE

The earliest mentions of X-ray technology used in an agricultural setting were most likely by Professor George L. Clark of the University of Illinois. (formerly of M.I.T.) He mentions the use of X-ray imaging by cheesemakers to track the development of eyes (i.e. holes) in Swiss cheese (Clark 1929). Not too long after, Clark and dairy scientist collaborators explored the use of powder X-ray diffractometry (PXRD) in dairy technology. Crystalline substances analyzed included: mineral deposits in dairy equipment and lactose crystals in milk powder (Tuckey et al. 1934). The same group performed PXRD studies on casein and cheddar cheese, and in the process generated reference patterns (d values) for crystals of calcium phosphate and calcium lactate (Tuckey et al. 1938a, b). This led to the first positive identification of the white specks in cheddar, *via* PXRD, to be calcium lactate (Tuckey et al. 1938c). McDowall and McDowell (1939) confirmed this result *via* chemical analysis, and further stated that the pentahydrate form of the calcium lactate was identified in cheddar cheese. Shock et al. (1941) published results that indicated the crystals found within cheddar, *via* PXRD, to be a mixture of calcium lactate and tyrosine. The presence of tyrosine may have been due to the advanced age (>2 years) of the samples they analyzed, which would have had large amounts of proteolytic products. Dorn and Dahlberg (1942) analyzed canned cheddar cheese and concluded that the crystals present throughout the body and cheese surface were tyrosine, with a possible calcium phosphate "impurity". They conjectured that the previous reports of calcium lactate may have been due to experimental error, or trace amounts being an impurity contained within the tyrosine crystals. Although no analytical techniques were used, Jacquet and Saingt (1952) observed crystals on the surface of a camembert cheese, embedded within a bacterial (*Brevibacterium linens*) smear that was present. This may be the first documented case of finding surface crystals in washed rind (i.e. smear ripened) cheese. They assumed the crystals to be tyrosine and leucine. Further PXRD studies confirmed the presence of calcium lactate and tyrosine in cheddar cheese, with the amino acid cysteine also being identified (Harper et al. 1953). Authors of the time indicated that the chief limitation of PXRD was the need for a crystal concentration of at

least 10% in order to be accurately identified (Harper et al. 1953), which is in stark contrast to the standards of today. This necessitated the need for chemical identification to be paired with PXRD studies. This prompted the work by Swiatek and Jaworski (1959), who used histochemical techniques to identify calcium phosphate in a wide variety of cheese types: Emmental, Tilsit, Trappist, and Roquefort. Conochie et al. (1960) further used PXRD to identify calcium lactate and tyrosine in well-aged cheddars. The first PXRD-confirmed presence of brushite was reported by the same group not too much later (Conochie and Sutherland 1965). They concluded that the presence of brushite was one of the principal causes of the *seaminess* defect in cheddar cheese. Scharpf and Michnick (1967) demonstrated the first use of PXRD to identify crystals *in situ*. Processed cheese was loaded into the diffractometer and a diffractogram was generated. Crystal peaks indicative of disodium phosphate dodecahydrate were identified, likely caused by the addition of emulsifying salts during the manufacture of processed cheese. By the 1970s and 1980s, techniques such as PXRD and electron microscopy were in use to identify cheese crystals (Brooker et al. 1975; Severn et al. 1986). The use of PXRD continues into the 21st century, with PXRD being used to confirm identities of cheese crystals—mainly calcium lactate in cheddar (Chou et al. 2003; Agarwal et al. 2006). Recent studies have demonstrated the utility of PXRD in analyzing many different crystal types (e.g. calcium lactate, tyrosine, leucine, ikaite, struvite, brushite, calcite), across a wide number of cheeses (e.g. cheddar, Parmesan, Gouda, washed rind) (Tansman et al. 2015a, 2017b, c).

Use of Single Crystal X-Ray Diffractometry on Cheese

The presence of single crystals in cheese is a recent discovery. Single crystal of struvite, ikaite, and calcite have been analyzed *via* single crystal X-ray diffractometry (SCXRD) (Tansman et al. 2015b, 2017a). They published the structures of ikaite and struvite. This is the first recorded instance of SCXRD being used in cheese, and in dairy products at-large. The diffraction artifacts present in the preliminary work of the aforementioned study demonstrated that the extracted crystals can dehydrate when exposed to atmospheric conditions. This lends valuable insight into the conditions in which these crystals grow. The surface smear of washed rind cheeses allows for the growth of metastable forms of single crystals, which may have use in other disciplines. On a related note, laboratory-grown single crystals of α-lactose monohydrate have been

analyzed *via* SCXRD. Beevers and Hansen (1971) found that α-lactose monohydrate is monoclinic.

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