

# Dairy Pipeline



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A Technical Resource for Dairy Manufacturers

## CRYSTALLIZATION IN CHEESE

### Introduction

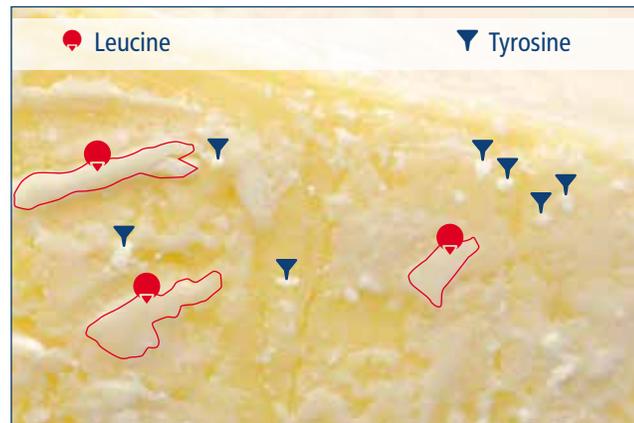
Crystal formation in cheese has been talked about in the literature for more than 100 years. From common calcium lactate crystals to the more obscure and less studied tyrosine crystals, cheesemakers have long discussed the impact that these crystals have on the visual and textural appeal of a cheese.

While the crystals are relatively harmless, consumers often confuse the crystals with yeast or mold growth as the appearance of the crystals implies a defect. In reality, calcium lactate and tyrosine crystals are actually seen as a desirable trait in certain aged cheeses as they often indicate that the cheeses will have a robust and hearty flavor. They are considered defects only if they take away from the acceptance of the cheese.

Traditionally, calcium lactate crystals have been found in aged Cheddar cheese, Colby, aged Parmesan and aged Gouda cheese, with tyrosine crystals most commonly seen in romano, Parmesan and Swiss cheeses and more recently in Gouda and Cheddar cheeses made with *Lactobacillus helveticus* used as a flavor enhancing adjunct culture.

We know that calcium lactate and tyrosine crystals generally require two conditions for development; concentrations exceeding solubility and expression of free serum, but most of the research regarding their development has revolved around documenting common observations under carefully

controlled experiments. While these experiments have been successful at reconstructing conditions that lead to the development of crystals the experiments have thus far been somewhat inadequate in explaining the fundamental chemistry (nucleation) leading to crystal appearance. An excellent summary of studies has been published by *Dybing et.al.1988, Agarwall et.al. 2008, and Rajbhandari and Kindstedt 2014.*



Smear of leucine crystals with distinct tyrosine crystals on a hard Italian cheese.

### How Crystals Form

The development of calcium lactate and tyrosine crystals is similar to the crystallization of any mineral in water. Essentially, in order for a crystal to develop there must be sufficient contact between reactants, in this case calcium and lactic acid, and collisions between calcium lactate or tyrosine molecules. At low concentrations of calcium lactate or tyrosine, the molecules are so far apart that collisions between the

molecules are rare. When the concentration of calcium lactate or tyrosine increases, however, there is greater chance of collision and the length of interactions between molecules is longer and stronger. Eventually, a critical size is reached and a nucleation site is formed. This site attracts more calcium lactate or tyrosine and when the site is large enough crystalline material falls out of solution and becomes visible to the naked eye. The crystal will continue to grow as long as there is enough calcium lactate or tyrosine in solution and the molecules can reach the nucleation site. This requires conditions that allow serum to move to where a crystal is forming or where serum can collect.



This explains why temperature abuse often results in calcium lactate crystals on the surface of the cheese. When cheese sweats or loses serum to the cheese surface the casein network of the cheese tightens and forms pools of serum. These pools serve as a conduit for the transport of lactic acid to the crystal site. Coincidentally, the free serum that collects in the packaged cheese can also promote the growth of yeasts and molds if the cheese has been contaminated with them.



Calcium lactate crystals occur at buckling sites.



Calcium lactate crystals on edge of film, package removed.

Another model for crystallization implies that calcium lactate or tyrosine adsorbs onto the surface of a solid, such as a bacterial cell or is attracted to the area around a highly charged site such as colloidal calcium phosphate (bound to casein). Colloidal calcium phosphate is also a source of calcium and is surrounded by serum (thus assuring a steady supply of lactic acid). These areas are in essence an accumulation site for both calcium and lactic acid and thus it greatly increases the chance for more calcium lactate interactions and crystal growth.

In both models there must be sufficient calcium and lactic acid or tyrosine to form the crystal. The solubility of lactic acid (no calcium attached) is 100 g per 100 ml of water (Sigma). It is available commercially in concentrations of 50-90 percent with water making up the difference. In

the presence of calcium, however, the solubility is greatly diminished because calcium lactate is formed and it is much less soluble than lactic acid.

There are different reports on the solubility of calcium lactate. *Kubantseva et al 2004* found that the solubility of calcium lactate at 10° C was 4.04 g per 100 g water. That limit is equivalent to 1.41 percent lactic acid in 35 percent moisture Cheddar, 1.49 percent in 37 percent moisture Cheddar and 1.53 percent lactic acid in 38 percent moisture Cheddar. At 4° C the solubility of calcium lactate was 3.38 g per 100 g water. That is equivalent to 1.18 percent lactic acid in 35 percent moisture Cheddar, 1.25 percent lactic acid in 37 percent moisture Cheddar and 1.28 percent lactic acid in 38 percent moisture. It is not uncommon for Cheddar and Colby cheeses to have lactic acid content that exceed these limits and many do not exhibit calcium lactate crystals.

Yet another observation was made by *Thomas and Crow, 1983*, who demonstrated that starter bacteria formed only L (+) lactic acid and that certain strains of "wild" *Lactobacilli* could convert or racemize L (+) lactic acid to D(-) lactic acid. They theorized that the racemization of L(+)-lactic acid to D(-) lactic acid and the lower solubility of a mixture of the racemic mixture was responsible for the formation of calcium lactate crystals in aged cheese. *Swearingen et. al 2004* reported that if the level of calcium lactate was high enough and if there was the necessary serum loss to the surface crystals containing only L(+) calcium lactate could be formed.

Tyrosine crystals develop under similar conditions but appear to be strongly associated with the metabolism of *Lactobacillus helveticus*. Since the formation of tyrosine crystals has not achieved the same level of scrutiny as calcium lactate much of our information may be circumstantial or coincidental. One possibility for the formation of sufficient tyrosine for crystallization involves the peptidase activity of *L. helveticus*. This bacteria is auxotrophic for many amino acids. Which means that *L. helveticus* can not catabolize or make tyrosine and other amino acids such as leucine and must rely on sources containing these amino acids. Rennet and other proteinases breakdown casein into large peptides. *L. helveticus* has a very robust peptidase activity which helps develop flavor but also releases tyrosine from the peptides. It is so active that it releases more than it or other bacteria can utilize. Consequently after sufficient breakdown of peptides substantial quantities of tyrosine are released which exceeds the limit of solubility and crystals develop. One reason tyrosine accumulates is that *L. helveticus* is not growing in the cheese when the crystals are formed but rather they have lysed and their peptidase enzymes remain active.

Tyrosine is very insoluble in serum (0.45 mg per ml). Since tyrosine is so insoluble it is likely that the crystals form solely around colonies of *L. helveticus* or where they once thrived. Indeed tyrosine crystals are generally found in discrete crystals and rarely form a smear of crystals as is common with calcium lactate.



## Do calcium lactate crystals contain other materials?

Absolutely, after all, the crystals are forming in a liquid environment full of other dissolved substances. Prior to crystallization calcium lactate was soluble in cheese serum; that is, the moisture phase of cheese. Crystals form in liquid and unless dried will contain much more moisture than just the five molecules of water that make up the actual crystal, calcium lactate pentahydrate. Consequently, there are a lot of possibilities that other substances are being caught near or in the crystal matrix. Upon drying, these substances are left with the crystal. Any serum minerals such as calcium phosphate, serum proteins, amino acids, peptides and salt (sodium chloride) can be trapped in the matrix. CDR has analyzed calcium lactate crystals found on the edges of packages and calculated that less than 50 percent of the dried crystal was actually calcium lactate pentahydrate.

We have also observed calcium lactate crystals in reduced fat cheeses that were made from whole milk standardized with reconstituted non-fat dry milk (9 percent total solids) but not in cheeses made from partially skimmed milk. The significance of this is that both milks contained the same level of lactose and the cheese had almost identical levels of total lactic acid (L and D- lactic acid combined). The only difference was the heat-treatment given to the non-fat dry milk during its manufacture.

The crystals were evenly distributed in the interior of the cheese. They were extremely numerous, very tiny and were only found inside the cheese and not in aggregates at the surface of the cheese. We speculated that during the drying process calcium phosphate crystals were formed and associated with casein molecules at the outer regions of the casein micelle. These crystals were trapped in the curd and became focal points around which moisture and soluble calcium and lactic acid would collect.

## Can you tell the difference between calcium lactate and tyrosine crystals by just looking at them?

With experience you can make a very good guess as to the type of crystal present on the cheese. Calcium lactate crystals are softer than tyrosine crystals and they seem to be wetter and more diffuse. Tyrosine crystals tend to be firmer (denser), more discrete and brighter white in appearance. Tyrosine crystals are rarely observed in Cheddar or Colby cheese unless *L. helveticus* is used as an adjunct. Indeed *L. helveticus* is always used in cheeses where tyrosine crystals have been observed. (Parmesan, romano and Swiss)

Calcium lactate crystals can be found both on the surface and in the interior of cheese but tyrosine crystals are almost always found only in the interior. Once the cheese is cut the interior crystals are exposed at the new surface. After wrapping cheeses calcium lactate crystals can develop along edges of the packaging material or where there is a rough or uneven surface on the cheese. These are areas where moisture can collect.

Recently CDR sent a cheese to Gil Tansman and Paul Kindstedt at the University of Vermont. This cheese exhibited



Interior tyrosine crystals on parmesan cheese.

a smear of crystals at the original surface of the wheel of cheese. The cheese contained tyrosine crystals in the interior but CDR did not think that the smear was tyrosine due to their atypical appearance. CDR thought that they might be calcium lactate. In 2014 the American Dairy Science Association had previously reported using powder X-ray diffraction methodology for verification of the identity of crystals found in a variety of cheeses at the annual meeting. They found that my smear of crystals did not contain tyrosine nor calcium lactate but rather an unusual form of leucine. This is interesting because *L. helveticus* is used in the same cheeses that develop both leucine and tyrosine crystals. Neither amino acid can be synthesized by *L. helveticus*. Both must be obtained through very active amino peptidase activity of this bacteria. Whether or not other *Lactobacillus* strains are capable of releasing sufficient quantities of these amino acids in order to form crystals is unknown.

## Prevention of Calcium Lactate Crystals

The prevention of calcium lactate crystals in cheese falls into three areas:

- 1 Reduce the level of calcium lactate in cheese
- 2 Reduce potential for serum to collect in pools either in the cheese or at the surface of cheese especially where there is loose packaging.
- 3 Reduce the level of soluble ionic calcium

Work done at CDR shows that there are several steps that can be taken to prevent the appearance of calcium lactate crystals. Although the obvious first step is to reduce the level of lactic acid in the cheese by rinsing the curd with warm water or whey dilution, doing so did not always prevent the appearance of crystals. At times the washing step was not done long enough to remove sufficient lactic acid and lactose which would eventually be fermented to lactic acid. Whey dilution was a better means of reducing both lactose and lactic acid. It required the removal of a portion of the whey (~25%), however, and replacing it with water as is done in the manufacture of Gouda and Baby Swiss cheese.

For cheese not vacuum packaged we recommend lactic acid of <1.2% in 36% moisture cheese. The use of slow starters is also recommended as well as a lower pH at drain. It does not mean

that a cheesemaker should develop acid at an extremely slow rate nor should it be interpreted that using a reduced amount of a fast acid producing starter will accomplish the same thing. It does not mean that the cheesemaker is doomed to eight hour make schedules. It is all about the attributes that a slow starter has and the reduced moisture content of the cheese. A slower rate of acid development is often accompanied by longer cook times to achieve the desired pH at drain. This can often result in lower moisture in the cheese and thus less residual lactose and lactic acid. Combined with a curd wash or rinsing, lactic acid values of less than 1.3 % were routinely achieved. Slow cultures often slow down acid development after whey drainage and are often salt sensitive. This combination would result in cheeses of lower acid content and a pH above 5.0.



Surface smear of leucine crystals on hard Italian cheese.

Another common observation associated with crystals was the accumulation of free moisture at the surface of the cheese. Free moisture or serum is strongly influenced by the pH history of the cheese. A low pH (< 5.0) at any time in the life of the cheese will result in it being more apt to release moisture in the package during storage. Of course there would also be more lactic acid and soluble calcium at this low pH. Serum release is due to the physical nature of the casein network that allows movement of serum. If the packaged cheese is warmed (temperature abuse in retail) the serum will more readily be released from the cheese especially if the cheese is acidic.

At the time when CDR and Norm Olson, Ph.D. made these recommendations, the cheese industry was undergoing a major change in the starters that were being used. The new cultures rapidly produced lactic acid after salting and the resulting cheese often reached a pH of < 5.0 within a few hours after salting. These cheeses were more prone to “sweating” and thus calcium crystallization at the cheese surface. Because the industry had a great reluctance to change back to the slower cultures and slightly longer make times, cheesemakers made adjustments to their manufacturing schedules that included higher pH at rennet addition, drain, and salt addition. The idea was to increase the buffering

capacity of the cheese and this would prevent a low pH from occurring in the finished cheese. It generally worked so far as preventing an excessively low pH but it strongly depended upon the culture used. The level of lactic acid produced, however, was often greater than the cheeses made with the slow starters making it potentially more susceptible to develop calcium lactate crystals as the cheese aged.

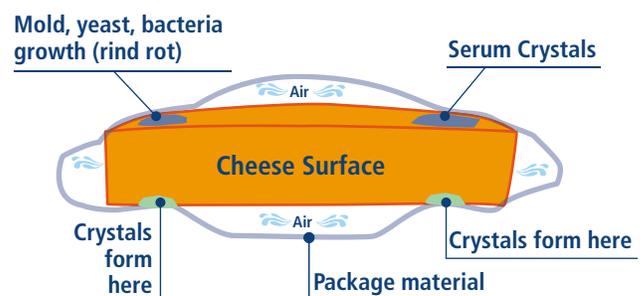
Vacuum packaging was found key to prevent crystals forming at the surface of cheese. Since free moisture easily collects at the interface between packaging material and the cheese if there is not a tight fit to the cheese, it is recommended that vacuum packaging be used. We found that unless the package was compromised crystallization was avoided. If there was a wrinkle or fold in the package, an uneven surface or any site where the cheese was not in complete contact with the packaging material, serum collected and crystals formed. Nutriceps, Inc introduced CrystalBan™ (sodium gluconate) as a chelator (ties to calcium) to reduce the availability of calcium to react with lactic acid. Consequently less calcium lactate crystals are formed.

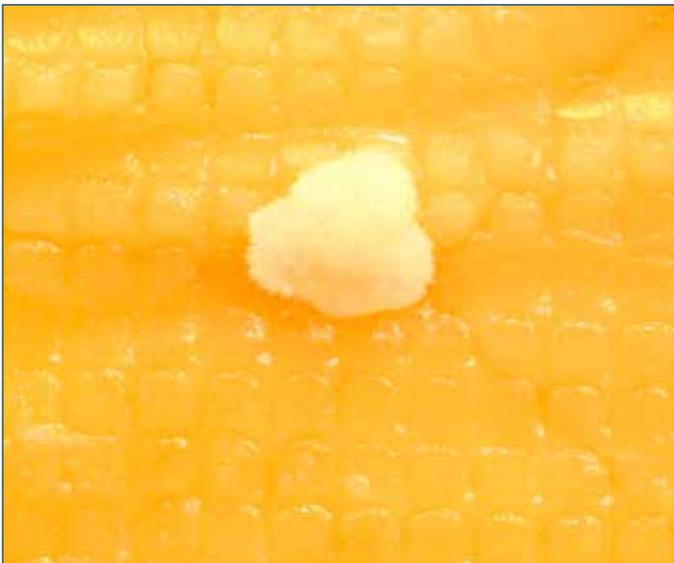
### Prevention or Reduction in the Appearance of Tyrosine Crystals

Currently the only means of preventing tyrosine (or leucine) from forming crystals is to avoid the use of *L. helveticus* as a starter or flavor adjunct. Of course, prevention of temperature abuse which induces sweating, greatly reduces the chance of forming a smear of crystals at the surface of a packaged cheese. It is rare to see tyrosine crystals in Cheddar that is less than three months old and in young Italian cheeses (Parmesan and romano) where it may take eight months or longer for tyrosine crystals to develop. The reason for the delay in crystal formation is that tyrosine is a product of protein hydrolysis or breakdown. Since this bacterium is a thermophile and the activity of its enzymes are slower at relatively cold ripening temperature used to cure these cheeses, it will take longer to accumulate enough tyrosine to form the crystals.

We cut blocks of Parmesan in half and aged one portion at 45° F and another at 50° F for 12 months. The cheese stored at 50° F had larger tyrosine crystals than cheese stored at 45° F (more proteolysis at 50° F than at 45° F). This was also in accordance with the concept that cheeses that sweat will have a greater potential for developing crystals since the cheese stored at 50° F had significant free moisture in the package

### Impact of loose packaging

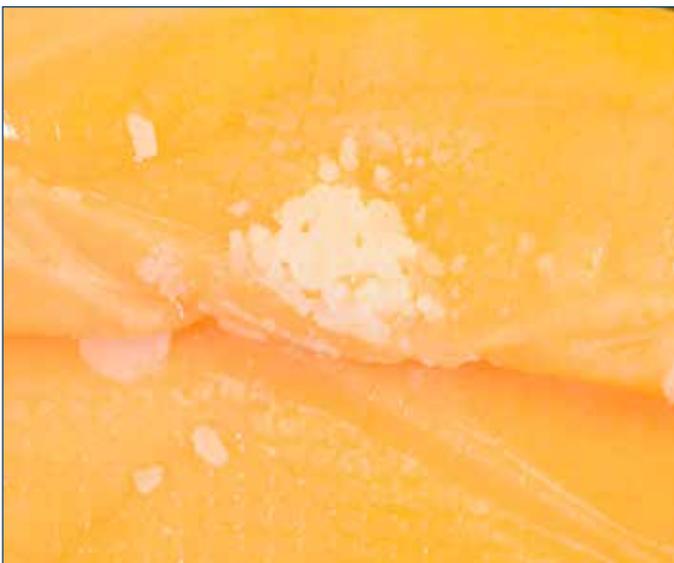




Magnified calcium lactate crystals.



Dots of calcium lactate crystals.



Calcium lactate crystals.

and inside the cheese. The cheese stored at 45° F did not show sweating on the outside but did exhibit only a tiny amount of free moisture in the cheese. Free serum in the cheese or on the surface of packaged cheese is normal for aged cheeses including Parmesan. Cheeses showing the smear of crystals at the surface have been stored at warmer temperatures or temperature abused in retail. In addition cheeses stored at higher temperatures also develop gas which causes a loosening of the package and accumulation of free serum at the cheese surface.

### Conclusion

There is still more to learn regarding the science behind crystal formation, but researchers have made many useful observations that can help the industry avoid crystal formation in their cheese. Being aware of the affect that temperature, acid, moisture and starter culture (or adjunct) selection can have on crystal formation in cheese is key to develop strategies to avoid crystal issues. Also, don't overlook the value of educating cheese mongers, grocery store managers and other members of the distribution chain about the role of proper cheese handling and temperature control in preventing unwanted crystal formation in cheese. It must also be recognized that some consumers demand that the cheeses that they purchase contain crystals. It's a harbinger of things to come. 🍌

Contributed by: Mark Johnson Ph.D, CDR

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## A LOOK AT A GROWING MARKET: MEXICAN CHEESES

Contributed by: Luis A. Jiménez-Maroto, CDR

According to the Wisconsin Milk Marketing Board, U.S. production of Hispanic cheeses totaled 241 million pounds in 2013. Of the cheese sold in 2013, cheeses that trace their origins to Mexico (Cotija, Oaxaca, queso fresco/ranchero, queso panela, queso quesadilla/Chihuahua) accounted for 30.07 percent of sales while cheeses labeled as "Mexican" (mostly multi-cheese shredded varieties) accounted for 64 percent of sales.

As is clear by the numbers, Hispanic and Latin American style cheeses are a popular choice among consumers. As this emerging market and the Hispanic/Latin American population within the U.S. continues to grow, these cheese varieties are worth considering when adding new and unique products to your line.

### History of Cheese in Mexico

Before 1492, pre-Columbian civilizations in the Mexican Plateau did not have any large domesticated mammals that produced milk in sufficient quantities to use as a source of food, let alone to create the need to transform milk into a longer shelf life product like cheese. It was not until the arrival of the European powers to the Americas in the late 15th century and the decades of conquest that followed, a period known as the Conquista (1492-1650), that dairy cattle, sheep, goats, and other domesticated animals, along with cheesemaking techniques, were brought from Europe to the then-called New Spain to meet the needs and demands of the the European immigrants. What occurred during the next 200 years was a continuously evolving, fusing, and mingling of both European and Amerindian cultures in a process referred to as mestizaje (from Latin mixticius, meaning mixed); the wide-ranging effects of which include Mexican cuisine and, with it, cheese. Dairy production and cheesemaking techniques were adapted to climate and local tastes of the different regions in Mexico, over time generating new cheese varieties.

### Cheesemaking in Mexico

Since colonial times, cheesemaking has been a part of the Mexican culture, but two regions have a long standing history of cheesemaking: the Comarca Lagunera area between the states of Coahuila and Durango, and the Altos de Jalisco area in the northeast of the state of Jalisco. Nowadays, the states of Aguascalientes, Chiapas, Chihuahua, Guanajuato, Michoacán, Oaxaca, Puebla, Querétaro, San Luis Potosi, State of Mexico, and Tlaxcala are also prominent cheese producing areas.



The exact number of cheeses varieties made in Mexico is uncertain due to different regions using different names for the same type of cheese, but it is believed that there are about

30 to 40 varieties of cheese indigenous to Mexico. Only about a dozen are well-known, however, and made in large volumes for national consumption; the rest are produced regionally in small quantities and are not very popular outside of their area (Espinoza-Ortega et al, 2008). Most of these cheeses are made with cow milk, with a few being made using sheep or goat milk.

Although legislation exists to regulate the quality and safety of cheeses in Mexico, there is no standard or hard reference for most Mexican cheeses, so product and manufacturing variability is the norm rather than the exception.

### Popular varieties of Mexican cheese

Mexican cheeses are part of the cuisine, and more often than not used as an ingredient and not as a standalone dish. Their functionality is as important as their flavor, considering that there is an expectation in the mind of the consumer regarding the melt, break, crumble, grate, or stretch. While the overwhelming majority of the popular varieties are unripened, a few do undergo months or years of maturation.

### Cheese Making Regions in Mexico



### Major Cheeses made in Mexico

**Fresco** ▶ Its name literally means fresh. It is a white, crumbly, unripened cheese made with whole milk that undergoes minimal pressing. Its main use is to be crumbled over dishes.

**Panela** ▶ Its name comes from the name of the basket molds used in its manufacture, which leave a characteristic imprint on its surface. Due to this, in some regions, it is also referred to as queso de canasta (basket cheese). It is a white, unripened, self-pressed cheese made with whole or part skim milk, typically from cow but can be mixed milk of cow and goat, with a high moisture content and relatively high pH, which causes significant wheying-off and make the cheese non-melting. Cheese sizes range from 0.5 lb to rarely larger than four lbs, as the soft curd would deform. It is used as an appetizer, in salads, sandwiches or where a non-melting cheese is required.

**Blanco** ▶ This cheese is popular among some Latin American groups in the United States, especially those with Caribbean origins, but is not commonly found in Mexico. It is a white, ▶▶

non-melting cheese made with cow milk by direct acid addition, usually with acetic acid (Villegas de Gante, 2004).

**Requesón** ▶▶ An unsalted whey cheese very similar to the Italian ricotta, it has a slightly sweet flavor and is used sprinkled on top of typical Mexican fare.

**Ranchero** ▶▶ Meaning “from the ranch”, it is also known in some regions of Mexico as Queso Molido, due to its crumbly, mealy texture caused by a grinding step during manufacturing, or Queso de Aro, referring to the hoop mold used in its manufacture. It is, perhaps, the most pervasive style of cheese in Mexico due to the simplicity of its manufacturing steps. Most often made artisanally in small batches using raw milk, it is also made industrially using pasteurized milk and starter cultures. A fresh, soft, unpressed cheese with a milky flavor and a crumbly texture, Ranchero is often used in the same way as Queso Fresco.

**Oaxaca** ▶▶ It is one of the most popular cheeses in Mexico and is manufactured throughout the country in sizes that range from a few ounces to several pounds (Villegas de Gante, 2004). Made with cow milk in a wide range of fat content, from whole milk to part skim, it is a white or slightly yellow cheese similar to a Monterey Jack but with the string-like texture of Mozzarella. It undergoes a cooking and washing step using hot water, from which a strand is pulled and salted by rubbing, then shaped into a yarn-ball. Along with queso Chihuahua, it is the quintessential melting cheese for quesadillas.

**Asadero** ▶▶ Very similar to, and often confused with, queso Oaxaca, with whom it shares very similar organoleptic and functional properties. The main differences are in the cheesemaking: Asadero undergoes a cooking step using whey instead of water, is salted during this cooking step, and is shaped as a block or a ball, but not as a yarn ball. It is used in melting applications and is the main cheese used for the dish queso fundido.



Selection of Mexican cheeses

**Chihuahua** ▶▶ This popular cheese is mostly known for the name of the northern Mexican state where it originated, but it also goes by other names. In the state of Chihuahua it is also known as Queso Menonita, for it was the Mennonite communities in the state who first commercialized it, but the Mennonite communities call it Queso Chester, for it is thought that it is a modified version of an English Cheddar. Furthermore, in the United States, the name Queso Chihuahua is trademarked, so it is sold as Queso Quesadilla. It is a semi-hard, pressed-curd, non-cooked cheese made with whole or part-skim cow milk. The cheese is ripened from a few weeks to months and its flavor develops over time. A very versatile cheese, Chihuahua can be used as a snack, in desserts, or as an ingredient in a variety of dishes. Its melting properties and pleasant flavor make it a very common cheese to use in quesadillas.

**Mexican Manchego** ▶▶ Similarities between this cheese and the original Manchego from the La Mancha region in Spain begin and end with the name. Mexican Manchego is a semi-hard, pressed-curd cheese made from cow milk and ripened for 2-3 weeks, whereas the original Manchego is made from sheep milk and ripened for several months. The flavor and texture of Mexican Manchego is somewhat similar to a Monterey Jack, with good melting properties that make it a popular cheese for use in melting applications in Mexican cuisine.

**Cotija** ▶▶ The most popular ripened cheese in Mexico, Cotija was originally made in the mountainous region between the states of Jalisco and Michoacán. It is a hard, pressed-curd cheese made with whole or part skim cow milk, with a high salt content (4-6%) and ripened a minimum of 3 months. As with all other Mexican cheeses, it is made with raw milk at the artisanal level, and with pasteurized milk and starter cultures at the industrialized level. The resulting cheese is strongly flavored and grated as topping in many Mexican dishes. In recent years, this cheese has received a Collective Brand designation, which states it must be made from raw milk obtained from free-range cross-bred cows (Holstein-Zebu or Brown Swiss-Zebu) from the region between the states of Jalisco and Michoacán, set using calf rennet, hand salted, and aged at least 100 days and up to five years. 🍷

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## PERMEATE UPDATE

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### Overview

Permeate (which can be labeled as dairy product solids) is a by-product of the ultra-filtration process of milk or whey. The composition of permeate will vary depending upon the original milk composition, but essentially, permeate includes products that have a minimum of 59 percent lactose, a maximum of 10 percent protein and 27 percent ash. Permeate contains milk salts (minerals), lactose, amino acids and peptides, all of which contribute to permeate's functional properties.

Baked goods, soups, sauces, confections, dry mixes, meats and beverages can all benefit from the use of permeate as a cost-effective and functional ingredient. Permeate can enhance the browning and moisture retention of baked goods. It can also provide the functionality of sucrose but with less sweetness to a baked product or confection. Permeate provides a clean label approach for replacement of other carbohydrates such as maltodextrin or dextrose as well. As sweet whey availability declines, permeate can also be used as a sweet whey substitute at a cost benefit. The high mineral content of permeate also provides a natural source of the milk minerals; calcium, potassium, and magnesium to enhance the nutritional properties of foods. This mineral content also aids in helping to reduce sodium in foods by providing some salty character while enhancing other flavors in the food.

In general, 10 grams of permeate will replace 1 gram of salt, however, other ingredients such as flour, fat, eggs and sugar can be reduced to balance the formulation. Overall, a total cost-reduction can occur as permeate will reduce the amounts of other ingredients needed in a formula.

### Types of Permeate:

- ▶ Whey Permeate, which is sometimes called deproteinized whey, is known for its good solubility and pleasant dairy flavor.
- ▶ Milk Permeate has fewer processing steps because it comes directly from milk and is known for its clean, consistent flavor.

### Utilization

The American Dairy Products Institute (ADPI) has been tracking permeate production and utilization for a few years now. While the product is relatively new, ADPI's data shows a significant amount of growth in permeate production. For example, in 2010, there was record amount of permeate sold for use in human food, but the majority, 51.7 million pounds, was sold for animal feed. By 2013, 18.2 million pounds of whey permeate was used for food and 135.2 million pounds for animal feed. The dairy industry used 61 percent of the permeate while 34.1 percent was used in prepared dry mixes & dry blends and 4.4 percent was used by beverage manufacturers.

### Export and What's Next for Permeate

CDR continues to support permeate suppliers by evaluating their ingredients in different food applications. We are also helping to increase permeate awareness by showcasing products that use permeate at various events. For example, CDR has developed permeate prototypes for the U.S. Dairy Export Council (USDEC) booth at the Institute of Food Technologists annual meeting eight of the last 14 years.

Efforts to share our permeate knowledge have also expanded overseas in cooperation with USDEC. CDR staff have given technical seminars in Ho Chi Minh City and Hanoi, Vietnam, contributing to efforts that have aided in the growth of U.S. whey permeate exports, which have increased by over 45 percent in the last five years (ADPI, 2013). Since these seminars, which included one-on-one visits with food companies, the U.S. has started exporting permeate to Vietnam for food use. Technical seminars will also be given in Beijing and Shanghai, China in October 2014, though China currently does not allow permeate in foods. The permeate story will also be told in Japan and Korea in November 2014.

Educating food scientists on the benefits of permeate is key in creating opportunities for the regulations to change in China and for other countries to adopt the use of permeate. Though utilization of dairy protein ingredients has been the most recent success story for sports nutrition, weight management, healthy aging and other nutritional products, permeate has a lot of potential to be a success story in food applications as well. 🍌



VeggieMoo Dumpling,  
Reduced Sodium Sauce



Sweet and Savory  
Butternut Squash Soup



Cucumber Lemon  
Yogurt Drink



Cheesy Cruncher



Products developed at CDR using permeate.

## COMMERCIAL COLORANTS IN WHEY AND CHEESE: AN EVOLVING INDUSTRY

### Annatto as a color source

Annatto and similar carotenoids have been used in cheesemaking since the 16th century and have generally proven to be a safe and effective way to create an orange tint in Cheddar and other similar style cheeses. Carotenoids, which include annatto compounds and beta-carotene, are a group of pigments found in fruits, vegetables and plants like grasses. Bixin, an oil soluble carotenoid present in annatto is processed from the extract of the achiote seeds and converted to the water soluble compound norbixin, which works well in coloring natural cheese. While annatto remains an extremely popular colorant within the cheese industry, the annatto color is quite stable and studies show that between 10 to 26 percent of the annatto added to milk enters the whey stream. Thus, whey products made from colored cheese also have some yellow color, which is undesirable for many of its food applications.

### Eliminating Color in Cheese Whey

Several methods have been developed to remove annatto from the whey via filtration processing, however, many companies use hydrogen peroxide and benzoyl peroxide to oxidize the residual annatto. This process, which is commonplace within the food industry, is often nicknamed “bleaching” because the oxidation reaction causes the orange tinted whey to turn a bright white color. Of course, the process is more complicated than we’ve outline here so for more on the pros and cons of color removal please review the upcoming Wisconsin Center for Dairy Research handbook, Annatto and Color Removal which will be published in late 2014. Essentially, companies should note that the U.S. permits the use of both peroxides in food as a bleaching agent. CODEX, however, only permits benzoyl peroxide INS No 928 as a bleaching agent, but this excludes use in whey powders for infant food. Additionally, the European Union and most Asian markets do not permit the use of benzoyl peroxide in any whey products whatsoever.

### Beta-Carotene as a color source

Beta-carotene is a naturally occurring pigment in milk and whey, but historically, the pigment alone was not widely used in cheesemaking. Recently, however, companies including Chr. Hansen and Socius Ingredients have developed several beta-carotene based commercial colorants which are more suitable for use in cheesemaking. Early studies (referenced by Chr. Hansen) show that some of these newly developed beta-carotene colors may leave behind as little as one to three percent in the whey stream, allowing for an end product that has the desired white color but does not require bleaching. These early studies suggest that this type of colorant may be an excellent alternative for U.S. companies looking to avoid the bleaching step in whey processing.

### The European Union’s position on added color

It should be noted, for those companies that are interested in exporting their whey for use in infant formulas that the EU does not allow any addition of colorant (annatto or beta-carotene) to cheese for whey that is destined for infant formula.

In fact, the EU recently updated its stance on colorants in whey used for infant formula.

It states *“Colourings shall not be present in ingredients supplied to Baby Nutrition. Regulation (EU) 1333/2008 in combination with Regulation (EU) 1129/2011 does not permit the carryover of a food additive including food colours to infant formulae, follow on formulae, processed cereal based baby food and baby food for infants and young children.”*

Essentially, this means that while colorant will still be allowed in cheese, that colorant cannot be present in the whey stream if the end product is to be used in infant nutrition products.

In fact, Michael Hickey, Chair of the Science and Program Coordination Committee, International Dairy Federation and Principal at Michael Hickey Associates, a food and dairy consulting agency, states that “the EU does not allow the use of beta-carotene (or any other color) as an additive in infant formula. Additionally, no carry-over of color is allowed. The EU is aware of the potential use of beta-carotene in dairy products and it is anticipated that testing for this ingredient will be carried out.”



Whey Protein Concentrate (WPC 34) processed with beta-carotene (left). WPC 34 processed with Annatto (right).

### Asia’s position on added color

According to the American Dairy Products Institute (ADPI), the Far East imported 343,981 metric tons of dry whey from the U.S. last year. Given that this market is quickly growing, it is important to note Asia’s regulations in regards to colorants in food.

The regulatory environment in China is rapidly changing, but (currently) permitted food additives can be found in their new document, National Food Safety Standard for Uses of Food Additives (GB 2760), which, according to the United States Dairy Export Council (USDEC) states that “beta-carotene (160a) is permitted for use in all foods so long as the appropriate amount as required in production is used.”



It's important to note, however, that infant formula does not fall under the "all foods" legislation in China. In regards to infant formula and China's carry-over policy, "a food additive can be introduced through ingredients according to the national standard" if the level of the additive in food ingredients does "not exceed the allowable maximum level" and ingredients were applied in the normal production process. Additionally, the content of a specific additive in the food should "not exceed the level that is carried over by the ingredients and the content of this additive introduced into the food by ingredients shall be notably lower than the required level of such additive through direct adding into the food."

Beta-carotene testing in whey for infant formula is likely to be done according to Chinese standard GB 5413.35-2010. All products are subject to any such testing, to ensure regulation compliance is upheld. Beta-carotene now has its own food safety standard in China (GB 28310-2012). Note that beta-carotene is permitted in China as an additive in different types of cheese up to a maximum level of 1.0 g/kg.

Annatto, on the other hand, is permitted in processed cheese up to a maximum level of 0.6 g/kg (according to GB 2760) and the Chinese Ministry of Health (MOH) issued formal

notice No. 23, 2010 that indicated that annatto could be used in natural/ripened cheese up to a maximum level of 0.6 g/kg.

### Conclusion

These newly developed beta-carotene colorants offer cheesemakers the potential to produce white whey without the use of a bleaching process which provides many benefits. Work, however, is ongoing to evaluate the stability of these new colorants in cheese. Additionally, their use in whey destined for use in infant formula remains controversial. At this time, companies exporting whey to the EU will need to either supply whey from uncolored cheese, or develop technologies to color the cheese itself (not add color to cheesemilk) and thus avoid the issue of any color carryover into the whey. On the other hand, it appears that low levels of beta-carotene carry over in the whey is acceptable for the Chinese infant formula sector, however, China does not allow for the presence of benzoyl peroxide and benzoic acid in whey powders and will be testing imported whey powders for these materials. As long as these regulations are kept in mind, cheesemakers should feel comfortable using the colorant method of their choice. 🍌

*Please contact Dr. John Lucey with any questions regarding this material [jlucey@cdr.wisc.edu](mailto:jlucey@cdr.wisc.edu)*



## TURBO BOOSTING THE DAIRY INDUSTRY

The CDR TURBO program would like to extend a thank you to all of the individuals and companies who have supported the program's efforts thus far. We have received a number of inquiries regarding our technologies and we truly appreciate the partnerships that have been formed.

In particular, the CDR TURBO program is proud to announce that we have accepted "Letters of Intent" for three of our TURBO technologies in addition to continuing our work with Dairyvative on their own technology.

While the details regarding each partnership are not available for disclosure, we can share with you that work has begun on "Fun Flavored" Process Cheese. Dana Wolle, CDR Assistant Coordinator of Cheese Industry and Applications is working with Whitehall Specialties to develop a "Fun Flavored" processed cheese suitable for export to Southeast Asia and China.

While a few technologies have been licensed, there are still a number of technologies available for technology transfer. All of our technologies are listed within our tech transfer portfolio which is available on the CDR TURBO website. We are also excited to announce that CDR will be making several additional technologies available over the next few months. So, be sure to check back often for updates on the TURBO program and its available technologies.

We are pleased that so many of you see the potential for growth in our industry and we look forward to working with many of you on these projects. If you have any questions or wish to participate in the program, please feel free to contact CDR TURBO coordinator, Vic Grassman at [vgrassman@cdr.wisc.edu](mailto:vgrassman@cdr.wisc.edu) or 608-512-6661. 🍌

## INDUSTRY EXPERT INSIGHT

As a part of our short courses, CDR often invites dairy industry experts from around the world to assist us in providing the very best education for our students. CDR is honored to host these individuals and we are excited to share with you a few insights from our visitors. **This quarter we are highlighting Anne-Claire Bauquis of Chr. Hansen France who joined us for the Master Artisan Short Course, Cheeses of the Alpine Region, September 23-25, 2014.**



*What is your current title/position at your company?*  
Marketing Manager for Cheese Cultures at Chr.Hansen

*What is your background? Education? Areas of Expertise?*  
I have a Food Engineer degree from ENSAIA (France) and a Masters degree in Microbiology from Heriott Watt University (UK).

My areas of expertise are:

- ▶ Food Biochemistry
- ▶ Antibiotics from marine environment
- ▶ Microbiology of fungi, aerobic & anaerobic bacteria
- ▶ Interactions between microorganisms
- ▶ Fermentation processes

*How did you become interested in cultures?*

As a microbiologist, it is very natural to be interested in cultures. Microorganisms can be both a source of spoilage or diseases and thus, highly undesirable or else essential in all processes of fermentation and highly beneficial for the production of metabolites. I like to be able to understand and get the best benefits from each type of cultures: Molds, yeast and bacteria. Each type, each species and even each strain has its own specificity and requirements for growth. Each one can be either inhibited or boosted according to the environmental conditions and according to positive or negative interactions. It is "mother nature" in all its diversity.

*Why do you feel that it is important to educate cheesemakers about cultures? Microorganisms as part of a microscopic world are complex to understand. Cheesemakers are users of cultures, and sometimes grow the cultures themselves but are not specialists of the art. When you start to explain about microorganisms, about all the effects the cultures have in cheese in concrete words, they get immediately very interested.*

*If you could tell the cheese industry one thing about cultures, what would it be?*  
"Cultures are like a tool box for you: Choose the tool that you need, and get the best out of it!" 🍷



## 2015 DAIRY SHORT COURSES



January 6-7, 2015  
Milk Pasteurization



January 13-15, 2015  
Batch Freezer Workshop



February 10-11, 2015  
Wisconsin Dairy Field Reps



February 24-25, 2015  
Wisconsin Process Cheese



March 3-5, 2015  
Buttermakers



March 16-20, 2015  
Cheese Technology



April 26-30, 2015  
World of Cheese



May 5, 2015  
Wisconsin Cleaning & Sanitation



May 6, 2015  
HACCP Workshop



May 12-13, 2015  
Applied Dairy Chemistry



June 2-4, 2015  
Cheese Grading



August 4-5, 2015  
Milk Pasteurization



September 8-9, 2015  
Cultured Dairy Products



September 22-24, 2015  
Master Artisan Course



October 12-16, 2015  
Cheese Technology



October 13-14, 2015  
Dairy Ingredient Applications



November 4-6, 2015  
Cheese Grading



November 10-11, 2015  
Waste Water



December 2-4, 2015  
Ice Cream Makers

For more information on each short course see  
[www.cdr.wisc.edu/shortcourses](http://www.cdr.wisc.edu/shortcourses)

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## Short Course Calendar:

- Cheese Grading Short Course, November 5-7
- Ice Cream Makers Short Course, December 3-5
- Milk Pasteurization, January 6-7, 2015

**For detailed information on each CDR short course:**  
[www.cdr.wisc.edu/shortcourses](http://www.cdr.wisc.edu/shortcourses)

## Events:



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