A Brief View to Spores in Dry Dairy Ingredients

Rafael Jiménez-Flores, Prof.
Dairy Products Technology Center
California Polytechnic State University
e-mail: rjimenez@calpoly.edu

The international trade of milk powders holds big hopes in the future growth of our industry. Most predictors indicate an increase on demand of milk, milk protein and dairy components in various forms. Most popular and of current demand are skim milk powder (SMP), and non-fat dry milk (NFDM). In national and international markets, the traditional classification has been modified by a very specific microbiological quality specification: spore count.

Besides basic descriptors and specifications for SMP or NFDM, some buyers of these commodities are asking for certain levels of spore counts. Low spore counts are ill defined and mostly left to the buyer's definition. One big factor that further complicates this specification by the industry is the biological aspects of spore counting. Therefore, this article aims to give some insight as to what and why we find spores in milk powder. This may also apply to other dairy powders, and it is a nascent concern for dry products such as whey powder, WPC and even MPC.

<u>Spore definition</u>. Unlike many of the other food industries, the dairy powder industry is concerned today with bacterial spores or endospores, rather than the more ubiquitous fungal spores. The endospore is a dormant, tough, non-reproductive structure produced by a small number of bacteria from the Firmicute family such as *Bacillus* or *Clostridia*. These spores present structures that are very resistant to dehydration, heat and many chemicals. They have been studied for a long time, and are unique to the dairy industry. The spores directly isolated from milk, are much more thermally resistant than those from the same microbe but grown in laboratory media.

<u>Origin of spores in dairy products.</u> Common questions from the people that work in industry are: Where do the spores come from? How do they get into the processing plant? The answer to each question is not a single or an easy one. As to where the spores come from, it is well known that endospores from bacteria are ubiquitous in the soils and most environments. In particular the *Bacillus* germs live in soils and plants; the different bacillus and clostridia therefore are present in dust and agricultural products. This is particularly important for the dairy producers, because most materials used in making the feeds for cows, are a source of spore-forming bacteria. Silage is a good example; although the inoculation for silage is aimed to promote lactic acid bacteria growth and lowering of pH for prevention of mold and other pathogens, many bacilli can grow and sporulate during silage production. Therefore we find large amounts of spores in silage. It is well known that makers of Swiss cheese prefer to buy milk that comes from cows fed grass (or at least not silage).

This empirical observation leads us to think what is particular about cows fed silage. While we do not have specific answers, some research at DPTC, and elsewhere, points towards the environment. It is unlikely that the spores make their way into the milk via the cow and its milk producing biological system. Raw milk samples directly taken from cows show no spores even after concentration of solids. No spores were found even concentrating one liter of milk. On the other hand, environmental samples of feed, bedding, water (especially drinking water shared with birds), and air around milking parlor all showed spores in different abundance. We are conducting a study (one year long) to monitor the fluctuation of spores around the cows at Cal Poly Dairy Farm. So we can say with some assurance, that spores get into the milk through the course of handling milk after milking. Of particular importance for milking parlors in dry and dusty places, is the air surrounding milking parlors, since endospores are naturally airborne.

The answer to the second question, (How do spores get into the processing plant?) is a most complicated one. The spores normally would come in and out the processing plant without much alteration of milk quality. The endospores of most bacilli and clostridia survive pasteurization, and take some time before germination. In fluid milk, the presence of some spores has been identified as culprits of reduction of shelf life. Especially the bacteria known as *Paenibacillus* spp. is of concern, because the viable (or alive) organisms can grow at very low temperatures, such as those of pasteurized milk in our refrigerators.

In the case of milk powders, it has been recognized, that the problem with spores is not large when considering raw milk, where experts calculate that raw milk has between 10 to 50 spores per liter of milk (USDEC recently edited a summary on spore-formers in milk). However, sometimes powders or dry dairy products can contain spore counts in excess of 1000 spores per gram of solid. Concentration alone does not account for such counts. One of the most favored explanations from experts in the field is the production of biofilms in the processing plants. These biofilms are complex aggregation of microorganisms growing on a solid substrate, and stainless steel has been proven to be a good substrate for bacilli. Biofilms are characterized by a complex structure composition (carbohydrates, proteins and fats), genetic diversity is common since several organisms can accomplish different roles, complex community interactions. It is not very clear what the initial steps of biofilm formation are in dairy processing plants. In the laboratory, the biofilm formation begins with the attachment of free-floating spores or microorganisms to a stainless steel surface that may show abrasions or certain nucleation points. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. If the colonists (either live bacteria or spores) are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion molecules or organelles such as pili.

The biofilm grows in size and complexity as time elapses without any cleaning intervention. As we mentioned before, the attachment of the biofilm is extraordinarily complex, but thorough industrial cleaning does a good job of removing these residues from most stainless steel surfaces. Exceptions may be found depending upon configurations and ease of access to the chemicals used in cleaning-in-place practices.

Spore Enumeration Challenges. Once the dairy fluid reaches a concentrated or dried stage, the spore population will not grow, and most bacteria do not survive or thrive in dry powder. The spores will remain dormant until favorable conditions induce their germination. Here again, there is a lack of understanding of the clear requirements for efficient and total germination of all the spores present in any sample. Scientist are still studying and trying to understand this so far mysterious step in the emerging of life of a microbe from its spore. However, in most food and dairy quality control laboratories, we rely on a 'HEAT SHOCK'. Through many experiments, we have found out that giving our milk samples a heat treatment, causes two microbial reactions; kill some live bacteria that can't stand the heat, and induce spores to germinate. While this is one way to induce germination, the problem for quantitation resides in that not all endospores germinate after the same heat shock!

Some species of spores, germinate after a heat treatment of 80˚ C (176˚ F) for 12 minutes (however, some live bacteria can survive such treatment). Some other organisms need (absolutely) a treatment of 100˚ C (212˚ F) for 30 minutes, before they germinate! When several of these kinds of spores are found in the same milk sample (remember that biofilm is complex and can have many kinds of organisms), the absolute quantification of spores becomes extremely difficult. An added factor is that in order for us to become aware of the presence of these organisms, it is not enough to induce their germination, we must allow them to grow so we can count their colony forming units (cfu in the QA lingo). Here, again, variety indicates that even at optimal nutritional conditions some organisms grow at 32˚ C (90˚ F) but not at 55˚ C (131˚ F) and vice versa.

What is next? Perhaps now it is clear to the reader why there are so many different protocols for detecting endospores in milk. Many international technical laboratories and scientist have focused on the microbes that are most heat resistant, both for germination as well as for growth, as problematic organisms. This may hold true for uses of milk where very high temperatures will be used, such as UHT processes. However, we do not have much information as to how other spores, those that may have milder germination conditions, or those that may be sensitive to nutrient signals regarding temperature induction, may affect the foods containing dairy products.

It is our belief in DPTC that with more judicious analysis of this phenomenon, we can design better preventative measures. It is clear that close collaboration between producers and processors would be ideal in finding solutions.