Thank you. It is an honor and a privilege to be able to come today and speak to you regarding something I am very passionate about. I am going to tell you right out of the chute, I am not a bakery expert, I am a true microbiologist and for those of you who do not know the definition of a true microbiologist, it is those individuals who wash their hands before they go to the bathroom.

So today I am going to talk about some new natural mold inhibition technology for bakery products and some of the things I am going to run through are, why natural...why is it becoming such a big trend and we heard earlier today that the definition of natural is vague and not well defined. I will talk about hurdle technology and the importance of a series of steps, ingredients or processes that you can do to increase the stability and safety of your product. I will then talk on natural solutions, focusing on fermentates, natamycin and natamycin applications (Slide 3).

If we look at natural claims for new foods and beverages introduced globally, from 2001 to 2008, you can see that the largest number of new products hitting the market during this time were those containing no additives/no preservatives, followed by organic, all natural and whole grain (Slide 4).

So, why is natural such a big deal? Well, society has really come to understand the importance of health and nutrition. Consumers are looking for better-for-you foods, which includes fortified or functional foods or those foods which have lower salt, sugar, higher fiber, things of that nature. This is part of what is driving this market with the second being a cleaner label declaration. So we want to reduce the allergens and remove the chemical preservatives. This has really created these natural product brands and is ultimately what is driving this sort of thing in the market (Slide 5).

If we look at Hurdle technology, it is basically a series of steps, processes and ingredients that are going to help maintain the stability, quality and safety of your food. Obviously, the goal is to minimize the sensory effects and it is generally set up to inhibit unwanted organisms. This becomes an extremely powerful tool when you think in the U.S. that millions get sick every year and globally about 25% of the food is discarded due to spoilage (Slide 6).

How does hurdle technology really work? As you continue to add hurdles, it becomes increasingly more difficult for the bugs to overcome the situation. One such hurdle could be moisture. Sometimes we have
bread products with high moisture levels. You try to manage or reduce it as best as you can, this could be one way to increasing the shelf life. When you go and add, for example, pH, in some cases for bakery products we are kind of stuck in a specific range and obviously if you go too low you are going to have some sensory issues. That again assists in extending the shelf life. If you add temperature to the mix, that is another hurdle. This is followed by our final hurdle example which is antimicrobials. The goal is to continue to add hurdles until the unwanted organisms can no longer overcome those hurdles and we reach statis or cidal activity (Slide 7).

If we look at what is currently available or is used in the bakery industry as natural antimycotics, we have the following: Vinegar, Raisin Juice Extract, Plum Powder, Fermentates, and Natamycin. It is important to note that some of these options can impart a color difference, which is the case for raisin juice extract and plum powder. These are typically used in whole wheat or whole grain products. Things like vinegar could impart a flavor. When switching to a natural antimicrobial system, it is always recommended that a sensory analysis be performed on your product before moving forward with your products (Slide 8).

Fermentate technology is not new and has been around for thousands of years. Fermentation is used to produce things like yogurt, cheese, butter, beer, bread, and wine. This is the basis for commercial fermentate antimicrobials(Slide 9).

How do you make a fermentate? Well, you take a GRAS Organism, add it to a substrate, let’s say sugar, and you let them grow up. You take the product of that growth, which could contain things like organic acids, peptides, and other antimicrobial compounds, run it through a kill step to get rid of the organisms, and spray dry into a powder (Slide 10).

Many of the fermentates used in the bakery industry rely on organic acids to inhibit mold. Organic acids are pH dependent; thus, the activity is influenced by the pH of the final product. The active portion is related to the undissociated form of the acid. When the pH increases, the amount of undissociated acid decreases; thereby negatively influencing activity. So, the lower you can get the pH of a product, the more effective the fermentates can be (Slide 11).

When you look at how this works on a cellular level, the normal cell will maintain homostatis by moving H+ ions into and out of the cell. Now, the undissociated form of the acid comes along and is taken into the cell. It is allowed to pass into the cell because it is neutral in charge. Once inside the cell, the acid encounters a more neutral pH, resulting in the dissociation of the acid. The cell now has an abundance of H+ ions that it needs to get rid of to maintain homostatis. The cell spends its time trying to maintain homostatis and as a result, is not focused on growth (Slide 12).

If you are thinking about going with a fermentate to control yeast and mold there are many things to consider. These include what carrier is being used, if there are allergen concerns, how is it labeled, what is the flavor impact, and what is the cost in use (Slide 13).

One can take the fermentate and go through a purification step to pull the specific compounds of interest out, like Natamycin (Slide 14).

Natamycin has been used in the food industry for over 30 years with research demonstrating no development of resistant strains. It is effective at very low concentrations in the 1-20 ppm range. In addition, it is active over a wide range of pH between 3-9. It also does not impact flavor (Slide 15).

A general mode of action for peptide and similar compounds is as follows: The peptide binds to the cell wall; the cellular membrane is destabilized; molecular leakage occurs and the cells lysis. Recent research suggests the mode of action of natamycin is based on the inhibition of growth by binding to the ergosterol on the cellular membrane with no disruption to the membrane(Slide 16).

When you compare Natamycin to chemical preservatives you can really see the difference. Natamycin is natural and is cidal, Propionate is chemical and is fungistatic. Natamycin has no effect on bacteria; whereas, propionate is bactericidal. Additionally, due to its low solubility in water, natamycin stays on the surface of the bakery product. This is important because the mold will be on the surface but propionate tends to penetrate into the food. Natamycin has no flavor impact on the product while propionates tend to leave a bitter flavor. For propionates to be effective,levels need to be in the 1000-2000 ppm range however natamycin can be used
at much lower concentrations in the product, between 1-20 ppm. Natamycin, unlike propionates, are not pH dependent and work over a wider pH range (Slide 17).

When applying natamycin on the surface of bakery goods, it is best to spray it on the surface of the product immediately after baking and depanning. Due to its low solubility in water, natamycin requires constant mixing to maintain the suspension in water. Natamycin can be applied as a single pass or recirculating system depending on the product and type of process. It is important that you choose the best spray system for your needs (slide 18).

When looking at various spraying systems, it is important to note the advantages and disadvantages of each of these options. For example, let’s look at the spinning disk or spinning drum in a recirculating system. The advantage would be, no concern of overspray loss and it works well with products that are distributed randomly through the spray zone. This may be seen with products like tortillas or English muffins. On the flip side, contamination of the recirculated suspension can be an issue and needs to be monitored. The system needs to be monitored for potential settling points where natamycin could fall out of suspension (Slide 19).

The next type of spraying system one could look at is a single pass system with nozzles that utilize air pressure to spray the fluid. The advantages to this system is that the suspension is less likely to become contaminated, it’s easier to clean, and works well when products flow orderly through the spray zone. The difficulties include getting adequate coverage without excessive overspray and keeping the nozzles from clogging (Slide 20).

Another single pass option could be a system with Ultrasonic Nozzles. The advantage of this system is the nozzles do not clog, you in theory have more coverage with less material and like the previous system, it works better when products pass orderly through the spray zone. The disadvantage is there is excessive overspray and the nozzles are very expensive (Slide 21).

So, when choosing a spray system you must keep these 5 items in mind. 1 – The repeatability of the sprayed liquid volume, 2 – Even distribution across the surface of the product, 3 – Accurate flow rate compensation to accommodate conveyor speed changes, 4 – Spray validation for each spray cycle of every nozzle, 5 – Efficacy of the process proven by lab results (Slide 22).

In this slide, we have the results of a commercial trial using natamycin and various levels of fermentates. As you can see, the control product started molding in about 7 days and all the loaves molded in roughly 20 days. The 1% cultured wheat flour started molding on day 12 and only 8 loaves molded over the 30 day study. The 2% cultured wheat flour started molding after 18 days and only 5 loaves molded. The 14 ppm of natamycin started molding around day 20 and 3 loaves were molded over the course of the study. When you added the natamycin at 14 ppm and cultured wheat flour together, you start to see mold around 17 days, with only 2 loaves molding over the study. Finally, with 14 ppm natamycin and 2% cultured wheat flour, none of the loaves molded. As you descend down the variables, mold starts at various times but you see that less and less loaves are molding over the 30 day study (Slide 23).

In summary, today we talked about why natural has become so important, the value of hurdle technology, some of the natural solutions available in the market place, how fermentates and natamycin work, the ins and outs of natamycin application, followed up with the results of a commercial bread trial (Slide 24).

Thank you.
Jerry Erdmann
Team Manager – Food Protection

New Natural Mold Inhibition Technology for Bakery Products

Why Natural?
Health & Nutrition –
Better-for-you Foods
Reducing Allergens
Removal of Chemical Preservatives
Supporting Natural and/or Organic Product Brands

How They Work – Multiple Barriers

Hurdle Technology
Combinations of treatments and/or ingredients used to enhance shelf life, safety, and quality of foods
Minimize effects on sensory qualities of foods
Generally, meant to eliminate or inhibit growth of unwanted microorganisms!

Natural Antimycotics - Bakery
Vinegar
Raisin Juice Extract
Plum Powder
Fermentates
Natamycin

‘Natural’ Claims Over Time
New food & beverage introductions globally - 2001-2008
All Natural Mold Inhibitors

Commercial Microbial Fermentation by Lactic Acid Bacteria

The basis for commercial fermentate antimicrobials

LACTIC FERMENTATION

FRUITS & VEGS

Lb, Leu, Ped

Dairy

Lactococci, Lb, Prop, Leu

MEATS

Pediococci, Lb

SILAGE

Lb

BREADS

Lb

The basis for commercial fermentate antimicrobials

Mode of Action: Organic Acids

Percentage undissociated at pH:

\[ \text{Mol wt.} \times \text{pKa} = 5.0 \times 6.0 \times 7.0 \]

<table>
<thead>
<tr>
<th>Acid</th>
<th>pKa</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>60.1</td>
<td>4.76</td>
<td>35</td>
<td>5.2</td>
</tr>
<tr>
<td>Propionic</td>
<td>70.1</td>
<td>4.87</td>
<td>43</td>
<td>6.9</td>
</tr>
<tr>
<td>Lactic</td>
<td>90.1</td>
<td>3.85</td>
<td>0.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>

- Antimicrobial activity: acetic > propionic > lactic

Fermentates Considerations

- Carrier
- Allergen concerns
- Label
- Flavor Impact
- Cost in Use

Fermentates

Purified Antimicrobials

General Mode of Action

Natamycin

- Used in the food industry for over 30 years
- No development of resistant strains
- Effective at low concentrations (1 - 20 ppm)
- Active over a wide pH range (pH 3 - 9)
- No flavor impact

Cell Wall Binding

Cellular Membrane Destabilization

Molecular Leakage

Bacteriocidal Functionality

Bacteriostatic Functionality
### Natamycin versus Propionate

<table>
<thead>
<tr>
<th>Natamycin</th>
<th>Propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Chemical</td>
</tr>
<tr>
<td>Fungicidal</td>
<td>Fungistatic</td>
</tr>
<tr>
<td>No effect on bacteria</td>
<td>Bactericidal</td>
</tr>
<tr>
<td>No migration into food</td>
<td>Penetrates into food</td>
</tr>
<tr>
<td>Bitter flavor</td>
<td>No flavor</td>
</tr>
<tr>
<td>Effective at 1-20 ppm</td>
<td>Effective at 1000 – 2000 ppm</td>
</tr>
<tr>
<td>Effective only at acidic pH</td>
<td>Effective at pH 3 - 9</td>
</tr>
</tbody>
</table>

### Application on Bakery Products

- Spraying on the surface immediately after baking and depanning
- Natamycin requires constant mixing
- Applied as single pass or recirculating
- Determination of appropriate spraying system

### Types of Spray Systems

- **Spinning disk/drum – Recirculating system**
  - Advantage: No concern of overspray loss
  - Random distribution through the spray zone
  - Disadvantage: More contamination of the recycled solution
  - Settling points for Natamycin suspension

- **Nozzle with air pressure/Pressurized fluid – Single Pass**
  - Advantage: Solution less likely to become contaminated
  - System easier to clean
  - Disadvantage: Difficulty with coverage without excess overspray
  - Nozzles can become clogged

- **Ultrasonic Nozzles**
  - Advantage: No clogging of nozzles
  - More even coverage with less material (In theory)
  - Disadvantage: Spray pattern directed by air jets
  - Excess overspray
  - Nozzles very expensive

### Commercial Trial

- **Commercial Bakery Natamycin Trial**
  - Control (vinegar)
  - 1% Cultured Wheat Flour (CWF)
  - 2% CWF
  - Natamycin 14 ppm
  - 1% CWF + Natamycin 14 ppm
  - 2% CWF + Natamycin 14 ppm

### Application Choice

- Repeatability of sprayed liquid volume
- Even distribution of across the surface of the product
- Accurate flow rate compensation to accommodate conveyor speed changes
- Spray validation for each spray cycle of each nozzle
- Efficacy of the process proven by lab results

### Summary

- Why Natural
- Hurdle Technology
- Natural Solutions
- Fermentates
- Natamycin
- Natamycin Application
Thank You

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