Glad to be here and thank you for coming out in such great numbers and I hope you will not be disappointed.

(Slide 2) When this title was proposed, one realizes very quickly what a broad subject it is, so what to do? It is possible to focus on a specific subject, which we haven’t done, we hope to give you a flavor of what is currently going on in the area of enzymes.

I will not be talking about details of enzymes because everybody knows that they are proteins and that they catalyze enzyme reactions in the dough and in the bread.

(Slide 3) In order to make this an even-keeled and multi-angled presentation, many people have contributed to this presentation. Some of them are here from the bakery industry: Flora Lau, Wheizu Yu and Theresa Cogswell, I am very grateful for that. Also, for my colleagues at Caravan Ingredients and CSM in Europe and furthermore inputs from AB Enzymes, DSM and Novozymes. Several graphs and the pictures and data of baking experiments that you will see later on in the presentations we kindly provided my these co-authors. Thank you for that.

Where shall we start ? Well, first of all we thought, let’s work on the core theme of this conference: it is to have the past in order to fortify the future. For this reason we’ll give a fairly brief overview as to what kind of journey the ever increasing use of enzymes in baking is on.

(Slide 4) We selected four topics, which we think are currently the hot buttons that really drive the development of enzymes in baking. Then you can ask, if enzymes are so good, why are we still using monoglycerides, why are we still using chemicals, answering that question will conclude this presentation.

(Slide 5) Okay, going to the real past. And for anybody who loves Wikipedia, you should turn to Plinius the elder, we are talking first century A.D. in Rome. He wrote the first encyclopedia, the “Historia Naturalis” in one of the volumes, he writes of everything that is known in the Roman Empire about food, including bread making. It is amazing to see that he already writes about synergistic blending effects of different varieties of wheat (from the Island of Crete and flour from Egypt) to produce a better baking result, in this case water binding, as bread was sold by weight in the Roman days.

Plinius was a great observer and in a time where most of the breads were unfermented and he observed that the Iberians from current day Spain and the Gaul’s from
current day France, had a much lighter bread. He wrote that they were using the froth of beer and the left over beer in order to produce that lighter bread. Adding beer would add yeast, but it also some enzymes in the dough. Later this evolved in traditional sours. Only in the last two centuries the breads we eat nowadays became the prominent type.

(Slide 6) So where are we right now? We are right now in era where in the last 20, 30 years the enzyme usage is on a significant increase. About every five to ten years we have seen a significant step in enzymes use when a new enzyme (functionalities) enters the bread market. In the years after such introduction companies are working in order to find the right applications and the right usage of those enzymes. And that journey we are going to describe.

(Slide 7) The following slide describes the flow of enzymes from their primary production to their ultimate application in baking. About every conceivable route can be found in the industry, which accounts for double counting, making it difficult to put a monetary size on the bakery enzyme market.

(Slide 8) This slide requires some more time to go through. Preparing this slide, with the co-authors required the best part of a full day on the phone, discussing with the sequence, significance, use level and whether an enzyme use was trending up or down.

So if we looked at the first enzymes used, it’s the use of malt, already more than 100 years. The amylase in the malt results in improved fermentation, color and volume. Still today the fast majority of the breads has some usage of malt.

Around the Second World War, enzyme active soy was increasingly used for its bleaching action, producing a nice white crumb. It will also give some stability. As result of the recent allergen legislation, we see the use of enzyme active soy decreasing, as the bread industry judges the technological benefit to small, preferring not to have soy listed as allergen on the package.

Fungal alpha amylase was the start of the bakery enzyme industry in the ’60s, offering the benefit of dosing amylase to a much higher level, not having the undesired (sticky dough) side effects of the presence of protease in malts. The usage of alpha amylase is considered stable.

With better wheat varieties and the need for faster dough mixing processes, increasingly proteases were used for enzymatic dough development. The use of proteases is trending stable, we think, in a minority of the bread production. The improvement of dough mixing equipment has reduced the need for non-mechanical means to develop the dough.

Bacterial alpha amylase was introduced for producing really soft breads, straight forward (non-resilient) softness and moist breads. It started to replace the fungal alpha amylase and I think that the use of bacterial amylase is trending down at the moment, because of the increase in use of maltogenic amylase.

In the 80’s xylanase was introduced bringing: volume, tolerance, machine ability. In the same decade enzymes get introduced, produced by genetic engineered micro organisms. This is where worlds go apart and in America one talks about genetic engineering; in Europe about genetic modification; in the U.K.: Frankenstein foods. You are laughing about it and it would be a laugh if this wasn’t the situation pretty much.

The reduced usage of bromate resulted in an increase of oxidases. Glucose oxidase, is being used for dough strengthening, but not in the majority of baking. Part of it and we will discuss that later is still cost and part of it is still the robustness of the application and its sensitivity to overdosing.

A big step in the’90s was the introduction of maltogenic amylase as it brought extended shelf life, both in softness and resilience of the bread.

Also bacterial xylanase was introduced in the 90-ties. Whilst bacterial xylanase have a tendency in European flours to give a tacky dough and fungal xylanases not, we see the opposite effect in American flours where fungal xylanases give tacky doughs and bacterial xylanase not. If there is anybody from grain science in the audience, this would be a very interesting subject to study being one of the big questions that I truly do not understand the nature of.

At the beginning of the 21st century, we many lipases coming into the market. These lipases are geared towards production of lyso-lipids, having a higher functionality in bread making for datem replacement.
We see here a journey, were still enzymes that were introduced in the ’90s are still on the way up and the broadness of the enzyme applications are still everyday developed by bakery supply companies, enzyme companies, and the bakery industry.

(Slide 9) To visualize the previous slide, you can see here the steps forward enabled by enzymes, going from the blanc, and the subsequent improvement form malt, alpha amylase and amylase/xylanase addition.

(Slide 10) The big four themes that are currently driving the change towards enzymes used in baking:

First theme is natural. Natural has been the subject of lengthy and sometimes not very fruit full debates and definitions. I think that yesterday the movie we saw about high fructose corn syrup captured the essence of natural. Natural is what consumers understand or that they think they understand, so the "natural theme" has got to deliver shorter and more understandable labels. This is where enzymes can play a role.

The second theme is still shelf life and when thinking shelf life, it is long, longer and I will show you that we have reached a point where we have a point that bread softness can be guaranteed for more then 100 days. Now that softness can be guaranteed for long times, the bread shelf life is increasingly dependent of the mold control, a subject that Jerry is going to talk about later.

For the last 20 years the boundary of shelf life has been increased, from 4 to 20 days to 40 days now to 100 days. The extension of shelf life has been a huge enabler for the bakery industry to completely modify its logistics and then we are looking for shelf life, but we are looking for shelf under all conditions, so not only ambient, but also under frozen and refrigerated conditions. It is very funny for anybody here who comes with a cereal science background who knows in terms of bread staling, the worse conditions to store your bread it is in the refrigerator. However, from consumer research we conducted it showed that 28% of the people store their bread in the refrigerator. And then you ask these people for reasons why they are storing their bread in the refrigerator? And they say " if you want to keep something fresh you store it in the refrigerator " You think...oh, yes there logic to that. To find a solution for bread fresh keeping, even under refrigerated conditions has been a challenge that my company has been working on for the last years and we consider that barrier to be taken right now.

The third big theme is wholegrain. I am almost a very profound believer in whole grain and subject of fibers in general. If there is one mission for the bakery industry contributing to consumers health it is this. If you talk to a nutritionist asking what they really worry about in western diets? They will say it is the lack of fibers. There is 10% of the population in America that eat a fiber-sufficient diet. If you look at the top 3 food products delivering most fibers in the US diet, you will find white bread and French fries amongst the main vehicles for delivering fiber in the American diet and you know that there is some business to be done.

As bakery industry we should be triggered by the efforts other industries try to make, for example fiber rich beverages with 0.2% fiber, which is a very easy amount of fiber to add to a diet through a bakery product. If there is one role to play in the health and nutrition arena for bakery it is this one. We have seen many introductions in whole grain bakery products. But...the use of whole grain comes with challenges to over come for machinability, water management in the dough and product, overall product quality, which are illustrated by some pictures on what we have got to work on.

The fourth big subject is the extension of enzyme applications to non-bread applications, where, most enzyme applications have been developed for bread historically. Currently the enzyme industry and the bakery supplies companies are working on the crossover into fine bakery. We now see enzyme applications and dedicated enzymes for freshkeeping and ingredient replacement in cakes and pastry.

Are there any other issues being worked on? Yes, we are working on various other subjects. “The green issues” for example, subject of a separate session at this years ASB. Can enzymes have an impact there? Yes, because enzymes can have an impact on water management, water management during baking and the amount of water being baked-off and therefore the energy use during baking.

Enzymes can have an impact in the area of food safety through the reduction of acrylamide.
To show some examples, let's have a look at the "natural" theme and take some main themes of the last years: bromate replacement, ADA replacement, datem replacements. Enzyme enabled solutions for these replacement are technically feasible, either already in use or still being worked on.

What is the conclusion of the work so far? Well, when applied in a dough an enzyme continues and it catalyses reactions and it basically means that the functionality is very dependent on time and temperature and it does not stop and therefore require a very high level of process control.

In many brain storms we have been dreaming about enzymes with a switch that you could start and could stop at the desired moment, unfortunately these do not exist (yet?).

When thorough process control is difficult to establish, the proven ingredients will show their robustness, as they will react on a known and finite basis. This reformulates the question: What is the bigger issue: the cleaner label or the required ingredient robustness?

The next slide shows some developments in the area of ESL, extended shelf life & freshness. This experiment shows the staling of two bread under refrigerated conditions, as mentioned earlier, about the harshest conditions that bread can be stored at. For the bread produced with a standard shelf life improvement system, we see it firming pretty quickly, reaching the limit of consumer acceptability in about 10 to 20 days. The other bread, produced with a recently developed ESL enzyme shows hardly any firming up to 80 days. Some of the bread of these trial are still in the testing phase, reaching 200 days, still having nice, very soft texture. This means that the basic mechanisms of staling are now so-well understood that they can be effectively counteracted through emulsifiers for the amylose related staling and enzymes for your amylopectin related staling. The latest generations of ESL technology might not be the cheapest of all, but it is the frontier of fresh keeping at present.

The next slide shows examples on the role of enzymes can play in the area of whole grains. Enzymes counteract the way the cellulose is interfering with the gluten network and help the generation of soluble pentosans strengthening the network. The addition of seeds and larger husk particles require the dough to be even stronger than a normal white bread. A third and very major subject is the management and availability (in time) of water in the dough and the final product. The science and mode of action is reasonably well understood, but how many varieties of whole grain bakery products are imaginable – there are many ways to selects grains, mill, formulate flours and seed mixes, all requiring a dedicated solutions for consumer acceptance and appreciation.

The final effectiveness of cake in its turn will depend on many things of which the formulation of the cake recipe is seen as the most important. Will we see more enzyme application in fine bakery? Yes. And that is currently what is being worked on.

When working with enzymes, we are working with a protein that continues its action in time and is very dependent on temperature – in the majority of cases there is no self limiting mechanism on its action. Glucose oxidase might be one of the exceptions.

Many enzymes and protease is very well-known because of its truly catastrophic result it can cause if you do not control time and temperature. And I can tell you, having seen a plan where 100 square foot crackers came out of the oven.

The enzyme action is related to flour quality. This dependency is sometimes quite poorly sometimes fairly well understood. The action of lipases in dough has some variation that is assumed to be related to accessibility / binding of the lipids, which is not fully understood yet and is being worked on.
Many companies are working on robust and more forgiving enzyme applications. Enzymes act very specific and that is both good and that is very bad. That means that they act on a fairly specific substrate, but I can give you an example from around in the mid-'90s when about 70% of the area in France was planted with a wheat variety called “Soissons” and this new variety did not respond to xylanase the same way other wheat varieties did. Fairly intense research work revealed the high level of di-substituted xylan units in the arabinoxylane was hindering the xylanase action. Addition of arabinofuranosidase to the enzyme resolved the issue and resulted in a robust enzyme application, underpinning that robust applications can be achieved, but not rarely require quite a sizable R&D effort.

Lastly the wide use of some enzyme is hindered by its cost-in-use level, against a background that many chemicals we use in baking are very, very cheap.

(Slide 16) That brings me to the conclusions of this presentation. We see an ever-increasing importance in the role of enzymes in baking. The enzymes are truly our key enablers for some important market trends. Of these trends natural, as into a short, friendly and an understood label by the consumer. Secondly it is product freshness, for an ever increasing shelf life, under all storage conditions in all bakery and fine bakery products. Thirdly enzyme development in baking are driven by the increase of whole grain products, where we as bakery industry have a mission to address the fiber deficiency in the Western diets.

Enzymes will respond to time, to temperature, flour quality. Therefore, application of enzymes requires very strict process control. So if you are not controlling this, I can tell you that will be a very painful experience.

During this presentation we have been looking at many factors determining the speed of the increase of use of enzyme in baking and the developments in the area of enzyme R&D and application development. This speed will be determined by the speed, extent and quality of process control improvement in the bakery enabling the use of more enzyme solutions. The strength of the market trends, as driver for making investments or acceptance of higher cost in use. And finally of the developments in the bakery R&D arena for developing new and more robust enzymes, and enzyme based solutions.

And this brings me to the end of the talk and I would like to repeat my thanks for all the eight contributors to this presentation. Thank you for your attention.
Roel Orsel
GSM - Vice President R&D

New Ingredient Technology
Advances in enzyme technology

Contributors & Acknowledgements
- Frank Rittig
- Troy Boule
- Udo Scharf
- Diane Sadowsky
- Sara Lee
- Berrie Bruinam
- Wei Zhu Yu
- Larry Skogerson
- Bimbo USA
- Guohua Feng
- Theresa Cogswell
- BakerCogs

Outline
- A brief historic & actual overview of enzyme use in baking
- Overview of the 4 big themes driving enzyme technology currently
- Limitations of enzyme technology
- Conclusion

Where are we right now?
- Enzyme usage on the increase
- New enzymes & enzyme applications

Use of enzymes in baking

<table>
<thead>
<tr>
<th>Year</th>
<th>Enzyme</th>
<th>Improved bread production quality</th>
<th>Baking</th>
<th>Main benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>Malt</td>
<td>&gt; 75%</td>
<td>↑</td>
<td>Improved fermentations, color &amp; volume</td>
</tr>
<tr>
<td>1990</td>
<td>Enzyme active soy</td>
<td>&gt; 50%</td>
<td>↓</td>
<td>Chromaticity</td>
</tr>
<tr>
<td>2000</td>
<td>Gluten protein</td>
<td>&gt; 75%</td>
<td>↑</td>
<td>Improved fermentation, color &amp; volume, dough development</td>
</tr>
<tr>
<td>2010</td>
<td>Protease</td>
<td>&gt; 10%</td>
<td>↑</td>
<td>Dough development</td>
</tr>
<tr>
<td>2015</td>
<td>Aminopeptidase</td>
<td>&gt; 10%</td>
<td>↓</td>
<td>Straight softness &amp; thickness</td>
</tr>
<tr>
<td>2020</td>
<td>Amylase</td>
<td>&gt; 15%</td>
<td>↑</td>
<td>Volume, tolerance &amp; roundness</td>
</tr>
<tr>
<td>2030</td>
<td>Glucose oxidase</td>
<td>&gt; 15%</td>
<td>↑</td>
<td>Dough strengthening</td>
</tr>
<tr>
<td>2040</td>
<td>Malto-amylopectin</td>
<td>&gt; 15%</td>
<td>↑</td>
<td>Softness &amp; resilience</td>
</tr>
<tr>
<td>2050</td>
<td>Pentose oxidase</td>
<td>&gt; 15%</td>
<td>↑</td>
<td>Volume, tolerance &amp; roundness</td>
</tr>
<tr>
<td>2060</td>
<td>Cellulases</td>
<td>&gt; 15%</td>
<td>↑</td>
<td>Dough improvement</td>
</tr>
</tbody>
</table>

Structure of the industry

Bread making & enzymes in ancient times
- Pliny the Elder: "the Gauls and Iberians make a lighter kind of bread than any people by adding froth of beer to the dough"
Enzyme Technology

Slide #9
Shifting the boundaries of baking

Control
6.6% whole wheat
99.5% Anywhere
100% Anywhere + Enzymes

Slide #10
The 4 big drivers of enzyme technology in baking
1. Natural
   Cleaner & Understandable labels
   Regular EPL: Based on the regular enzyme.
   Limit EPL: Derived enzymes, often not yet on the market.

2. Shelflife
   Long = longer,
   Regular EPL: Baking shelflife, even under refrigerated conditions is possible.

3. Whole Grain
   Product quality
   Bakeability
   Water Management
   “Green issues”
   CO2 fixation
   Food safety
   Acceptance

4. Cakes & Pastry
   Dedicated enzyme functionality for Cakes & pastry

Slide #11
1. Natural
   Cleaner & Understandable labels
   • Conductive: possible, requires very good process & environmental controls
   • More in demand
   • Shorter shelflife

Slide #12
2. Future of freshness in baking
   Refrigerated & extreme shelflife
   • Practically unlimited shelf keeping, even under refrigerated conditions is possible.
   • Regular EPL: Based on the regular enzyme.
   • Limit EPL: Derived enzymes, often not yet on the market.

Slide #13
3. Enzymes & Whole Grain Products
   Product Quality & Processability
   • Without enzymes
   • With enzymes
   • 100% whole wheat flour
   • 60/40 whole wheat/white flour
   • 100% whole wheat flour
   • 100% white flour + enzyme

Slide #14
4. Dedicated enzyme technology applied for cake & pastry
   Freshkeeping & Ingredient replacement

Slide #15
Intrinsic to enzyme technology:
• Dependency on kinetics
  • Time
  • Temperature
  • Often no self-limiting mechanism
• Action is related to flour quality
• Robustness = less “foregone”
• Enzymes act specific
  • Good & bad
• Cost
  • Limiting for some enzymes

Slide #16
Conclusions
• Enzymes play an ever-increasing role in baking
• Enzymes are the major technology enablers for key market trends:
  • Natural
  • Freshness
  • Wholegrain
• Enzymes will respond to time, temperature, flour quality and therefore require strict process control
• The wider use of enzymes is determined by:
  • Strength of market trends
  • Cost
  • Process control in the baking industry
  • Development of robust enzymes (applications)