

Catching the PERSISTENT BACTERIA

New research shall predict the effects of microbiological enzymes on stability and quality of UHT milk using omics-technologies.

Background

One of the most important quality problems related to UHT milk can be traced back to the activity of quality-deteriorating enzymes. These enzymes can come from the cow or from contaminating microorganisms.

Bovine plasmin has been identified as one of the main contributing factors, together with enzymes from especially psychrotrophic bacteria. In the 2000s, a pre-heating step was conceived as a tool for plasmin inactivation. Nevertheless, UHT milk stability regained interest in the 2010s due to a general growth in the international demand at long-distance export markets and renewed focus on food waste. With the necessity of a more stable product, other potential spoiling agents must be addressed. Bacterial enzymes and, more specifically, the AprX-type proteases were pointed as the next issue to tackle.

The current industry problem

Protein and fat degrading enzymes are produced by bacteria from the Pseudomonas genus. Even though the bacteria are killed by the UHT treatment, these quality deteriorating enzymes resist high temperatures and, therefore, remain active in the final product. One out of five hundred batches of UHT milk are estimated to become organoleptically unacceptable due to the presence of exogenous enzymes.

Building the basis of MicroLink

In order to build a basis for prediction methods of UHT milk stability, it is essential to understand the biochemical mechanics behind the spoilage of UHT milk by exogenous enzymes. Literature seems to point to a correlation between the genetic structure of the Pseudomonas species and their proteolytic effect. AprX is one of the well-known protein-degrading

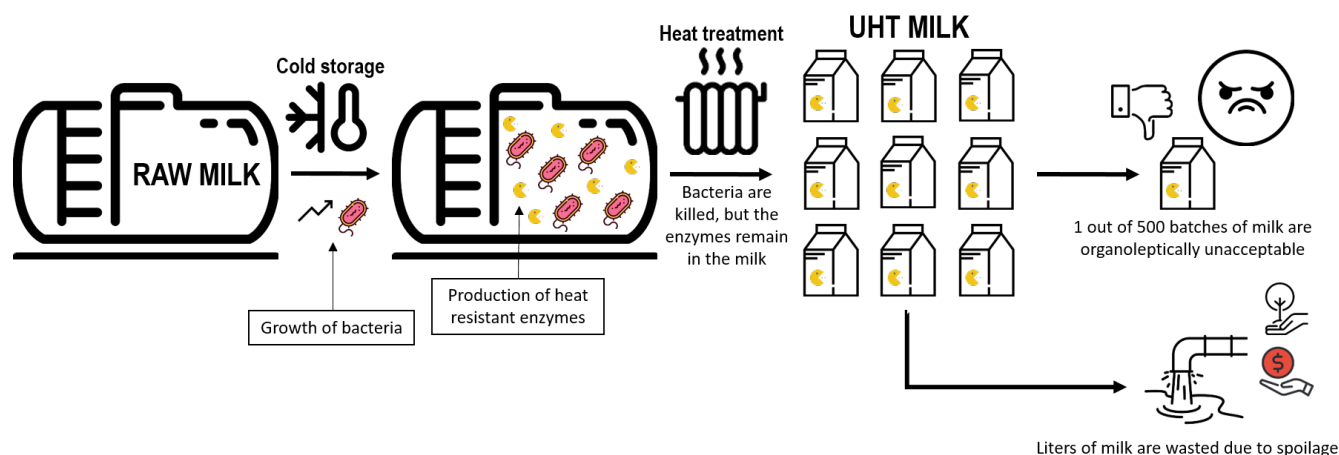


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Summary

The aim of the project, which is supported by the Danish Dairy Research Foundation, is to establish knowledge enabling prediction of UHT milk stability by linking microbiological enzymes to changes in quality and composition during storage using omics-technologies. A PhD study is embedded in the project.





enzymes produced by *Pseudomonas* spp. The AprX protease is known to be expressed by the homonym gene, *aprX*. At the same time, this gene is part of a bigger gene cluster named *aprX-lipA* operon, which also codes lipases. It has been reported that differences in the operon structure influence the protein and fat degrading activities of the bacteria. The complexity of the project resides on the fact that several *Pseudomonas* species exist, with many differences at the inter- and intraspecies level regarding their genetic organization. The project seeks to investigate the cleavage patterns of the enzymes in order to find common molecular biomarkers that can be linked to specific genetic structures and proteolytic activity.

MicroLink project content

The MicroLink project consists of four work packages (WPs). The first WP focus on the establishment of screening methods using known strains of psychrotrophic *Pseudomonas*, and thereby establish links between inoculation level, quality deterioration and enzyme activities. This is done by inoculation of heat resistant enzyme-producing bacteria into milk, followed by storage tests. The second WP is centered around collection and characterization of effects of unidentified *Pseudomonas* obtained from dairies and their spoilage potential in shelf-life tests. The third WP is about the characterization of effects of the heat resistant enzymes by addition of these to milk and isolated milk proteins and examine their spoilage potential before and after laboratory scale UHT heat treatment. The protein degradation pattern is determined by omics-technologies. The fourth WP is about characterization of bacteria that can produce heat-resistant spoiling enzymes and their enzymes using bioinformatics, as well as the identification of DNA/RNA biomark-

Project Info

Project title: MicroLink – Prediction of effects of microbiological enzymes on stability and quality of UHT milk using omics-technologies.

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Project period: 2020-2022.

Aim: to establish the link between heat-surviving enzymes from microorganisms and stability/quality of UHT milk during storage.

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ers for *Pseudomonas* species with the perspective of establishing a fast method for detection.

Benefits for the industry

The knowledge generated in the project will form the basis for the development of DNA/RNA based methods for fast detection of spoilage bacteria that could contribute to the spoilage of milk batches intended for UHT production. A future method for the identification of spoilage bacteria will allow the dairy industry to be more efficient when handling, processing, storing and distributing UHT milk. This production optimization would reduce the volume of wasted product and the overall environmental impact of the process. ●