Histamine reduction, but not elimination in cheese

New research reveals that histamine production in cheese can be reduced using a nisin-producing bioprotective culture.

This main objective of the PushLim project was to facilitate innovation in two important areas, namely, (i) producing a healthier cheese (reduced salt content) and (ii) production cost reduction (elevated ripening temperature). This was achieved by, firstly mapping the microflora of normal cheese (control), reduced salt cheese and elevated temperature ripened cheeses. Secondly, the safety risks associated with salt reduction and elevated temperature ripening of cheese, with particular focus on the production of the biogenic amines, was studied. Thirdly, bio-protective cultures were applied in cheese trials to extend the "no defect" limit in cheese ripening.

High levels of biogenic amines

Biogenic amines are produced by the decarboxylation of amino acids such as histidine and tyrosine, with the production of histamine and tyramine, respectively. Production of these toxic compounds needs to be minimized to produce safe cheeses. The risk factors associate with high levels of biogenic amines in cheese, include, poor hygiene, low salt, and long ripening times. The responsible bacteria for the production of these compounds in cheese are certain species of lactic acid bacteria such as *Lentilactobacillus parabuchneri, Levilactobacillus brevis,* and *Latilactobacillus curvatus.* Amino acids released from casein as a result of proteolysis may be taken up by the microflora and subsequently decarboxylated as a response to the acid stress conditions found in cheese (Figure 1).

Reduced salt level

In the first cheese trial, a baseline was established to determine the biogenic amine risk associated with reducing salt levels in Cheddar cheese. Cheese was manufactured in pilot scale at the University of Copenhagen. Normal and reduced salt cheeses with salt levels of 1.98 % (normal) and 1.42 % (reduced) salt were produced, to which the known histamine-producing strain, Lb. parabuchneri was added. The cheeses were ripened for 6 months at 10ºC and 15ºC. As shown in Figure 2, reduction of salt and increase in ripening temperature resulted in significantly higher levels of histamine in the

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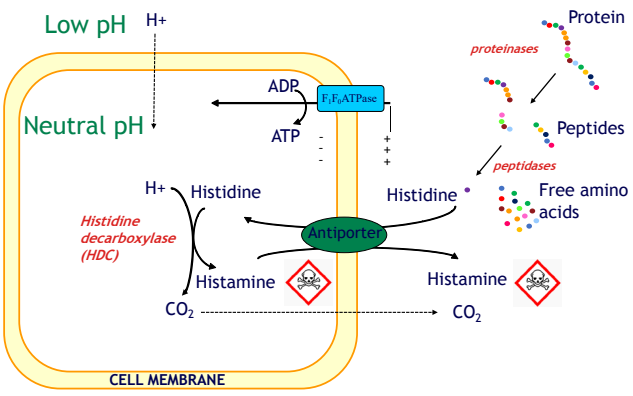

Figure 1. Decarboxylation of histidine and formation of histamine and carbon dioxide by a lactic acid bacteria.

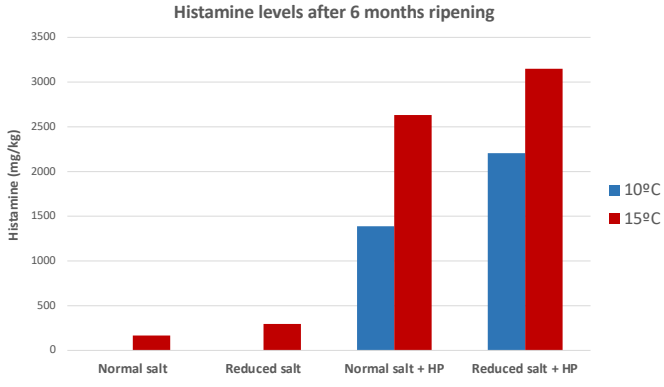

Figure 2. Histamine-forming ability of Lb. parabuchneri in reduced salt Cheddar cheese ripened at 10ºC (blue) and 15ºC (red). +HP: Histamine-producing strain added.

cheeses. Indeed, extremely high levels (in excess of 3000 ppm!) histamine was present in the reduced salt cheese ripened at 15ºC.

Bioprotective cultures

Once this baseline was established, the next phase of the project was to examine whether bioprotective cultures could be used to inhibit histamine production by L*b. parabuchneri*. Seventeen different commercially available bioprotective cultures were screened using an agar well diffusion assay for their ability to inhibit the growth of *Lb. parabuchneri*. The commercial bioprotective cultures used were obtained from the main culture suppliers (Chr. Hansen A/S., SACCO, DSM, CSK and IFF). Surprisingly, only one of the commercial bioprotective cultures was able to effectively inhibit the growth of the histamine producer. This bioprotective culture was a nisin producer, which is normally used to prevent late blowing in cheese. The nisin-producing culture was then tested in pilot scale Cheddar cheese production to establish if it was also effective under cheese ripening conditions. Reduced salt Cheddar cheeses (1.2 % salt) were produced to which the histamine producer was added to the cheese milk. In addition, a *Lactobacillus helveticus* ripening culture was added to boost the amount of free amino acids in the cheese to "stress test" the system. Encouragingly, the results showed that the cheeses with the nisin-producing culture had approximately half the level of histamine present compared to the cheeses without the nisin producer. However, the mode of action was indirect, the nisin producer was able to inhibit the *Lb. helveticus* ripening culture, but not the *Lb. parabuchneri* histamine producer. Inhibition of *Lb. helveticus* resulted in a lower pool of free histidine, and thus lower histamine production.

Early detection method

An additional aspect of the project was to develop a rapid early detection method for histamine producers in milk. Development of such a tool would enable the dairy industry to measure the level of histamine producers in the cheese milk prior to production. Inspired by the COVID-19 pandemic, a **L**oop-mediated isothermal **amp**lification (LAMP) was developed. This method is often compared to PCR, but the two main differences are that **loop** mediated refers to the way the DNA is replicated and **isothermal** means that only one fixed temperature is used. LAMP is both faster and more robust than conventional PCR. Results can be obtained within 15 minutes and no advanced equipment is required. A colour change from red to yellow indicates a positive result. A highly selective primer set was successfully designed for detecting the histidine decarboxylase *(hdcA)* gene in *Lb. parabuchneri*.

A final aspect of the project was an investigation into tryptamine formation in cheese. Tryptamine is produced by the decarboxylation of tryptophan, and unlike histamine, levels found in cheese are low. Interestingly, tryptophan decarboxylase is not found in lactic acid bacteria, however, recently a tryptamine producing *Clostridium* sporogenes strain has been isolated. *Cl. sporogenes* is a well-known cause of late blowing in cheese. In this part of the project, its ability to produce tryptamine was investigated. Surprisingly, it was found that decarboxylation of tryptophan by *Cl. sporogenes* was not an acid resistance mechanism, as only 15 % of the amino acid was decarboxylated. This contrasts with the situation for lactic acid bacteria, in which all of the precursor amino acid is decarboxylated to the respective biogenic amine. ●

Summary:

The research reveals that histamine production in Cheddar cheese can be reduced using a nisin-producing bioprotective culture. Only one of the 17 tested, commercial bioprotective cultures were able to reduce histamine production. A rapid early detection method for histamine producers in milk was developed based on a **L**oop-mediated isothermal **amp**lification (LAMP). The method takes less than 15 min and requires no advanced equipment.

Project info

Project: Pushlim - Pushing the limits in cheese manufacture and ripening; minimising the risks through the use of bio-protective cultures.
Project leader: Professor Fergal P. Rattray, Department of Food Science, University of Copenhagen (KU).
Project participants: Associate Professor Finn K. Vogensen, KU, Assistant Professor Cleide Oliveira de Almeida Møller, KU, PhD Student Elif Fatma Ucok, KU, MSc Student Geoffrey Johansen, KU, MSc Student, Mathias Engholm Hørstedt Madsen, and Søren Soren Lillevang, Cheese Expert, Arla Foods, Anders Hauge Okholm, Research Scientist, Arla Foods.
Project period: 1 February 2018 - 31 January 2021.
Project objective: To experimentally map the detrimental microflora using genotypic and phenotypic analysis methods, and to use this critical information to select the most suitable bioprotective cultures for pushing the "no defect" limit beyond what is normally possible. Cheese technologies in focus were salt reduction and elevated ripening temperature.
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