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Reduction of Tyramine in Fermented Foods

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Key words
Biogenic amines, tyramine producing bacteria, fermented milk and meat, tyrosine degrading lactic acid bacteria, biocontrol

Aim of the study
Tyramine, a biogenic amine (BA) can often be detected in Fermented Food (FF) such as cheese and dry fermented sausages (salami-type) due to endogenous tyrosine decarboxylating microorganisms (MO) and might represent a consumer health-risk to be reduced in future. This project aims (1) to identify tyramine producing MO in FF and identify the most health risky FF on the Swiss market and to proof or neglect the hypothesis of enterococci as main tyramine producers; (2) to improve mechanistic knowledge on tyramine production in MO and in FF; (3) to screen *in vitro* antagonistic *Lactobacillus* strains against tyramine producers or alternatively MO for tyramine degradation and (4) to deliver data which enable Swiss Food Safety authorities to propose measures and recommendations for tyramine reduction in food. The final summarizing goal of the project is a contribution for low-tyramine containing FF of the future.

Material and methods
Sampling of different cheese varieties and fermented dry sausages (salami-type) as FF examples produced in Switzerland was done at Swiss retailing places. Quantitative analysis of FF for dominant bacteria was carried out by using selective agar-media. Prevalence of tyramine production of single isolates was assessed by phenotypic growth analysis and presence of a tyrosine decarboxylase gene (*tyr* gene) by PCR. For quantitative determination of tyramine and tyrosine, ion chromatography (IC) was used. Reduction of tyramine was performed in 2-ml cheese models in the presence of tyramine-producing bacteria and a tyrosine-utilising *Lactobacillus plantarum* strain.

Results and significance
We investigated 274 cheeses and 62 salami-type sausages, which all represent the Swiss market, for biogenic amines (BAs), dominant bacteria and their prevalence for BA production. In cheeses, tyramine was with a concentration up to 984 mg kg^{-1} by far the most abundant BA followed by cadaverine with a concentration up to 872 mg kg^{-1}. Histamine and putrescine were found in less cheese samples with a concentration up to 860 mg kg^{--1} and 539 mg kg^{-1}, respectively. Tyrosine decarboxylase-positive enterococci were with an incidence of 78.04% the major tyramine producers in cheese and showed a significant correlation to tyramine concentration. In addition, the factors of milk treatment, milk origin and ripening time affect BA biosynthesis and accumulation in cheese. The highest amount of all four measured BAs were found in semi-hard cheese made from cow's raw milk.
In sausages, concentrations of all four BAs were higher in industrially-produced sausages compared to artisanally-produced ones. Tyramine was found in 46 out of 62 sausages, with a maximum observed amount of 785 mg kg^{-1}, while putrescine was found in 20 out of 62 sausages, with a maximum amount of 708 mg kg^{-1}. Both cadaverine and histamine were found in fewer sausages. Cadaverine was detected in nine sausages and was found in significantly higher levels (P = 0.019) in industrial sausages, with a mean of 131 mg kg^{-1} compared to the level found in artisanal sausages, which had a mean of 2.7 mg kg^{-1}. Histamine was detected in eight samples, with a mean value of 67.7 mg kg^{-1} in industrial sausages. This was significantly higher (P = 0.036) than the concentration found in artisanal sausages, which had a mean concentration of 4.1 mg kg^{-1}. Contrary to the finding

in cheeses, enterococci titers do not correlate with tyramine concentrations and coagulase-negative staphylococci belonging to starters had a high prevalence for the *tdc* gene. Taking into account the significantly higher (P = 0.035) pH after maturation in industrially-produced sausages, it can be said that both the amount of tyrosine-decarboxylase-positive resident microbiota of the meat and the pH of the system have an influence on the tyramine concentration in the meat product.
We tested a micro-cheese model in order to modulate tyramine formation of different tyramine-producing bacteria at different salt concentrations. An increase in salt concentration up to 4.5% resulted in an increase in tyramine concentration up to 755%. In the biocontrol screening among lactic acid bacteria a *Lactobacillus plantarum* strain was found that could consume in successful competition with other resident bacteria tyrosine for a source of energy gain. Thereby tyramine accumulation was reduced up to 54%.

The evaluation of the situation regarding tyramine in Swiss cheeses and salami-type sausages revealed that cheese products contain only in less than about 6.57% (18 of 274) tyramine at health-concerning concentrations whereas 27.78% (10 of 36) of artisanally and 33.33% (12 of 36) of industrially produced salami-type sausages contain health-concerning tyramine concentrations.
It was shown that in cheese enterococci and salt concentration have major influence on tyramine concentration. Therefore, measures to reduce tyramine in cheese are pasteurisation of milk as well as the reduction of salt concentration. In fermented salami-type sausages enterococci along with staphylococci and pH value influence tyramine concentration. Therefore, it is important to use starter cultures, which are unable to produce tyramine.
Finally, the study disclosed, with the use of a *Lactobacillus plantarum* strain, a feasible strategy to reduce tyramine concentration and increasing the safety level of fermented food.

Publications, posters and <u>selected</u> presentations

Meile, L. (2017). «Enterokokken in fermentierten Lebensmitteln: Reduktion dieser vernachlässigten Bakterien als Beitrag zur Lebensmittelsicherheit». Invited talk at the 50th SGLH Symposium «Lebensmittelsicherheit und – hygiene in der Schweiz», June 22-23, ETH Zurich.

Anderegg, J. (2017). «Identifizierung von Tyramin produzierenden Mikroorganismen in fermentierten Lebensmitteln». Invited talk at the 50th SGLH Symposium «Lebensmittelsicherheit und – hygiene in der Schweiz», June 22-23, ETH Zurich.

Anderegg, J., Dürig J., Die A., Meile L. (2017). «Reduction of tyramine in fermented food» Poster at the 7th Congress of European Microbiologists FEMS, July 9-13, Valencia, Spain.

Anderegg, J., Gürber S., Hug D., Fischer M., Häusermann I., Plüss S., Ly S., Dürig J., Borbely S., Die A., Lacroix C., Meile L. (2018) «Identification of tyramine production microorganisms in fermented food» Poster at 26. FoodMicro Congress, September 3-9, Berlin, Germany.

Anderegg, J. (2019). «Role of the microbial community in production of biogenic amines in fermented foods and characterization of a tyramine reducing *Lactobacillus plantarum* strain» Dissertation, ETH Zurich, December 2019.

Anderegg, J., Fischer M., Dürig J., Die A., Lacroix C., Meile L. (2019). «Detection of biogenic amines and tyramine producing bacteria in fermented sausages from Switzerland» J. Food Protect (submitted).

Anderegg, J., Hug D., Jenni C., Die A., Lacroix C., Meile L. (2019). «Determination of biogenic amines, tyramine producing bacteria and factors influencing tyramine accumulation in cheeses from Switzerland» (ready to submit)

Anderegg, J., Meile L. (2019). «Effects of NaCl on tyramine production in fermented food and its suppression by tyrosine degrading lactobacilli» (in preparation).

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