Risk of salicylic acid formation in milk during sample storage

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Introduction

For a correct qualification and quantification of residues in matrices, proper handling of samples is required. Improper storage conditions could be a potential risk for the stability of analytes in samples. Reanalysis of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in milk samples showed an increase of the extractable salicylic acid concentration during storage outside the freezer (e.g., transport). Even within a day, the levels increased and some exceeded the MRL, which could lead to false non-compliant findings for salicylic acid.

Salicylic acid:
- is a phenolic plant hormone;
- occurs in animal feed, like alfalfa, rapeseed, citrus pulp, wheat;
- is the basic substance of salicylates;
- can be used as an NSAID in e.g. cattle, sheep and goats;
- maximum residue limit (MRL) is 9 µg/kg in milk (EU/2010/37).

Results

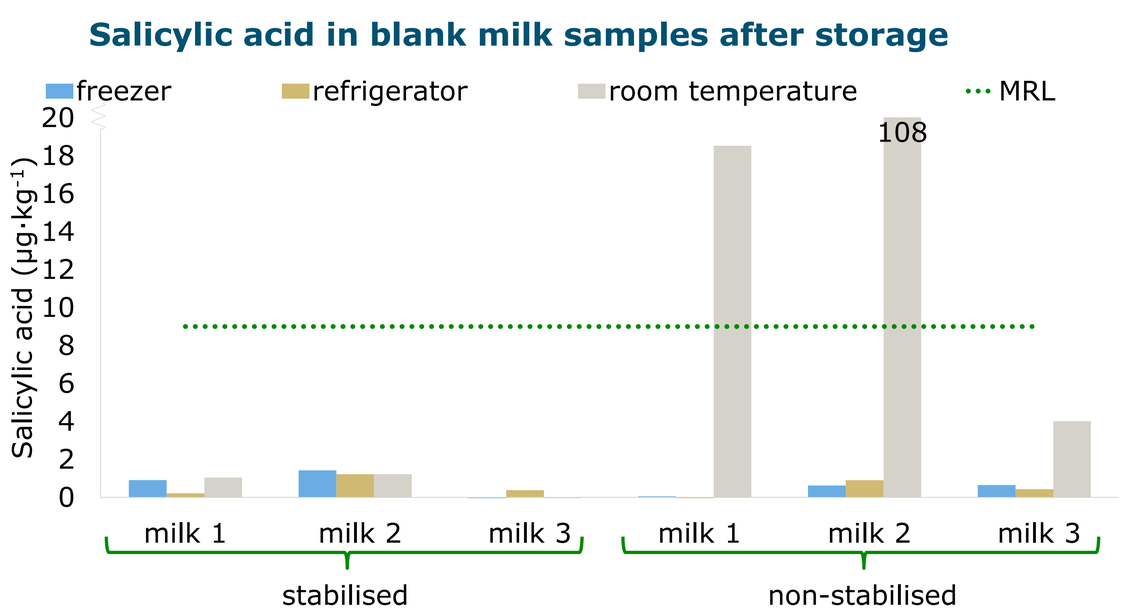

Figure 1. Three blank milk samples (above) and three positive milk samples (below) were stored over the weekend stabilised with 0.5 (v/v)% acetic acid and non-stabilised. After the incubation time, the salicylic acid concentration was determined.

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Identification of the problem: (In)stability of milk

A raw (non-stabilised) milk sample which was screened negative (< 0.5*MRL) for the presence of salicylic acid can show an increase of the salicylic acid levels even above the MRL (Figure 1), during storage at room temperature from a few hours to a few days. This complicates enforcement. In order to solve the problem, the origin of the instability was investigated.

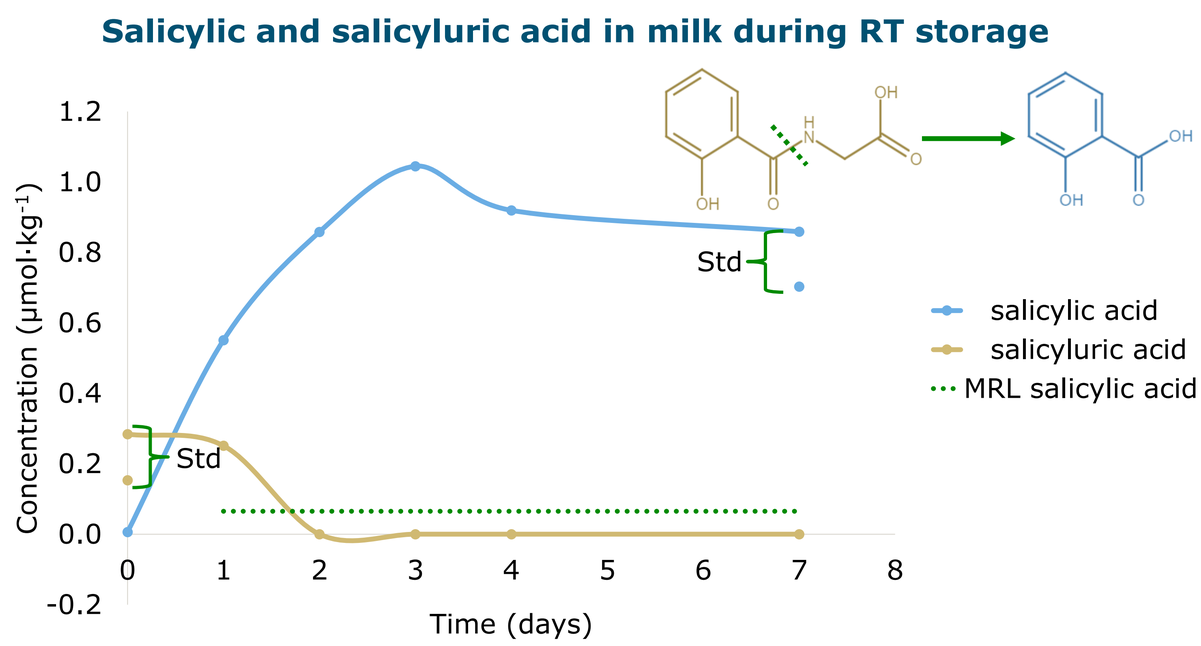

Figure 2. During storage at room temperature, the salicylic acid and salicyluric acid concentration was monitored in a sample spiked with 25 µg·kg^{-1} (0.13 µmol·kg^{-1}) salicyluric acid (n=2). The contribution of this standard of salicyluric acid (std) before and after hydrolyse is shown at t=0 and t=7 days with the braces.

The origin of the problem: Salicyluric acid

Different mass spectrometric techniques (parent ion scan and high-resolution MS/MS) were used to identify an over time decreasing signal that seemed to be related to an increasing signal of salicylic acid. Results show that the hydrolysis of salicyluric acid, a metabolite of salicylic acid, which is naturally present in milk, is partly the origin of the increasing salicylic acid levels. Even additional added salicyluric acid was hydrolysed during storage into salicylic acid (Figure 2).

Solution of the problem: Acid stabilisation

During research, several indications were found that the hydrolysis is microbial or enzymatic rather than chemical. This led to stabilisation opportunities, namely inactivation of microbial/enzymatic activity by lowering the pH. The stabilisation with 0.5% acetic acid showed to be successful (Figure 1).

Conclusions

In conclusion, the hydrolysis of salicyluric acid into salicylic acid (partly) explains the increasing salicylic acid levels in milk samples, during insufficiently cooled storage. An in-house stabilisation procedure was developed using 0.5 (v/v) % acetic acid for the prevention of salicylic acid formation in milk samples. This study clearly shows the importance of proper stabilisation and storage of samples for residue analysis.

Acknowledgements

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