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# LC-MS/MS-DETECTION OF MILK PROTEINS IN MEAT PRODUCTS

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## INTRODUCTION

Milk allergy is one of the most common food allergies in infants up to one year of age. Milk protein is used in a multitude of meat products like liver sausages or Frankfurter-type sausages and is subdivided into the two fractions casein (80 %) and whey (20 %).

The objective of this study was to develop an analytical method for mass spectrometrical detection of milk allergens in commercially available meat products (LOD 1 ppm) after tryptic digestion using the two marker peptides YLGYLEQLLR and FFVAPFPEVFGK originating from alpha-S1-casein. Furthermore, the influence of thermal processing of the meat product on the detectability of milk protein was investigated.

## MATERIALS AND METHODS

*Production of emulsion-type sausages*

The basic formulation was 49.1% pork, 26.4% back fat, 22.5% ice, 1.8% salt (0.4% $NaNO_3$), and 0.2% $K_2HPO_4$. Skimmed milk powder (36% protein) was added with milk protein contents of 0, 1, 3, 5, 10, 25, and 50 ppm.
Cans (200 g) of each batch were heated as home cannings (F = 0.41), full stable cans (F = 5.02), and cans to be used under tropical conditions (F = 14.78).

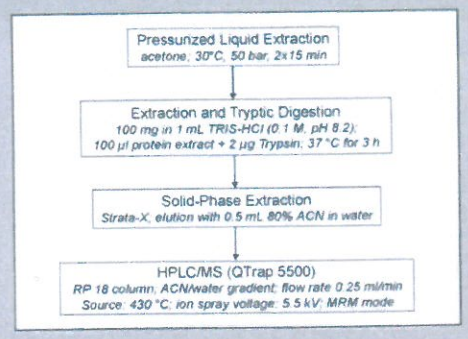


Fig. 1: Isolation and detection of milk proteins

| | YLGYLEQLLR | FFVAPFPEVFGK |
|---|---|---|
| RT [min] | 16.01 | 16.65 |
| Precursor [m/z] | 634.4 (+2) | 692.9 (+2) |
| Product1 [m/z] | 249.2 (b2) | 970.5 (y8) |
| Product2 [m/z] | 991.6 (y8) | 465.2 (b4) |
| Prod1/Prod2 | 1:9 | 1:3 |
| Prod1 (CE/DP) | 28/28 | 26/28 |
| Prod2 (CE/DP) | 27/28 | 22/24 |

Tab. 1: Parameters of the MRM method

## RESULTS AND DISCUSSION

A rapid LC-MS/MS method for the detection of milk proteins in meat products was developed, applying short protein extraction and digestion times (1h and 3h, respectively) (Fig. 1).

A chromatogram of the two marker peptides in sausages with 0 ppm and 5 ppm milk protein is shown in Fig. 2.

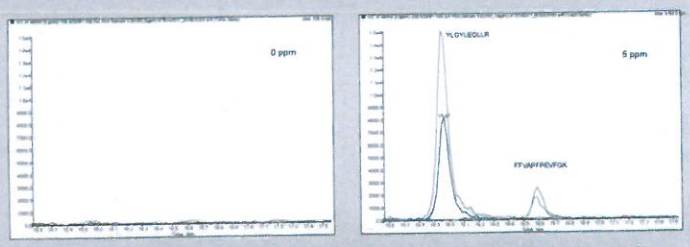


Fig. 2: Chromatograms of the two marker peptides in sausages (full stable cans) with 0 ppm (control) and 5 ppm milk protein

The limit of detection (LOD) of the method was significantly below 1 ppm milk protein for all types of cans. The signal-to-noise (S/N) ratio of marker peptide 1 (YLGYLEQLLR) in the lowest concentration (1 ppm milk protein) was about 50:1 for product 1 (m/z 249.2) and about 80:1 for product 2 (m/z 991.6).

The correlations between peak area and content of milk protein [ppm] for the two marker peptides 1 (YLGYLEQLLR) and 2 (FFVAPFPEVFGK) for the different types of cans are shown in Fig. 3. The determination coefficients ranged between $R^2$=0.9899 and $R^2$=0.9997. No false positive and false negative results were obtained. Between the different thermal treatments of the meat products no relevant differences were observed.

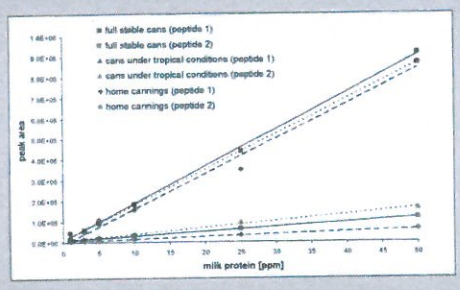


Fig. 3: Correlation between peak area and content of milk protein [ppm] for the marker peptides 1 and 2 in different types of cans

## CONCLUSION

The developed analytical method is suitable for the detection of traces of milk protein in meat products below 1 ppm. Thermal processing did not negatively influence the detection of the marker peptides. Based on the presented method a LC-MS/MS multi allergen screening method for meat products should be developed. Such a screening method can also be adapted to a detection method for foreign proteins in meat products.

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