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HPLC-MS/MS-Detection of caseins and whey proteins in meat products

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Abstract

Screening methods for the mass spectrometric detection of caseins and whey proteins in meat products have been developed. After tryptic digestion, two α-S1-casein and two β-lactoglobulin marker peptides were measured by HPLC-MS/MS. For matrix calibrations, emulsion-type sausages with different concentrations of milk and whey protein (ppm level) were produced. The limits of detection (LODs) were below 1 ppm for milk protein and about 3 ppm for whey protein. The determination coefficients for the correlation between peak area of the marker peptides and the concentrations of milk and whey proteins were $R^2 \geq 0.9899$.

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Keywords: milk proteins; caseins; whey proteins; allergens; foreign proteins; HPLC-MS/MS

1. Introduction

Milk proteins are used in various meat products such as liver sausages or Frankfurter-type sausages, in which their use is usually declared. Besides the declared use, small amounts of milk proteins can also get into meat products inadvertently, for example due to cross contamination or via spice ingredients. This can be a problem for persons suffering from allergies, because milk is one of the 14 food ingredients which must be indicated on the label of foodstuffs as they are likely to cause adverse reactions in susceptible individuals[1]. Furthermore the deliberate and

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non-declared use of milk protein as foreign protein in meat products is possible, because milk protein is cost-efficient and produced in high amounts.

A number of Enzyme-linked Immunosorbent Assays (ELISAs), specific for caseins and/or whey proteins[2], are available for the detection of milk in food[3]. Although there are many advantages of ELISAs, including specificity, sensitivity and simplicity, thermal processing of food may have an adverse influence on the detection of milk proteins, because a possible alteration of the protein structure may lead to an alteration of antibody binding[4]. The frequently used detection by means of polymerase chain reaction (PCR) cannot be used for the detection of milk proteins in meat products, as a differentiation between beef and milk is not possible at the DNA level.

The main objective of this study was to develop analytical methods for the HPLC-MS/MS-detection of casein[5] and whey peptides in meat products. Emulsion-type sausages with different contents of caseins and whey proteins were produced and the LODs as well as linearity and repeatability of the developed methods were determined.

2. Materials and methods

2.1. Production of emulsion-type sausages

The formulations of the batches (milk powder/whey powder) applied to a 3l bowl chopper were 49.1/53.3% pork, 26.4/25% fat, 22.5/20% ice, 1.8/1.5% salt ($NaNO_2$: 0.4%), and 0.2% K_2HPO_4. Skimmed milk powder (36 % protein) and whey powder (16 % protein) were added as follows (Table 1):

Table 1. Batches of sausages with milk and whey protein.

Batch	Milk powder [ppm]	Whey powder [ppm]
0 (control)	0	-
1	3	-
2	8	-
3	14	-
4	28	-
5	69	-
6	139	-
0 (control)	-	0
A	-	16
B	-	64
C	-	250
D	-	1000
E	-	4000

Batches with milk powder were heated as home cannings, full stable cans, and cans under tropical conditions. Batches with whey powder were stuffed into casings and heated for 70 min at 75 °C.

2.2. HPLC-MS/MS-Detection of casein and whey peptides

Homogenized meat products (2 g) were extracted with acetone in a Speed Extractor E-916 in two cycles (30°C, 50 bar, 15 min) and the defatted sample was dried at room temperature (RT). 100 mg of the defatted sample were solubilized in 1 ml TRIS-HCl (100 mM, pH 8.2) and the samples were shaken for 1 h (caseins) or 0.5 h (whey proteins) at 60°C, cooled to RT and centrifuged. To 100 µl of the protein extract, 20 µl Trypsin solution (0.1 µg/ml) were added and incubated at 37°C for 3 h (caseins) or 18 h (whey proteins). After addition of 2 µl concentrated