Optimization of a GC-MS method for the quantification of CPFA in milk

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Introduction

Milk is a nutrient-rich, white and cloudy emulsion which is one of the basic foodstuffs for humans [1]. Haymilk comes from cows fed exclusively on fresh grass or hay and a limited amount (maximum 25%) of concentrated feed in the ration [2]. Cyclopropyl fatty acids (CPFA) are components of bacterial membranes present in silage and detectable as biomarker in the milk of cows fed maize silage, but not in milk produced without addition of maize silage [3]. One aim of the project HEUMILCH is to optimize a GC-MS method for the application in milk obtained from grass silage feeding, which is mainly used in the alpine regions.

Materials and methods

Sample preparation

The samples were taken from 23 farms (5 haymilk, 9 feeding only maize silage and 10 feeding only grass silage) for three weeks in a row (69 samples in total). The fat was obtained by a modified method based on Feng et al. [4] and was transesterified for analysis according to ISO 15884:2002 [5].

GC-MS analysis

For the CPFA (9,10-methylene-octadecanoic acid) analysis, a gas chromatography-mass spectrometry (GC-MS) was used (QP2010 SE Shimadzu, Kyoto, Japan). Chromatographic separation was carried out using a SLB-5ms (30 m x 0.25 mm x 0.25 µm) capillary GC-Column (Sigma Aldrich, St. Louis, Missori, USA). The mass spectrometer was operated in full scan and SIM mode.

Results

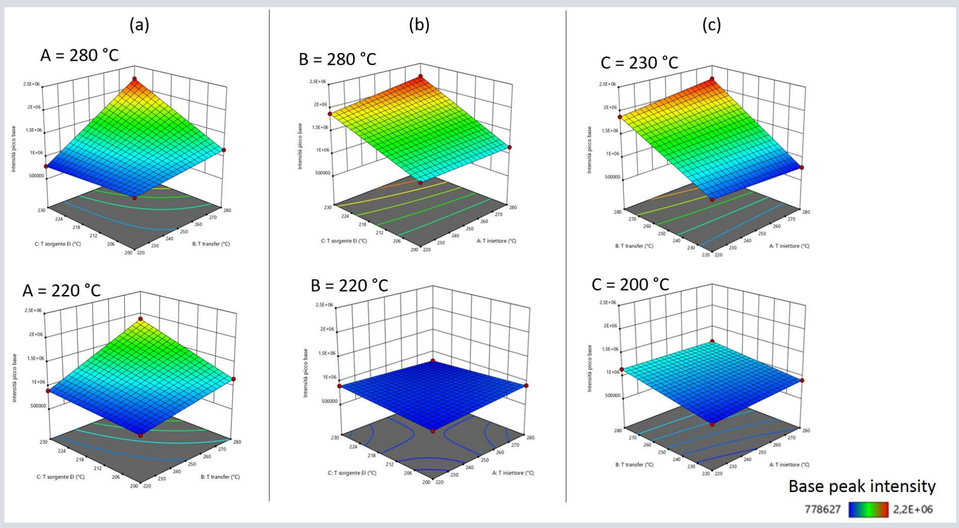

Fig.1: Response surface plots of the base peak intensity (m/z 55) for the injector (a), transfer line (b), and ion source temperatures (c)

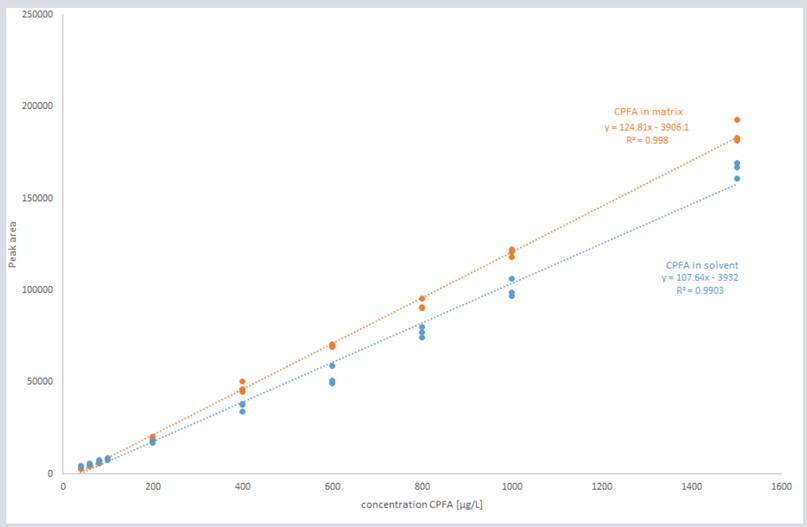

Fig.2: Calibration curves of CPFA in solvent (hexane) and matrix (haymilk)

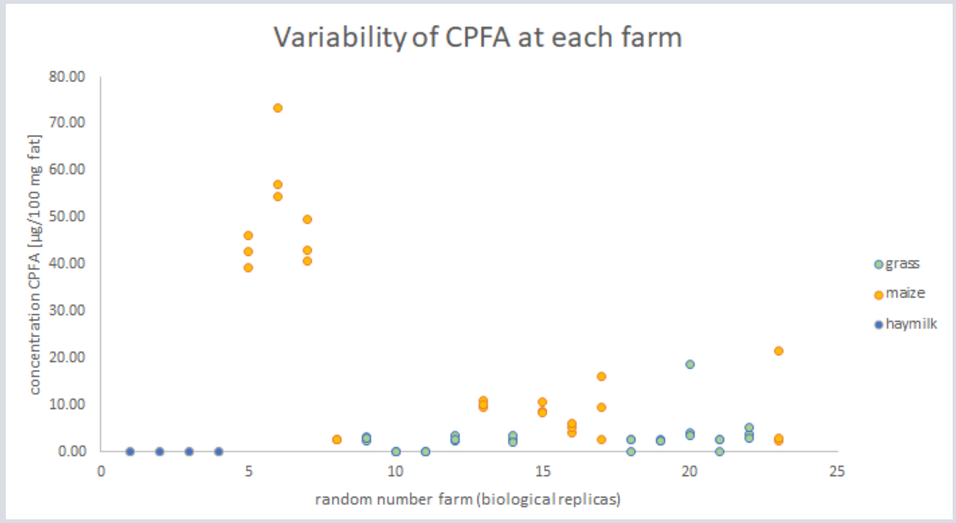

Fig.3: Measured content of CPFA [µg/100 mg fat] in milk samples from various farms. Blue=haymilk, yellow=milk from maize silage feeding and green=milk from grass silage feeding. Biological replicated from each farmer are reported.

- The optimal temperatures for the GC-oven ramp, injector, transfer line, and ion source have been determined (Fig. 1).
- Method validation was carried out assessing linearity, LOD, LOQ, precision, recovery, accuracy, and matrix effect (Fig. 2).
- Figure 3 suggests a high biological variability.
- No CPFA could be found in all haymilk samples.
- CPFA could not be detected in some samples in which grass silage was fed.
- CPFA was always detected if maize silage was fed.

Aknowledgements

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