CYCLOPROPANE FATTY ACIDS AS QUALITY MARKERS IN MEAT

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Abstract – Cyclopropane fatty acids (CPFA) are unusual fatty acids of microbial origins and, recently, their presence was demonstrated in milk and dairy products. Information is lacking on the presence of cyclic fatty acids in meat. Therefore, an analytical procedure, based on GC-MS analysis and ^{1}H NMR spectroscopy, to detect them in meat was developed. The GC-MS method allowed to detect cyclopropane fatty acids, but in some cases, the presence of interfering peaks required the confirmation by ^{1}H NMR spectroscopy. CPFA (mainly dihydrosterculic acid) were detected in several commercial bovine meat samples but not in meat of certified origins from cows not fed with silages; moreover, they were absent in pork, chicken and rabbit meats. Cyclopropane fatty acids can be proposed as a marker in meat of the use of silage in animal feeding.

Key Words – cyclic fatty acids, ensiled feed, GC-MS.

I. INTRODUCTION

Counterfeit of costly meat with cheaper counterparts is a serious global problem, so there is the need for new, rapid and reliable analytical methodologies and easily quantifiable markers to be used for meat authentication. Current methods for detection of different meat species in beef are based on DNA and ELISA, but also UPLC, Raman spectroscopy, low-field NMR, mass spectrometry have been considered [1]. Cyclopropane fatty acids (mainly dihydrosterculic and lactobacillic acid) are unusual fatty acids found in microorganisms, seed oils of some tropical plants and protozoa. In plants CPFA are usually minor components, where cyclopropene fatty acids are the most abundant. Sterculia foetida seed oil contains 65–78% cyclopropene fatty acids, principally sterculic acid [2]. Recently, CPFA were detected in milk and dairy products from cows fed with silages, and their determination has been demonstrated to be a powerful tool for the authentication of Protected Designation of Origin cheeses, as Parmigiano Reggiano, where the use of silages is forbidden [3]. Because no data are reported in literature on the presence of cyclic fatty acids in meat, we developed analytical procedures, combining GC-MS and ^{1}H NMR techniques, in order to detect them in different meat samples. The occurrence and content of cyclopropane fatty acids in foods were mainly correlated with the presence in forage of ensiled feeds. Therefore, cyclopropane fatty acids could be used as a marker of silage feedings and as a tool for the authentication of high quality costly meat from cows not fed with fermented.

II. MATERIALS AND METHODS

Forty samples of meat of different species were purchased on the market (Parma, Italy). Twelve beef meat samples were obtained from a farm certified for the absence of ensiled feeds in the animal diet.
Lipid extraction from muscular tissue following the Folch et al. [4] method was performed. For GC-MS analysis, 200 mg of fat were dissolved in hexane (4 ml) and added to 1 ml of the internal standard solution (tetracosane) at 50 mg/l, then mixed for 1 min with 0,2 ml of KOH 10% in methanol. After phases separation, the organic phase was injected (1 µl, split mode) on an Agilent Technologies 6890N gas-chromatograph coupled to an Agilent Technologies 5973 mass spectrometer. A low-polarity capillary column (SLB-5ms) was used. The chromatogram was recorded in the scan mode (40-500 m/z) with a programmed temperature from 40°C to 280°C. For ^{1}H NMR analysis, 100 mg of fat were dissolved in 1 ml of $CDCl_3$. ^{1}H-NMR spectra were recorded on a VARIAN INOVA-600MHz spectrometer, equipped with a 5-mm triple resonance inverse probe. Data were collected at 298 K, with 32K complex points, using a 90◦ pulse length. 1024 scans were acquired with an acquisition time of 1.707s and a recycle delay of 2 s. Presaturation of the fatty acids $–CH_2-$ signal (1.25 ppm) was performed. The NMR spectra were processed by MestReC software 6.0.2: spectra were Fourier transformed with FT size of 64k and 0.2 Hz line-broadening factor, phased and baseline corrected, and referenced to the chloroform signal (7.26 ppm).

III. RESULTS AND DISCUSSION

A quantitative GC-MS method was developed based on the method previously applied for cheese [3]. CPFA were detected in the GC–MS profiles of most of the commercial bovine meat samples, on the contrary CPFA were absent in all the twelve samples of certified meat from cows not fed with fermented forages. The amount of the cyclopropane fatty acids detected in the commercial bovine meat samples varied from 100-400 mg/kg of the total fatty acid methyl esters. GC-MS analysis of the other meat samples (pork, horse, chicken, turkey and lamb) generally showed the presence of a signal at the retention time of CPFA and with the corresponding mass spectrum, with amounts between 60 and 100 mg/kg of the total fat. In some cases, the signal at the same retention time showed a little different mass spectrum, indicating the co-elution of another unknown substance. This interfering peak was also resistant to oxidation as a saturated fatty acid, but it has not been identified yet. Therefore GC-MS analysis was not able to confirm the presence or absence of CPFA in meat samples, but it required in some cases ^{1}HNMR analysis. The presence of the cyclopropane unit was confirmed by ^{1}HNMR analysis of meat fat that evidenced characteristic signals in the region from -0.30 to -0.35 ppm, due to the methylene of the propane ring [5]. The presence of CPFA was confirmed only in bovine meat samples resulted positive by the GC-MS analysis, on the contrary CPFA resulted absent in pork, chicken, horse and rabbit meats as shown in Table 1.

Table 1 Presence of CPFA in meat samples

Meat Samples	N° Samples	CPFA Range (mg/kg total fat)
Commercial beef	14	100-400
Beef of certified origin (not fed with silages)	12	Negative
Other meats (pork, horse, rabbit, chicken, lamb, turkey, goose)	26	Negative

IV. CONCLUSION

CPFA were present in several bovine meat samples, and not in pork, chicken, horse and rabbit meats, reflecting the use of silages in animal feeding. Therefore, they can be considered interesting molecular markers, able to distinguish meat from cows fed with silage-based diets from those fed with hay-based diets. The analysis of CPFA could be a simple and useful tool for the authentication of high quality costly meat, whose specifications of production declare the absence of silages in the feeding.

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