Rapid MALDI-TOF-based Proteomics approach for fast and reliable detection of Feta cheese adulteration

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Introduction

"Feta" is a Protected Designation of Origin (PDO) Greek cheese, produced exclusively from pasteurized sheep's milk or sheep's and goat's milk (up to 30%) and ripened in brine. Similar white brined cheeses are found in many countries of the eastern Mediterranean Sea, which are mainly made of cow's milk, and cannot legally be called Feta. However, in this industry, ovine cheeses, like feta cheese, are in the spotlight of adulteration, because often cow's milk is added during their manufacture. This is the result of the lower milk yield of ewes and combined with the much lower price of cow's milk. The worldwide recognition of feta cheese combined with its high economic impact, as one the of Greece's most important exports, has brought feta fraud investigation into the frontline of research. This is to meet both the established legal standards and to reassure the public on the authenticity of the product and prevent consumers deception.

Methods

In the present work, a fast and reliable matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)-based methodology has been developed for the detection of feta cheese adulteration. For fast MALDI-TOF analysis a microfleX LRF (Bruker Daltonics) was used in linear mode operating in the mass range of 3.5 – 40 kDa. Analysis was carried out in positive ionization mode. Exploiting the total protein profile (caseins and whey proteins), an integrated proteomics-based workflow has been developed for the detection of the potential feta cheese adulteration with cow's milk. Statistical treatment using advanced in-house developed chemometric tools, such as unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) recognition techniques were utilized for the discrimination/classification of the cheese samples.

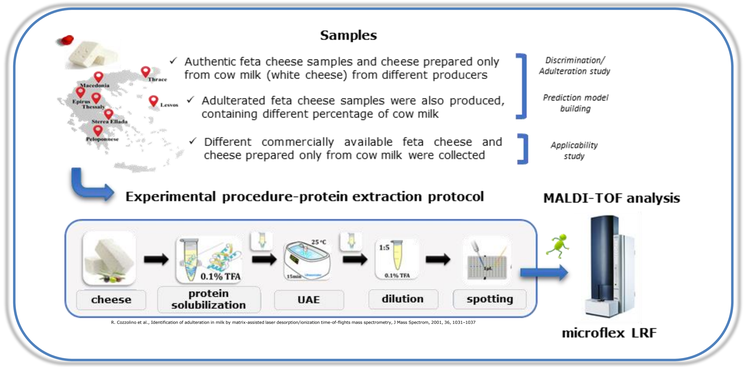

Fig. 1: Cheese samples produced in different areas of Greece collected for the study and processed with a simple SLE extraction protocol.

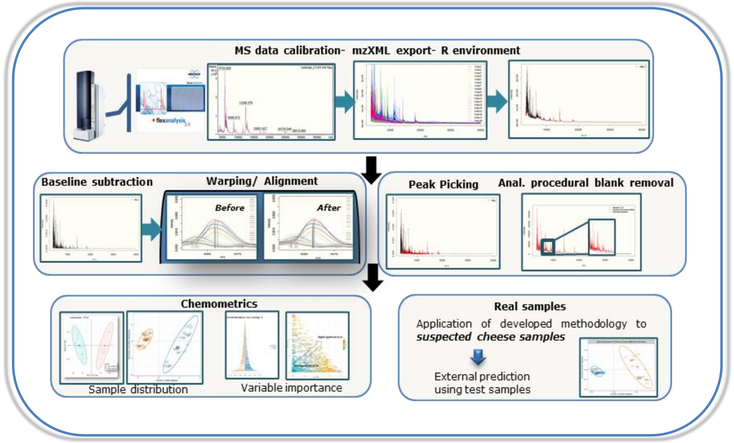

Fig. 2: Schematic workflow of the key-steps in data processing.

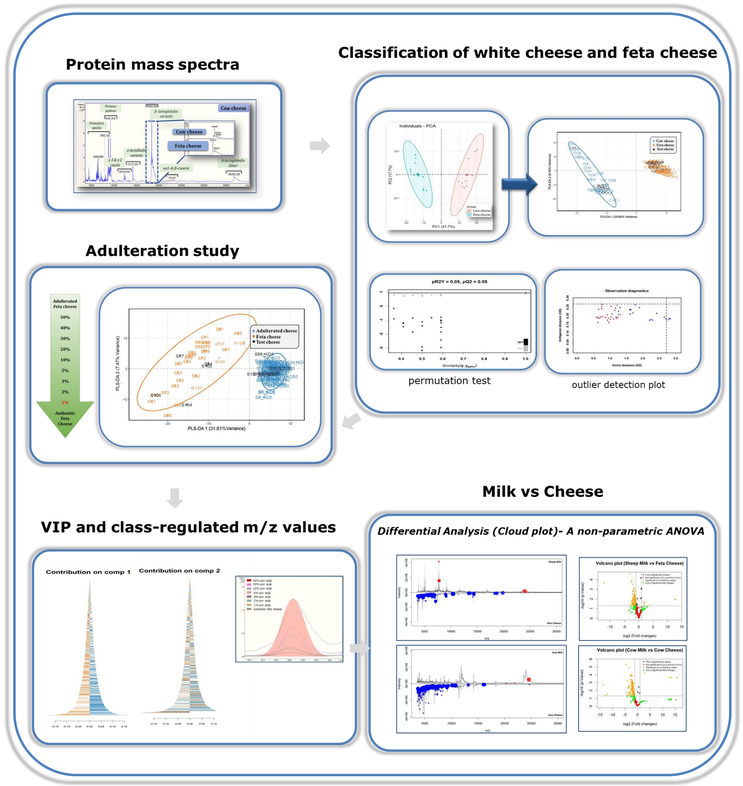

Fig. 3: Summary of the most significant results of the study using non-targeted data extraction approach. Prediction models based on the exploitation of the total protein profile were built and VIP values were investigated. Comparing to milk, different specific markers were detected in cheese.

Results

A rapid and reliable method for the adulteration of PDO Feta cheese using cow's milk as adulterant has been developed. Authentic feta cheese samples and 100% cow cheese were collected from different producers. A variety of different commercially available feta cheeses and cheeses prepared solely from cow's milk were collected. As part of this study adulterated feta cheese was also produced, containing different percentages of cow's milk. The feta cheeses were prepared using protein solubilization, ultrasonic assisted extraction (UAE) and dilution. Sinapinic acid was used as the matrix for all MALDI experiments. Different adulteration levels were studied and characteristic m/z (species-specific markers) were detected, indicating cow's milk addition during the manufacture of feta cheese. To the best of our knowledge, this is the first study reported in the literature using MALDI technology, to rapidly reveal illegal adulterations in feta cheese and achieving detection of cow's milk at substantially low adulteration levels. The total analysis time including sample preparation is significantly faster than the IEF-based European Reference Methodology (ERM) for cow's casein detection in dairy products (Annex IX (Reg. 273/2008)).

Conclusions

- The combination of the MALDI-TOF technology and chemometrics can be applied for reliable detection of feta cheese adulteration down to 1% cow's milk and feta cheese and white cheese can be fully discriminated
- Pairwise chemometric prediction models have been built with the aim of pointing out "feta" markers and cow-specific markers
- Optimizing data extraction parameters (e.g. procedural blank removal, mass alignment), the workflow enhances identification confidence and lowers false positives incidence
- Towards the simplicity and rapidity of the analytical methodology, the MALDI-TOF-MS technique presented is shown to be a powerful analytical technique for the detection of dairy product adulteration and can be applied as a routine methodology in dairy industry

MALDI / Foodomics