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Application News

iD*plus*

No. MO398 Milk and Dairy Product Profiling Using iD*plus*

- Foodstuff authenticity screening
- Effective detection of adulteration in dairy products
- Molecular profiling with minimal sample preparation
- Patented SuperSpectrum™ concept
- High degree of flexibility: customizable open database
- Visualization, clustering and dendrogramming tools for simple interpretation of results

Introduction

iD*plus*™ is an established MALDI-TOF MS based platform in microbial identification but it is far from limited to this application. The flexibility of the open database associated with iD*plus* allows the use of the platform for molecular profiling experiments and differentiation of related samples based on the unique features in their profile. New custom sample-specific entries (SuperSpectra) can be added to the existing microbial database to create a sub-database relevant to a particular area of research. This has been reported in areas as diverse as cell line identification, entomology, zooplankton research, fish speciation and the study of food-borne bacteria (ref 1-7).

Adulteration of dairy products is a significant problem in the food industry. Methods for the detection of fraudulent addition of cows' milk to other more expensive types of milk, such as goat or sheep, is important to eradicate economically motivated milk adulteration. This illegal practice, however, is not limited solely to milk production: other areas of the dairy industry such as cheese manufacture have also been targeted.

For example: the European protected designation of origin (PDO) legislation protects highly sought-after buffalo mozzarella from the Campania region of Italy (Mozzarella di Bufala Campana). While mozzarella can be made from cows' milk, it would not receive PDO certification and would be a significantly cheaper product. This has led to widespread fraudulent attempts to misrepresent cow mozzarella as buffalo mozzarella, a practice highlighted in 2010 when checks of PDO-protected Mozzarella di Bufala Campana by the ministry of agriculture in Italy found that at least 25% contained cows' milk.

This work demonstrates the effectiveness of the iD*plus* platform for rapid differentiation of dairy products and identification of fraudulent practices. Proof of principle of this technique is demonstrated using milk profiling from several different species. The established method is then applied to foodstuff authentication using mozzarella cheese as a model product.

The SuperSpectrum Concept

SuperSpectra are database entries within the iD^{plus} database (SARAMIS™) that represent a typical population of a species or sample. They are computed from multiple mass spectra (Reference Spectra) acquired from a given sample that are combined into a consensus spectrum weighted by specificity. The weighting algorithm favors sample-specific peaks and devalues common-feature peaks, further increasing confidence when using database matching. The diagram in figure 1 illustrates the process used to create a SuperSpectrum.

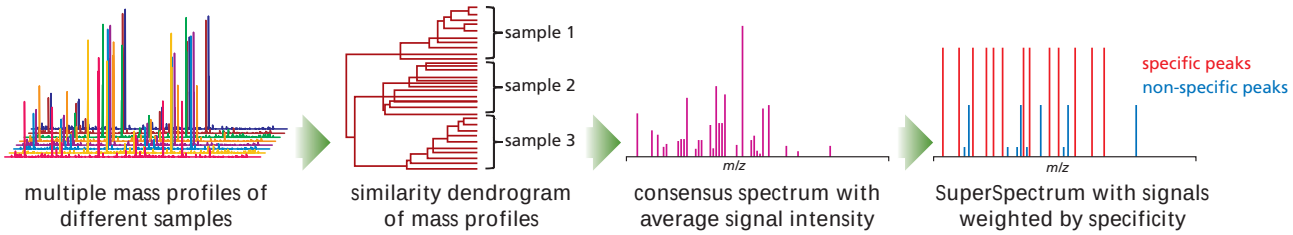

Figure 1: Creation of a SuperSpectrum

Experimental

Three types of milk (cow, buffalo and goat) and two types of mozzarella cheese (cow and buffalo) were obtained from several sources (table 1). Multiple reference spectra were acquired and combined into a characteristic SuperSpectrum for each milk product to populate a custom dairy-specific database.

Briefly, each of the milk samples was diluted 10-fold into 0.1% aqueous TFA. 1 µL of this solution was then deposited onto the target plate and left until almost dry before adding 1 µL of CHCA matrix. For the mozzarella samples, a small amount of the cheese was smeared directly onto the FlexiMass-DS™ target surface using an inoculation loop before adding 1 µL of CHCA matrix. Spectra were acquired across the m/z range 2000 to 20 000.

Product Name	Product Type	Species
Tesco Semi-skimmed	Milk	Cow
Tesco Pure Filtered Semi-skimmed	Milk	Cow
Tesco Jersey & Guernsey Cow	Milk	Cow
Delamere Sterilized Whole	Milk	Cow
Laverstock Park Semi-skimmed	Milk	Buffalo
Laverstock Park Whole	Milk	Buffalo
St Helen's Farm Semi-skimmed	Milk	Goat
Galbani	Mozzarella	Cow
Waitrose Italian	Mozzarella	Cow
Cantile	Mozzarella	Buffalo
Laverstock Park	Mozzarella	Buffalo
Garofalo	Mozzarella	Buffalo

Table 1: Summary of the milk and mozzarella samples

Results

Proof of Principle

The different types of milk analyzed (cow, goat and buffalo) generated highly taxon-specific mass profiles exhibiting many species-specific masses. Figure 2 highlights the differences observed between these profiles.

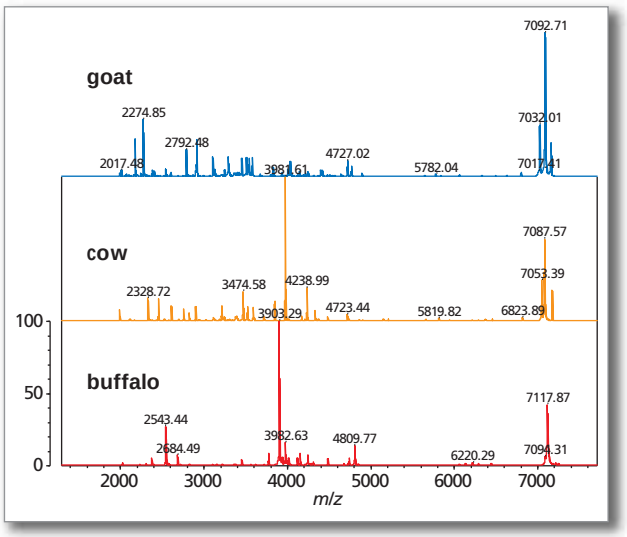

Figure 2: iD^{plus} mass profiles for goat, cow and buffalo milk

Additional samples of cow, buffalo and goat milk were analyzed and processed for cluster analysis. The dendrogram results obtained confirmed effective differentiation of the milk samples (figure 3).

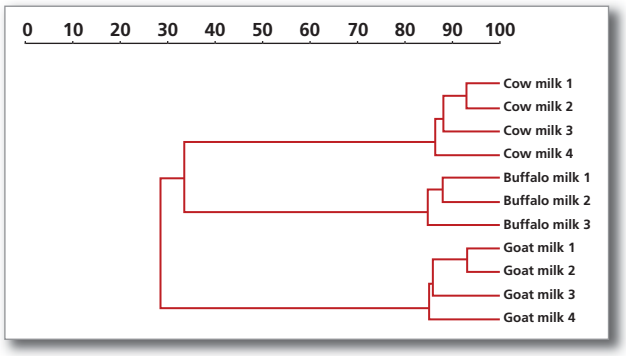

Figure 3: Cluster analysis of milk originating from cow, buffalo and goat

Food Adulteration Detection

Similarly to milk, the different types of mozzarella cheese generated distinguishable mass profiles exhibiting many product specific masses (figure 4).

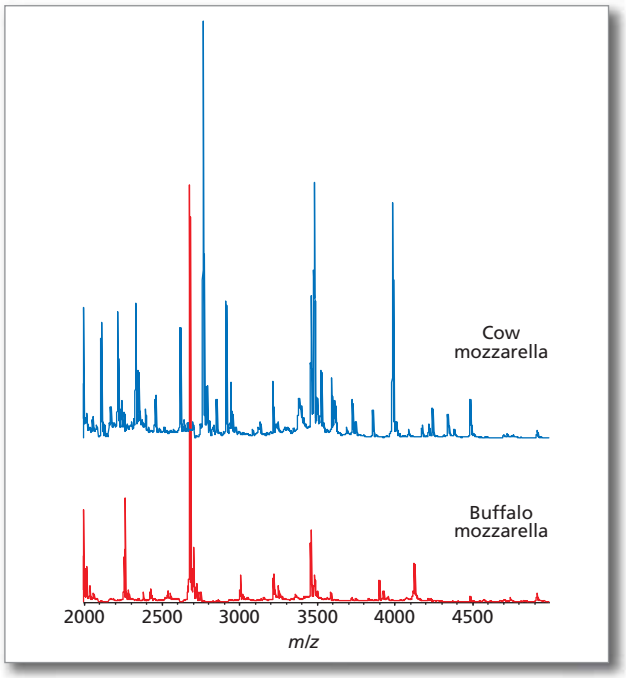

Figure 4: iD^{plus} mass profiles for cow and buffalo mozzarella cheese

To simulate adulteration of a mozzarella sample, Mozzarella di Bufala Campana cheese was mixed with cow mozzarella and prepared in the same manner as previously. The mass profile obtained for the adulterated sample is shown in figure 5 and clearly exhibits mass signals from both taxa.

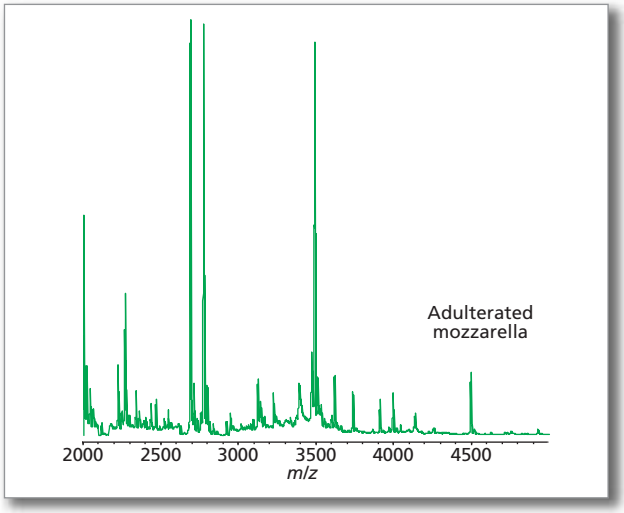

Figure 5: iD^{plus} mass profiles for adulterated mozzarella cheese

When the adulterated mozzarella sample results were submitted to search the custom dairy database an unambiguous identification was not achieved (result flagged in red in figure 6). Further inspection of the detailed result showed that the sample had matched both cow and buffalo mozzarella in the database, a clear indication of an adulterated sample (figure 6).

Figure 6: Search result confirming mozzarella adulteration

Further adulterated mozzarella samples were analyzed. Cluster analysis of the results clearly displayed three distinct groups: buffalo mozzarella, cow mozzarella and adulterated mozzarella (cow and buffalo mix) confirming that the iD*plus* system can be applied to dairy foodstuff adulteration detection (figure 7).

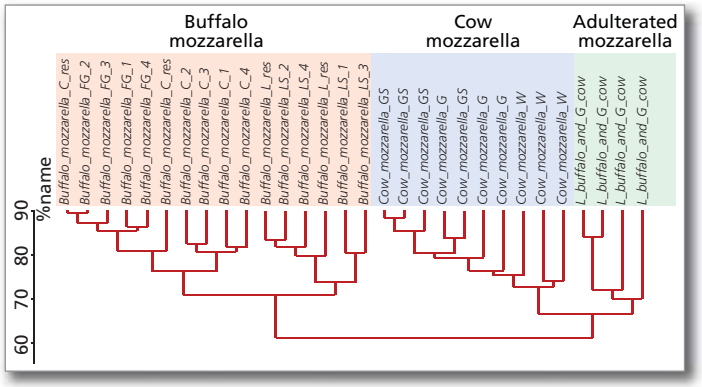

Figure 7: Cluster analysis of buffalo, cow and adulterated mozzarella

Conclusion

Foodstuff adulteration is a prevalent problem within the industry. It comprises both misrepresentation of products and deliberate contamination with lesser ingredients. The results shown here demonstrate effective detection of adulteration in dairy products. Construction of custom databases is straightforward and provides a very high level of confidence due to the use of the patented SuperSpectrum concept and the cluster analysis tool provides a simple graphical representation of the results obtained. The iD*plus* is an ideal platform for simple and efficient foodstuff adulteration detection and authenticity screening.

References

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