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Note d'application

A Complete Discovery Workflow for Species-Specific Gelatin Identification

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The species of animals used to prepare gelatin, a common ingredient in both foods and cosmetics, is often restricted and regulated for both health and religious grounds. For example, gelatin analysis is increasingly important for halal authentication. Proteomics offers an alternative approach for the evaluation of gelatin origin using the determination of species-specific peptide markers by liquid chromatography-mass spectrometry (LC-MS). Herein, we have developed a robust sample preparation and ultra-performance liquid chromatography coupled with an electrospray ionization quadrupole time-of-flight mass spectrometry operating in MS^E mode (UPLC™-QTof-MS^E) method that allows the identification of peptide markers representing bovine and porcine gelatin, respectively. ProteinWorks™ eXpress Digest Kit is a ready-to-use kit which consists of pre-weighed reagents and simple stepwise instruction. It can be applied to different types of samples without the need for further method development, hence simplifying and speeding up the sample preparation. The workflow includes sample preparation using ProteinWorks eXpress Digest Kit, separation using ACQUITY™ Premier UPLC Column and determination using UPLC-QTof-MS^E, ultimately providing a reliable approach for the identification of species-specific markers for gelatin.

Benefits

- Easy-to-follow sample preparation protocol using ProteinWorks eXpress Digest Kits
- Specific peptide markers identified and separated on an ACQUITY Premier HSS T3 UPLC Column with optimized instrumentation method using an ACQUITY UPLC I-Class PLUS System
- Progenesis™ QI for Proteomics provides a unique co-detection workflow to determine unique peptide markers to assign the species origin of gelatin

Introduction

Gelatin is widely used in the food and pharmaceutical industries due to its unique gelling properties. It is a mixture of peptides produced from collagens which are often derived from various animal species. The primary raw materials of commercial gelatins are bovine and porcine body parts, such as their hides, skin, and bone. Other sources such as fish can also be found but is less commonly available.

The outbreak of bovine spongiform encephalopathy (BSE) in 1986 led to the use of bovine gelatin being restricted in products for human consumption. There are also restrictions by some religions and cultures banning the consumption of porcine products. For example, Muslim halal and Jewish kosher dietary laws require food items free from porcine derivatives, whereas the majority of Hindus are lacto-vegetarian (avoiding meat and eggs) and beef is always avoided.[1] Manufacturers and importers may be required to submit laboratory analysis reports to confirm the products are compliant with religious and regulatory labelling requirements.[2] Accordingly, gelatin authentication is central to ensure the products are free of bovine or porcine derivatives.

A variety of methods for gelatin authentication have been developed including enzyme-linked immunosorbent assays (ELISA), DNA-based methods using Polymerase Chain Reaction (PCR) techniques, as well as those employing spectroscopy and mass spectrometry.[3-5] These methods all have their own limitations. ELISA is thought to be less effective because some antibodies show poor species specificity because of high homology in the collagen sequences among various species. DNA-based techniques have shown their potential to distinguish different sources of gelatin, but concern remains as gelatin DNA is present in low quantities in the finished products and there is possibility of fragmentation of DNA under severe heat and other processing treatments. Spectroscopic techniques, such as Fourier Transmission Infrared Spectroscopy (FTIR), can discriminate various gelatin sources based on differences in their spectral signatures using chemometrics but have shown low sensitivity and have yet to be used to determine gelatins or products containing a mixture of species. Over the last

decade, mass spectrometry-based protein and peptide profiling for food frauds has gained popularity in the area of traceability and authenticity assessment.[6] This approach utilizes the determination of species-specific peptide markers by LC-MS for the evaluation of gelatin origin.[7-10] In contrast to DNA, the amino acid sequence of protein is highly consistent in the processing of gelatin and therefore, methods based upon LC-MS have the advantage of being able to detect multiple gelatin species in a single analysis.

This approach utilizes differences on the protein level between collagen of different animal species to detect gelatin specifically per animal species, after solubilization, reduction, alkylation, and tryptic digestion. In this work, we demonstrate the first step of the workflow; the initial identification of species-specific peptides, from tryptic digests of porcine and bovine gelatin, by a shotgun proteomics. The novelty of the UPLC-MS^E is that it uses a data-independent acquisition mode and gives both product and precursor ion information from alternating high and low collision energies that generates information-rich mass spectra.

Results and Discussion

Six commercially available bovine and porcine gelatin standards (Sigma Aldrich) were pre-treated with 50 mM ammonium bicarbonate (NH_4HCO_3). This is followed by a 5-step protocol using ProteinWorks eXpress Digest Kits, as indicated in Figure 1.

The analysis was performed on ACQUITY UPLC I-Class PLUS System coupled with Xevo™ G2-XS QTof Mass Spectrometer using a water/acetonitrile gradient on a 1.8 µm ACQUITY Premier HSS T3 Analytical Column (p/n: 186009468 <https://www.waters.com/nextgen/global/shop/columns/186009468-acquity-premier-hss-t3-column-18--m-21-x-100-mm-1-pk.html>).

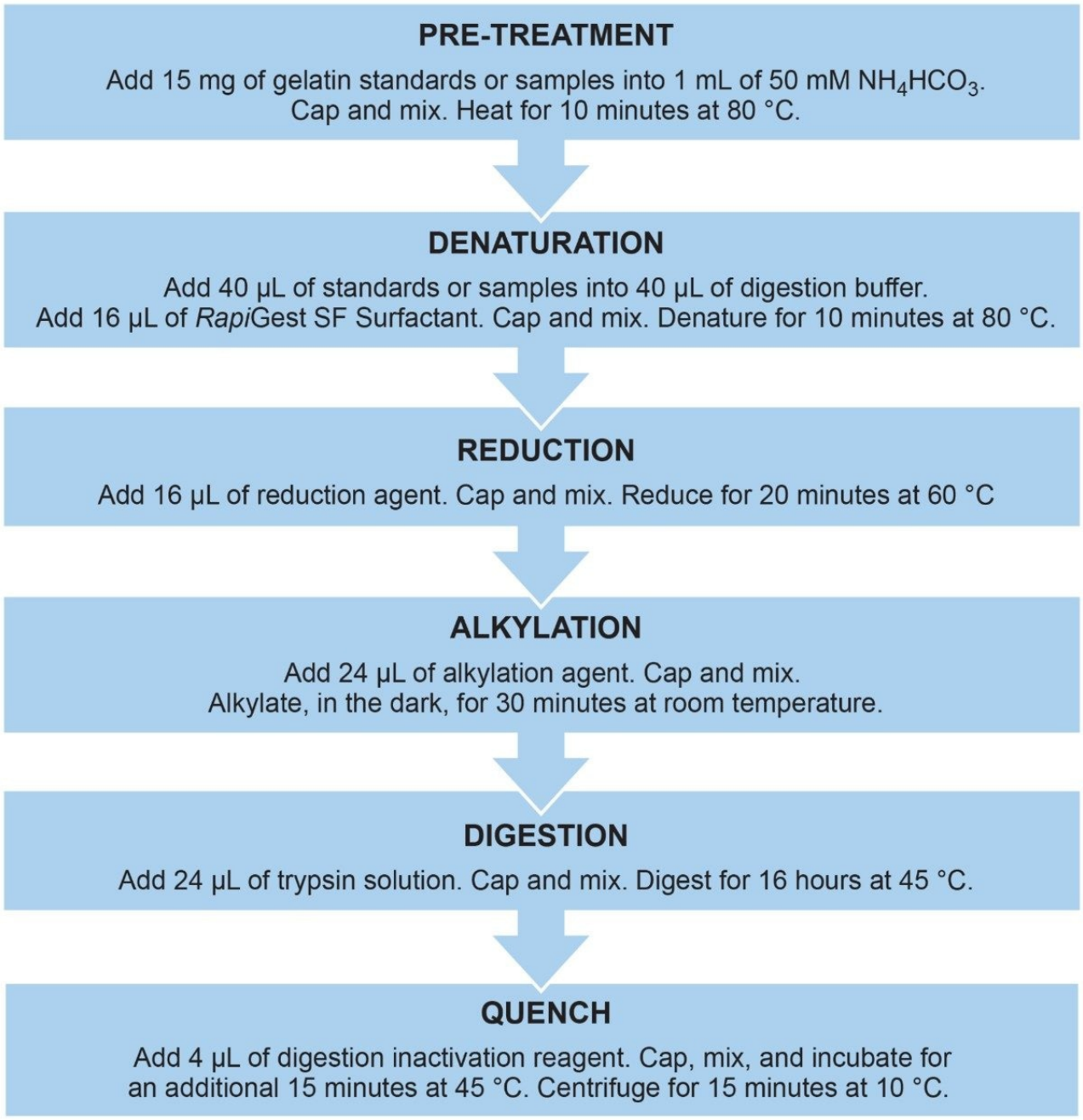

Figure 1. 5-step digestion protocol.

Triplicate injections were performed for each gelatin standard. The raw data was analyzed using Progenesis QI for Proteomics, which is a discovery analysis software for LC-MS data. This software was used to identify

characteristic markers for gelatin speciation. It follows a menu-guided workflow which performed chromatographic alignment, data normalization, and peak picking automatically. The peptide data obtained was exported to EZInfo, and the S-plot from the orthogonal partial least square discriminant analysis (OPLS-DA) model (Figure 2) highlights the discriminatory compounds associated with the bovine and porcine gelatin. The features with the highest confidence and importance (boxed up in Figure 2) were selected and imported back into Progenesis QI for Proteomics, for verification and evaluation of identity. An identification example of the peptide SGDRGETGPAGPAGPIGPVGAR is shown in Figure 3, using the Ion Accounting identification workflow with the settings: FDR less than 1%, fixed modification (carbamidomethylation of cysteines), variable modifications (oxidation of methionines), and *Sus scrofa domesticus* and *Bos taurus* UniProt databases. When comparing the normalized abundance profiles of the selected peptides, it can clearly be seen that the choices are unique and specific to the respective species (Figure 4). The shortlisted signature peptide markers are summarized in Table 1 and can be used as targets for quantitative LC-MS/MS analysis of commercial products after validation.

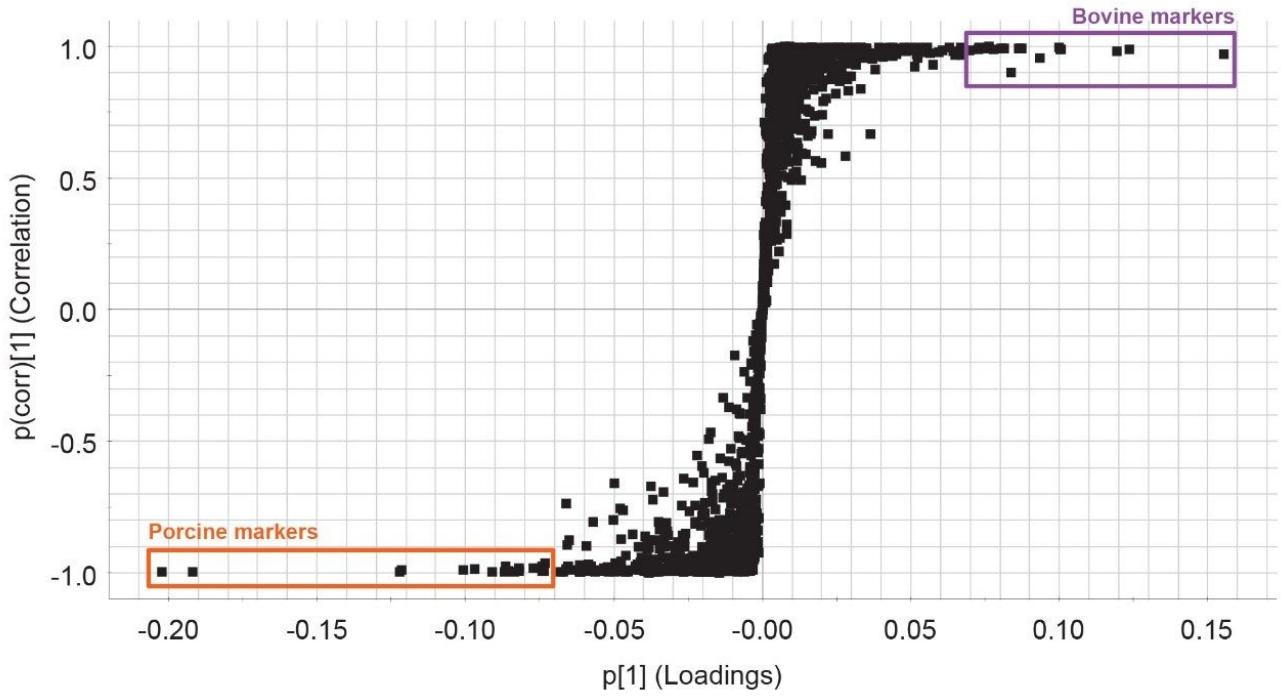

Figure 2. S-plot that shows discrimination of bovine versus porcine gelatin.

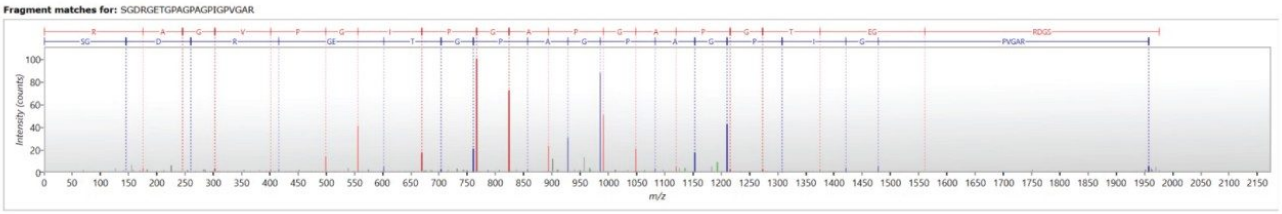

Figure 3. Identification of peptide SGDRGETGPAGPAGPIGPVGAR.

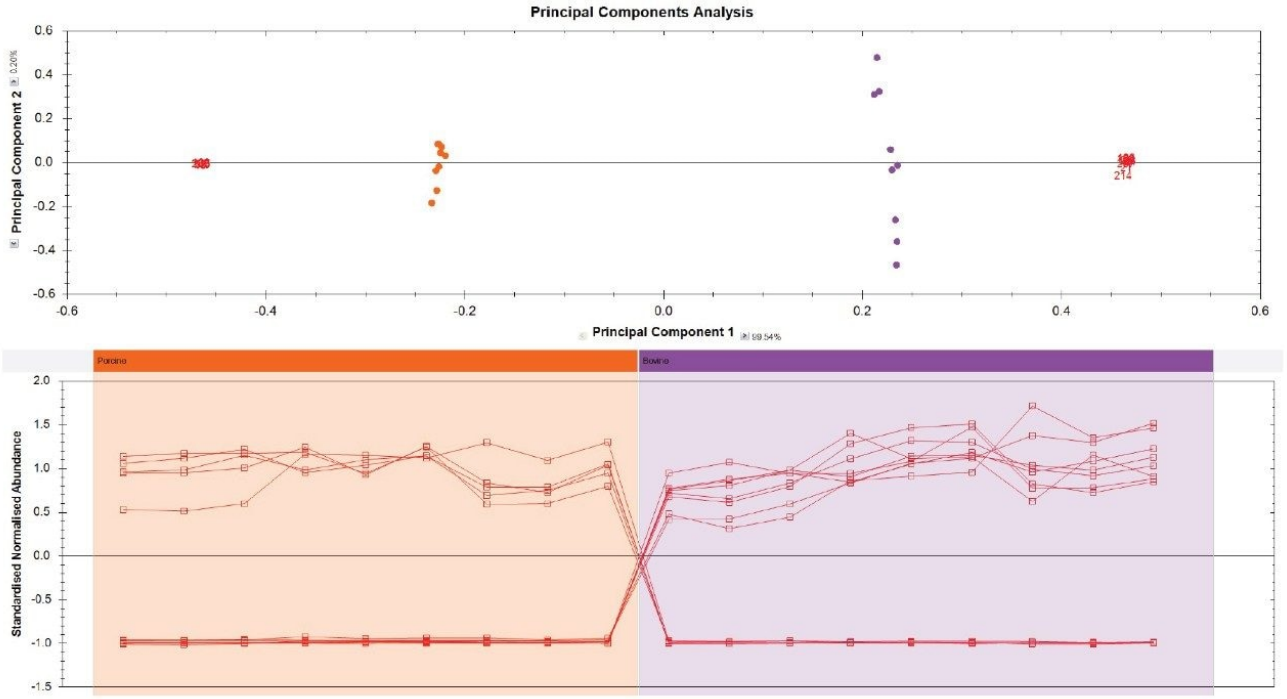

Figure 4. Principal components analysis (PCA) and the standardized normalized abundance plots for porcine (orange, left) and bovine (purple, right).

Class	Sequences	Mass	*m/z*	Charge	Mass error (ppm)
Bovine	QGPSGASGER	944.4297	473.2221	2	-1.5
	GSPGPAGPK	766.3970	767.4043	1	-0.5
	GATGPAGVR	784.4193	785.4265	1	0.2
	GEPGPAGSVGPAGAVGPR	1531.7728	766.8937	2	-1.0
	SGDRGETGPAGPAGPIGPVGAR	1974.9881	659.3366	3	0.5
		1974.9770	988.4958	2	-5.1
	IGQPGAVGPAGIR	1191.6740	596.8443	2	1.3
	GETGPAGPAGPIGPVGAR	1559.8054	780.9100	2	-0.2
Porcine	TGQPGAVGPAGIR	1179.6356	590.8251	2	-0.4
		1179.6339	1180.6412	1	-1.8
	SGDRGETGPAGPAGPVGPVGAR	1960.9776	654.6665	3	3.1
		1960.9691	981.4918	2	-1.3
	GETGPAGPAGPVGPVGAR	1545.7902	773.9024	2	0.2

Table 1. Bovine and porcine peptide markers.

Conclusion

This work demonstrates how an intuitive proteomics discovery workflow, using UPLC-QTof MS and Progenesis QI for Proteomics, can be used to determine a series of different peptide markers for gelatin of porcine and bovine origin. The use of universal kits streamlines and simplifies the process, aids the implementation of such proteomics methods, and ensures they are reproducible between laboratories and instrument platforms. The next step is to create targeted methods for these markers to verify their sensitivity and selectivity for classifying the species origin of gelatin as a food ingredient and in finished products.

References

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