Detection of Gelatin Adulteration using Bio-informatics, Proteomics, and High-Resolution Mass Spectrometry

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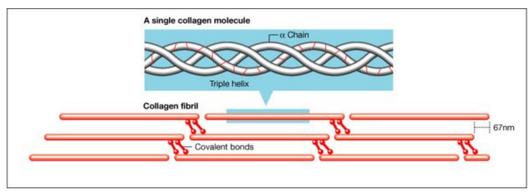
Introduction

Gelatin is composed of highly processed proteins and is widely used as a gelling and thickening agent in a variety of food products, including meat, confectionery products, and water-based desserts. It is also widely used in the pharmaceutical industry. Gelatin is obtained by hydrolysis of collagen, which is extracted from materials such as bone, hide and skin from animal slaughterhouses. Nearly 80% of gelatin is produced from pig by-products. However, vegetarian, Halal, and Kosher gelatin are normally prepared from seaweed, fish, or bovine skin or bones. The objective of this study was to demonstrate that detection of specific peptide biomarkers in the digested gelatin samples using HPLC–QqOrbitrap™ is an effective strategy to detect gelatin adulteration.



Collagen polypeptide primary structure

-G-X-A-G-A-A-G-Y-A-G-A-A-G-X-A-G

G – Glycine
X- proline or hydroxyprolinie
Y – Lysine or hydroxylisine
A- other amino acids

Figure 1. Collagen structure. Type I collagen protein sequence is highly conserved between animal species; there are substantial residue substitutions and the level and locations of post-translational modification (proline) are variable. Collagen has an unusual amino acid sequence. Glycine (G) is found at almost every third residue, and collagen contains large amounts of proline (P) as well as hydroxyproline.

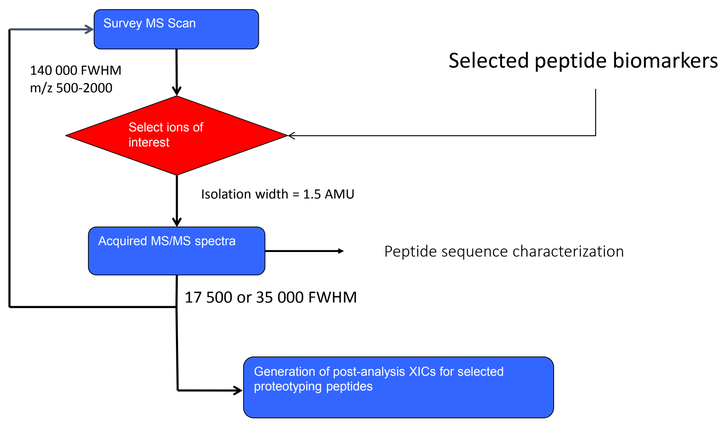


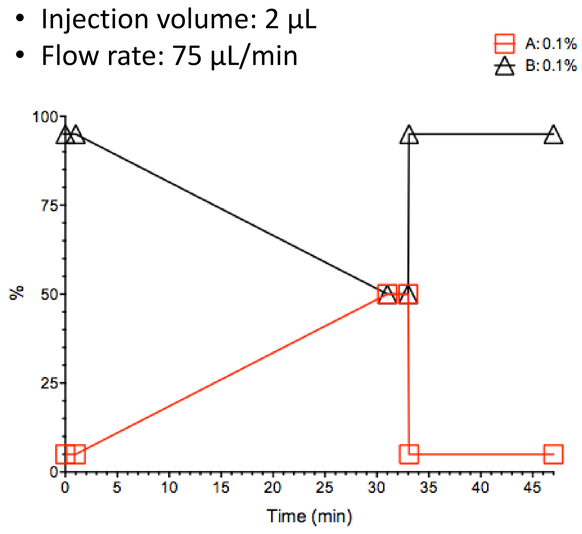
Figure 2. Analytical strategy to identify and detect species-specific gelatin biomarkers.

Sample preperation

- 0.1 g of gelatin was mixed with 0.5 mL of 100 mM ammonium bicarbonate (pH 8.5). Sample was vortexed and the mixture was sonicated for 30 minutes.
- 200 µL of the suspension was transferred to a microcentrifuge tube. Proteins were precipitated with 200 µL of ethanol.
- Ethanol was discarded and the protein pellet was evaporated for 20 min to remove traces of ethanol.
- Protein pellets were dissolved in 200 µL of 100 mM ammonium bicarbonate (pH 8.5). Only soluble proteins were dissolved.
- Proteins were denatured by heating at 120˚C for 10 min (close glass tubes necessary).
- Reduction and alkylation is not necessary since collagen proteins contain disulfide bonds only at the end of the polypeptide chains (propeptide)
- 2 µg of proteomic-grade trypsin was added and the reaction was performed at 40˚C for 24 h.
- Digestion was stopped by adding 500 µL (or 200 µL if ethanol precipitation is used) of a 1% TFA solution. Samples were centrifuged at 12,000 g for 10 min. 200 µL of the supernatant was transferred into injection vials.

HPLC conditions

- Column: Thermo Scientific™ BioBasic™ C_8 100 x 1 mm (5 µm)
- Injection volume: 2 µL
- Flow rate: 75 µL/min



Results

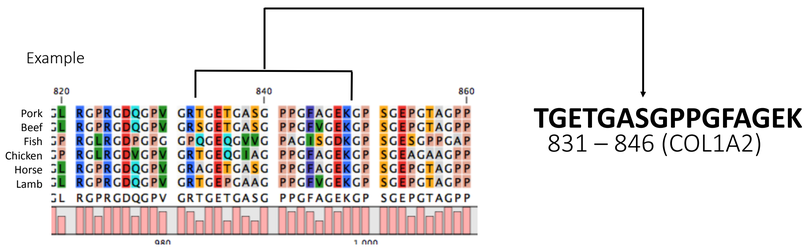


Figure 3. *In silico* analysis of collagen type 1 sequence. Species-specific tryptic peptides can be identified.

Table 1. Identified pork specific tryptic peptides.

Start		
975	GNPGPAGAAGAPGPQGAVGPAGK	Complementary peptides [831-846] / [847-879] [949-974] / [975-996] were chosen for a targeted method.

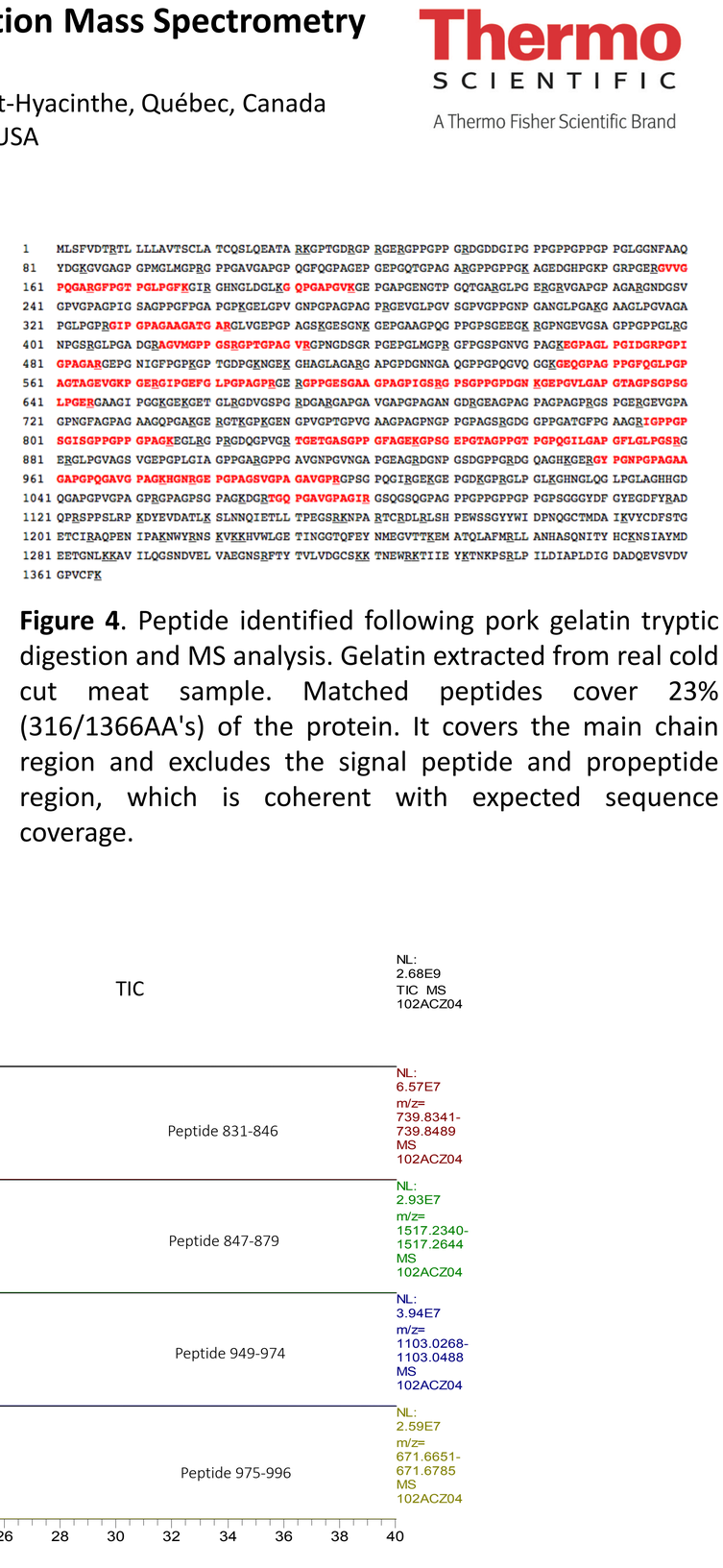


Figure 5. Analysis of gelatin extracted from pork cold cut sample. All four targeted peptides were detected.

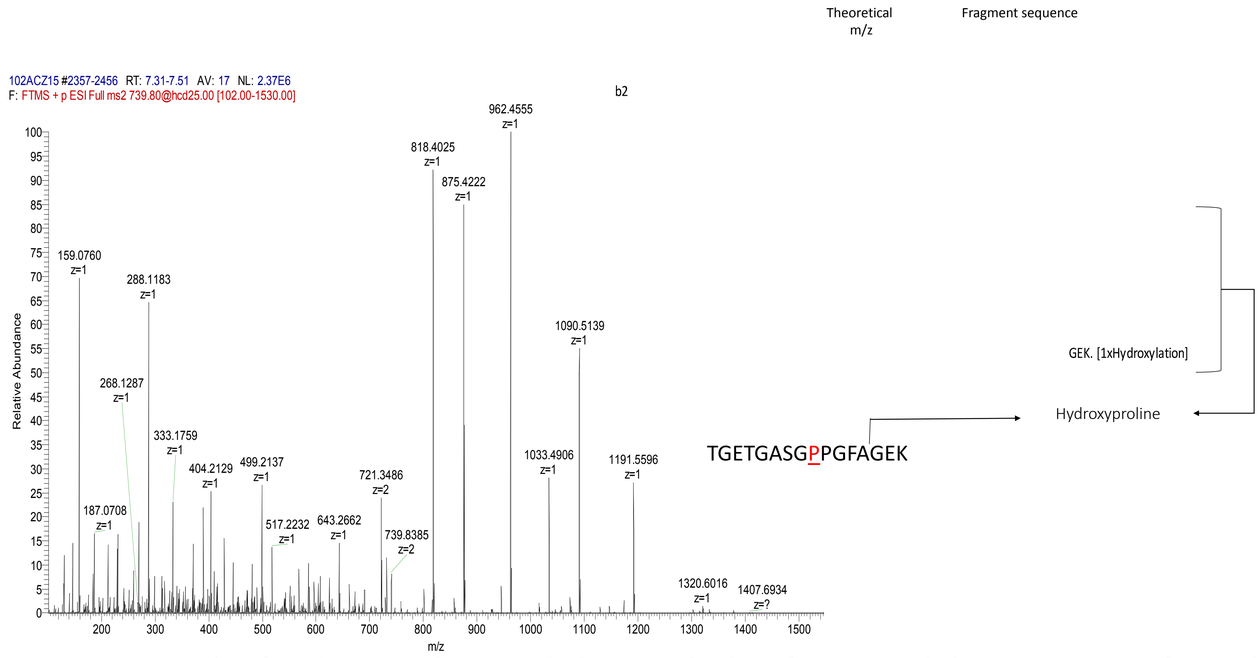


Figure 6. Example of MS^2 spectra for peptide [831-846]. Identification and characterization of peptide sequence.

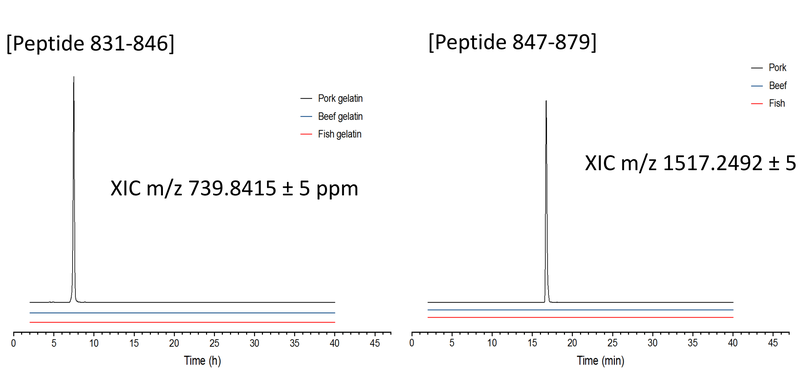


Figure 7. XICs for specific signature pork gelatin peptides. These peptides can be used for gelatin speciation.

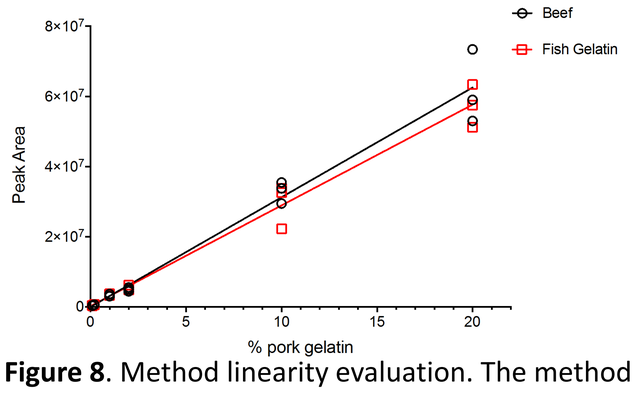


Figure 8. Method linearity evaluation. The method was able to detect 0.1% pork gelatin biomarkers in gelatin samples. Therefore, contaminated or adulteration of gelatin samples can be detected down to 0.1% (w/w) using this experimental approach and potentially better.

Conclusion

Species-specific peptide biomarkers were identified, characterized, and quantified using an HPLC-QqOrbitrap MS analytical strategy. The method was tested with real samples (animal cold cuts obtained from grocery stores) and peptides were clearly detected. Moreover, we were able to detect down to 0.1% (w/w) of pork gelatin fortified in bovine or fish gelatin. Using labeled internal standards, this analytical method could be extensively validated for routine food analysis.

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