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**Mass Spectrometry Approach for Identification of Porcine and Bovine Gelatin Biomarkers in
Gelatin Food Ingredient**

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ABSTRACT

Gelatin is a common food additive that is obtained by hydrolysis of collagen primarily from bovine and porcine skin and bones. The similarity between bovine and porcine gelatin makes it difficult to trace their animal origin. In this work, a combination of quadrupole time-of-flight mass spectrometry (Q-TOF MS) coupled with chemometric based statistical analysis is used to profile and distinguish the unique chemical fingerprint origin from porcine and bovine gelatin in a few commercial gelatin ingredients. Using standard gelatins made from porcine and bovine origin, the gelatine were reduced and alkylated before subjected to trypsin digestion. The digested gelatin was dried and reconstituted into small volume before injecting into a Q-TOF LC/MS system. Each sample was analyzed in multiple replicates to eliminate technical errors. Data analysis was carried out using a chemometric software, Mass Profiler Professional. Spectrum Mill was used as a protein database search engine for the identification of protein/peptide markers. This workflow was then tested by using commercially available gelatins. Data analysis were done by using molecular features/peaks finding based on grouping together corresponding ions including isotope, adduct and charge state. After molecular feature extraction on all of the samples, a chemometric software was used for statistical analysis of the differential features of each of the gelatin samples. The porcine and bovine gelatin samples can be distinguished from the statistical difference according to PCA and ANOVA analysis. The unique peptides found in the bovine and porcine gelatin were matched against the porcine and bovine peptide database to identify their amino acid sequences. This study described a workflow for profiling and identification of porcine and bovine gelatin markers using Q-TOF LC/MS and statistical analysis which could be used as a method for detection and authentication of gelatin animal origin.

Keywords: gelatin, mass spectrometry, liquid chromatography, Q-TOF