Determination of Animal Species Origin from Gelatin in Food and Pharmaceutical Products by LC-MS/MS

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ABSTRACT

Gelatin has many applications in the food, pharmaceutical and nutritional industries supported by its excellent properties and functionalities. Pig skins and bovine hide and bones are the largest commercial sources of gelatin. For religious reasons, Muslims, Jews, Hindus etc. in the world would need to ensure that their gelatin based food products do not contain pork or beef hence the development a method for determination of animal species origin from gelatin products is necessary. In this paper we present a fast, robust, and reliable method, which has been validated for determination of animal species origin from gelatin products. The LC-MS/MS method using the Multiple Reaction Monitoring (MRM) detects gelatin with Limits of Quantitation (LOQ) of 1% w/w in gelatin mixture.

INTRODUCTION

Gelatin is a protein based product derived from the fibrous protein collagen and produced by partial denaturation of native collagen extracted from skins, bones and connective tissues of animals like bovine and porcine.[1] In its production, dilute acid or alkali is used to treat raw animal material to achieve partial cleavage of crosslink and break the structure, resulting in formation of ''warm-water-soluble collagen'', namely gelatin.[2] It is widely used as a gelling and thickening agent in a variety of foodstuffs such confectionary products, water-based desserts and in the pharmaceutical industry e.g. in gel capsules for medicines. Pig skin was the largest commercial source of gelatin, followed by bovine hide and bones as sources of gelatin.

In the mid-1980s, the world was shaken by the emergence of bovine spongiform encephalopathy (“mad cow disease”) epidemic that swept the European countries. Since then, there has been much concern about using the gelatin from the infected animals. In addition, religious and socio-cultural factors have influenced the need for a method to identify the species origin of gelatin to fulfil the halal and kosher markets.

Under the conditions of gelatin production, species-specific DNA present from the original animal is often denatured or removed making the use of the polymerase chain reaction (PCR), often used in species identification, difficult[3-5] or impossible6. ELISA (enzyme-linked immunosorbent assay) has been used for speciation[7] but this approach has limitations due to it’s risk of false negatives and positives. Some nano UHPLC-ESI-Q-TOF based methods have also been reported for speciation of gelatin[8], but this approach has issues due to the inherent complexities of nano UHPLC as well as long run times. Triple Quad/TRAP systems have excellent sensitivity, and the combination of UHPLC can quickly and accurately identify markers in a short time, making it more suitable for deployment in a routine setting.

MATERIALS AND METHODS

Sample Preparation:

5 mg of gelatin or gelatin production sample was dissolved using 50 mM ammonium bicarbonate solution at 37˚C to the final concentration of 1mg/ml. Digestion took place either overnight at 37˚C (10-15 hours) or using a microwave burst technique.

HPLC Conditions:

An EXIONLC™ LC system with an Phenomenex Kinetex® C18 , 100 Å, 50x3 mm, 2.6 µm column at 40˚C with a gradient of eluent A 0.1% formic acid in water and eluent B 0.1% formic acid in acetonitrile was used at a flow rate of 250 µL/min. The injection volume was set to 20 µL. The LC gradient conditions are shown in Table 1.

MS/MS Conditions:

A SCIEX QTRAP™ 4500 LC-MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe was used. Porcine and bovine gelatin were detected using 3 MRM transitions per species to allow identification based on the number of detected markers.

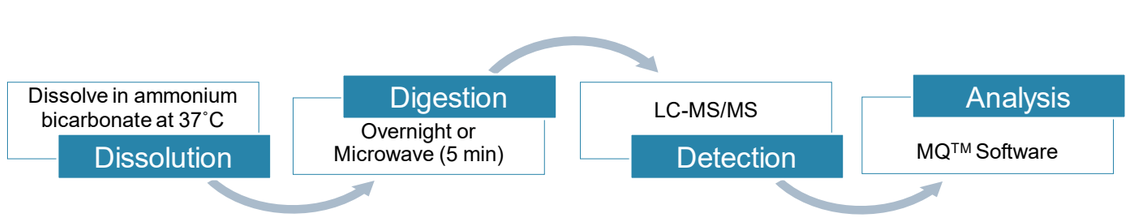

Figure 1. Work flow of the method to determine the animal species origin from gelatin by LC-MS/MS.

Step	Total Time (min)	Module	Event	Parameter (%)
1	2	Pumps	Pump B Conc.*	5
2	12	Pumps	Pump B Conc.*	40
3	12.5	Pumps	Pump B Conc.*	90
4	13.5	Pumps	Pump B Conc.*	90
5	14	Pumps	Pump B Conc.*	5
6	19	Controller	Stop*	-

Table 1. LC gradient conditions used for separation at a flow rate of 250 µL/min

RESULTS

A quick, simple and effective method for identification of porcine and bovine gelatin was developed (Figure 1). Three markers for each species of gelatin are using for identification (Figure 2). As shown in Figure 3 and 4, 1% contamination of bovine gelatin with porcine gelatin or porcine gelatin with bovine gelatin could be easily identified by microwave digestion, and the overnight digestion shows the same result.

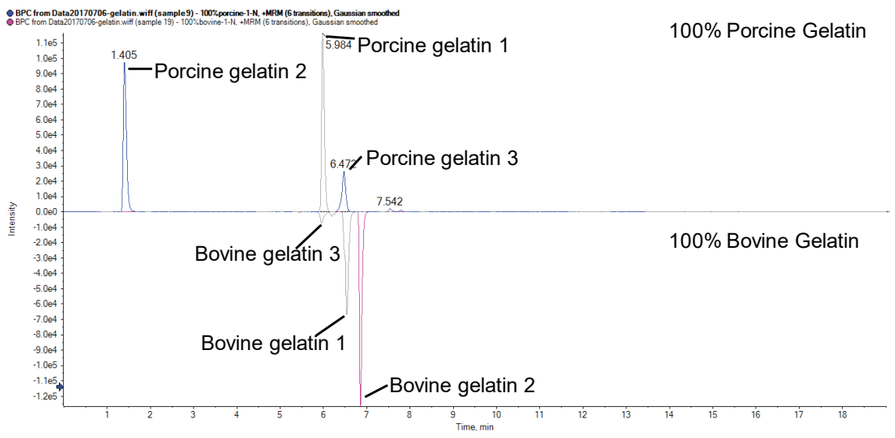

Figure 2. Porcine gelatin markers and bovine gelatin markers for identification the animal species origin from gelatin.

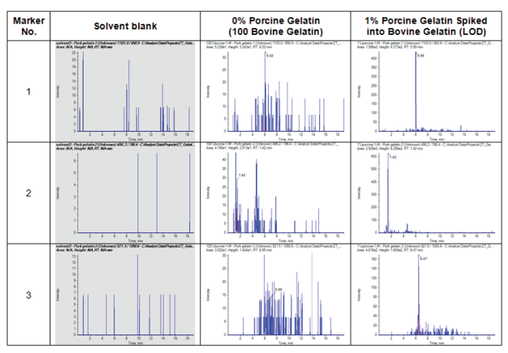

Figure 3. 1% porcine gelatin could be easily identified by microwave digestion

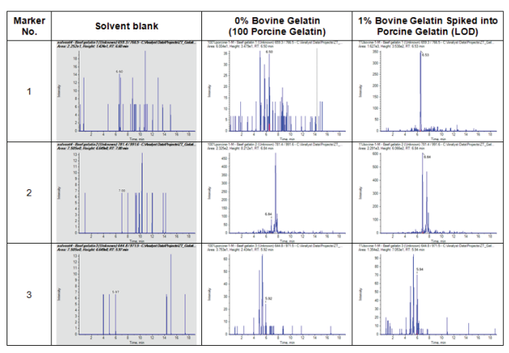

Figure 4. 1% bovine gelatin could be easily identified by microwave digestion

System stability was evaluated using the 10% added gelatin samples. As shown in Figure 3, the method has good system stability. A total of 14 commercial gelatin products (raw gelatin, dairy products, candies, sausages and capsules) were used as unknown samples to assess the feasibility of this method for screening the species of gelatin in food or pharmaceutical products. As shown in Table 2 and Figure 5, the method was able to accurately screen for the species of gelatin both in raw and processed gelatin products using both digestion methods.

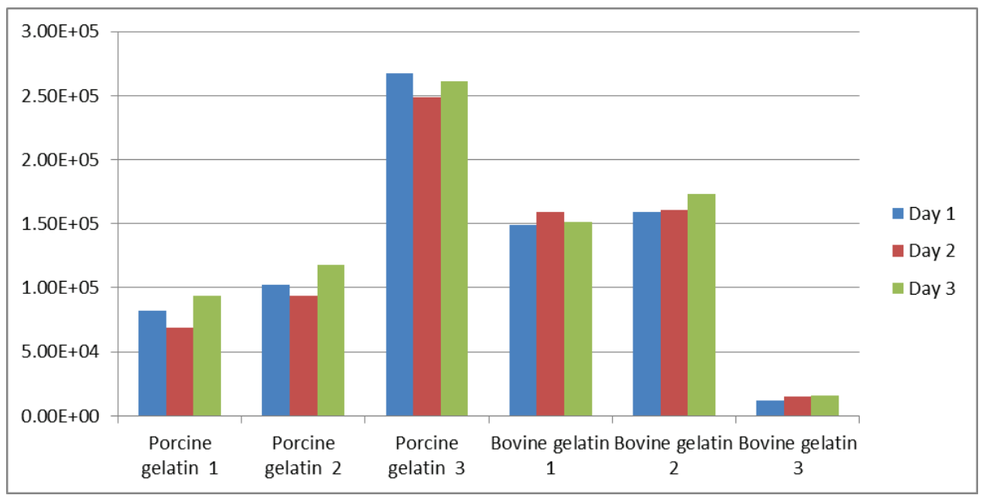

Figure 5. System stability test result. all the markers were found to be stable up to 3 days at 10°C in the autosampler.

ID	Description	Raw or Processed	Number of gelatin marker with positive identification (n of 3)			
			Overnight Digestion		Microwave Digestion	
			Porcine	Bovine	Porcine	Bovine
1	Raw Gelatin 1	Raw	3 of 3	3 of 3	3 of 3	3 of 3
2	Raw Gelatin 2	Raw	3 of 3	3 of 3	3 of 3	3 of 3
3	Raw Gelatin 3	Raw	3 of 3	3 of 3	3 of 3	3 of 3
4	Yoghourt Brand 1 (Halal)	Processed	N/A	N/A	0 of 3	0 of 3
5	Yoghourt Brand 2	Processed	3 of 3	3 of 3	3 of 3	3 of 3
6	Yoghourt Brand 3	Processed	3 of 3	3 of 3		
7	Dairy Product Brand 1 (Halal)	Processed			0 of 3	0 of 3
8	Beef Sausage Brand 1 (Halal)	Processed			0 of 3	3 of 3
9	Capsules 1	Processed	3 of 3	3 of 3	3 of 3	3 of 3
10	Capsules 2	Processed	3 of 3	3 of 3	3 of 3	3 of 3
11	Candy Brand 1	Processed	3 of 3	3 of 3		
12	Candy Brand 2	Processed	3 of 3	1 of 3		
13	Candy Brand 3	Processed	1 of 3	2 of 3		
14	Candy Brand 4	Processed	3 of 3	1 of 3		

Table 2. Screening of commercial gelatin products. For gelatin to be identified as present, 2 out of 3 markers will have to be positively identified. It did not demonstrate false positive where gelatin is not present, such as the absence of gelatin in Halal certified products (sample 4 and 7, sample 8 also absence of gelatin but it is a kind of beef product so bovine gelatin marker can be detected in it).

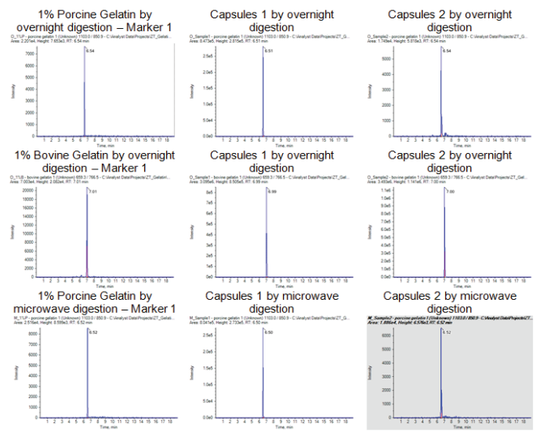

Figure 6i. A part of example XICs for gelatin markers in commercial gelatin products..

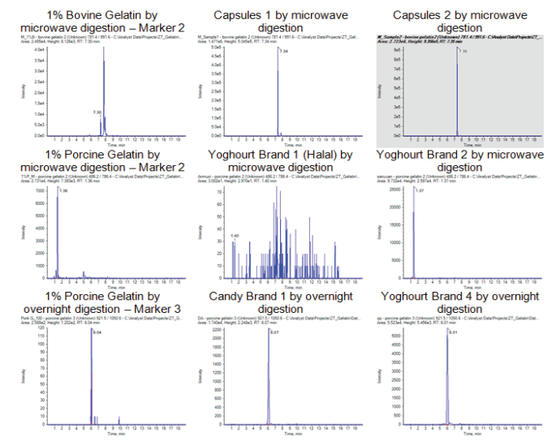

Figure 6ii. A part of example XICs for gelatin markers in commercial gelatin products..

CONCLUSIONS

A fast, robust, and reliable method, for the determination of animal species origin from gelatin in food and pharmaceutical products by LC-MS/MS was developed and validated. This method has the advantages of simple operation, quick analysis and accurate identification. High resolution LC using a small particle size column was combined with high sensitivity detection using an SCIEX Triple le Quad/QTRAP™ 4500 LC-MS/MS system. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity.

By using this method, 1% contamination of gelatin could be easily identified. The method was validated in 14 commercial gelatin products include raw gelatin, dairy products, candies, sausages and capsules. It can accurately identify the animal species origin of gelatin in the gelatin product and avoid false positives.

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TRADEMARKS/LICENSING

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