A Novel Sensitive LC-MSMS Method for Porcine Gelatin Detection in Cosmetic and Confectionary Products

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ABSTRACT

Purpose: This study aims to develop a sensitive, specific, and robust LC-MS/MS method to detect the presence of porcine gelatin in cosmetic and confectionary products..

Methods: Commercial cosmetic and confectionary specimens spiked with porcine gelatin were subjected to protein extraction and tryptic digestion. The extracts were analyzed by a HPLC system coupled to triple quadrupole tandem mass spectrometer. A reverse phase C18 150 x 1 mm, 3 µm HPLC column with a water:acetonitrile mixture containing 0.1 % formic acid was used. The quantitative analysis was performed using selective reaction monitoring (SRM) to detect multiple porcine-specific peptide markers. The food and cosmetic specimens were spiked with known amounts of porcine gelatin to assess the limit of quantitation (LOQ) for different sample matrices. The inter-day reproducibility of the established method was examined by comparing the results of the extracts conducted on different days.

Results: Eleven peptides were found to be specific to porcine gelatin and they were not detected in bovine reference materials. Among the eleven peptides, the five most sensitive and robust peptides were employed for the SRM method development. The SRM transitions for each peptide marker were optimized to predict the SRM transitions and respective collision energies (CE) using the porcine reference material. The method was then tested on hair cream and facial gel specimens spiked with known amount of porcine gelatin. All the porcine-specific markers were successfully detected in the spiked hair cream and facial gel specimens with good repeatability (CV < 20 %) and linearity (R2 > 0.98). The method was capable of detecting 0.01 % (LOQ) of porcine gelatin in the hair cream specimen, and 0.02 % (LOQ) of porcine gelatin in the facial gel specimen. The results of the extracts conducted on different days also showed good inter-day precision. In-house prepared marshmallow, made by porcine gelatin, was also tested to assess the applicability of this method for detection of porcine gelatin in confectionery products. All the SRM transitions for the porcine-specific markers were detected in the in-house prepared marshmallow, demonstrating excellent specificity of the established method. A broad range of commercially available cosmetic and confectionery products, such as fruit gum candies, collagen drinks, lipstick, facial masks, etc., were also analyzed by the established method.

INTRODUCTION

Gelatin is a mixture of polypeptides derived from hydrolysis of collagen extracted from skins, bones and connective tissues of animals. Gelatin has been widely used in food, cosmetic and pharmaceutical industries. Nearly 80% of gelatin are made from pork because of the cost-effectiveness and availability. However, consumption of pork and/or pork-based byproducts is strictly forbidden in certain religions. Thus, it is necessary to develop a detection method that can identify and quantify the biomarkers specific for porcine gelatin. In this work, we present a novel and robust LC-MSMS method for sensitive and specific detection of porcine gelatin in cosmetic and confectionary products. The established method can be easily applicable for routine laboratory testing to verify halal authenticity of gelatin.

MATERIALS AND METHODS

Sample Preparation

0.2 g each of the cosmetic and confectionary samples were weighed into microcentrifuge tubes and dissolved in 800 µL of sodium bicarbonate buffer. The mixture was vortexed and sonicated for 30 mins. Hexane was added into the mixture and sonicated, subsequently the mixture was centrifuged and the top supernatant layer was discarded to remove the lipid from the sample. The hexane extraction was repeated twice. The extract was then digested with trypsin at 37 ºC for 16 hrs. Afterwards, the sample was centrifuged and 200 µL of the supernatant was transferred into vial for LC-MS analysis.

Test Method
HPLC Conditions:
Thermo Scientific™ Vanquish™ Flex Binary UHPLC system
Column: Acclaim Pepmap, 150 x 1 mm, 3 µm
Column Temperature: 45°C
Injection Volume : 2 µL
Mobile Phase A: Water + 0.1 % Formic Acid
Mobile Phase B: Acetonitrile + 0.1 % Formic Acid
Flow Rate : 100 µL/min

Gradient: 0.0 min 5 % B
2.0 min 5 % B
13.0 min 50 % B
15.0 min 50 % B
15.1 min 5 % B
25.0 min 5 % B
Run Time : 25 mins

MS and Ion Source Conditions:
Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer
Ion Mode: Positive Electrospray (H-ESI) Mode
Spray Voltage: 3800 V (+)
Vaporizer Temperature: 250°C
Ion Transfer Tube Temperature: 325°C
Sheath Gas: 20
Aux Gas: 10
Sweep Gas: 0
Q1/Q3 Resolution: 0.7/0.7 FWHM
Cycle time (sec): 0.8
CID Gas (mTorr): 1.5
Chromatographic Peak Width: 30 s

Table 1. Scan parameter - SRM table

Name	RT (min)	Polarity	Transition	Precursor (m/z)	Product (m/z)	CE (V)
P1	2.15	+	Quan	472.7	432.2	25
			Qual 1st	472.7	529.2	18
			Qual 2nd	472.7	586.3	18
			Qual 3rd	472.7	717.3	18
P2	1.56	+	Quan	406.2	499.3	18
			Qual 1st	406.2	556.3	10
			Qual 2nd	406.2	657.4	25
P3	6.17	+	Quan	739.8	730.8	25
			Qual 1st	739.8	818.4	25
			Qual 2nd	739.8	875.4	18
			Qual 3rd	739.8	962.4	18
P4	6.62	+	Quan	735.7	429.2	25
			Qual 1st	735.7	850.9	18
			Qual 2nd	735.7	992.9	18
			Qual 3rd	735.7	1051.6	18
P5	6.88	+	Quan	774.9	602.6	18
			Qual 1st	774.9	978.4	25
			Qual 2nd	774.9	1035.5	25

Thermo Scientific™ TraceFinder™ 4.1 Environmental and Food Safety software was used for data acquisition,processing and reporting.

RESULTS

Figure 1. Extracted ion chromatograms of the five porcine-specific peptide markers detected in trypsin-digested extract of porcine gelatin reference (top) and bovine gelatin reference (bottom). The five peptide markers were detected in porcine gelatin reference but not detected in bovine gelatin reference, demonstrating the specificity of these peptides. These peptides can be used for halal gelatin speciation.

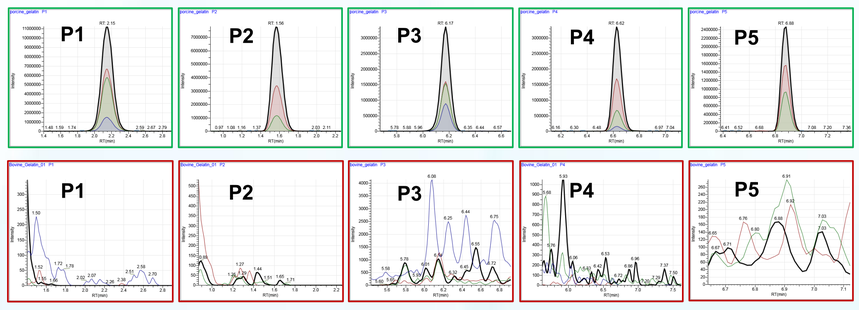

Figure 2. Results from the analysis of hair cream samples. Chromatograms from hair cream samples without spiking (top) and with spiking of 1 % porcine gelatin standard (bottom).

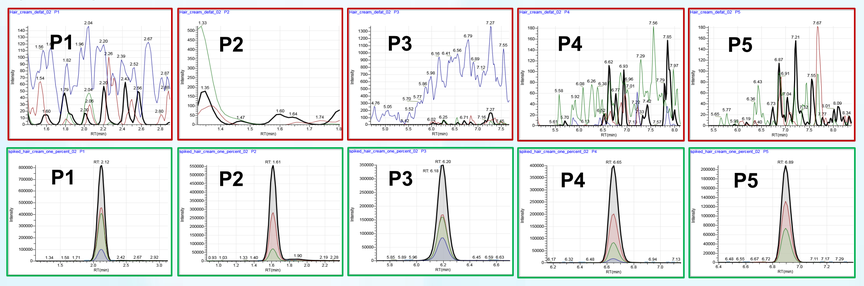

Figure 3. Method linearity and precision was evaluated by spiking porcine gelatin in hair cream at different concentration levels (0.01, 0.05, 0.1, 0.5, 1.0, and 5.0 %). The method was able to detect 0.01 % porcine gelatin in the hair cream samples with $R^2 > 0.98$. The inter-day and intra-day replicates analysis (n = 6) of the samples also showed good reproducibility with % RSD < 20 %. The results showed adequate linearity and precision.

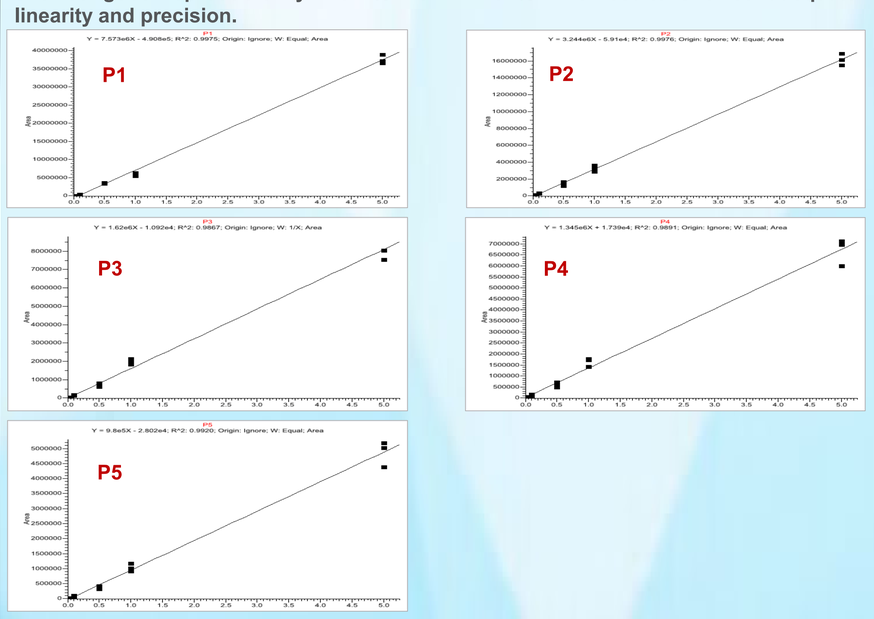

Figure 4. Results from the analysis of facial gel samples. Chromatograms from facial gel samples without spiking (top) and with spiking of 0.5 % porcine gelatin standard (bottom).

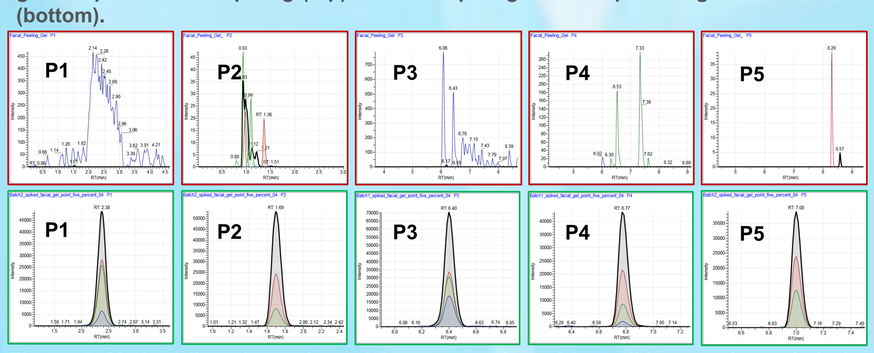

Figure 5. Method linearity and precision was evaluated by spiking porcine gelatin in facial gel at different concentration levels (0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 %). The method was able to detect 0.02 % porcine gelatin in the facial gel samples with $R^2 > 0.98$. The inter-day and intra-day replicates analysis (n = 6) of the samples also showed good reproducibility with % RSD < 20 %. The results showed adequate linearity and precision.

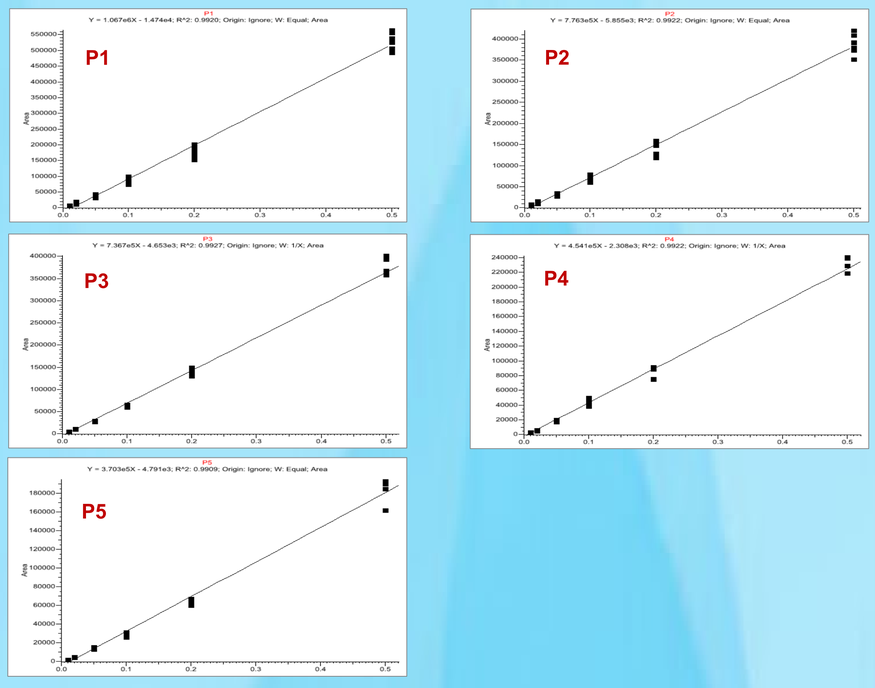

Figure 6. Results from the analysis of in-house prepared marshmallow. Chromatograms of the five porcine-specific peptide markers detected from in-house prepared marshmallow. All the SRM transitions were detected, showing the method can be used not only for cosmetic products but also for food confectionery products.

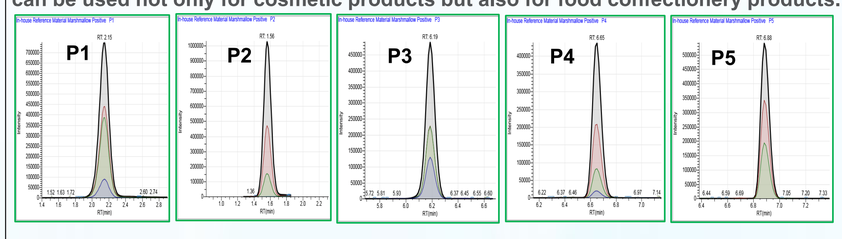

Table 2. Results from the analysis of cosmetic and food confectionery products for the presence of porcine peptide markers. The accuracy of the LC-MSMS method was determined by comparing the results against the conventional technique, ELISA. Coherent results were obtained for most of the analyzed samples, except for hair cream and hair conditioner samples which the ELISA method provided inconclusive results due to the interference from heavy matrix. The consistent results between LC-MSMS and ELISA, demonstrating this LC-MSMS method has enormous potential as an alternative analytical technique for halal gelatin speciation for cosmetic and food confectionary products.

No.	Sample	ELISA	LC-MSMS
1	Facial Moisturizer Gel (Brand K)	Positive	Positive
2	Hydrolysed Collagen (Brand U)	Positive	Positive
3	Pork Gelatin from Skin (Brand S)	Positive	Positive
4	Face Serum (Brand ES)	Negative	Negative
5	Face Toner (Brand ES)	Negative	Negative
6	Facial Peeling Gel (Brand MC)	Negative	Negative
7	Hair Conditoner (Brand O)	Inconsistent	Negative
8	Hair Shampoo (Brand O)	Negative	Negative
9	Whitening Cream (Brand BS)	Positive	Positive
10	Hair Moisturizer Cream (Brand M)	Inconsistent	Negative
11	Facial Gel (Brand E)	Positive	Positive
12	Foundation Face Serum (Brand H)	Negative	Negative
13	Face Cream (Brand T)	Negative	Negative
14	Foundation Facial Cream (Brand WG)	Negative	Negative
15	Beauty Jelly Collagen Supplement (Brand J)	Positive	Positive
16	Cosmetic Beauty Drinks (Brand M)	Positive	Positive
17	Gummy Candy (Brand P)	Positive	Positive
18	Marshmallow (Brand P)	Positive	Positive
19	Marshmallow (Brand T)	Negative	Negative
20	Marshmallow (Brand RM)	Positive	Positive
21	Marshmallow (Brand E)	Positive	Positive
22	Gummy Candy (Brand H)	Negative	Negative
23	Jelly (Band B)	Positive	Positive
24	Jelly (Band W)	Positive	Positive
25	Milk Candy (Brand R)	Positive	Positive
26	In house Reference Material marshmallow (negative)	Negative	Negative
27	In house Reference Material marshmallow (positive)	Positive	Positive

* Positive = Porcine gelatin detected
* Negative = Porcine gelatin not detected
* Inconsistent = Porcine gelatin detection is not consistent

Conclusion

In this study, a sensitive, robust and reliable method for the detection of porcine gelatin in cosmetic and food confectionery products has been demonstrated. The method can confidently detect the presence of porcine gelatin peptides in various sample matrix down to 0.01 % of contamination level. By combining simple sample preparation procedure and rapid SRM-based LC/MS approach, this method allows for rapid identification with high accuracy and can be adopted by testing laboratories to complement existing testing protocols.

Reference

1. Charles T. Yang, Dipankar Ghosh & Francis Beaudry (2018) Detection of gelatin adulteration using bio-informatics, proteomics and high-resolution mass spectrometry, Food Additives & Contaminants: Part A, 35:4, 599-608, DOI: 10.1080/19440049.2017.1416680

Acknowledgement

The author would like to thank Thermo Fisher Scientific for supporting this project with Vanquish Flex UHPLC system and TSQ Altis mass spectrometer.