Food Chemistry 274 (2019) 16–19

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Differentiation of bovine from porcine gelatin capsules using gel electrophoresis method

Beow Keat Yap, Lay-Harn Gam*

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Penang, Malaysia

ARTICLE INFO

Keywords:
Gelatin
Gel electrophoresis
Halal
Capsule
Ammonium sulfate precipitation

ABSTRACT

Gelatin is commonly used in food supplements and in the form of soft or hard capsules. The source of gelatins is usually from porcine and bovine, and less commonly from vegetable and fish. Nevertheless, these different origins of gelatin have much similarity in term of structures, physicochemical properties and amino acid sequences. Due to these reasons, differentiation of the source of gelatins has been very difficult. In our present study, differentiation of sources of gelatin was made possible in a simplified yet economical method. Sample was prepared using ammonium sulfate precipitation and subjected to gel electrophoresis for protein separation. We have found a fraction of proteins which is able to differentiate porcine and bovine gelatins accurately, with distinctive protein bands in SDS-PAGE at 140 kDa and 110 kDa for bovine and porcine samples, respectively. This method was verified by 13 double-blinded gelatin samples, all the 13 samples were accurately identified.

1. Introduction

Gelatin, a collagen derivative, has been used widely as ingredient in both soft and hard capsules and as food supplements. However, the source of gelatin has become a debated issue globally. For instance, Muslim communities are concerned of halal issue where consuming porcine gelatins is considered non-halal, whereas Hindus are concerned of bovine gelatins particularly from the cow. On the other hand, bovine spongiform encephalopathy or 'mad cow disease' is another concern among the general population. All these issues give rise to the need of analysis methods to differentiate the source of gelatins particularly from bovine or porcine.

Previous studies have shown that bovine gelatin can be differentiated from porcine gelatin by physicochemical methods such as principal component analysis of amino acid content (Nemati, Oveisi, Abdollahi, & Sabzevari, 2004) and calcium phosphate precipitation test (Hidaka & Liu, 2003). These methods can only be applied on pure gelatin samples, whereas mixture of gelatins in a sample cannot be analyzed accurately. Although immunological method using polyclonal anti-peptide antibodies in indirect and competitive indirect enzyme-linked immunosorbent assay (ELISA) (Venien & Levieux, 2005), and polymerase chain reaction (PCR) technique which was based on amplification of bovine and porcine DNA (Cai, Gu, Scanlan, Ramatlapeng, & Lively, 2012) were claimed to be able to overcome this issue, these methods are highly complex and expensive.

Therefore, in this present work, we aimed to develop a much simpler, reliable and cheaper method to distinguish bovine and porcine gelatins, which are the main sources of gelatin ingredients in capsule and also in food containing gelatin, such as chewable sweets, jelly and etc. To our knowledge, the present method, which uses ammonium sulfate precipitation to selectively precipitate the specific fraction of useful protein for determination the sources of gelatin is the first that was reported.

2. Materials and methods

2.1. Chemicals

Ammonium sulfate, Tris base, sodium dodecyl sulfate (SDS), acrylamide, bisacrylamide, ammonium persulfate (≥98% purity), tetramethylethylenediamine (TEMED) (~99% purity), butanol, glycine, ethylenediaminetetraacetic acid (EDTA), glycerol, β-mercaptoethanol, bromophenol blue, porcine and bovine gelatin standards were purchased from Sigma (St Louis, Missouri, USA) (with at least 99% purity, unless specified), whereas methanol and acetic acid were purchased from R&M Chemicals (Essex, UK). Bovine gelatin samples (i.e. capsulated supplements) were both purchased over-the-counter from pharmacies, and obtained from a supplier of gelatin (Halagel) in Malaysia, while porcine gelatin sample was kindly provided by National Pharmaceutical Regulatory Agency (formerly known as the National

* Corresponding author.
E-mail address: layharn@usm.my (L.-H. Gam).

https://doi.org/10.1016/j.foodchem.2018.08.111
Received 16 March 2018; Received in revised form 17 July 2018; Accepted 24 August 2018
Available online 25 August 2018
0308-8146/

Pharmaceutical Control Bureau). The protein marker, i.e. unstained Precision Plus Protein™ Standards, containing ten highly purified recombinant proteins with molecular masses from 10 to 250 kDa, and the Coomasie Brilliant Blue R250 powder, were purchased from Bio-Rad (Hercules, California, USA).

2.2. Polyacrylamide gel preparation (7.5%, 8 cm × 10 cm × 1.0 mm)

The 7.5% polyacrylamide gel for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was prepared in-house. Briefly, 10 mL of resolving gel solution [375 mM Tris-HCl, pH 8.8, 0.1% (w/v) SDS, 7.5% (w/v) acrylamide/bis-acrylamide, 0.05% (w/v) ammonium persulfate, 0.05% (v/v) TEMED] was swirled gently and transferred quickly into gel casting glass plates to a level of 5 cm from the top. Then, a small volume of water saturated in butanol solution [water: butanol 1:1] was filled on the surface of the solution to level the gel surface layer. When the gel had completely polymerized (20 min), the water-saturated butanol solution was removed and the gel was rinsed with distilled water.

Approximately 5 mL of the stacking gel solution [125 mM Tris-HCl, pH 6.8, 0.1% (w/v) SDS, 4% (w/v) acrylamide/bis-acrylamide, 0.05% (w/v) ammonium persulfate, 0.1% (v/v) TEMED] was transferred into the polymerized resolving gel until the brim. Then, a well-forming comb was inserted immediately. After the stacking gel had polymerized completely (50 min), the comb was removed. The wells formed were filled with electrode buffer [25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS] before samples were loaded.

2.3. Sample preparation

Capsule was cut using a clean scissors, its content was removed and the capsule was cleaned using a piece of blotter paper. 150 mg of soft gelatin capsule or 400 mg of hard gelatin capsule and 0.835 g of ammonium sulfate were weighed. After the gelatin dissolved, ammonium sulfate salt was added little by little into the solution that is under constant stirring using a magnetic bar. After added all the salt, the solution was left for 30 min under constant stirring. After 30 min, 1 mL of the supernatant was collected into 1 mL microcentrifuge tube. The solution must be under constant stirring when the supernatant is pipetted out. This is important to ensure there is no precipitated protein sediment at the base of the beaker. The tube was then centrifuged at 18,729 × *g* RCF for 20 min at 35 °C. This is to spin down the precipitated protein.

After centrifugation, the supernatant was decanted from the tube. The pellet was then resuspended with 70 µL of TSE buffer [10 mM Tris, pH 8.8, 1% (w/v) SDS, 1 mM EDTA]. This allows the protein to dissolve in the buffer. After resuspension, the tube was vortexed for about 10 s to mix the protein in the buffer homogenously. In colored hard gelatin capsules where the pellets did not dissolve completely in the buffer, the solutions were centrifuged at 24 × *g* RCF for 4 min at 35 °C to spin down the undissolved impurities. The supernatant was collected. 20 µL of solution was then transferred from the tube into a new microcentrifuge tube containing 5 µL of sample buffer [62.5 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol (added freshly), 0.0025% (w/v) bromophenol blue]. The mixture was vortexed briefly and then heated at 95 °C for 4 min.

2.4. Protein separation with SDS-PAGE

SDS-PAGE was performed as described by the modified method of Laemmli (Laemmli, 1970). 15 µL of each sample and standards were loaded into the wells of the 7.5% (8 cm × 10 cm) polyacrylamide gel prepared in-house. After all samples and standards were loaded, the gel was subjected to electrophoresis separation at 200 V for 50 min.

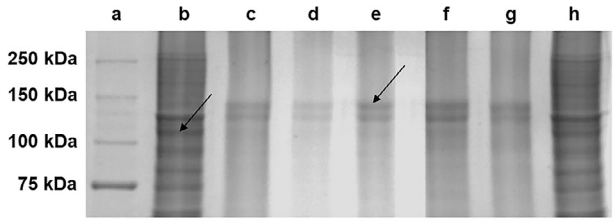

Fig. 1. Image of bands of bovine and porcine gelatin after gel electrophoresis (a: unstained Precision Plus Protein™ Standards (protein marker); b and h: porcine gelatin standard; e: bovine gelatin standard; c and d: bovine gelatin samples (soft gelatin); f and g: bovine gelatin samples (hard gelatin) obtained from Halagel). The bands that are able to distinguish between porcine and bovine gelatin are 140 kDa and 110 kDa in bovine and porcine samples, respectively (shown in arrows).

2.5. Imaging and data analysis

Once the run ended, the gel was transferred immediately into a container and immersed in Commassie blue staining solution [0.1% (w/v) Coomasie Blue R250, 40% (v/v) methanol, 10% (v/v) acetic acid] for 1 h. After 1 h, the staining solution was discarded and the gel was soaked in the destaining solution [40% (v/v) methanol, 10% (v/v) acetic acid] for 30 min. It was then replaced with fresh destaining solution for another 30 min and finally the gel was soaked in distilled water. The image of the gel was then captured using VersaDoc Imaging System and analyzed with the Quantity One 1-D Analysis software (Bio-Rad, Hercules, California, USA).

3. Results

3.1. Characteristics of bands of a bovine versus porcine gelatin

Image of the gel in Fig. 1 shows the characteristic of bands of bovine and porcine gelatins. From Fig. 1, it is clear that more bands are observed in porcine gelatin compared to bovine gelatin. In addition, the two bands between 100 and 150 kDa (Fig. 1) are with different mobility distance in SDS-PAGE for bovine and porcine gelatin whereby in bovine gelatin, the two bands are of significantly at higher molecular weights (125 kDa and 140 kDa) than those in porcine gelatin (110 kDa and 125 kDa). Thus, through the difference in band profiles between bovine and porcine gelatin, bovine gelatin can be easily distinguished from porcine gelatin and vice versa. This is illustrated in Fig. 1 (lane c, d, f and g) which clearly showed bands similar to those of bovine gelatin standard (Fig. 1, lane e).

3.2. Cross-validation of the developed method by an authorized pharmaceutical body

The current method has also undergone a blind test by an authorized pharmaceutical body in Malaysia, where a total of 13 samples (8 capsule shells, 5 finished product over-the-counter) were tested using the gel electrophoresis method developed in this study. The result of such blind test is shown in Table 1 and in Supplementary Fig. S1, where our method has been shown to be better than the PCR methods carried out by other laboratories.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.08.111.

4. Discussion

In general, there are many methods that can be used to purify protein such as immunological methods, chromatography and etc. In our present method, ammonium sulfate precipitation was used to prepare sample for gel electrophoresis. This is because ammonium sulfate