Analytical Methods

Halal authenticity of gelatin using species-specific PCR

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A B S T R A C T

Consumption of food products derived from porcine sources is strictly prohibited in Islam. Gelatin, mostly derived from bovine and porcine sources, has many applications in the food and pharmaceutical industries. To ensure that food products comply with halal regulations, development of valid and reliable analytical methods is very much required. In this study, a species-specific polymerase chain reaction (PCR) assay using conserved regions of mitochondrial DNA (cytochrome b gene) was performed to evaluate the halal authenticity of gelatin. After isolation of DNA from gelatin powders with known origin, conventional PCR using species-specific primers was carried out on the extracted DNA. The amplified expected PCR products of 212 and 271 bp were observed for porcine and bovine gelatin, respectively. The sensitivity of the method was tested on binary gelatin mixtures containing 0.1%, 1%, 10%, and 100% (w/w) of porcine gelatin within bovine gelatin and vice versa. Although most of the DNA is degraded due to the severe processing steps of gelatin production, the minimum level of 0.1% w/w of both porcine and bovine gelatin was detected. Moreover, eight food products labeled as containing bovine gelatin and eight capsule shells were subjected to PCR examination. The results showed that all samples contained bovine gelatin, and the absence of porcine gelatin was verified. This method of species authenticity is very useful to verify whether gelatin and gelatin-containing food products are derived from halal ingredients.

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1. Introduction

At present, gelatin is a very popular ingredient in various food and pharmaceutical products. Gelatin is, by far, most widely used in confectionery products and pharmaceutical capsules around the world (Schrieber & Gareis, 2007). Gelatin is a mixture of polypeptides obtained by the partial hydrolysis of collagen (Cheng et al., 2012). In the large-scale manufacture of gelatin, the primary raw material used is the collagen found in cattle and pig (Schrieber & Gareis, 2007). Commercial forms of gelatin such as sheets, granules, or powders are mainly sourced from bovine bone and hides, pig skin, and, more recently, pig bone (Hermanto & Fatimah, 2013; Jaswir, Mirghani, Mohd Salleh, Hassan, & Yaakob, 2009). Other sources such as fish and poultry are very new and are mostly processed to cater to specific religious consumer groups (Gómez-Estaca, Montero, Fernández-Martín, & Gómez-Guillén, 2009; Karim & Bhat, 2009).

In Islam, consumption of products considered non-halal, such as those containing porcine gelatin, is strictly forbidden (Widyaninggar, Triyana, & Rohman, 2012). Some Islamic countries have established strict regulations for producers and importers to stamp their products with a halal certificate to distinguish them from non-halal products (Hermanto & Fatimah, 2013). In this regard, development of reliable and sensitive analytical methods for evaluating halal authenticity is very critical. Methods that rely on protein analysis or physicochemical properties, such as chemical precipitation (Hidaka & Liu, 2003), Fourier transform infrared spectroscopy (Hashim et al., 2010), electrophoretic analysis (Hermanto & Fatimah, 2013), high-performance liquid chromatography (Nemati, Oveisi, Abdollahi, & Sabzevari, 2004), mass-spectrometer detection (Zhang et al., 2009), and enzyme-linked immunosorbent assay (Venien & Levieux, 2005), have been applied to differentiate bovine gelatin from porcine gelatin. These methods proved to be unsuitable for differentiating a mixture of gelatin as well identifying the origin of gelatin in food products. This is mainly because of the similarities in structure and physicochemical properties of gelatin derived from different sources. Moreover, the presence of highly degraded proteins due to extreme
temperature and pH treatments during gelatin production render these methods almost unreliable (Tasara, Schumacher, & Stephan, 2005).

Polymerase chain reaction (PCR) can be used as an alternative method for rapid and sensitive detection of porcine DNA in gelatin due to the higher stability of DNA compared to protein (Aida, Man, Yaakob, Raha, & Son, 2007). Earlier reports have also described PCR using species-specific primers in highly processed food products (Calvo, Zaragoza, & Osta, 2001; Ghovvati, Nassiri, Mirhoseini, Moussavi, & Javadmanesh, 2009; Hidaka & Liu, 2003; Jia-qin et al., 2008; Murugaiah, Mustakin, Mohd Noor, & Radu, 2010). There are a limited number of reports available on animal species-specific identification of gelatin, gelatin-containing food, and pharmaceutical products using PCR techniques (Cai, Gu, Scanlan, & Oliveira, 2008; Martín et al., 2007). The essential prerequisite to 2 g of the sample, vortexed briefly, and incubated for 30 min at 60°C in a water bath (Memmert, Schwabach, Germany) with constant shaking. After incubation, the solution was cooled to room temperature (15–25°C) on ice and then centrifuged (Eppendorf, Hamburg, Germany) for 5 min at 2500 × g. Subsequently, 700 μl of the clear supernatant was transferred to a microcentrifuge tube containing 500 μl of chloroform, vortexed vigorously for 15 s, and centrifuged at 14,000 × g for 15 min. After centrifugation, 250 μl of the upper aqueous phase was added into a fresh microcentrifuge tube containing 1 ml of binding buffer and mixed thoroughly by vortexing. Then, 600 μl of the mixture was pipetted into a spin column placed in a collection tube, centrifuged at 17,900 × g for 1 min, and the flow-through was discarded. This step was repeated with the remaining sample. Afterwards, 500 μl of the wash buffer was added to the spin column, followed by centrifugation at 17,900 × g for 1 min, and the flow-through was discarded. The collection tube was centrifuged again at 17,900 × g for 1 min to dry the membrane. Finally, the spin column was transferred to a 1.5-ml fresh microcentrifuge tube, and 30 μl of elution buffer was added onto the membrane, incubated for 1 min at room temperature (15–25°C), and then centrifuged at 17,900 × g for 1 min to elute. The purified DNA solution (30 μl) was immediately used.

2.2. DNA extraction

DNA was extracted from pure gelatin powders, capsule shells, and gelatin-containing food products, using a DNeasy mericon Food Kit (Qiagen, Hilden, Germany), Small Fragment Protocol (2 g), recommended for highly processed foods. Initially, 10 ml of lysis buffer and 25 μl of proteinase K (20 mg) solution were added to 2 g of the sample, vortexed briefly, and incubated for 30 min at 60°C in a water bath (Memmert, Schwabach, Germany)
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