

**Mini Review**

## Halal authentication in Malaysia context: potential adulteration of non-Halal ingredients in meatballs and surimi products

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**Abstract**

Halal is a term that describes substances that are deemed 'pure and clean' which Muslims are allowed to consume according to Islamic law. The industrialization of food processing in the 20<sup>th</sup> and 21<sup>st</sup> centuries has exposed Muslims community to various ingredients such as blood plasma, transglutaminase and gelatin introduced in meatballs and surimi product. Muslims are facing difficulties to ascertain which products are permitted or not under the Islamic law. Thus, this paper is to give knowledge of non-halal ingredients being introduced in meatballs and surimi products for consumers, researchers and policy makers. Local halal logo issued by Department of Islamic Development Malaysia (JAKIM) is needed to imply that all ingredients used in the food production and processing are Syariah compliance. The scientific evidence to substantiate any claim on Halal issue was developed based on several methods including PCR-based methods with different mitochondria and chromosomal DNA (MtDNA and cDNA) primers, real-time PCR with different probes and DNA binding agent, loop-mediated isothermal amplification (LAMP) with different primers developed, PCR- RFLP, ELISA and etc.

**Keywords**

*Halal authentication  
Non-Halal ingredients  
Meatballs  
Surimi products*

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**Introduction**

Islam has emphasized its followers to choose wholesome, clean and Halal foods in their daily lives. This awareness is strengthened by the widespread knowledge, extolling the virtues of consuming clean and Halal foods, and its relationship to their daily religious practices. The concept of Halal should be observed since Syariah laws generally guide the lives of Muslims. Syariah laws means Islamic law based on Al-Qur'an, the Sunnah, consensus of opinions of Muslims jurists (ijma') and analogy (qiyas) and other modes of legal reasoning (ijtihad). In Malaysia, Syariah rulings based on ijtihad or 'fatwa' on Halal matters should be in line with the Shafie Mazhab of Ahlul-Sunnah wa al-Jamaah. Hence, a particular food becomes Halal and non-Halal by Syariah laws if it is considered so by any of these sources. With regard to foods and beverages, Islam has provided three very important guidelines, namely whether the consumption of foodstuff is prohibited by Allah; whether the foodstuff is obtained through Halal or Haram (prohibited) means, and whether or not the material is harmful to health (El-Mouelhy, 1997).

The Codex general guidelines for the use of the term Halal were adopted by the Codex Alimentarius

Commission at its 22<sup>nd</sup> Session, 1997 (CAC/GL 24-1997) (Codex Alimentarius Commission, 1997). Malaysian Standard on Preparation and Production of Halal Food, MS 1500:2009 was introduced by Department of Islamic Development Malaysia (JAKIM) to assure the concept of Halal is fundamentally applied from farm to table. To receive the Halal certificate from JAKIM the manufacturer necessarily ensures preparation, slaughtering, ingredients, cleaning, handling, processing, transportation and distribution is truly comply with Syariah laws. A local Halal logo issued by JAKIM implies that all ingredients used in the food production and processing should comply with Syariah laws. Now, the Halal logo is globally accepted by more than fifty countries due to its stringent criteria for Halal certification (Nestle Malaysia, 2014). In order to maintain the Syariah value of Halal and safe, cleanliness aspects should also be observed to ascertain that Muslims' consumption of certain food products is free from non-Halal ingredients and hazardous materials. Nevertheless, to inspire Malaysian as world Halal Hub, the Halal logo should in line with hazard analysis and critical control point (HACCP) certification, high quality standards of ISO9001 certified, attention to environment

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issue of ISO14001 certified; and infrastructure and technologies support for Halal authentication analysis to provide scientific evidence to substantiate any claims regarding the presence or absence of the non-Halal ingredients and/or additives.

Those criteria implied that the Halal logo has high quality of hygienic, safety and Halal assurance of products for consumption. This will attract the non-Muslim consumers towards the Halal concept and earn a great opportunity to lucrative Halal business in local and international markets. Muslims nowadays are exposed to various kinds of ingredients and manufactured foods, arising from the advancement of science and technology which extend their concerned about food ingredients and additives produced by food industries in their daily use. The food ingredients are defined as ingredients which are listed in descending order by weight on a food label. Food has to be described in a way which is not misleading, the name prescribed by law, and precise description to inform consumers of its true nature and application (JAKIM, 2010). While, food additives are defined as any safe substance that is intentionally introduced into or on a food in small quantity in order to affect the quality, such as texture, appearance, odor, taste, alkalinity, or acidity. It also may serve as technological function in the manufacture, processing, preparation, treatment, packaging, transport, or storage of the food. It includes any preservatives, coloring substances, flavorings, flavor enhancer, antioxidant and other food conditioners, but shall not include nutrient supplement, an incidental constituent (JAKIM, 2010). For examples, some of food ingredients may either contain pork gelatin, blood plasma, lard, alcohol, whey, rennet, transglutaminase (meat gum) and hormones; or food colors (E100-199), preservatives (E200-299), oxidant and antioxidant (E300-399), gelling agent, emulsifiers, anti-caking, stabilizers (E400-499), enzymes, glycerin/glycerol (E422) and flavor enhancers (E600-E699) (Ismail, 2006). The Muslim communities would like to know whether or not the ingredients or the finished food products contain any haram/non-Halal substances.

#### *Blood plasma and proteins*

Meatballs are produced from a mixture of finely ground meat and it can be prepared using beef, chicken, fish or pork (Arief *et al.*, 2012; Purmono and Rahadian, 2008). Those products are popular across various cultures in the world with different name. In Malaysia, the meatballs are usually from beef or locally recognized as bebola daging. If it is made of fish, it is known as fish ball. Commonly, fish balls are produced using surimi as the main ingredient. Surimi

is referred to the myofibrils protein concentrate extracted from fish meat by mechanical separation, washed with water and mixed with a cryoprotectant (Okada, 1992). Surimi is popular not only in Japan but also in many other countries due to its unique texture and nutritive value. It has been estimated that about 315,800 million tons of surimi products have been produced in the Southeast Asian region in 2005 (Laong and Siriraksophon, 2007).

Gel strength was most commonly used to assess the qualities of surimi which involve the three dimensional protein gel network formation. The protein gel network was formed by cross-linkage between actin and myosin of fish muscle proteins, which solidifies during heat treatment (Park, 2005). Surimi gelling is a process that involves decomposition and aggregation of protein. During heat treatment, the protein breaks down exposing the reactive clusters. On exposure, there will be formation of bonds between the neighboring protein molecules. When sufficient bonding force have been obtained, a series of three-dimensional networking will be created producing gel (Lanier *et al.*, 2005). Food ingredients such as pork gelatin are also possibly mixed as the best gelling protein and a water holding capacity in the meatballs that contribute to the juiciness of the meatball products (Aravindran *et al.*, 2014). However, gelatin may cause weakening of myofibril protein gelatins in surimi products (Sun and Holley, 2011) and this will lead to less elastic texture of surimi. As an alternative, some protein additives have been used in surimi and surimi based products to improve the characteristics of the gel as well to reduce protein degradation caused by endogenous proteinases. Thus, to increase the surimi gel strength protein supplements have been widely used due to its function as a proteinase inhibitor and increase the protein-protein interactions for the formation of 3-dimensional network (Alina *et al.*, 2012; Benjakul *et al.*, 2004). They are a number of protein supplements being added into surimi or meatballs products. Such as plasma protein, whey protein, egg whites (EW) and soy protein isolates (Benjakul *et al.*, 2004). The EW and soy protein isolates are considered as Halal but not plasma protein. While, whey protein is depend on the enzymes used in the cheese production. It is Halal if the rennet comes from slaughtered calf according to Syariah law. Whey protein is a liquid protein expressed out during cheese production. The protein is collected and dried to form whey protein concentrate or isolate.

Plasma protein is derived from blood and prohibited for Muslim consumers. The plasma protein has been reported being used as emulsifiers

and stabilizers to enhance the stability of food (Rawdkuen *et al.*, 2004). It contains a complex mixture of various proteins such as albumin (3.3%), globulin (4.2%) and fibrinogen (0.4%), where the serum is the major protein involved in the formation of heat-induced gels (Howell and Lawrie, 1983). Food grade enhancer such as bovine protein plasma (BPP), pig protein plasma (PPP), egg white (EW) and potato powder may potentially be used in surimi production (Lee *et al.*, 2000; Benjakul *et al.*, 2004). A research conducted by Benjakul and Visessanguan (2000) found that PPP successfully inhibits proteolysis of specific proteinase whitening activity and surimi autolytic activities. Besides PPP, chicken protein plasma (CPP) is able to increase the gel strength by acting as surimi gel filler in the matrix as well as proteinase enhancer. Blood plasma can increase or decrease myofibril protein gel strength, and this differs with different meat products (Sun and Holley, 2011).

Market surveillance on non-Halal additives incorporated in surimi based products using polymerase chain reaction (PCR)–southern hybridization analysis by Aravindran *et al.* (2014) at Klang, Malaysia indicated that industries are also using alternative sources of other protein such as chicken and goat plasma proteins. The researchers used the Olipro Bitotechnology Meat ID kits to detect a total of 7 known species of the gene sequence encoding the mitochondrial cytochrome b for *Bos taurus* (202 bp), *Gallus gallus domesticus* (206 bp), Anatidae (311 bp), *Capra hircus* (175 bp), *Bison bison* (565 bp), *Ovis aries* (390 bp), *Sus scrofa* (265 bp) and Internal control (439 bp). A leguminous plant gene was used as internal control as to avoid cross-reactivity among animal species studied. None, out of 17 (n = 17\*3) brands were positive towards porcine DNA, but 4 (n = 4\*3) brands showed positive for the presence of DNA species. Three brands showed the presence of cytochrome b that codes for chicken (*Gallus gallus domesticus*) and one brand showed the presence of cytochrome b for goat (*Capra hircus*). The results suggested that the awareness of surimi manufacturers in current issues associated with surimi is increased and led them to find an alternative to shroud from certain parties. As such, it is recommended that the authorities monitoring the surimi based food product marketed in Malaysia take more stern action to combat fraud in the use of non-Halal additives and giving away the Halal certification. Thus, the potential of Muslim consumer's expose to plasma proteins added into surimi and meatballs are greater. Not by just concerning the 1.6 billion Muslims population acceptance and suitability, the

Halal standard is as well said to be practiced by the non-Muslim community (Sahilah *et al.*, 2012).

#### *Transglutaminase (TGase)*

Transglutaminase (TGase) is food additive and recognized as meat glue, an enzyme that catalyzes the formation of a covalent bond between a free amine groups. It can be found in the animal, vegetable and yeast cells. Although the transglutaminase may come from different sources, the mechanism of the reaction is essentially similar. TGase (protein-glutamine  $\gamma$ -glutamyltransferase, EC2.3.2.13) has a specific reaction where this enzyme catalyzes post-translational modifications of proteins, producing covalent amide bonds between a primary amine group in a polyamine or lysine (amine donor) and  $\gamma$ -carboxamide groups of the glutamyl residue of some proteins (amine receptor) (Santos and Torne, 2009). Thus, the isopeptide bond of  $\epsilon$ -( $\gamma$  - Glu) lys will be formed by inter- and intramolecular bonding (Ando *et al.*, 1989).

The reaction has allowed great potential utilization of the enzyme in the manufacture of foods, cosmetic products, biopharmaceuticals, biosensors and textile products. Besides, exhibiting high resistance to proteolytic degradation its application to meat products such as meatballs, steak, seafood-based meatball product and surimi is greatly improved the taste, flavor, texture and nutrition value of the products. The TGase has been shown to improve the stability, water holding capacity, nutrient content of protein based products through the formation of protein-protein crosslinks and hence, improving the mechanical structure and texture (Gerrard, 2006; Shleikin *et al.*, 2011). It also reported that the addition of TGase in fish coated soy product increased the elasticity and density of the product (Shleikin *et al.*, 2011). In dairy products, the addition of TGase will increase the viscosity and strength of yoghurt, reduce syneresis, give a smooth surface, and improve creaminess of low fat yoghurts. While, its application in flour especially in bakery products will improve flavor, enhance the sticky, flexibility and crystal-clear of the dumpling skin, avoid noodle become thick, and extend shelf life. Currently, the potential of TGase being added in various food products such as steamboats, surimi, meatballs, noodles, milk, bakery and gelatin is possible (Gauche *et al.*, 2009; Gerrard, 2006). The use of TGase with its diverse applications in food industries exposes Muslim society to Haram TGase. This is because a large number of TGase have been isolated from mammalian organ such as blood, liver and placenta of bovine and pig (Ikura *et al.*, 1981; Ando *et al.*, 1989; Rain, 2011).

Although the TGase can be isolated from other Halal animal such as bovine, Muslim consumers are still threatening with this additive due the non-Halal slaughtering practices and possibly produced from the bovine blood plasma. The blood plasma of Halal animal is considered Najjis. Besides, TGase can be isolated from animal and plants it also can be obtained from microorganisms such as bacteria and fungi (Ando *et al.*, 1989; Del Duca and Serafini-Fracassini, 2005). Reported Actinomycetes producing transglutaminase are *Streptomyces* sp., *Streptoverticillium griseocarneum*, *S. cinnamoneum* subsp. *cinnamoneum* and *S. mobaraense* (Washizu *et al.*, 1994). Isolation and screening of bacterial- and fungal- transglutaminase were determined by hydroxamate assay as described by Ando *et al.* (1992). MTGase from microorganisms can be produced in a large-scale through the continuous or chemostate fermentation which leads to a simple purification protocol and cheaper than plant or animal cells. The large scale TGase production from mammals is reported to be difficult due to the isolation and purification process. This in turn affects the final product price. Not only that, the enzyme obtained is depending on the availability of Ca<sup>2+</sup> ions for its catalytic activity.

### Gelatin

Food ingredients such as pork gelatin are possible being added into meatballs and surimi products due to gelatin serves as gelling protein and able to hold water component which in turn contribute to the juiciness of the meatballs and surimi products. Gelatin is widely used as raw materials in foods and beverages, pharmaceuticals and cosmetic products. Gelatin is a protein based hydrocolloid which has special and unique characteristics, serving multiple functions with a wide range of applications in various industries including food ingredient as gelling, foaming agent, thickener, plasticizer, emulsifier, foaming agent, moisture retention, improve texture and binding agent. Due to these characters, gelatin is widely used in dairy and bakery products especially in ice creams, yogurt, cheese and cakes (Karim and Rajeev, 2009). Besides that gelatin is also applied in other food industries of jelly desserts, gummy jelly, chocolate, marshmallow, soft candy, toffees, chewing gum, butter, meat products and pet foods. In fitness products, gelatin has been used due to its easily digestible, low in calories and contains no cholesterol. In pharmaceutical industry, gelatin is used as hard and soft capsules, sugarcoated pills, tablets, serum substitute and vitamin encapsulation. The use of gelatin in pharmaceutical is inevitable

because it helps to protect the medicines against harmful influences, such as light and oxygen. The soft capsules for instance are mainly used for liquid fillings, while hard capsules are used for powders. Thus, the use of gelatin is an all-rounder. Thus, the chances of Muslims to be exposed to non-Halal gelatin are becoming greater (Sahilah and Aminah, 2010; Aravindran *et al.*, 2014).

Gelatin can be extracted from bones, fat, meat waste, used cooking fats and oils of animals. There are a few types of gelatins, and the most widely available are from porcine and bovine sources (Morrison *et al.*, 1999; Karim and Rajeev, 2009; Sahilah and Aminah, 2010; Sahilah *et al.*, 2015). The alternative gelatin sources are gum arabic, seaweeds (carrageenan) and fishes (fish gelatin) but these could not fulfill various industries demand. The various use of porcine gelatin in industries is expanding and the exposure of haram (non-Halal) gelatin is not only towards Muslims but also other communities such as Jews, vegetarians and a number of people who are allergic towards hidden porcine ingredients and meat sources in processed foods (Tanabe *et al.*, 2007a).

Halal gelatin production in the world is estimated less than 1% (Halalgel®) and become a challenge toward Muslims globally. Porcine gelatin is the most preferred than bovine for several reasons. One of the issue using bovine gelatins due to the emergences of bovine spongiform encephalopathy (BSE) or mad cow disease in the 1980s, thus the use of bovine gelatin is reduced and increased the use of porcine gelatin (Morrison *et al.*, 1999; Karim and Rajeev, 2008). The other reason is to produce one batch of bovine gelatin will take about 60-80 days when compared to porcine gelatin which only takes about 30 days (Sahilah *et al.*, 2015). Thus, Halal gelatin has also faced a challenge to provide gelatin and its related products at a competitive price.

### Methods of DNA detection

To avoid the misuse of Halal certification, some scientific methods have been developed for the detection of intentionally added ingredients such as plasma proteins and gelatin in meatballs and surimi products. Currently, the most reliable method to overcome the disputability of Halal food products is the molecular technique that based on the detection of porcine DNA in food samples (Matsunaga *et al.*, 1999; Montiel-Sosa *et al.*, 2000; Aida *et al.*, 2005; Yoshida *et al.*, 2009). Most early techniques were based on hybridization to specific probes, which are time consuming (Chikuni *et al.*, 1990; Ebbehøj and Thomsen, 1991). However, the PCR techniques using DNA amplification of specific target gene



of mitochondria DNA (mtDNA) is the method of choice due to its rapidity, specificity, sensitivity and reproducibility (Tanabe *et al.*, 2007). MtDNA based methods are considered more reliable because the DNA template is stable and resistant under conditions associated with high temperature, pressure and chemical treatments used in food processing in which DNA has mostly been degraded (Spsychaj *et al.*, 2009; Madesis *et al.*, 2014). The availability of mtDNA as DNA templates is abundant due to its multiple copies in the cells that lead to be used as a potential sensitive genetic marker (Murugaiah *et al.*, 2009). Besides, the variations of target sequences in mtDNA enable them to be used for designing species-specific PCR primers (Kortbaoui *et al.*, 2009). Among frequent target sequence used for porcine DNA detection is the cytochrome b gene. In addition to the cyt b gene, other target primers used in conventional PCR for detecting porcine DNA include tRNA-ATP8, D-loop, 12S rRNA, 16s DNA, ATP8 and ATP6 (Tartaglia *et al.*, 1998; Partis *et al.*, 2000; Cheng *et al.*, 2003; Corona *et al.*, 2007; Yoshida *et al.*, 2009). The PCR amplification using primers Sine-F and Sine-R for short interspersed nuclear elements (SINE) at 161 bp sizes was reported by Calvo *et al.* (2001).

Other technique for porcine DNA detection in meatballs is using real-time PCR as reported by Ali *et al.* (2012) and Farrokhi and Jafari (2011), PCR-RFLP (Ali *et al.*, 2011; Sahilah *et al.*, 2012a). While, Alina *et al.* (2012) used sandwich enzyme-linked immunosorbant assay (ELISA) for detection non-Halal plasma transglutaminase in selected surimi based products. The other promising technique is the PCR- southern hybridization analysis. This technique is a combination of PCR and southern hybridization analysis that were reported by Sahilah *et al.* (2012b) and (2015) in Halal market surveillance of gelatin capsules in pharmaceuticals market in Malaysia. This technique is also potentially used in porcine DNA detection in meatballs and surimi-based products that are considered as highly processed foods (Aravindran *et al.*, 2014). The PCR-southern hybridization on chip has been developed to detect the presence of porcine DNA by hybridizing the denatured biotinylated amplicons with specific probes immobilized onto membrane. The biotin-labeled amplicons bind to streptavidin-alkaline phosphatase and subsequently detected by the colorimetric substrate of nitro blue tetrazolium/5-bromo-4-chloro-3-indoyl-phosphate (NBT/BCIP). The colored signal was captured by Scanner System that allows the species-specific identification (Sahilah *et al.*, 2015). The other technique recently applies for Halal authentication is using loop-mediated isothermal amplification

(LAMP) which is claimed has excellent sensitivity and specificity for porcine DNA detection (Ran *et al.*, 2015). LAMP is a new nucleic acid amplification method. This method relies on four specific primers, inner primers (FIP and BIP) and outer primers (F3 and B3) that recognize six distinct regions of the target DNA and have high sensitivity and specificity (Notomi *et al.*, 2000). No, study has been made to detect porcine DNA from meatballs and surimi from this technique. However, this technique is potentially used on such products.

## Conclusion

Halal is a term that describes substances that are deemed 'pure and clean' which Muslims are allowed to consume according to Islamic law. Halal (permissible) quality of food is required to abide in Muslims daily live. However, the industrialization of food processing in the 20<sup>th</sup> and 21<sup>st</sup> centuries has exposed Muslims community to various ingredients such as blood plasma, transglutaminase and gelatin introduced in meatballs and surimi product arising from the advancement of food science technology. As a result in many cases, the Muslims are facing difficulties to ascertain which products are permitted or not under the Islamic law. It is also influence the other communities such as Jews, allergic toward porcine ingredient and vegetarian. Thus, this review paper is to expose and give knowledge to the consumers, researchers and policy makers of potential of non-Halal ingredients added into meatballs and surimi products.

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