HALALSPHERE

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Halal Concerns on Lard in Food Products and its Detection Methods

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Abstract

Lard refers to a creamy, white, soft substance made from pig fat and used in cooking and baking. Lard not only has the risk of biological complications and health problems associated with regular consumption, but also has other issues such as the restriction on use in the food industry from the perspective of the Muslim religion. There are few literature studies that show several issues related to the adulteration of food products, especially halal meat. This issue must be resolved to avoid confusion for Muslims. Therefore, laboratory analysis on meat species authentication is highly necessary. There are different available methods to detect different species in food samples, including Polymerase Chain Reaction (PCR), Electronic Nose (e-nose), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimeter (DSC), and Rapid PCR. This article aims to review the application of lard in food, cases related to halal and methods to detect the presence of lard.

1. Introduction

Food is one of the fundamental and essential needs for human survival; hence, natural and raw foods will be subjected to multiple industrial processes to retain its nutrient and functionality while maintaining it to fit for human consumption. However, its complex matrix and issues such as health and religion bound to it have made it a subject of interest worldwide among researchers and scholars. Recently, food has been processed based on modern science and technology advancements, and the ingredients used can be permitted or prohibited (Fadzlillah *et al.*, 2011). As technology improves, a few problems occur, and one of the most frequent problems is adulteration in food. Adulteration is the addition of undeclared substances to boost the bulk product or weight and make the product more valuable than the actual product. In the case of meat products, adulteration refers to substituting ingredients and unsuitable information regarding the origin of raw materials. The choice of food typically represents aspects of lifestyle, culture, faith, nutrition and health issues. From the perspective of Muslims, choosing one food over another is solely based on its halal status, as Muslims observe strict dietary rules expressed in the *Qur'an*.

Historically, meat was not commonly associated with adulteration for Muslim consumption, and this may be due to the fact that it was sold fresh at joints that were easily identifiable. Today, the food supply chain is too long, cross geographical origin, and people's lifestyles have changed greatly (Nakyinsige *et al.*, 2012). The issue with meat products is that the meat is adulterated with lard. This is because lard is one of the cheapest edible oils subsequently added to food products to reduce production costs (Mohamad *et al.*, 2015). Lard or industrially modified lard could be effectively blended with other vegetable oils to produce shortenings, margarine, and other food oils. In addition to the risk of biological complications and health problems associated with regular consumption, there is a restriction on the use of this animal product in the food industry from the perspective of the Muslim religion (Azir *et al.*, 2017).

The most frequent food fraud cases, found in 95% of reports, are replacing the real ingredient with an ingredient that is akin but has a cheaper cost, hard to notice by the consumer and hard to identify via usual analytical methods. The species authentication is vital to the user as there is an economic loss since the food frauds happen, and health issues are also affected, such as food allergies and religious reasons (Abbas *et al.*, 2018).

Halal foods are foods free from any ingredients or components that Muslims are forbidden from eating. Both good and clean foods are halal, which has been stated in the *Qur'an*. In comparison, haram means anything that is forbidden in Islam. Consequently, excluding those explicitly forbidden by the al-*Qur'an* and *Sunnah*, almost all plant and animal-origin foods are considered halal (Ariff *et al.,* 2018). Therefore, any food products that are contaminated or have contact with haram food are considered haram food and cannot be consumed by Muslims. Few literature studies show several issues related to the adulteration of food products, especially in halal meat (Salahudin *et al.,* 2018). From Mahpar *et al.* (2016), few restaurants in Malaysia displayed signs of 'no pork'; however, it is not guaranteed that the vendors do not use other pig derivatives. For example, the author mentions a woman working at a restaurant that displays the sign 'no pork' and says that sometimes the vendors use the same utensil to cook pork and other meat or food. This will cause an issue as Muslims do not know the vendor uses the same utensil to cook pork and other food, which is unacceptable for Muslims. This issue must be resolved to avoid confusion for Muslims.

Therefore, laboratory analysis on meat species authentication is highly necessary. Among the available method to detect different species in food samples are Polymerase Chain Reaction (PCR), Electronic Nose (e-nose), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimeter (DSC) and Rapid PCR. Enzyme-linked Immunosorbent Assay (ELISA) can also be concluded in meat species authentication due to the specificity, simplicity and sensitivity (Kök & Atalay, 2018).

The studies on the detection of lard were mostly conducted using either infrared spectroscopy, high-performance liquid chromatography, gas chromatography or differential scanning calorimetry (Rohman & Man, 2008). Research by Saeed *et al.* (1986) determines the presence of lard in beef and mutton mixtures by gas chromatography. Most detection methods used ELISA were applied to samples like milk (Chughtai *et al.,* 2017), meat, pasta, flour and egg (Febo *et al.,* 2018). Therefore, the present paper will review the application of lard in food, cases related to halal, and methods to detect lard's presence. The study will be a significant endeavour for the food industry to the consumer as it protects the interest of consumers and public health, scientist and technologies are continuously established and advanced to check the validity of various food products (Abbas *et al.,* 2018).

2. Lard

From the Oxford Dictionary, lard is defined as the abdomen fat of a swine that is rendered and refined to be used in cooking. It can also be characterised as a soft, unctuous white substance with a bland taste and a distinctive odour (Bergfeld *et al.,* 2017). According to Cambridge Dictionary, lard is a creamy, white, soft substance made from pig fat and used in cooking and baking. It leads to a flakier finished product because lard has especially large fat crystals, which is one reason why people choose to use lard instead of other fat or oil. That is also why traditional empanadas are so delicious, based on the same crust logic. Many savoury uses abound. Typically lard for homemade sausage, *paté*, Mexican homemade tamales or carnitas. It also has a high smoke point so that it can be used without problems at elevated temperatures, and it is a perfect fat for deep and shallow frying (Reisner, 2020).

Lard contains about 200 fatty acids, and stearic, palmitic, and oleic acids are the main constituents (Bergfeld *et al.,* 2017). Lard is made of 100 % fat from pork. Via a method called rendering, it is extracted from the fatty parts of a pig. Portions such as pork belly, butt of pork, or shoulder of pork would make the most lard. At room temperature, the separated fat is solid and opaque and, depending on its purity, transforms into a transparent liquid at about 95 to 113 degrees Fahrenheit (Ferguson, 2019). Lard is obtained shortly after slaughter by a dry or wet rendering of fresh fatty porcine tissues from cuttings and trimmings. Lard produced by processes of wet-rendering is known as prime steam lard. Rendered lard can be bleached or deodorised and bleached (Bergfeld *et al.,* 2017). There are several advantages to cooking with lard; more chefs prefer lard over conventional cooking oils or shortenings. It does not contain trans fats, which makes it a healthier choice than hydrogenated fats, has fewer saturated fat and cholesterol than butter, like olive oil, contains healthy monounsaturated fats, and has a high smoke point, making it perfect for frying food (Ferguson, 2019). Lard contains less polyunsaturated fatty acids, which implies that when exposed to heat, it is more stable, less likely to turn bad and cause free radicals. Thus, to make it last longer, there is no need to add any chemical components (Pisuthipan, 2015).

In addition, it is cheaper than other consumable oil; therefore, it is purposely added to food products to lower the production cost (Mohamad *et al.,* 2015). Some religions, such as Islam and Judaism, believe that the existence of lard in any food products is prohibited. Besides that, Henry (2013) stated that although lard is healthier than butter, it still contains saturated fat. Therefore, it cannot be consumed in a huge amounts as saturated fat increases low-density lipoproteins, also known as LDLs, which are poor cholesterol and decreases healthy cholesterol, which is high-density lipoproteins (HDLs). It is associated with heart disease, hypertension, diabetes, and obesity, but metabolism and cell function are also important (Henry, 2013). Consequently, some analytical procedures, either physical or chemical-based methods, were developed to determine lard in food products (Rohman *et al.,* 2012).

2.1 Lard composition

Most fats and oils have different fatty acid compositions. In determining the source of lipids, the structure and configuration of fatty acids in fats and oils can act as an indicator (Fadzlillah *et al.,* 2016). The most important element of edible fats and oils is fatty acids (FAs). The fatty acids are commonly discovered in the ester form with a glycerol backbone called triglycerides. Lard consists of four main fatty acids, which are palmitic, stearic, oleic and linoleic (Rohman *et al.,* 2012). The facts on fatty acid composition are crucial for health consciousness and religious commitment. Nevertheless, the fatty acids compositions, particularly from animal fats, are difficult to determine because of the complicated length of the chain, branching, degree of unsaturation, geometry and position of the double bonds (Fadzlillah *et al.,* 2016). The fatty acids identified in lard are myristic, palmitic, palmitoleic, margaric, stearic, oleic and linoleic (Fadzlillah *et al.,* 2016), linolenic, arachidic and gadoleic acids (Rohman *et al.,* 2012). The fatty acids detected in lard are 7.2% palmitic, 39.7% elaidic, 16.5% oleic, 17.6% linoleic, and 4.28% linolenic acid (Sairin *et al.,* 2019).

According to Rohman *et al.* (2012), most fats and oils were mostly composed of triacylglycerols (TAG), diacylglycerols (DAGs), free fatty acids and other minor components like phospholipids, sterols, tocopherols, carotenoids, and
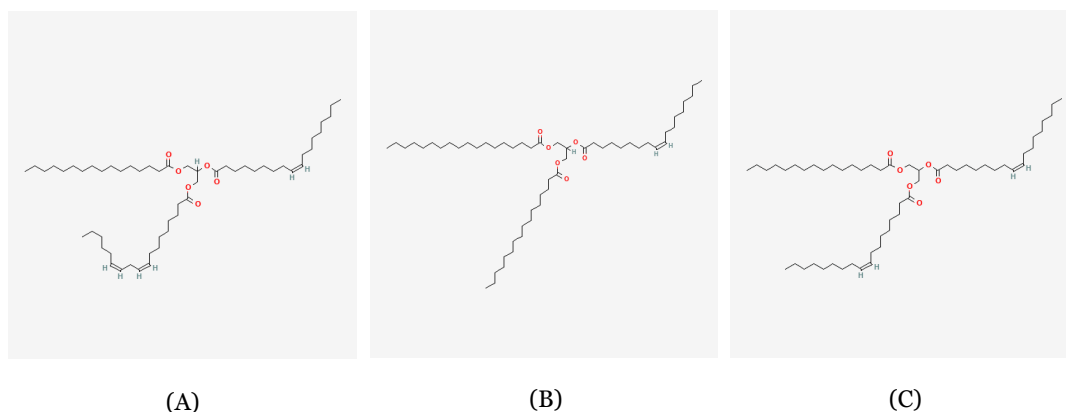
Figure 1: (A)-linoleoyloleoylpalmitoylglycerol (POL), (B)-palmitooleostearin (POS), (C)-dioleoypalmitoylglycerol (POO)
Source: National Centre for Biotechnology Information (NCBI)

fat-soluble vitamins. The major TAGs that make up lard are palmitooleoolein (POO), palmitooleostearin (POS), and palmitooleopalmitin (POP) (Rohman *et al.,* 2012). Azir *et al.* (2017) reported that lard contain 20.21% of linoleoyloleoylpalmitoylglycerol (POL), 18.58% of palmitooleostearin (POS), and 17.25% of dioleoypalmitoylglycerol (POO) as can be seen in figure 1.

2.1.2 Pig and lard in Islam

Muslims are taught to avoid eating pork flesh and its derivatives. These prohibitions have been mentioned strictly in the *Qur'an* and have been a Muslim guideline. As stated in the *Qur'an surah al-Maidah, Allah* says:

> *"Forbidden to you is anything that dies by itself, and blood and pork, as well as whatever has been consecrated to something besides Allah, and whatever has been strangled, beaten to death, trapped in a pit, gorged, and what some beast of prey has begun to eat unless you give it the final blow; and what has been slaughtered before some idol, or what you divide up in a raffle; (all) that is immoral!". (Al-Ma'dah, 5:3)*

Therefore, as referred to *Qur'an* and *Sunnah,* consensus Muslim jurist *(Ijma')* has forbidden pork and anything related. Among other verses that highlight the ban on pigs can be seen in *surah al-Baqarah, Allah* says:

> *"Indeed, Allah only forbids you to eat carrion, and blood, and pork, and animals that were slaughtered not for the sake of Allah, so anyone is forced (to eat it due to emergency) while he does not desire it and does not exceed the limit (in the amount of things eaten), then it is not a sin. Indeed, Allah is Forgiving, Most Merciful". (Al-Baqarah, 2:173)*

As stated earlier, lard is a pork fat extracted from pork belly, butt and shoulder and subsequently subjected to the rendering process to churn out the final product. In the food industry, its characteristic of large fat crystal, stable fat structure and high smoke point makes it the perfect choice to imply in food than conventional fat and oils (Ferguson, 2019). Also, lard is less likely to cause free radicals when heated due to less polyunsaturated fatty acid structure. Nevertheless, despite all the advantages demonstrated by the lard, Muslims must abide by the rules prescribed in the *Qur'an.* Prohibiting pork is only meant to protect Muslims from all possible harm, and only *Allah* knows the wisdom behind it. The advent of material like lard has raised much concern among Muslims and other parties like Judaism. Therefore, transparency in the food industry is vital to halt the concern about halal authenticity.

2.2 Application of lard in food

Many applications of lard in food make it a popular substance for food products. Among the justification for using lard in food is 1) the glycerolized lard, which is glycerolysed into diacylglyceride and monoacylglycerol, can increase the emulsifying activity and emulsion stability in meat products, 2) lard-based diacylglycerol can increase water holding capacity of meat product, 3) lard can reduce the hardness of cookie dough and 4) lard aids in controlling pests such as mite in dry-cured ham. This section is not intended to highlight the superiority of lard, however, it is meant to set the benefits of lard that are favoured by food manufacturers worldwide. Thus, justifies the need to have substantial techniques to detect its presence in food.

2.2.1 The glycerolized lard and purified glycerolized lard emulsions increase emulsifying activity and emulsion stability in meat products

Emulsion-type meat products such as sausages, frankfurters, bolognas, and wieners are very common in the meat industry and are produced in the presence of water and lipids by pounding the raw meat and creating an aqueous protein stage in which fat droplets are dispersed. Myofibrillar proteins have stronger emulsion properties in meat products to keep water and oil together. In contrast, immobilised fat droplets in the protein matrix play a crucial role in reducing purge and cooking loss, increasing water holding capacity and offering outstanding mouthfeel and juiciness.

Scientists proposed the use of lard to create the emulsion complex. Lard is inexpensive and very significant for meat product processing, and lard glycerolysis can be used to prepare DAGs. Diacylglycerols (DAGs) are glycerol esters in which fatty acids esterify two hydroxyl groups. Since DAGs have hydrophilic polar groups in their molecular structure, it shows higher surface activity as well as interfacial properties, DAGs can be emulsified more easily than TAGs. Therefore, DAGs in emulsions have beneficial effects and are ideal for use as emulsifiers. Furthermore, because of their higher melting points compared with TAGs, DAGs can enhance food texture (Diao *et al.,* 2016). In lard, glycerolysis was done to obtain the DAGs part used as an emulsion stabiliser that the authors did in their research (Diao *et al.,* 2016).

In general, emulsifying activity indices are determined by protein-lipid and protein-protein interactions and continuous and scattered phases are correlated with emulsion stability indices. The probability of creating a stable emulsion depends on the protein molecules remaining to stabilise oil droplets at the interface after adsorption. The emulsifying activity index and emulsion stability index of emulsion with lard, glycerolized lard, and purified glycerolized lard are shown in Figure 2.
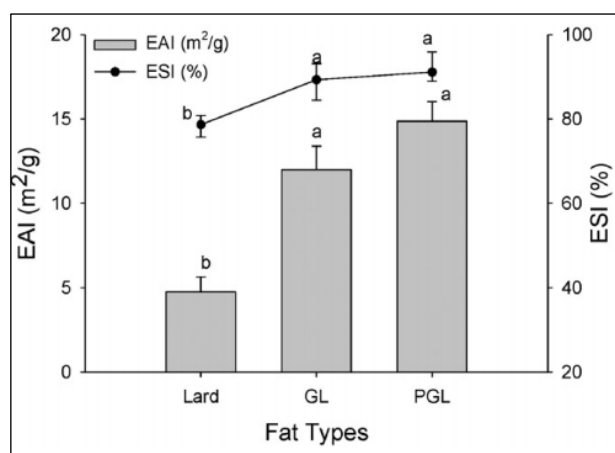

Figure 2: Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions prepared with myofibrillar proteins and lard, glycerolized lard (GL), or purified glycerolized lard (PGL).

Source: (Diao et al., 2016).

The emulsion emulsifying activity index for lard was 4.76 (m^2/g), which was slightly lower than for glycerolized lard, which is 12.0 (m^2/g) and purified glycerolized lard is 14.9 (m^2/g), respectively. The findings showed that in emulsions, glycerolized lard and purified glycerolized lard could disperse more effectively than lard, which can be due to the hydrophilic groups in DAGs that are correlated more strongly with water droplets scattered in the emulsions. Glycerolized lard and purified glycerolized lard's acylglycerol structures are more versatile than lard and can interact more strongly with proteins at the water-oil interface. To conclude, the study conducted by Diao et al. (2016) reported that DAGs can greatly increase emulsion properties. Compared with the emulsion prepared with lard, the emulsion prepared with myofibrillar proteins and purified glycerolized lard had higher emulsifying behaviour and emulsion stability. Thus, in emulsion-type meat products, such as frankfurters, bolognas, and wieners, lard DAGs have very good application prospects (Diao et al., 2016).

2.2.2 Lard-based diacylglycerol can increase water holding capacity of meat product

The rheological and physicochemical properties of pork myofibrillar protein gels at different pH levels are influenced by lard-based diacylglycerol. Myofibrillar protein is an essential functional protein primarily responsible for meat products' physicochemical and thermally mediated gelation properties. In the characteristics of processed meat products, the shaped myofibrillar protein gels play a crucial role, such as microstructure, texture, and sensory properties. Adding fat to meat products affects the physicochemical properties of meat products. In the three-dimensional protein gel matrix,

emulsified fats as fillers occupy voids, reducing the porosity of processed meat products (Zhou et al., 2020).

According to Zhou et al. (2020), the addition of impurified diacylglycerol or purified diacylglycerol of lard to the gel of pork myofibrillar protein has a higher water-holding capacity than the control gel of pork myofibrillar protein. The enhanced water holding capacity can be due to the increased number of hydrogen bonding sites between protein and water with increased pH (Zhou et al., 2020). The increased water-holding capacity of the gel of pork myofibrillar protein was correlated with myofibril's swelling resulting from increased pH since swelled myofibrils promote retaining moisture and fat during heat treatment. The space-filling effect of the fat globules and the interaction between the membrane proteins of the fat globules and the proteins in the emulsions can result in increasing the water-holding capacity of the gel of pork myofibrillar protein treated with fats. In addition, due to the free hydroxyl group in their structures, the improved water-holding capacity of the gel of pork myofibrillar protein can be obtained by the addition of unpurified diacylglycerol and purified diacylglycerol (Zhou et al., 2020).

Furthermore, the addition of lard-based diacylglycerol and the shift in pH had a major effect on the physicochemical properties of the thermally induced gel of pork myofibrillar protein. As the pH increases or there is an addition of impurified diacylglycerol or purified diacylglycerol, the water-holding capacity of the myofibrillar protein gels increases (Zhou et al., 2020). As the authors used composite gel in their research, they stated that the results obtained give a theoretical basis for using lard-based diacylglycerol in emulsified meat products (Zhou et al., 2020).

2.2.3 Lard reduces hardness in cookie dough

Cookies are among the most popular snacks people of all ages, races and cultures enjoy. They are products made from wheat flour, sugar and fat that are baked. Most cookies are formulated from either plant or animal fat, with around 10 to 30% of the shortening made (Manaf et al., 2019). Shortening plays an important role in dough production as it interacts efficiently with the matrix of protein and starch to avoid excessive gluten development throughout mixing. Shortening is also believed to act as a lubricating agent during dough making, allowing aeration to capture and hold air. Shortening could also impart desirable quality attributes to the texture and flavour of baked products.

Fat plays a significant role in the mechanical properties and fracturing behaviour of cookies. It is widely accepted that various types of fats, such as butter, lard, palm olein, palm stearin, and mid-fraction palm, have different effects on the textural properties of cookie dough. For instance, solid fat softens the dough and reduces viscosity (Manaf et al., 2019). Hardness plays an important role in baked goods, as it can lead to the freshness of products (Noor Raihana et al., 2017). They further claimed that this led to an increase in the length and a decrease in cookies' weight and thickness (Manaf et al., 2019).

In a previous study, Yanty et al. (2014) noted that cookie lipids formulated with palm-based fats and lard showed different thermal behaviours due to their compositional variations. Manaf et al. (2019) have attempted to create binary, ternary, and quaternary plant-fat mixtures as alternative plant-fat mixtures since halal and kosher food regulations commonly forbid the use of lard in cookie formulations. The maximum force has been reported to determine the hardness of the

dough. Based on the authors ' research results, cookie dough made from binary shortening was the hardest, which is 231.56 g. This is because it requires more force to compress. This may be attributed to the components of palm stearin and Mee (Madhucalongifolia) fat, which are difficult due to the dominance of di-saturated molecules, 40.97% and 1.38% tri-saturated molecules. The softest was dough made from lard which is 218.33 g because its compression needed the least force. The presence of low concentrations of di-saturated and tri-saturated molecules, which are 26.6% and 2.50%, respectively, may be attributed to this soft existence.

Cookie dough made of lard and quaternary shortenings should have been more cohesive and viscous, contributing to a smoother texture (Manaf *et al.,* 2019). The set times for Mee (Madhucalongifolia) fat, palm stearin (99:1), and lard-prepared cookies were 7 mins, while those for other shortening forms were 6 mins. However, no major variations in width and thickness between the cookies of all fat mixtures have been found. This may be attributed to the standardised expansion of cookies made from formulated plant-based shortenings and lard shortening during baking (Manaf *et al.,* 2019).

2.2.4 Lard aids in controlling pests in dry cured ham

In the ageing process, dry-cured hams can become infested with ham mites, red-legged beetles, cheese skippers, and larger beetles. Methyl bromide is the only fumigant available that is effective in controlling ham mites in dry-cured ham plants in the United States, although other methods can be used for beetles and cheese skippers (Zhou *et al.,* 2016). Nonetheless, methyl bromide will be phased out of all industries by roughly 2015. In the European Union, methyl bromide cannot be used to regulate mites. In the European Union, mites are considered a quality issue regulated by relative humidity, the use of lard on the surface and other good production practices (Zhou *et al.,* 2016).

In the United States, industry and university scientists are exploring possible solutions to methyl bromide as it is being phased out of use in the United States by the industry. These potential alternatives may include certain methods, such as using hot lard and relative humidity control, currently used in Europe to manage mite populations (Zhou *et al.,* 2016). A popular practice in Spain is coating hams with vegetable oils or hot lard to control mite infestations in dry-cured ham. Some vegetable oils protect cured fish from pests and give fish a more appealing look, including coconut, sunflower, groundnut palm, maise and sesame oil. As dipping on dry-cured ham cubes, a list of animal and vegetable oils, including soybean oil, canola oil, corn oil, olive oil, mineral oil and lard, sorbic salts like sodium, potassium and calcium propionate, iodide salts such as sodium, potassium and calcium iodide, sodium, potassium, calcium citrate which is an example of citrate salts, short-chain alcohols (1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol), organic acids like maleic acid, citric acid, 3,3-thiodipropionic acid and butylated phenol preservatives including BHT and BHA have been analysed. According to Zhou *et al.* (2016), approximately 2.5 cm³ cubes of dry-cured ham were immersed in a test compound of a given concentration for 1 min for food dips, then put in a ventilated glass jar and inoculated with 20 adults consisting mainly of female mites. As a result, ham cubes dipped in water from 20 parents created around 600 mites per container. 100% lard, 50 % propylene glycol (1, 2-propanediol) and 10 % butylated hydroxytoluene (BHT) were successful in regulating mite reproduction under laboratory conditions among all substances tested (Zhou *et al.,* 2016).

2.2.5 Adulteration issues related to lard

The global halal market accounts for more than 20% of the entire food industry, and by 2050, demand for halal products is estimated to continue to rise by up to 70% (Ruslan *et al.,* 2018). *Halal* food products are generally manufactured in Malaysia by certified Multinational companies and Small and Medium Enterprises (SMEs). As per the Syariah Legislation, the halal food industry involves manufacturing, preparation, preservatives, distribution, food service, and beverages. The industry embodies a dynamic and multinational array of diverse businesses that supply Muslim consumers worldwide with most halal food products (Ruslan *et al.,* 2018). Recently, the regular reports in the mass media of food fraud controversies in the food supply chain have raised serious concerns among Muslim consumers regarding halal food products. Several examples of food fraud are the contamination of undeclared porcine species meat products in sausages and burger patties, the detection of porcine DNA in commercial candy products such as marshmallows, gummies, hard candies and complex candies and issues in halal slaughterhouses where they do not follow the halal practices in the slaughtering process (Ruslan *et al.,* 2018).

Adulteration is a legal concept for a food product that fails to meet certain requirements. According to the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA), adulteration commonly applies to non-compliance with health or safety requirements. Food adulteration is purposely lowering the quality of the food offered for sale by combining or replacing inferior substances or withdrawing some important ingredients. Fats and oils are important nutrients for humans. Industrially, manufacturing has played an important role in developing the various fields of chemicals, pharmaceuticals, cosmetics and foodstuffs. Using animal fats in foods was very popular in the first 50 years of the twentieth century (Fadzlillah *et al.,* 2011). For example, lard or pig fat was the most commonly used commodity in the mass production of breads and cakes for domestic frying and raw material. Lard remains an essential ingredient for the food industry in formulating some food products, mainly embedded products. Usually, the label of the ingredients does not list the origin of the ingredients. A serious problem for Muslim buyers is secret ingredients from different sources (Fadzlillah *et al.,* 2011). However, lard can be replaced by other fat such as butter, shortening, margarine and coconut oil (Jaron, 2020).

Halal requires numerous procedures, such as slaughter, storage, display, preparation, hygiene, and sanitation. Thus, Muslim consumers are worried since most halal food items are imported from non-Muslim countries. Generally, non-Muslim countries such as Brazil, Australia, India, France, China, the Netherlands, and Spain are responsible for global halal meat supplies (Ruslan *et al.,* 2018). Some cases have reported a high risk of cross-contamination between halal and haram products. Cases such as when halal meats and non-halal meats are stored together. As reported, there has been a case of the Malaysian Quarantine and Inspection Service (MAQIS) Department seizing cargo shipments of halal and non-halal frozen meat stored together at Tanjung Pelepas Port (Said, 2017). Due to complex food supply chains, halal meat adulteration can occur in many forms. In the case of meat adulteration, in addition to the slaughter of animals in a manner that does not comply with the Syariah law as being addressed in the halal food industry, it includes not only substituting the ingredients but mislabelling these items from the country of origin (Ruslan *et al.,* 2018). Zakaria (2008) argued that it is hard to check the halal status of food items when the product is pre-packaged or processed.

Thus, the production of methods for studying food ingredients has been improved by the high demand for transparency in the food industry (Fadzlillah *et al.,* 2011).

3. Detection method of lard in food

The wide application of lard in food has been a concern for certain parties like Muslims and vegans. Undeclared substances in food products nowadays have catalysed the need and importance of detection methods for food adulteration. Many methods have been developed to distinguish the presence of lard or pork in meat or food products. For instance, Polymerase Chain Reaction (PCR), Gas Chromatography (GC), Enzyme-linked Immunosorbent Assay (ELISA), Duplex PCR-Enzyme-linked Oligonucleotide Assay (ELONA), Rapid PCR, Electronic Nose technology (E-nose) and Fourier Transform Infrared (FTIR) are the methods that have been employed to detect lard in foods and food products where the summarised information can be seen in table 1. Mohamad *et al.* (2015) summarised that few techniques can be used to detect the existence of lard in food products, and it involves labelling and non-labelling-based techniques. A labelling-based technique characterises Polymerase Chain Reaction (PCR), and the non-labelling technique are Electronic Nose (E-Nose), Fourier Transform Infrared (FTIR) Spectroscopy and Gas Chromatography (GC).

3.1 Polymerase chain reaction (PCR)

Several procedures supported by PCR are planned as advantageous means for recognising species of origin in foods due to their high specificity and sensitivity from qualitative PCR over restriction fragment length polymorphism (RFLP) to quantitative PCR, referred to as real-time PCR (Rohman *et al.,* 2016). Consistent with Perestam *et al.* (2017), the recognition of species within treated meat products uses the procedures of Polymerase Chain Reaction (PCR), a DNA-based method. The PCR assay was an alternative method for species recognition in treated meat products when a small recognition limit is needed. Furthermore, Ulca *et al.* (2013) stated that the polymerase chain reaction could distinguish less than 0.1% porcine DNA from meat mixture and recognise the right meat species. Lubis *et al.* (2016) noted that PCR-based DNA analysis has several drawbacks, such as the expense and time required for DNA extraction. DNA extraction can be labelled as slow due to the longer extraction time required. The extraction process might take up to 6 hours or more for certain samples due to different compositions. Other than that, DNA is prone to deterioration in treated food, which promotes difficulty in detecting the targeted DNA and possibly gives rise to false negatives.

Moreover, Rosman *et al.* (2016) investigated the inhibitory effect exerted by each of the chocolate components, four basic chocolate components, sugar, milk powder, cocoa butter, and cocoa powder, whereby they found that cocoa powder, as the only component that prevents DNA extraction of lard in chocolate. No substantial polymerase chain reaction inhibition was detected, and thus confirms the cocoa powder's inhibition on DNA extraction of lard from lard-adulterated chocolate.

3.2 Rapid PCR

Previous studies have reported that meat adulteration is ruining users' attraction towards food. Wu *et al.* (2020) suggested that it is crucial to establish a fast, uncomplicated, cost-effective and sensitive methodology to recognise meat species. In addition, according to Wu *et al.* (2020), in the experiment that was conducted, as little as 0.01% pork contents

in binary mixes can be identified, and the entire identification method manages to be completed in 20 mins from the sampling steps to the step where the outcome is obtained. The developed methodology has great potential for fast identifying pork meat and recognising meat species (Wu *et al.,* 2020). To create a quick, easy and cost-effective method for extracting meat nucleic acids, a new meat extraction method was developed and carried out. The method would be completed in 5 mins, where the changing time between water baths is also considered (Wu *et al.,* 2020). Previous studies have reported that nucleic acids can be magnified in mins. This can be done by rotating the plastic capillary between the two water baths (Zhuo *et al.,* 2018). The author uses the glass capillary as the reaction vessel to transfer heat efficiently between water baths and the reaction solution (Wu *et al.,* 2020). Moreover, the molarity of polymerase and primers were correspondingly raised 10 times and 2 times to equal the kinetics of primer annealing and polymerase extension under faster temperature cycling. To verify the period consumed in each water bath, a thermocouple was utilised to measure the temperature change of the reaction solution (Wu *et al.,* 2020).

Next, rapid PCR linked with the portable device was utilised to distinguish pork meat in binary mixes. The strong green fluorescence with 520 nm wavelength was formed to identify raw beef meat or cooked beef meatball containing 100%, 10%, 1%, 0.1%, and 0.01% pork meat. It was found that the colour of negative samples continued to be black. The fluorescence signal was comparatively low in recognising 0.01% of pork meats in beef meat, however, the identification of outcomes could still be distinguished by the naked eye if compared with the negative samples (Wu *et al.,* 2020). The process of visual recognition could be completed in 30 seconds, and the risk of carryover contamination could be prevented as there is no uncapping step (Wu *et al.,* 2020). Rapid PCR is used to amplify pork meat DNA, which can be achieved using two water baths in 5 mins. A SYTO 9-based visual detection system is created to make it simpler and more convenient to classify the amplification effects (Wu *et al.,* 2020).

3.3 Enzyme-linked immunosorbent assay (ELISA)

Other methods to determine lard's existence in food products are Enzyme-linked Immunosorbent Assay, also known as ELISA. The simplicity of sample preparation, the absence of complex equipment, trained staff and the high productivity of serial testing define immune techniques. Indeed, electrochemical immunosensors are an alternative detection method for food authentication and are highly viable for on-site use (Mandli *et al.,* 2018). Perestam *et al.* (2017) research shows that in terms of sensitivity, ELISA could persistently identify pork in the binary mixture at levels down to 10.0% w/w. Even though the pork was identified at levels as low as 5.00% w/w in another sample prepared with different concentrations of pork spiked in beef, this outcome was only detected with one of the duplicate samples. The ELISA test showed better sensitivity than the pork-specific test, with the lowest recognition at 0.50% w/w and the lowest uniform recognition level at 1.00% w/w for beef within a binary mixture (Perestam *et al.,* 2017). Mandli *et al.* (2018) used the anti-pig IgG (whole molecule), a peroxidase antibody produced in rabbits, and pure IgG from porcine serum used as a standard to detect pork in meat. Compared to the competitive ELISA, the developed direct ELISA detects low adulteration levels of 0.01% in 14 hours and 15 mins compared to the competitive ELISA, which allows the determination of 0.1% as the adulteration level in 45 mins. However, with the developed immunosensor, 0.1% of pork adulteration can be detected in 2 hours, and a low level of pork

Table 1: Comparison method for detection of lard in food

Method	Limit of Detection	Reliability	Drawbacks	Types of samples
Polymerase Chain Reaction (PCR)	• Able to distinguish <0.1% porcine DNA (Ulca *et al.,* 2013).	1. Portray high sensitivity method. 2. Required only small number of samples to analyse.	1. Slow method. 2. Required longer time for DNA extraction depending on composition of food matrices. 3. Susceptible to DNA deterioration in process food.	• Preferably only on treated meat products. Eg; nugget and sausage.
Rapid PCR	• Study reported as low as 0.01% pork content needed for recognition of adulteration (Wu *et al.,* 2020).	1. Highly sensitive method available. 2. Cost effective. 3. Fast method. Time required to analyse is only 20 minutes (Wu *et al.,* 2020).	• Susceptible to DNA deterioration in process food. Eg; chocolate-based product (Rosman *et al.,* 2016).	• Suits for treated meat products. Eg; patties
Enzyme-linked Immunosorbent Assay (ELISA)	• Capable of detection 0.01% pork adulteration (Mandli *et al.,* 2018).	1. High sensitivity. 2. Uncomplicated procedures. 3. Easily operated. No qualified personnel and equipment needed	1. Expensive due to antibody involved. 2. Antibody instability due to improper care of storage procedure. 3. Possibility of false positive and negative results (Sakamoto *et al.,* 2018).	• Applicable for treated meat products. Eg, minced meat
Gas Chromatography	• Previously reported only 0.5% of or lard adulteration could be detected (Hussain 2022).	• Capable of providing fine details of fatty acid composition and quality.	1. Laborious 2. Complex procedures 3. Dependent. Method like PCA analysis required to distinguish types of animals presents.	• High fat content food. Eg, biscuits
Duplex PCR Enzyme-linked Oligonucleotide Assay (ELONA)	• Pork adulteration range from 0.5% - 1% w/w (Skouridou *et al,* 2019).	1. Sensitive and cost-effective 2. Small number of samples required	1. Multiple procedures involved 2. Trained personnel required	• Raw meat and processed meat products.

adulteration can be detected with the competitive immunosensor (0.01%) in only 20 mins. The developed immunosensor was effective in detecting IgG pork in processed foods and can detect pork in a studied range of 1 to 100% in boiling beef meatballs and was successfully applied to species screening tests. Due to its sensitivity, specificity, simplicity and low cost, the assay is suitable for food authentication (Mandli *et al.,* 2018).

3.4 Gas chromatography

Research by Hussain (2022) reported that the long-wave NIR (LW-NIR) spectroscopy system at 1350 – 2450 nm region in combination with chemometrics analysis was used for detecting and quantifying lard adulteration in palm oil (PO). The result has shown that the samples with a minimum level of adulteration as low as 0.5% could still be easily detected with an overall correct classification rate using linear discriminant analysis (LDA) in the Open-source R software.

Meanwhile, Marikkar *et al.* (2021) differentiate fatty acid and triacylglycerol compositions of native lard (NL), beef tallow (BT), mutton tallow (MT), and chicken fat (CF) by using gas-liquid chromatography (GLC). GLC analysis showed that comparing the overall fatty acid data might not be suitable for discriminating different animal fats, but using the principal component analysis and the % palmitic acid enrichment factor [PAEF (%)] calculations were useful.

The study by Azizan *et al.* (2021) was conducted to detect lard adulterated wheat biscuits using chemometrics and machine learning–assisted GCMS. Oil was extracted from the laboratory-prepared wheat biscuits using the Soxhlet extraction method, converted to fatty acid methyl ester and analysed using GCMS. The result shows that principal component analysis (PCA) and hierarchical cluster analysis (HCA) could categorise lard, wheat biscuits and lard-adulterated samples based on their fatty acid distribution. Random forest outperformed partial least squares-discriminant analysis (PLS-DA) in sample classification. Feature selection using random forest identified two fatty acids as potential biomarkers. The researcher proposed C18:3n6 as the potential biomarker to differentiate pure wheat and lard-adulterated biscuits.

3.5 Duplex PCR enzyme-linked oligonucleotide assay (ELONA)

Skouridou *et al.* (2019) have pursued an alternative and flexible platform for the identification of meat adulteration. It was based on PCR amplification and direct post-PCR detection with a colourimetric enzyme-linked oligonucleotide assay (ELONA) using specially developed species-specific tailed primers. The authors have informed that PCR–Enzyme Linked Oligonucleotide Assay (ELONA) is a susceptible and decent procedure for determining the presence of pork in beef and chicken products (Skouridou *et al.,* 2019). The design of the primers was based on incorporating the tails of ssDNA and a PCR stopper to receive amplicons at each end with separate ssDNA tails. This design aimed to facilitate the detection of ssDNA tails by hybridisation with capture and detection probes. Control genomic DNA was used from each species in PCR-ELONA assays and was collected from the various animal tissues using the High Pure PCR Template Preparation Kit following the manufacturer's instructions (Skouridou *et al.,* 2019). Meat from cows, chicken and pigs was obtained from local commercial sources to test the sensitivity of the developed assay and used after grinding for the preparation of binary

mixtures containing 0.1, 1 and 10% pork in beef or chicken (w/w) (Skouridou *et al.,* 2019). Using the Gene JET Genomic DNA Purification Kit according to the manufacturer's protocol, DNA was extracted from approximately 20 mg of each mixture and analysed by agarose gel electrophoresis (Skouridou *et al.,* 2019). To detect pig DNA in mixtures with cow or chicken DNA, duplex PCR amplification using species-specific tailed primer pairs was optimised, followed by Enzyme-Linked Oligonucleotide Assay (ELONA) (Skouridou *et al.,* 2019). For duplex amplification, the primers utilised were the species-specific tailed primers. To allow for simultaneous verification of the origin of the principal species comprising the food product, duplex amplification was sought, bypassing the additional use of universal eukaryotic primers (Skouridou *et al.,* 2019). Therefore, the approach's potential flexibility and consumer-friendly features are highlighted at a lower cost relative to commercially available kits, without needing costly reagents or sacrificing detection sensitivity. It was authenticated using DNA add mixtures and DNA extracted from raw meat mixtures, and 0.5 – 1% *w/w* pork could be easily distinguished if mixed with beef or chicken. Skouridou *et al.* (2019) demonstrated that in preparing PCR-ELONA assays, each species' control genomic DNA was implemented, and the animal's animal tissue was extracted using the High Pure PCR Template Preparation kit. The two duplex PCR-ELONA assays were evaluated by adding mixtures of pig DNA with cow or chicken DNA. Mixtures containing 0.1 – 25% pig genomic DNA in cow or chicken DNA solutions were prepared using each species' control genomic DNA (Skouridou *et al.,* 2019). A PCR reaction followed by duplex amplification and ELONA detection of the pig amplicon was then applied to each DNA pool. The detection of pig DNA combined with cow DNA was more sensitive than that of the chicken/pig duplex. According to Skouridou *et al.* (2019), the circumstances that provide optimal performance of the two duplex PCR–ELONA assays were then employed for the building of calibration curves for all the targets via both synthetic ssDNA and genomic DNA (Skouridou *et al.,* 2019). The sensitivity achieved was high, detecting pig genomic DNA as low as 71 pg, while the presence of 0.5 - 1% pig DNA in mixtures with cow or chicken DNA could easily be visually identified compared to pure DNA-containing samples from only one animal. It also successfully identified the inclusion of 1% pork in beef or chicken raw meat mixtures. The output of the strategy established demonstrated its suitability for food product screening and the identification of adulterant levels of tissue extracted from pork (Skouridou *et al.,* 2019).

3.6 Electronic nose technology (E-nose)

The electronic nose acts like a biological nose in detecting the form of vapour that reaches its 'receptors'. An electronic nose's sensors can be selected to recognise an interesting odour. The electronic nose is typically a combination of an odour delivery system, a sensor (array), a data acquisition and a data processing unit (Latief *et al.,* 2017). In environmental and medical investigations, electronic noses have different applications. They also play a significant role in the food sector. The identification of various flavours of milk, meat, tea, spoiled beef and spoiled fish are some applications of electronic noses (Latief *et al.,* 2017).

According to Che Man *et al.* (2005), the electronic nose was shown to be able to differentiate between different types of vegetable oils and control the storage stability of RBD palm olein and the authors decided to use an electronic nose to detect lard. The gas sensor used is specified to be sensitive to volatile organic compounds (Latief *et al.,* 2017). There are many

chemical compositions of animal fats, which mainly contain triglycerides. A triglyceride is a chemical compound formed from one glycerol molecule and three fatty acids. The principal component of animal fat, like pig fat, is triglycerides. Moreover, lard primarily comprises saturated fatty acids (Latief et al., 2017). Lard is reported to contain up to 10% linoleic acid and small amounts of arachidonic acid, both of which are in the unsaturated fatty acid group. For this analysis, the gas sensor selected can quickly detect volatile organic compounds (Latief et al., 2017).

Metal oxide gas sensors are widely used in electronic noses, including pork adulteration, quality control, and a formaldehyde sensor. Tin dioxide [SnO_2] and tungsten trioxide [WO_3] are sensing materials used in metal oxide sensors, as both materials are said to be extremely sensitive to different forms of volatile compounds (Latief et al., 2017). When exposed to the sensor, it was revealed that decanal, an aldehyde, gives a major signal shift (Latief et al., 2017). As such, predicted to be significantly more sensitive to lard than other fats. A SnO_2 layer is coated with the sensor used in the author's report (Latief et al., 2017).

On the other hand, an electronic nose was used to authenticate lard in refined, bleached, deodorised palm olein (Che Man et al., 2005). The samples were prepared in proportions ranging from 1% to 10% of animal fat, in 1% increments (w/w) and from 10% to 20% of animal fat, in 5% increments (w/w), refined, bleached, deodorised palm olein and lard were blended. The electronic nose result is displayed as a chromatogram, the derivative of the frequency shift versus time shown graphically. The peak area was correlated to the concentration of the compound and was expressed in the count. RBD palm olein contained compounds that were less reactive than lard. This was due to the different nature of the processing methodology between the two oils. The palm olein used was processed, bleached and deodorised, but the lard was crude oil with a strong and distinctive swine odour. The findings show that with the increase of lard in the samples, the peak area of these components substantially increased. There was a substantial difference between the detector counts of 18.00 and 21.00 for 0 % lard and 1 % lard. Although the fine details of fatty acid composition and consistency parameters of adulterated RBD palm olein could be given by gas chromatography and chemical tests, they are of very little use for the qualitative identification and quantitative determination of adulterants such as lard in RBD palm olein. In contrast, using the e-Nose in RBD palm olein provided a more sensitive tool for detecting lard.

3.7 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy was used in halal authentication for the analysis of lard in a binary mixture with other animal fats by means of multivariate calibration in conjunction with discriminant analysis and for the analysis of lard in cake and chocolate formulations by means of partial least square calibration (Kurniawati et al., 2014). Fourier transform infrared has also been used to distinguish the presence of lard in animal fat mixtures, other vegetable fats in cocoa butter, the characterisation of edible oils and lard, and the Spanish fatty acid shortening composition (Syahariza et al., 2005). Syahariza et al. (2005) used Fourier transform infrared to identify lard in the cake using Perkin–Elmer spectrum RXI Fourier transform infrared spectrometer equipped with a LITA (Lithium tantalate) detector. The results show a range of spectra. The entire range of spectra looks almost the same, but when it was observed closely at the frequency range of 3009-

2800 cm $^{-1}$ which is due to CH stretching absorption, the carbonyl absorption of the triacylglycerol ester relation at 1744-1739 cm $^{-1}$, the bands associated with the fingerprint area (1500-1000 cm $^{-1}$), the trans double bond C=CAH bending vibration at 990-950 cm $^{-1}$ and the overlap of the methylene rocking vibration and the out-of-plane bending vibration of cis-disubstituted olefins at 723 cm $^{-1}$ (Syahariza et al., 2005). This Fourier transform infrared analytical approach is likely to be adaptable to detect and quantify the adulterated lard level in cake formulation, particularly if the same type of shortening was used in the formulation. The authors said that using 1117 - 1097 and 990 - 950 cm-1 regions can verify the presence of lard when combined with this shortening in cake formulation. The authors have shown here that it is possible to extract meaningful information from mid-infrared spectroscopy (MIR) spectra by combining attenuated total reflectance (ATR) with partial least square (PLS) regression (Syahariza et al., 2005).

In another study, lard in 'rambak' cracker was detected using Fourier transform infrared (FTIR) spectroscopy combined with partial least square and principal component analysis chemometrics. FTIR spectroscopy at wavenumber regions of 1200 – 1000 cm−1 was successfully used to quantify and classify lard in 'rambak' crackers (Erwanto et al., 2016).

4. Conclusion

In conclusion, adulterating pig sources in food products is banned in Islam. From the Islamic point of view, this restriction involves all parts of pigs, such as meat, skin, and even their derivatives like lard, enzyme, and others. Products containing lard must also be indicated in the labelling of foods. Several detection approaches have been developed, for instance, FTIR, ELISA, ELONA, e-nose, DSC and PCR. The review shows few lard applications in food, especially when lard is in the diacylglyceride form. The diacylglyceride form of lard can increase the emulsion activity and stability and increase the water-holding capacity of meat products. At the same time, the unsaturated part of the lard plays a role in increasing the stability of lipid and protein oxidation in minced pork. Lard also has a role in making cookies. It will help reduce the hardness of cookie dough so that the cookie produced has the properties of a good cookie, as there is an ever-growing demand from consumers worldwide for knowledge and trust relating to the origin and content of food purchased. Food producers have no choice but to include and validate the genuineness of the sources of their food ingredients in this regard. It is no longer feasible to detect adulteration using physical properties such as the refractive index, viscosity, melting point, saponification, and iodine value, in view of the availability of more modern, sophisticated procedures and approaches. However, at a known stage, each oil and fat have a particular component, and their existence and quality should be considered as a method for detection. Therefore, it is important to consider advanced, sophisticated, highly sensitive methods to detect and measure adulteration. Many methods can be used to detect the adulteration of lard, and from the review, a few methods are PCR, rapid PCR, ELISA, ELONA, e-nose, FTIR and DSC.

As the Muslim population have increased worldwide, the authorised person should take the initiative to produce and commercialise kits for halal analysis. There is clearly a great demand for steps to support Muslims with the religious duty to ensure that halal is their food. As technology has improved from time to time, it will be easier to determine any contamination in food, particularly the adulteration of lard in the food product.

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