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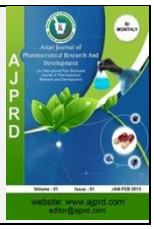

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Research Article

Chemical Analysis Methods to Detect Pork DNA and Derivative Contents in Food: A Review

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ABSTRACT

Purpose: Indonesia is a country which the most Muslim population in the world. In Indonesia, Halal in food product are mainly priority. Food adulteration with elements of pork or its derivatives become a very important thing to be analyzed. Therefore, There are several analytical methods that can be used such as molecular biology techniques including PCR, FTIR, chromatography, and electronic nose. This method can provide information to the public in choosing food products that are halal and tested. This review article describes the analytical methods that can be used for determination pork contents and its derivatives using various methods.

Selection of Data Sources: In preparing this article review, literature study techniques was used by using primary data from international journals. The data was searched by used online media such as Science Direct, NCBI, Research Gate, Google Scholar, Pub Med, and E-Resources.

Conclusion: PCR method was the best method. It was sensitive, specific, simple, and economical. This method was suitable for confirm authenticity of food and detected of pork DNA in food products.

Keywords: Pork DNA, Lard, Food products, PCR, FTIR, Chromatography, and Electronic Nose.

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INTRODUCTION

Indonesia is the country with the largest muslim population in the world with 87, 18% of the population are followers of Islam[1]. Halal lifestyle is sweeping the world, not only in countries with muslim majority populations, but also in countries with muslim minority populations[2]. So that the demand for halal products is quite large[3]. Halal has become a part of the life of a Muslim. The halal aspect is very broad, such as food, drinks, medicines, cosmetics, etc[4].

A Muslim's need for halal products should be supported by a halal guarantee. However, not all of the products circulating in Indonesia are guaranteed to be halal[5]. Globally, Muslim consumers are anxious about a number of issues regarding meat and meat products, especially, presence of pork-derived ingredients in food and authenticity of the halal label[6].

The authenticity of food can be seen from: (1) complete or incomplete important ingredients; (2) the whole or partial replacement of food components with alternative ingredients that are not announced (usually cheaper); (3) conceal damage to food or use of inferior foodstuffs; (4) adulteration (addition of an unannounced substance to increase the mass of the product or improve the taste of a product)[7]. Materials from pork derivatives are usually cheaper than halal animals such as cattle and buffalo[8]. Pork derivatives used in the meat processing industry include lard extracted from adipose tissue, Mechanically Recovered Meats (MRM), pork gelatin and pork blood plasma. The

consumption of pork derivatives have been prohibited in islamic law. Therefore, required the standardization of analytical methods have been proposed, developed, and tested to assist in testing their swine derivatives on food [9,10].

The analytical method used for detecting contamination of pork is based on the analysis of proteins and DNA. Protein-based methods include: Fourier Transform Infrared (FTIR) Spectroscopy[11], Electronic nose[12], and chromatography[13]. DNA-based methods include: Polymerase Chain Reaction (PCR) DNA mitochondrial [14], PCR-Restriction Fragment Length Polymorphism (RFLP) [15]and PCR Sequencing[16]. DNA-based methods developed rapidly. Therefore, protein analysis was changed to DNA analysis, because DNA-based techniques had more advantages over protein-based techniques. Protein-based techniques have a number of limitations. In addition, some immunoassays that use antibodies to specific proteins are often hindered by the cross-reactions that occur between adjacent species[17]. Conversely, DNA degeneration can differentiate between species using DNA analysis [18]. In addition, DNA is stable molecule that enables the analysis of the products processed and heated[19], and DNA can also be extracted from all types of tissue[20].Therefore, this review describes several methods that can be used as a reference, the development of new methods by researchers and scientists for testing pork derivatives in food products.

METHODS AND DATAS

The method that use by the authors in this review was by conducting literature studies. Primary data from international journals was used in this article. The literature was taken from various online journal search sites such as digital library, Science Direct, NCBI, Research gate, Google Scholar, Pub Med, and E-resources.

Result and Discussion

Table: 1 Identification of Pig DNA and its Derivates in Food by the PCR Method

S.No.	Sample	Purpose	Method	Primer	Result	Detection Limit	Author
1.	Softgellcandy	Detection of contaminants of DNA fragments coding pig cyt b in softgellcandy samples not labeled halal	PCR	Cty b	Positive 8 from 15 samples in 149 bp	-	23
2.	Pork, beef, lamb, and chicken meat	Applying the detection method of pork in a mixture of meat with beef, lamb, and chicken meatthrough PCR-RFLP cyt b gene and amylogenin gene specific primary PCR	PCR-RFLP	Cyt b	1% pig contamination colud be detected with PCR-RFLP (both for detection of halal products) and PCR specific genes have cross reactions.	1%	35
3.	Branded meatballs and bulk meatballs	looking for optimum annealing temperature with PCR using leptin and cyt b primers to detect pork in meatballs	PCR	leptin and cyt b	Optimum annealing temperature was 44 ° C	-	36
2.	Meatballs and sausages	Identification of pork DNA in meat	Multiplex PCR	Cyt b	Meatballs are 100% beef, and sausages are positive for pork	-	25
3.	Marshmallow, gum, and turkish delight	Detection of pork DNA in processed foods containing gelatin and gelatin halal authentication products	RT-PCR	Cyt b	2 samples of German food were positive for pork DNA, and 1 sample from Turkish delight	1%	37
4.	Meat from 24 kinds of animals	Pork counterfeit detection	Highly spesific PCR	Mitocondrial D-Loop	This technicue can be used for authenticating raw, processed and adulterated pork	Sensitivitas 0,1 %, LOD 10 pg	38
5.	Gelatin capsules, food products labeled beef gelatin, marshmallows, jellies, desserts and cakes	Halal gelatin authentication	species-spesific PCR	Cyt b	This method was affordable specific, and sensitive for routine analysis of foods containing gelatin and gelatin pharmaceutical products to protect consumer religion	0,1%	39
6.	Gelatin food products and dietary supplements,	DNA dtermination of bovine, porcine, and fish in gelatin mixtures, food products and dietary supplement	TaqManprobe based multiplex quantitative PCR	Cyt b dan 16S rRNA	Of the 35 samples, only two were found to be positive for porcine	0,005 ng/µL	40
7.	Pork, beef, chicken, lamb, and horse meat	Find out presence of pork DNA in food processing, product label verification, and consumer confidence	RT-qPCR	Cyt b	It is fast, specific, sensitive, high quantity method for detecting hidden cuts of meat during the food-making process	Loq 100fg / µl of mitochondrial DNA in 10ng / µl of mitochondrial DNA matrix	41

8.	Foods (gelatin, jelly, marshmellow, candy, pizza, cakes, and medicines) pork, beef, and fish	Identification and verification of pork DNA	Conv. PCR and Real Time PCR	Cyt b	5 samples of processed food tested positive for pork DNA. RT-PCR is more sensitive in halal authentication	-	42
9.	Halal and non-halal meat products	Determination of pork content with Eva Green RT-PCR	RT-PCR	Cyt b	Presence of undeclared pig species in 54% of the analyzed samples, with 40% of the halal product containing pork DNA, therefore not matching the label	0,0001% dan 0,01% (b / b)	43
10.	Commercial food	Identification of pork DNA in food	PCR and RT-PCR	Cyt b	Nine food samples tested positive for pork DNA	0,0001 ng/µl	44
11.	Processed meat	Identify the differences between pork, beef, chicken and lamb	PCR quadruplex	Cyt b and beta actin	There are 9 samples that do not include the origin of the meat	0,01 %	45
12.	Sausage, meat, animal feed	Determination of pork and chicken content, as well as testing methods	Droplet digital PCR	Beta-actin dan beta-3	The relationship between raw meat weight and DNA weight and between DNA weight and DNA copy number are both approximate linear. dPCR is particularly precise in measuring pork and chicken in meat products and therefore has the potential to be used in routine analysis by government regulators and quality control departments of commercial food and feed companies.	40 ng (chicken), 80 ng (pork)	46
13.	Meatballs and meat	Identification of pork adulteration	Taqman Probe RT- PCR	ATpase 6	There were 2 positive samples of pork DNA	5 pg	47
14.	Bread	Porcine derivative analysis	PCR	Mt- Cyt b	DNA strands, and especially, species specific markers of cyt b, can be effectively extracted and amplified from baked bread, resulting in a powerful bread analytical probe to reveal the use of animal material (porcine in this particular case).	0,5-0,1%	48
15.	Meatballs and sausages that are labeled halal and not labeled halal	Pork DNA detection	RT-PCR	Cyt b	Pork DNA positive in 3 samples that already have LPPOM MUI halal certificates	-	49
16.	Bread, nuggets, meatballs and sausages	Identification of pork adulteration	PCR	Mt-DNA	Positive 3 samples contain pork claiming to use beef / chicken	1%	50
17.	Various types of animal meat	Simultaneous testing and differences of different types of animal meat	Heptaplex PCR	Cytb, ND5,16s rRNA	86.7% of beef products (13 out of 15 tested) were adulterated. H-PCR is reliable in a single test platform due to its shortness of targets, which offers tight stability even with sample decomposition.	0,01−0,001 ng Pure DNA and 0.5% meat in meatball products	51
18.	Ground beef, roasts, dendeng, sausages, meatballs, nuggets, pressed ham	Predicting adulteration of pork in beef products	q-PCR based SYBR Green	Cyt b, 18S RNA	There were 3 samples containing pork, even though the label stated beef	0,01%	52
19.	Softgellcandy	Detection of contaminants of DNA fragments coding pig cyt b in softgellcandy samples not labeled halal	PCR	Cty b	Positive 8 from 15 samples in 149 bp	-	23
20.	Pork, beef, lamb, and chicken meat	Applying the detection method of pork in a mixture of meat with beef, lamb, and chicken meatthrough PCR-RFLP cyt b gene and	PCR-RFLP	Cyt b	1% pig contamination colud be detected with PCR-RFLP (both for detection of halal products) and PCR specific	1%	35

		amylogenin gene specific primary PCR			genes have cross reactions.		
21.	Branded meatballs and bulk meatballs	looking for optimum annealing temperature with PCR using leptin and cyt b primers to detect pork in meatballs	PCR	leptin and cyt b	Optimum annealing temperature was 44 ° C	-	36

Table: 2. Identification of pork DNA and derivatives in food using the FTIR method

No.	Sample	Result	Detection limit	Reference
1.	Lard, and animal body fat (beef, chicken, and lamb)	All samples containing lard formed a separate group from the samples free of lard.	-	70
2.	Pork, and other animals, and sausages	successfully used for sample identification and differentiation	-	71
3.	Refined, bleached, deodorized (RBD) palm olein, lard	zNose was a more sensitive method for detection of lard in RBD palm olein.	1%	69

Table: 3. Identification of pork DNA and derivatives in food using the electronic nose method

No.	Sample	Purpose	Chemometrics	Wave number (cm^{-1})	Result	Reference
1.	Meatball	Pork adulteration analysis	PLS	1200-1000	R2 (0.999), RMSEC(0.442)	33
2.	Chocolate and chocolate products	Lard adulteration analysis	PLS	4000-650	R2 (0,9872) SE (1,305)	12
3.	Vegetable oil	Lard adulteration analysis	PLS dan DA	1500-1000	PLS could calculate lard in vegetable oil mixtures, and DA could classify lard	61
4.	Meatball	Lard analysis	-	1000-1300	Tidak terdapat ikatanrangkap C=C	62
5.	Vitamin C gummy	Pork gelatin analysis	PCA	4000-750	Commercial vitamin C gummy contains gelatin sourced from bovine.	60
6.	Dendeng	Analysis of pork adulteration in Dendeng	LDA, SIMCA, and SVM	1500-600	LDA can classify and predict 100% accuracy of samples tested	63

Tabel: 4. Identification of pork DNA in food by chromatography method

No.	Sample	Method	Result	Detection limit	Reference
1.	Animal fat and vegetable oil	HPLC, CDA	Lard contamination in PKO is made possible by a visual comparison of the TAG profile of PKO adulterated with animal fat with animal fat	2%	75
2.	Meat	HPLC	The profile of pork triglycerides is different from that of cow or goat. Lard has more triglycerides than other meats.	1 % cow 3% goat	77
3.	Animal fat	LC-MS	The use of APCI-MS and Ag-HPLC facilitates analysis	5%	78

Polymerase Chain Reaction (PCR)

PCR was most common method to identify the presence of certain ingredients in food products, such as primers Species-specific PCR can be used to detect DNA count[21]. These method including qualitative PCR (conventional PCR), detection of Restriction Fragment Length Polymorphisms(RFLP), Real-TimePCR (quantitative PCR), and MultiplexPCR[22,23,24,25]. PCR is a highly sensitive technique that allows amplification of DNA segments quickly, and generate billions of copies of DNA fragments. PCR method is able to detect and identify gene sequences using visual techniques based on size and load 26[26]. However, PCR has disadvantages, such as : (1) DNA polymerase used in the PCR reaction is error prone and can cause mutations in the resulting fragments. (2) The specificity of the resulting PCR product may be altered by binding of non-specific primers to other similar sequences in the template DNA. (3) A primer design for producing PCR products, usually required some previous sequence

information[26].

PCR is performed on a DNA template, which can be single or double-helix, also requires two oligonucleotide primers that sandwich the DNA sequence to be amplified, dNTPs, which are four nucleotide triphosphate, heat-stable polymeration, and buffered magnesium ions for the quality of the PCR reaction. There are three steps of the PCR reaction, among them: (1) denaturation, (2) attachment, (3) extension. The reaction is carried out by means of a temperature rotation. High temperature is applied to separate (melt) the double helix of DNA. then temperature is lowered to let the primer adhere to the template, during attachment process, the complement hybridization sequence is also the temperature used based on the *melting temperature* (Tm) calculated from the primer (5 ° C in under primary Tm). During adhesion, the temperature is lowered (usually 45-60 ° C) to allow the primer to adhere to template strands. Unsuitable adhesion temperature can inhibit the primer-template interaction. Therefore, temperature should be optimized and finally set to around 72 ° C, it is optimal for primer lengthening polymeration by including dNTP [27,28,29].

PCR-Restriction Fragment Length Polymorphism (RFLP) is the amplification of a particular DNA fragment by using a pair of specific / universal primers to produce the intended fragment. The amplification product digested using restriction enzymes and produce a piece of fragments lenght can vary depending where restriction sites were identified[30].The principle of the PCR-RFLP method is the presence of polymorphic restriction endonucleases for each gene. The analyzed DNA fragments include amino acid coding regions (exons), introns or intergenic regions. The result of cutting is in the form of DNA fragments that have different sizes [31]. This method can be used to detect the presence of pork in other meat mixtures[32]. The weakness of the PCR-RFLP method is taking a long time for two stages important analysis, such as PCR processing and cutting of DNA as a results of PCR with restriction enzymes [33]. Pork adulteration can also be detected using PCR sequencing. PCR sequencing is direct way to get information from PCR products[20]. DNA amplification of mitochondrial sequences, especially cytochrome b[16], gene12S and 16S rRNAgenes[34] have been used to obtain information for identifying the animal origin of meat because of some advantages possessed by mitochondrial DNA[16]. Characterization of animal species by sequencing PCR relies on the availability of known sequences for comparison. The sequences have been available and could be downloaded from databases such as Gen Bank and information from the National Center for Biotechnology. PCR sequencing has a potential tool for detecting pork for halal authentication. However, this method may pose constraints in samples cooked or processed with degraded DNA and it is further restricted in the analysis of mixed species meat because the erogenous mix of sequences from different species precludes interpretation of results[17].

Pork DNA analysis in food could be used conventional PCR methods, such as meatballs[36] andbread[48]. Rahmawati[23] detected pork DNA contamination in softgellcandy. This research was conducted by testing 15 samples of Softgelcandy without halal label which are sold freely in Surabaya, with pork cytochrome b (cyt b) DNA fragment coding primers.The first step was DNA isolation which was carried out according to the protocol from the universal kit *Wizard KIT Promega*®. The results were tested for purity and concentration using the *BiochromBiodrop-DUO* spectrophotometer at λ260 and λ280 nm to determine the purity and concentration ofDNA *genome* present in the sample with results that met the requirements, that was around 1.8-2.0 and DNA below that purity number has the potential to interfere with the running process of PCR[23.53]. Visualization of PCR results using 2% agarose gel electrophoresis. The result showed that from 15 samples, 8 samples indicated that they contained / contaminated pig DNA were marked with DNA bands of ± 149bp. However, this thickness is not sufficient to prove whether or not the amount of DNA is contained because electrophoresis is a qualitative analysis. It is necessary to test with *Real Time* PCR (qPCR) to confirm the amount of DNA amplified, and with sequencing to ensure the correctness of the length / size of DNA. Mitochondrial DNA genes are commonly used as targets for various PCR tests in the detection of speciation of animal, plant and microbial cells. Mitochondrial DNA has a large number of copies in cells.Therefore, utilizing the mitochondrial DNA as a target will increase the chance to get the sufficient DNA for subsequent PCR testing, especially using products that are processed by heating, which can cause degradation / denaturation of genomic DNA[23].

Gelatin is an ingredient that sourced from bovine and porcine.Gelatin has been used in many foods and pharmaceutical products. Food products with halal regulations, need to be tested using reliable analytical methods, such as PCR which uses species-specific primers to evaluate halal authenticity of commercially pure gelatin and processed food products containing gelatin. Based on specificity and cross-reactivity results of the seven species-specific primers using conventional PCR, the pork species primer number 2 was selected and able to detect DNA species in 12 of 36 processed foods. Cloning, sequencing, and blasting at NCBI confirmed the presence of pork DNA in 5 of the 12 pork DNA positive food samples. The maximum identities (homology) with pork sequences available on the NCBI Gene Bank for the five samples ranged from 87% to 97% and Query Cover ranged from 94% to 100%. The RT-PCR test detected more positive samples (27 positive amplifications) compared to 12 positive samples with conventional PCR using no pork specific primers number 2. PCR using species-specific primers is a very useful and effective technique for the authenticity of halal gelatin and gelatin in food products[42]. Gelatin detection was also carried out by Demirhan[37] using RT-PCR with a detection limit of 1% based on multi-copy target cytochrome b (cyt b) using a pork specific primer. Karabasanavar[38] reported that by used a newly designed primer targeting pork mitochondrial displacement region (D-loop) produces a unique amplicon of 712 base pairs (bp) with a detection limit of 10 pg to detect pork adulteration. Raharjo[47] involved a specific primer and TaqMan probe targeting the pork mitochondria of the ATPase 6 gene, proving the specificity of the investigation by showing no

amplification of DNA isolated from six meat-providing species: cattle, dogs, rats, chickens, goats, and horses with detection limits of up to 5 pg total pig DNA, which equates to about 6.8 copies of pork mtDNA.

In RT-PCR, the amount of DNA products formed could be monitored in areal time, i.e. during the reaction, with accuracy and sensitivity height over distance dynamics. This monitoring can be assisted by the use of fluorescent dyes or a probe that is incorporated into the reaction. Intensitythe fluorescence of probe will be proportional to the amount of DNA productformed. In addition, in real-time the number of PCR amplification cycles is required to get that particular number of DNA molecules recorded. Assumption of efficiencyspecific amplification, which is usually close to double the number of molecules peramplification cycle, the number of DNA molecules carrying their initial target sequencepresent in the sample can be counted. A very detecting chemical figure efficient, sensitive instrumentation, and optimized testing is now available which allows the quantification of the DNA molecular number with a specific sequence in a complex sample with unprecedented accuracysensitivity which can detect one molecule[28].

The real-time PCR response curve divided into 4 separate phases: (1) the linear plain phase, (2) the initial exponential phase, (3) the log-linear phase (also known as exponential), and (4) thephase plateau[54]. Al Kahtani[44] has identified pork DNA in a mixture of meat and commercial food products using conventional PCR and RT-PCR methods, with a detection limit of 0.0001 ng/µl. Detection of pork adulteration in meat products using a special primer for pork DNA mitochondria. Mitochondrial DNA sequences for pork, bovine, chickens, and sheep were obtained from GenBank and aligned. The 294-bp mitochondrial DNA D-loop region was selected as the target pork DNA sequence and primers were designed using the MUSCLE program. The primers developed in this study were able to detect as little as 1% pork in pork-beef mixture with heat treated. PCR method could be used to detect adulteration of pork in various processed meat products for applications in maintaining religious food ethics, detecting allergens, and preventing food adulteration.

In testing for pork DNA contaminants using the RT-PCR method, it was found that three samples were positive for pork DNA. The three samples are A1, C1 and B2. This can be seen from the CP (*crossing point*) value of the three samples at <35 compared to positive control with a CP value of 32.63. The content of pork DNA in meatball and beef sausage products can be caused by cross-contamination in the production process, because there are processed meat products that have obtained a halal certificate from LPPOM MUI[49].

A quantitative approach is essential for distinguishing intentional adulteration from cross-contamination. This study is intended to develop and validate a novel specific and highly sensitive real-time PCR system for the quantification of pork in processed meat products. Normalized assays based on the DCt method were successfully developed and optimized, allowing detection and quantification down to 0.0001% and 0.01% (w / w) of pork, both in raw and thermal processing. The results were very surprising, namely the existence of pork species undeclaredin 54% of the samples analyzed, with 40% of the halal products containing traces of pork, therefore it did not match the labels they put[43].

Cross contamination of food products is prone to occur during food production in manufacturing facilities. Sausage products which states beef and pork as meat ingredients on the label.However, bovine DNA was not detected by used specific qPCR assay. Furthermore, estimated amount of pork contained was found to be up to 81.11%, this case indicated thatmislabeling would be an example of food fraud for economic gain[52].

Beside RT-PCR method, detection of pork contamination could also usedPCR-RFLP method. Because, cytochrome b gene produces better and clearer results for testing pork contamination compared to PCR with specific primers[35].

Multiplex PCR was a PCR technique by used several primers together in a reaction for amplification of several target regions. The genes most often used are chytochrome b (cyt b).Variation of the sequence in cyt b causes this gene to be widely used as a marker to distinguish material from different animal species. The samples analyzed were meatballs and sausages circulating in traditional markets in the Pandeglang Regency. The results showed thatcyt b gene was proven to amplify DNA from bovine and porcine with different fragment lengths in one reaction.Two bands were formed, among them 274 bp for bovine and 398 for pork. Sausage samples showed 398 bp amplification results. It is product contains pork[25].

Heptaplex PCR is another type of PCR method.heptaplex PCR have targets short amplicon lengths (73−198 bp) for the simultaneous detection and differentiation of species of bovine, buffalo, chickens, cats, dogs, pork and fish in raw and processed feed using species-specific primers targeting mitochondrial genes cytb, ND5, and 16 rRNA. The detection limit of heptaplex PCR was 0.01 to 0.001 ng DNA in a pure state and 0.5% in the meat meatball products. This test are efficient to detect target species simultaneously, even in highly degraded and processed food products in a shorter time[51],Digital droplet PCR (dPCR), calculations involved in dPCR are based on absolute data, not relative data (such as Ct values) and not require standard curves or reference materials, which improves the quantification accuracy [46], and quadruplex PCR[45].

Fourier Transform Infrared (FTIR) *Spectroscopy*

Fourier Transform-Infrared Spectroscopy(FTIR) is a technique used to obtain the infrared spectrum from absorption, emission, photoconductivity or scattering ratio of solid, liquid or gas[55]. Frequency, wavelength, or wavenumber where the sample absorbs IR radiation (x-axis) and the corresponding intensity (either transmittance or absorbance) (y axis) recorded in the IR spectrum[56]. Analysis using FTIR could be used to determine the halalness of a food product by looking at the spectrum

pattern in animal fat. The results of the FTIR can explain the functional group of the sample products. Various FTIR techniques are applied including near-infrared spectroscopy (14,000 to 4000 cm^{-1}) (NIR), center-infrared spectroscopy (4000 to 400 cm^{-1}) (MIR) and far-infrared spectroscopy (ATR) (400 to 50 cm^{-1})[57].

There are two types of instruments such as dispersive and FTIR instruments. Instrument dispersive has been non-existent used in food analysis because of the difficulty in sample handling techniques and not coombined with spectal ones precise scanning and spectral processing to provide value information for quantitative analysis. As a result, the last three decades, FTIR spectroscopy has replaced dispersive IR spsctroscopy and appears to be an emerging techniques for confirmation, identification, and quantitative analysis[58]. FTIR spectroscopy combined with computer software and chemometry. Therefore, it can easily manipulate spectral information (subtraction, spectrum derivatives, and deconvolution) and it was a technical analysis to analyze the presence of pork derivatives in food products[59,60,61,12].

FTIR combined with multivariate calibration of partial least square regression (PLS) was used to measure the presence of lard in food products, namely meatballs [33], chocolate[12] and vegetable oil[61]. Semiquantitative approach is proposed to measure the percentage of a mixture of lard with a food system based on spectral data in the frequency region of 1200-1000 cm^{-1}(meatballs), 4000-650 cm^{-1}(chocolate), and 1500-1000cm^{-1}(vegetable oil). The equation shows that the relationship between the actual value of lard (x-axis) and the predicted value of FTIR (y-axis) is y = 0.999x + 0.004, with the coefficient of determination (R2) and the root mean square error of the calibration are 0.999 and 0.442 (meatball), respectively. , y = 0.9225x + 0.5539, with the coefficient of determination (R2) of 0.9872 and the standard error of measurement (SEM) of 1.31% (brown)[33,12,61]. Apart from using PLS chemometrics, FTIR analysis can also use *Principal Component Analysis* / PCA[60]chemometrics. chemometrics*Linear Discriminant Analysis*/ LDA, *Soft Independent Modeling of Class Analogy*/ SIMCA, and *Support Vector Machines*/ SVM[63].

Analysis of lard in food could be determined by the number of unsaturated double bonds compared to beef fat. The characteristics of unsaturated fatty acids are that they have double bonds on the carbon[64]. In Islamic research[62] with meatball samples, it was found that there was absorption (CO) in the area of wave numbers 1000-1300 cm^{-1}, O-H knock vibration in the 1300-1440 area, carbonyl bonds (C = O) in the area 1680-1750 cm^{-1}, and C-H bonds in the area between 2853-2962 cm^{-1}, and the results of the FTIR spectra not showed any carbon double bonds.It is identified the presence of unsaturated fatty acids which are characteristic of lard from the results of the spectra analysis. In addition, the resulting spectra showed no overlap between the two regions wave numbers so that the analysis results indicate that the sample meatballs refined products not contain of lard

The FTIR method could be used for analysis of porcine DNA and its derivatives. FTIR spectroscopy combined with chemometric classification and multivariate calibration was a powerful analytical technique for the authentication analysis of high-priced meats such as beef with low-value meats such as pork. Chemometric FTIR spectroscopy screening of adulterated meat quickly.

Electronic Nose (EN)

E-noseis a system that is able to recognize a substance based on the smell and aroma response received[65]. *E-nose* works on principle of smell in animals and humans. Receptor cells in animals and humans are replaced by gas sensors which are very sensitive. Gas sensor works on the principle that changes in the composition of the gas around the sensor will be captured by the sensor and converted into an electrical signal through the transducer[66].

The mechanism of the electronic olfactory system (E-nose) was similar to the mechanism smell in humans and animals. The receptor cells in animals and humans are replaced by gas sensors which are very sensitive to certain gases. The main body of the *e-nose* is a gas sensor array which receives odor and odor responses and is converted into an electric signal via atransducer[66,67]. The type of gas sensor used on the *e-nose* generally uses mixed metal oxide semiconductors Sn, Zn, Cu and others with oxygen. The sample detection process is carried out by exposing volatile organic compounds from the sample to the gas sensor array. In general, samples are exposed to the sensor array without going through a preparation process. The absorption of oxygen atoms exposed to the volatile organic compounds of the sample on the semiconductor surface causes a change in the resistivity of the sensor material[68].

Electronic Nose (E-nose) has been used to detect and differentiate lard from other types of animal fat and samples containing lard[69,70,71]. The result of detected was presented inVaporPrint™. It is an image of the polar plot of the odor amplitude from the surface acoustic wave detector frequency (SAW). In VaporPrint™,Radial angle files representing the sensor provide individual fingerprints based on the scent of different animal body fat. Principal Component Analysis (PCA) is used to interpret data and good samples grouping, with 61% of variation calculated by PC 1 and 29% by PC 2.All samples containing lard formed a separate group from free lard samples. This method developed into a method for detecting the presence of lard in food samples for Halal authentication[70].

Volatile compounds from pork, meat and other meat products were studied using nasal and electronic Gas Chromatographic Mass Spectrometers with a headspace analyzer (GCMS-HS) for halal verification. PCA analysis was able to classify and differentiate pork meat from other types of meat and sausages. This suggests that PCA can provide good sample separation with 67% of the total variance recorded by PC1[71].

Refined, bleached, and deodorized(RBD) palm olein spiked with lard at levels ranging from 1% to 20% (w / w) were analyzed. zNose produces a two-dimensional olfactory image called a Vapor Print. It is can be used for direct (qualitative) detection of lard in sample mixture. Lard

adulteration could be determined by several different peaks inzNose chromatogram. Association between lard percentage in adulterated palm olein RBD and SAW detector response was observed at the E adulterant peak (R2 = 0.906). The Pearson correlation coefficient (r) was calculated using this parameter. An ideal correlation was observed between zNose data and other chemical tests (r> 0.90)[69].

Chromatography

Chromatography is a fast and reliable technique for the separation and quantitative analysis of major and minor components with very similar structural chemicals in complex foods. [72]. Chromatography has favorable separation characteristics, many chromatographic techniques have been tested, accepted and used in the analysis of porcine derivatives in food products. The disadvantage of this technique is that several steps in sample preparation are required prior to chromatographic analysis [73].

High-Performance Liquid Chromatography (HPLC) is one of the most numerousused analytical techniques for food authentication studies[74]. Application of HPLC to detect adulteration in food has attracted a lot of attention, due to this technique could applied to almost all components in food system[75,76]. Pork and lard mixed processed beef and mutton were analyzed using reverse-phase HPLC and ultraviolet (UV) detection. Total trigycerides (TG) containing saturated fatty acids (FA) at position C-2 was larger than the other meat fat. The precense of pork in samples causes ratio (R) of saturated FA containing TG to unsaturated FA containing TG at position C-2 increase compared to R beef or mutton[77].

Liquid chromatography combined with a mass spectometer (LC-MS) for authentication analysis has been developed rapidly in last decade, due to its sensitivity, exceptional selectivity, and fast analysis. Dugo[78] has determined composition triglycerides of lard, fats, and it is mixtures using HPLC in combination with atmospheric pressure chemical ionization mass spectrometry (APCI-MS). Distribution of fatty acids along glycerol backbone by diglyceride ion ratio derived from an atmospheric pressure chemical ionization mass spectrometry (APCI-MS) analysis. Ratio between positional isomers 1,2-dioleoyl-3-palmitoyl trigliceride in lard, fat, and it is mixture have been used as an indication of presence of lard.

Marikkar[75] has identified lard contamination in vegetable oils such as palm oil (Pa-O), palm kernel oil (PKO), and canola oil (Ca-O) using HPLC that combined with chemometric Canonical Discriminant Analysis (CDA). Analyzes were carried out for monitor changes in the composition of TG in vegetable oil samples before and after adulteration with lard. The result showed that lard contamination in PKO could monitored by evaluating TG profile of PKO mixed with lard. This method was not useful for Pa-O and Ca-O. The authors reported that HPLC data combined with CDA could separate vegetable oils from those mixed with lard.

CONCLUSION

There are important efforts underway to develop new applications of existing analytical techniques for detection and quantification of pork DNA and its derivatives in food. FTIR spectroscopy combined with chemometric analysis has proven to be a reliable technique for analysis of porcine in food samples, or halal authenticity. Chromatography-based techniques have been used for analysis of lard, pork, and porcine gelatin by determining composition of fatty acids, triglycerides, and amino acids. Electronic nose (E-nose)could be used to qualitatively detect presence of porcine and lard in food. PCR method is a fast, sensitive, specific, simple, and economical method. This method was used to determine authenticity of food and to detect presence of pork DNA in food products. PCR was also method for identification of gelatin sources in food and pharmaceutical products. Therefore, PCR method was an ideal method for halal authentication.

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