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Identification of Fat in Pork Using Fourier Transform Infrared Spectrum and GC-MS

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ABSTRACT

Halal food safety issues receive serious attention. Methods for analyzing contamination of non-halal ingredients, such as lard, must be developed to assist in the halal authentication process for food. This study was conducted to compare the fat profile of pork thighs using FT-IR and GC-MS. The pork is dried in the oven and then extracted using n-hexane. The resulting fat was characterized by FT-IR. Determination of fatty acids was carried out by GC-MS using a derivatization technique. Based on the results of the study showed that the infrared pattern of lard can be identified from the difference in absorption intensity. Pork fat has a higher unsaturated fatty acid content than beef and chicken. Infrared spectra can confirm the presence of -C=C- bonds at 3005, 1745, 1116, 1550, and 722 cm^{-1}. The FTIR data shows that there are larger unsaturated fatty acid groups in pork fat than in beef and chicken fat. This procedure is a non-destructive method that can be carried out quickly, and cheaply, and can be developed into a routine procedure for halal authentication of food. Based on the results of unsaturated fatty acid testing with GC-MS it can show the content of oleic acid (C18:1) contained in pork. The results of mapping the content of free fatty acids in pork can be used to complete halal authentication data easily and quickly.

1. INTRODUCTION

Food safety and quality assurance along the food chain demand great attention from the food industry and consumers [1]. Consumer caution in choosing quality food products needs to be campaigned continuously. Regulatory policies must be able to provide protection to consumers and ensure producers apply food safety standards [1]. Food safety standards ensure physical, chemical, and biological food safety. In addition, food safety is also used to ensure that food products are free from contamination by non-halal materials. Food products consumed by the public must have halal authentication and be free from non-halal contamination [2]. Halal authentication is carried out to ensure food is free from prohibited ingredients according to Islamic law which is carried out using methods of detecting physical, chemical and biological properties [3]. The standard methods of quantifying non-halal components are urgent [2], [4].

One of the contaminations of non-halal materials comes from pork products in meat products and their derivatives. Pork fat can be identified through DNA analysis using polymerase chain reaction (PCR), DNA barcoding, loop-mediated isothermal amplification (LAMP), and biosensor method [5], [6]. The DNA method is reported as capable of accurately detecting pork DNA,

flexible use, fast detection, high sensitivity, and high selectivity [5]. However, not many food quality test laboratories have this instrumentation. Inaccessible instrumentation in some areas may cause sample damage during transit. The relatively expensive cost of analysis is an important consideration in ensuring food safety, especially for micro, small, and medium enterprises.

This study was conducted to study pork fat using fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS). The FTIR has benefits in the halal authentication process. The FTIR can detect the macromolecule structure of lard [7], [8]. Analysis with FT-IR requires low cost with fast analysis and reduces the use of chemicals. This method is not yet able to provide specific data [9], but can be used in halal authentication [10]–[12]. The IR spectrum can be used for qualitative and quantitative analysis of pork. Qualitative analysis is based on characteristic peaks in pork, while quantitative analysis is based on absorption intensity [11]–[13].

The FTIR analysis can be used to distinguish fatty acid profiles of pork and fish with significant differences in absorption patterns of the infrared spectrum, especially at wave numbers 3010-3000, 1680-1600, and 968-966 cm^{-1} [10], [14]. Differences in peak intensity in lard and beef fat are also shown at 1747 and 1744 cm^{-1} of the carbonyl (C=O) ester group of triacylglycerol [13]. This method can be used to differentiate mixtures of lard and beef [11], [13]. The infrared spectrum provides an approximation of the proportion of saturated acyl groups and oleic acyl groups reflected in lard and beef fat as indicated by the difference in peak absorption intensity at 1119 and 1100 cm^{-1} [12], [13]. The FTIR spectra of lard provide a sharp absorption in the wavenumber region between 3010-3000 cm^{-1} which is a stretching vibration of the C=CH cis absorption band [15].

Pork fat is distinguishable compared to chicken and beef fat [2]. Pork contains more unsaturated fatty acids than chicken or beef. The FTIR method makes it easy to detect pork from the absorption intensity of unsaturated fatty acids [10], [16], [17] This method is a fast [11], [18] and effective procedure to identify fatty acids in pork [11]. The FTIR provides measurements with high accuracy and resolution high resolution spectra [18]. This method is considered the most appropriate for widely scaled meat quality [7]. However, the process of ensuring quality and halal authentication of meat products and their derivatives requires a comprehensive analysis process. The FTIR method can be used to identify the presence of unsaturated fatty acids with a certain intensity. However, the type of fatty acids in pork cannot be identified with the infrared spectrum.

The fatty acid content in pork can be identified by GC-MS [17], [19], [20]. The GC-MS method was developed for the extraction, purification, identification, and quantitation of components in meat and animal fats [21]. This method can be used in the laboratory to detect compounds contained in pork. Gas chromatography (GC) method is often used for the analysis of non-halal components in food [4]. The GC-MS has been used to determine the fatty acid composition of foods to detect the presence of non-halal ingredients [3]. Higher content of unsaturated fatty acids. The GC-MS can be used to determine the fatty acid profile in pork [3]. The ratio of saturated fatty acids to monounsaturated fatty acids in beef fat is higher than pork fat. The ratio of saturated fatty acids and monounsaturated fatty acids can be used to identify the type of meat in food [22]. The GC and GC-MS methods can be used to distinguish the content of oleic, linoleic, and linolenic acid in pork with a higher content than beef [10], [17].

Gas chromatography is more suitable for the analysis of smaller, volatile, and heat-stable compounds [4]. Analysis with GC can be carried out with a derivatization step to produce derivative products that are more volatile and stable, although this derivatization process increases the complexity of the method and extends the time in sample preparation [4]. The availability of derivatives and their steric disturbances in the analytes, as well as the stability of the derived compounds, must also be considered [4]. The cost of analysis and the availability of instrumentation are the main considerations in choosing a chromatographic method. The FTIR and GC-MS methods can be proposed for determination of fatty acids contained in pork. Both methods can provide very useful data in halal authentication. The results of this study are expected to be one of the references in developing methods for measuring non-halal components.

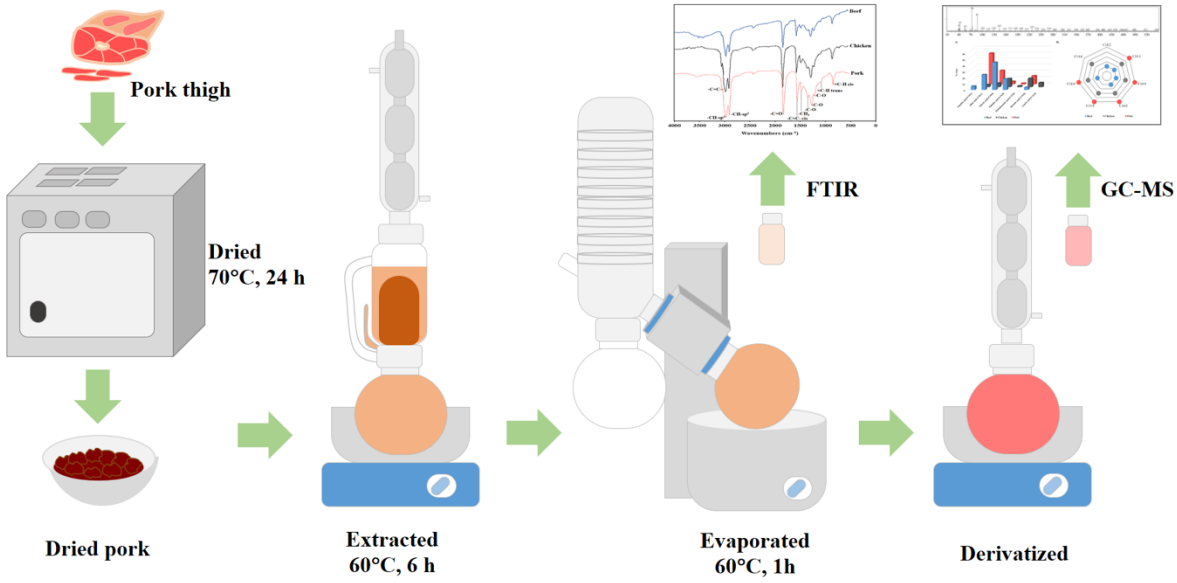

Figure 1. Pork detection procedure illustration

2. EXPERIMENTAL METHODS

2.1. Materials

The materials used in this study were pork, beef, and chicken thighs obtained from Kranggan Market, Yogyakarta, Indonesia. The chemical used is n-hexane, boron trifluoride, methanol, and sodium chloride produced by Merck. The equipment used in this study was laboratory glassware, soxhlet equipment, oven, Buchi evaporator, Fourier Transform Infrared Nicolet Avatar 360 IR, and GC-MS Shimadzu QP 2010 SE.

2.2. Extraction of Fat

Pork, beef, and chicken thighs were thinly sliced and dried in an oven at 70°C for 24 hours [10], [11]. Fat is separated by soxhlet extraction using petroleum ether solvent. Extraction was carried out at 60°C for 6 hours [10], [13]. The extract was concentrated with the evaporator Buchi.

2.3. Characterization using Fourier Transform Infrared

Pork, beef, and chicken fat characterization were directly carried out with Fourier Transform Infrared Nicolet Avatar 360 IR [16]. The fat extract was dripped onto the surface of the KBr cell using a polytetrafluoroethylene (PTFE) barrier to produce a layer thickness of 0.1 mm. Scanning was carried out with a wavelength range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1} [10], [11].

2.4. Detection using Gas Chromatography-Mass Spectrometry

Pork, beef, and chicken fat were dissolved in n-hexane and derivatized with a mixture of methanol and boron trifluoride according to the derivatization procedure [22]. The mixture was heated for 40 minutes at 80°C. The results obtained were added to a saturated NaCl solution and then centrifuged for 10 minutes. The n-hexane fraction was analyzed using GC-MS. Characterization of pork fat using GC-MS [4], [21]. As much as 1 µL is injected into the GC column. Separation was carried out in MXT-1 column, 30 m x 0.25 mm ID, 0.25 µm using the stationary phase poly dimethyl siloxane. The injector temperature was 300°C, and the column temperature was 80°C then increased to 300°C with 10°C/minute increments. The mobile phase used was helium (ultra-high purity) with a flow rate of 3.0 mL/minute. The MS detector used is a

70 MeV electron multiplier detector (EMD). Mass spectrum compared to the WILLEY147 and NIST47 libraries contained in the GC-MS post-run analysis software.

3. RESULTS AND DISCUSSIONS

3.1. Infrared Spectrum for Autenfification of Pork Fat

Identification of lard used in this study using samples of pork thighs. Pork thigh meat has little fiber and a juicy texture. Thigh meat is suitable for processed ground beef, ham, stir-fried, grilled, and various other processed foods. This section has less fat so that in the halal authentication process requires accuracy in sample preparation. The preparation is done by drying the pork which has been cut into small pieces to reduce the water content [10], [11] Fat separation was carried out by Soxhlet extraction using n-hexane solvent [10], [13] and fat separation was carried out by evaporation technique. Fatty acid authentication was carried out to study the absorption pattern and intensity of monounsaturated fatty acids using FTIR [16], [17], [22].

Figure 2 shows the infrared spectrum of lard using a comparison of beef and chicken fat. The FTIR spectrum can be used as a potential way to differentiate lard from beef and chicken fat [23]. The FTIR spectrum is considered a fingerprint, meaning no two fats have the same FTIR spectrum, in a number of peaks or peak intensity [23]. The FTIR spectrum in the fat extract describes the absorption of triglyceride compounds as the main component [23]. The pattern of infrared spectral peaks in lard is similar to that of beef and chicken fat, but these peaks have different intensity of absorption peaks [23]. This difference in absorption intensity shows that the fatty acid content in pork is different from chicken and beef.

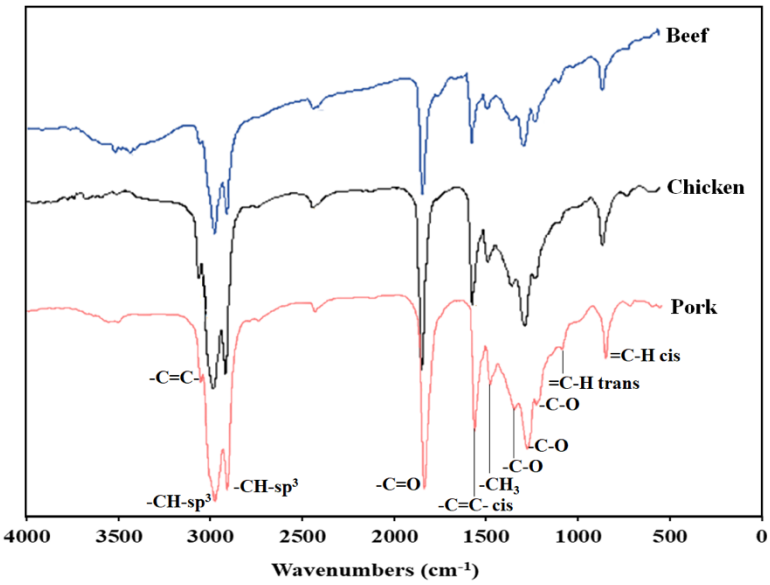

Figure 2. Infrared spectrum of pork, beef, and chicken fat

The results of the analysis showed that the FTIR spectrum profile of pork fatty acids had significant differences in absorption intensity at wave 3005, 1745, 1550, 1116, and 722 cm^{-1} [10], [14]. Differences in the peak intensity of pork fat and chicken beef fat were also shown at 1550 cm^{-1} of the carbonyl group (C=O) of the triacylglycerol ester [13]. The difference in intensity at 1116 cm^{-1} indicates that the number of saturated acyl groups and oleic acyl groups in lard is higher than in beef and chicken fat [12], [13]. The presence of unsaturated fatty acids in pork is also indicated by the difference in absorption intensity at 3005 cm^{-1} which is a stretching vibration of the C=CH cis absorption band [10], [15]. The stretching vibration at 1745 cm^{-1} indicates the carbonyl group of the fatty acid [8], [10], [11]. The presence of strong absorption at 2924 and 2855 cm^{-1} is characteristic of the absorption of alkyl groups by stretching vibrations of Csp^3-H [10], [11]. The

vibration is strengthened by the presence of absorption at 1459 cm^{-1} which shows the vibration of the C-H bending of the methylene group (-CH_2-) [10], [11].

Based on the difference in the intensity of the absorption peaks, these characteristics indicate that the FTIR spectrum is useful for identifying the presence of unsaturated fatty acids in pork fat which is greater than in beef and chicken fat. This data is useful in the halal authentication process for meat and food [11]. This test can be done in a fast, low-cost and includes a non-destructive detection method [9], [24]. The FTIR can only identify the presence of functional groups in unsaturated fatty acids and cannot be used to determine the type of fatty acid. Therefore, in halal authentication, it is necessary to support supporting data from the results of GC-MS analysis to identify the types of unsaturated fatty acids in pork fat.

3.2. Detection of Fatty Acid Using GC-MS

The GC-MS method is fast and provides high precision and sensitivity for detecting compounds contained in pork [25]. The study results show the fatty acid content presented in Figure 3. Figure 3. A) confirms that pork fat has a higher oleic acid content than beef and chicken fat [10], [22]. The fatty acid profile is in Figure 3. B) can be used as a mapping in halal authentication and ensuring that food products are free from contamination by non-halal ingredients, such as pork. Pork lard contains large amounts of free fatty acids [20]. The free fatty acid content of pork depends on the species [19], [26], pork sample tested [19], [26], and matrix effect [27]. Pork from China has a higher fatty acid content than European and hybrid porks [19]. Bali pork has a higher fatty acid content compared to hybrid pork [26]. Disturbance to the matrix background or analyte effects can arise from the presence of non-polar components dissolved in n-hexane solvent during the fat extraction process.

Generally, pork fat contains 8 types of unsaturated fatty acids and 6 types of saturated fatty acids [20], [28]. Oleic acid (C18:1) is the largest monounsaturated fatty acid contained in pork [22], [26]. The content of oleic acid in pork fat is very characteristic so it is useful in the halal authentication process [22], [28]. The main saturated fatty acid composition in pork is palmitic acid (C16:0), myristic (C14:0), and stearic acid (C18:0) [26], [28]. The detection of fatty acids in pork can be compared to beef and chicken. The content of fatty acids in beef and chicken can be used as a comparison to recognize the fundamental differences in authenticating halal food. Test data can be used as a mapping process to maintain traceability of test results. This mapping pattern can help to recognize the basic differences between pork and beef or chicken. Based on the data in Figure 3. B), the presence of pork can be identified from the content of oleic acid. This mapping can help to distinguish the fatty acid content of pork easily and quickly.

Besides mapping fatty acids, the GC-MS method can also be used to identify the volatile components contained in it. The distinctive aroma of pork comes from short-chain alkanes, alcohols, ethers, aldehydes, ketones, organic sulfur, and nitrogen oxides [19]. Lard contains triglycerides, phosphatidylcholine, and phosphatidylethanolamine [19]. Fat also contains compounds derived from fats such as methylated phosphatidylcholine, sphingomyelin, and acylcarnitine, and carboxylesterase [19]. These compounds can also affect the aroma and taste of pork.

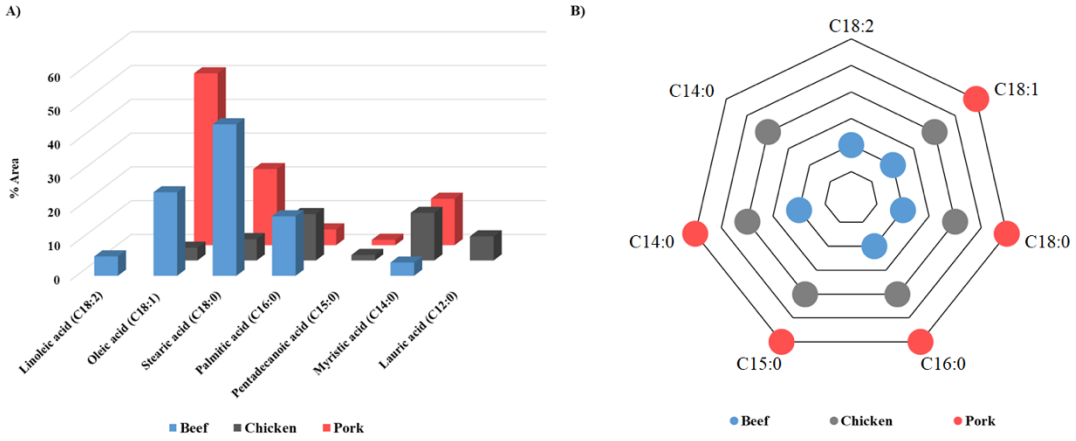

Figure 3. A) Free fatty acid content and B) The free fatty acid mapping on pork, beef, and chicken fat

4. CONCLUSIONS

Based on study, it shows that the FTIR absorption pattern in lard has characteristic peaks at 3005, 1745, 1550, 1116, and 722 cm-1 with higher intensity than beef and chicken fat. The difference in intensity at these characteristic peaks indicates the presence of -C=C- bonds from higher unsaturated fatty acids. The results of analysis with GC-MS showed that the unsaturated fatty acid content in pork fat came from oleic acid. Identification results with FTIR and GC-MS provide unsaturated fatty acid profiles and several fatty acids that can be used as fatty acid profile mapping in halal authentication. This method can continue to be improvised so that a validated procedure is produced in the qualitative and quantitative analysis of pork fat, especially as routine procedures in the halal authentication process for food products.

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