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Review

ATR-FTIR and chemometric method for the detection of pig-based derivatives in food products - A review

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Abstract

The authentication of halal products is important, especially for Muslim communities. Based on the Islamic perspective, pig-based derivatives such as pork, lard, and gelatine are considered haram, which is an Arabic term for "forbidden". Therefore, it is important to develop an analytical method for identifying and quantifying these compounds, which are sometimes found in some food products. The present work thus aimed to ascertain the potential of the attenuated total reflectance Fourier-transform infrared (ATR-FTIR) method for detecting pig-based derivatives in food products. ATR-FTIR spectrophotometry is recommended to be used to identify the presence of pig-based derivatives in some products, particularly processed food. In analytical chemistry, the method is generally used for the identification, characterisation, structure explanation, and monitoring of reactions. This analysis can be performed quickly, economically, easily, and does not require complicated sample preparation. ATR-FTIR can also be combined with principal component analysis (PCA), chemometric method, and multivariate partial least squares (PLS) calibration to accurately evaluate pig-based derivatives in beef meatballs. In combination with chemometric techniques, it can also provide the predictive and descriptive modelling in a combination with chemometric techniques by selecting the optimal frequency region. Furthermore, ATR-FTIR spectroscopy coupled with PLS and PCA chemometric regression models can be a potentially reliable, accurate, and precise method for determining pig-based derivatives in food.

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Introduction

The halal status of products is among the sensitive issues and concerns for Muslims around the world. Many countries with a large Muslim population have been developing these products such as foods, drinks, cosmetics, medicines, and other consumer goods (Siska *et al*., 2020). Nevertheless, it is sometimes difficult to determine the source of the materials since there are many ingredients contained in the products (Siska *et al*., 2020). Pork and its derivatives such as lard, gelatine, and other pig-based products, which are hard to identify with naked eyes, constitute the majority of non-halal ingredients found in the products (Siska *et al*., 2020),

Pig-based derivatives are any substances or compounds related to products derived from pigs (*Sus scrofa*) such as lard, pork, and any gelatine manufactured from their bones and skin. These products are typically less expensive than those obtained from sheep and cows; hence, they are often utilised as substitute components in consumable items (Hassan *et al*., 2018). Lard provides several advantages for the food industry, including the ability to stabilise vegetable oils. It is also commonly used as a substitute for saturated fats in food products to

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obtain a longer shelf life. Furthermore, food products containing lard slightly differ in terms of physical appearances such as colour, texture, taste, and odour. They are considered more flavourful, crispy, and do not become mushy when cooled (Harun, 2019). Lard also helps in the development of emulsions, and provides stability when manufacturing food products (Miklos *et al*., 2011). It can also be combined with other vegetable oils to produce special food oils such as shortening and margarine (Marikkar *et al*., 2002). Economic considerations play a significant role in the inclusion or substitution of pig-based derivates, particularly in meat products. For financial benefit, unethical farmers often mix expensive and cheap meat, such as replacing beef with pork (Ballin, 2010). This has become a problem for Muslims because the status of raw materials used for production may not meet the halal requirements.

Halal and haram for food products can be assessed by utilising the chemical analysis to identify the presence of pig-based derivatives based on their characteristics. The analytical methods for identifying pork-derived ingredients are essentially focused on the qualitative tests (identification) rather than quantitative. This is because the absence of pork-derived ingredients mainly determines a product's halal status. As a result, halal authentication of food products is not an easy task, particularly when non-halal materials are present in small quantities (Harun, 2019).

Several analytical techniques have been developed for detecting proteins/peptides in mixed samples of meat products, and also identifying the origin of raw meats. They include high-performance liquid chromatography, electrophoretic techniques, and enzyme-linked immunosorbent assays (Widyaninggar *et al*., 2012; Azira *et al*., 2014; Raraswati *et al*., 2014; Al-Mofarji *et al*., 2020). Some studies reported that these methods have low sensitivity for evaluating thermally processed foods due to epitope alterations (Rodríguez *et al*., 2005; Andriyani *et al*., 2019). Recently, DNA molecules have been selected as a target compound due to their high stability, as well as their ubiquity in all types of cells. DNA analysis combined with polymerase chain reaction (PCR) offers a fast, responsive, and highly precise method (Mafra *et al*., 2008). Real-time PCR is also suggested as the most frequently used method for quantifying DNA. However, the high cost of the equipment and reagents are drawbacks of this technique in most laboratories (Soares *et al*., 2010). An alternative, which is a promising and suitable method for quick and simple analysis of DNA, is the attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy (Rohman *et al*., 2016).

ATR-FTIR can generate fingerprint spectra from samples, and is suitable for the identification, characterisation, structure explanation, and reaction monitoring in chemical analysis (Rohman *et al*., 2016). Therefore, this method is a promising approach for identifying pig-based derivatives in food products.

The present review focuses on the application of ATR-FTIR spectroscopy coupled with isometric techniques for detecting non-halal content in foods. This combination is considered an ideal, simple, and fast spectroscopic method for halal product authentication. A literature study method was adopted, and the primary data were studies published in international scientific journals between 2015 and 2020. Online-based library search engines such as Google Scholar, NCBI-PubMed, ScienceDirect, ResearchGate, Scopus, and Springer were used to find related publications from April to June 2020 (Table 1). The keywords used were "ATR-FTIR spectroscopy", "authentication halal product using ATR-FTIR", "pork/lard/pig gelatine analysis using ATR-FTIR and chemometrics" and "pig derivatives analysis using ATR-FTIR and chemometrics".

Qualitative and quantitative analyses of pig-based derivatives using ATR-FTIR and chemometrics

The general procedures for analysing the spectral data of ATR-FTIR with the isometric method are shown in Figure 1. A sample of ATR-FTIR spectral data was analysed using available studies or databases before data processing using chemometric procedures to define the range of wave numbers employed for analysing the data, both qualitatively and quantitatively.

Quantitative analysis can be carried out using the partial least squares (PLS) model (Suparman *et al*., 2015; Erwanto *et al*., 2016; Sari and Guntarti, 2018; Utami *et al*., 2018). Linear regression is obtained from the spectral data of calibrated samples, and the values are then evaluated. Furthermore, the essential parameters evaluated such as the coefficient of correlation (R^2) and root mean square error (RMSE) are used for assessing the linear regression model. The R^2 values, which range from 0 to1 describe the variability of linearity in the dataset, and

Table 1. Articles of halal authentication issues.

Halal authentication issue	Reference
The principle of halal products	Siska *et al*. (2020)
The principle of food authentication	Mafra *et al*. (2008)
The principle of spectroscopy and chemometrics in the analysis of pig derivatives for halal authentication	Rohman *et al*. (2016)
Statistic and chemometrics for analytical chemist	Miller and Miller (2010)
The principle of spectroscopy and chemometrics in forensic chemistry	Silva *et al.* (2019)
Lard	Marikkar *et al*. (2002) Nurrulhidayah (2015) Suparman *et al*. (2015) Erwanto *et al*. (2016) Sari and Guntarti (2018) Saputra *et al*. (2018) Utami *et al*. (2018) Harun (2019)
Gelatine	Widyaninggar *et al*. (2012) Azira *et al*. (2014) Raraswati *et al*. (2014) Cebi *et al*. (2016; 2017; 2019) Hassan *et al*. (2018) Zilhadia *et al*. (2018)
Pork	Rodríguez *et al*. (2005) Ballin (2010) Soares *et al*. (2010) Miklos *et al*. (2011) Rohman and Che Man (2012) Guntarti (2018) Sari and Guntarti (2018) Andriyani *et al*. (2019) Rohman (2019) Al-Mofarji *et al*. (2020)

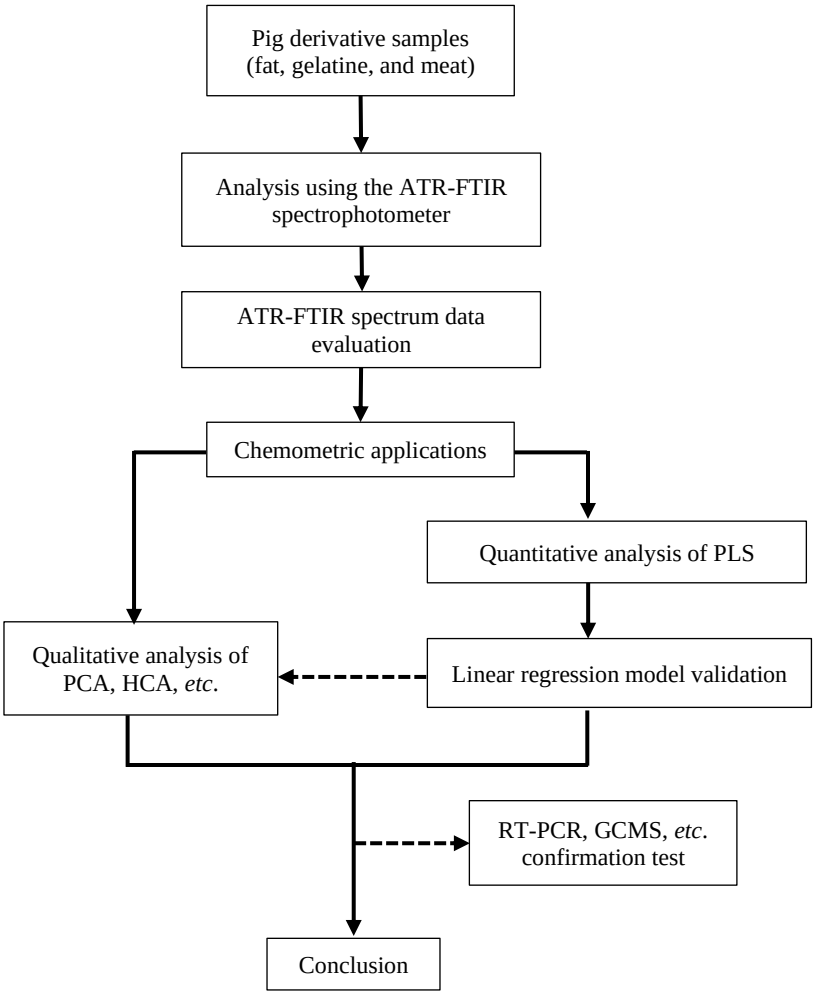

Figure 1. Flowchart illustrating the application of ATR-FTIR analysis method and chemometric in the detection of pig-based derivatives in food products.

the closer it gets to 1, the higher the accuracy of the results. On the other hand, RMSE is used for measuring global errors in the sample, and calculated for calibration. A smaller value of RMSE indicates a higher precision of the model used (Silva *et al*., 2019; Miller and Miller, 2010). It is important to note that quantitative analysis using the PLS model can only be performed on limited and calibrated samples.

The quantitative analysis uses the principal component analysis (PCA) or hierarchical cluster analysis (HCA) model to determine the presence of pig-based derivatives in commercial products (Cebi *et al*., 2016; 2017; 2019; Saputra *et al*., 2018). The range of wave numbers can also be calculated using the results of the PLS model study, and validated as a biomarker wave value. Afterward, the spectral data are analysed, and the data classification for each sample is generated in the form of a score plot and a dendrogram chart for PCA and HCA models, respectively. The PCA model can also provide characteristics/profiles of an object, while the HCA model is used for object classification and comparing traits between different groups.

In some studies, confirmation tests were added to validate the obtained results with the actual before the final stage of analysis using ATR-FTIR and the chemometric method. For pig-based derivatives samples, some studies used RT-PCR (Saputra *et al*., 2018) or GC-MS (Suparman *et al*., 2015) as a tool to confirm the results obtained from the ATR-FTIR and chemometric methods.

Analysis of lard

Nurrulhidayah *et al*. (2015) conducted a quantitative analysis of lard-containing butter using ATR-FTIR along with a chemometric method (Nurrulhidayah *et al*., 2015). Their results showed a difference between non-mixed and lard-containing butter in the wave numbers of 3005 - 725 cm^{-1}. The study used a chemometric technique, which is a

multivariate calibration model of PLS regression for predicting the amount of lard. The model produced the highest regression with R^2 = 0.999, root mean standard error of estimation (RMSEE) of 0.0947, and root mean standard error of prediction (RMSEP) of 0.0687. These results indicated that ATR-FTIR and PLS methods can be applied for the analysis of lard in butter.

Sari and Guntarti (2018) determined and grouped the profiles of wild lard and cow fat in sausage samples using ATR-FTIR instruments combined with PLS and PCA chemometrics (Sari and Guntarti, 2018). For quantitative purposes, the PLS model in wave numbers of 1250 - 900 cm^{-1} was used. The multivariate calibration model produced an R^2 value of 0.998, root mean standard error of calibration (RMSEC) of 1.22%, RMSEP of 0.11%, and root mean standard error of cross-validation (RMSECV) of 2.68%. Furthermore, the PCA model was used to classify the type of fat found in beef and wild boar sausage purchased in the market. The entire samples analysed exhibited no traces of boar meat, which was evidenced by the similar profile to the beef sausage.

Erwanto *et al*. (2016) employed ATR-FTIR combined with PLS and PCA for quantification and classification of lard in cracker "rambak" using a wave number areas of 1200 - 1000 cm^{-1}, (Erwanto *et al*., 2016). A high R^2 value of 0.946 was obtained, thus indicating that the calibration model represented an accuracy of 94.6%. The calibration errors obtained using the RMSEC were at a low value (2.77%); hence, the models were further assessed resulting in an R^2 and RMSEP ratings of 0.997 and 2.77%, respectively. For lard analysis in "rambak" crackers, ATR-FTIR spectroscopy integrated with PLS isometric models produced reliable and precise results with high R^2 values and low errors (RMSEC and RMSEP values).

Saputra *et al*. (2018) combined wave number biomarkers from ATR-FTIR with PCA chemometric techniques to identify pig-based derivatives in food samples (Saputra *et al*., 2018). Three food samples, namely non-halal food A (NHFA), halal food A (HFA), and non-halal food B (NHFB) were collected from the local market for that study. Results showed 16 different absorption bands along with the spectra at wave numbers of 3007 - 965.1 cm^{-1}. The analysis showed that the biomarkers for chicken fat and lard were at wave numbers of 1236 and 3007 cm^{-1}, respectively. The results further showed that NHFA and NHFB shared similarities with the PF (pig fat) wave number, thus indicating that the samples contained lard. On the other hand, HFA was closer to the CF (chicken fat) wave number, thus indicating that the sample might have contained chicken fat.

Utami *et al*. (2018) proved that lard could be identified in imported instant noodle seasoning oil using ATR-FTIR spectrophotometry combined with isometric model PCA and PLS (Utami *et al*., 2018). Calibration samples with two variations of lard and soybean oil were made, and each was analysed using ATR-FTIR at wave numbers of 4000 - 650 cm^{-1}. The ATR-FTIR spectrum produced two typical wave numbers of lard at 1226.73 and 1111 cm^{-1}. The PCA analysis produced a score plot indicating that the pork and soybean oil split from each other into different quadrants. Furthermore, PLS analysis of wave numbers ranging from 2924 to 717 cm^{-1} produced R^2, RMSEP, and RMSEC of 0.992103, 0.0072, and 1998, respectively. The results showed that the sample of imported instant noodles included no seasoning oil containing lard. This indicated that it could be inferred that the FTIR spectroscopy method coupled with PCA and PLS could be utilised both as qualitative and quantitative analyses for identifying lard contaminants in the seasoning oil of imported instant noodle products.

Suparman *et al*. (2015) used halal authentication techniques such as ATR-FTIR, GCMS, spectrophotometry, and isometric tonic on a variety of imported chocolate products (Suparman *et al*., 2015). The ATR-FTIR spectra were analysed using PCA and PLS, and calibration samples were made using a mixture of lard and cocoa oil at various concentrations ranging from 0 - 100% (v/v, %). The PCA and PLS calibration samples were then analysed in the fingerprint area 999.053 - 1190.638 cm^{-1}, which was used for the identification and quantification of lard in cocoa fat. Furthermore, the study of ATR-FTIR spectra and sample calibration of lard and cocoa in the wave number range 4000 - 65 cm^{-1} showed specific variations in the regions 3006.8, 1118.84, and 1097.42 cm^{-1}, respectively. The quantitative calibration sample analysis using the PLS model yielded the equation $y = 1000x - 0.0378$, R^2 of 0.997, and RMSEC of 1.563, with a minimum detection limit of 4% concentration. Based on the lard chromatogram, peaks appeared at a retention time of 38.8 min, and this result was compared with the available data from WILLEY7 library. The compound obtained was considered as eicosadienoic acid, a marker of the presence of lard. Furthermore, a

comprehensive PLS study was carried out on six different import lard containing chocolate from the market. Results showed that the fat-containing lard in the sample ranged between 30.4.6 and 73.5%. Therefore, it was concluded that the ATR-FTIR and GCMS spectroscopy methods could be utilised to detect the presence of lard in chocolate products simply and accurately.

Analysis of pig gelatine

Cebi *et al*. (2016) developed a technique that combined ATR-FTIR and chemometrics for identifying and authenticating gelatine sources in food products (Cebi *et al*., 2016). The samples used in that study were three types of food containing cow, pork, and fish gelatines, with a concentration range of 4 to 20%. Furthermore, Cebi *et al*. (2017) used HCA and three-dimensional PCA chemometric techniques in two wave ranges, namely at 1722 - 1487 cm^{-1} and 1313 - 1124 cm^{-1} (Cebi *et al*., 2017). The HCA technique was used to observe the similarities and differences in each sample from the spectrum results. The three-dimensional PCA technique was utilised to classify each sample based on its gelatine source. From both analyses, the gelatine was successfully classified based on the source, namely cow, pork, and fish. In addition, the results were reinforced with both HCA and three-dimensional PCA chemo techniques for verification.

Cebi *et al*. (2017) also used ATR-FTIR with the chemometric of HCA and PCA models to differentiate and authenticate gelatine sources of food products. Cebi *et al*. (2019) further used ATR-FTIR in combination with three isometric analysis models, including HCA and PCA, for sample classification to determine the gelatine source. The least-squares discriminant partial analysis model (PLS-DA) was used for calibration and cross-validation. In that study, 20 gelatine samples from gummy candies were analysed. The selected wave numbers for chemometric analysis were 1734 and 1528 cm^{-1}. The results were accurate and coherent across all isometric models and test samples. Subsequently, the results of ATR-FTIR spectroscopy were evaluated using the real-time polymerase chain reaction (RT-PCR) technique. A total of 20 commercial gummy candies from different sources were compared. The result was 100% accurate, with no errors or inaccurate estimates, as confirmed by the PCR technique. It showed that the FTIR-ATR method could be used for differentiating and identifying gummy candies based on the source of gelatine.

Zilhadia *et al*. (2018) differentiated cow and pork gelatines from vitamin C-containing gummy products using a combination method of ATR-FTIR and PCA (Zilhadia *et al*., 2018). They used four types of samples, namely cow and pig gelatines extracted from simulated vitamin C gummy, gelatine extracted from commercial products, and gelatine extracted from commercial products. The variable used to score the PCA plot was the number of waves in the specific area of gelatine which amounted to eight, namely 3296.3 cm^{-1} (amide A); 1633.7 cm^{-1} (amide I); 1552.7, 1454.3, 1 408.04, 1338.6 cm^{-1} (amide II); and 1244.09, 698.23 cm^{-1} (amide III). After plotting the results of a 4-quadrant diagram, cow and pig gelatines were in quadrants I and III, respectively. The pig and cow gelatines extracted from vitamin C gummy were in quadrant II and IV, respectively. The gelatine extracted from commercial vitamin C gummy was further observed in the same quadrant as the cow gelatine extracted from the experiment. Therefore, it was suspected that cow gelatine was contained in commercial vitamin C gummy products.

Pork meat analysis

Guntarti (2018) developed ATR-FTIR spectroscopy in combination with the PCA chemometric method and multivariate calibration of PLS calibration for analysing wild boar in beef meatballs and sausages (Guntarti, 2018; Sari and Guntarti, 2018). Furthermore, a PLS chemometric method was used to test pork in beef meatballs quantitatively. The PCA model described that the pork and beef in meatballs were distinctively observed in wave numbers ranging from 1022 to 833 cm^{-1} on each model. From the quantitative analysis using PLS, the linear regression equation $y = 0.9984x + 0.0758$ was obtained with a good R^2 of 0.9984. The RMSE, RMSEC, and RMSECV values were 1.09, 0.04, and 0.48%, respectively. Furthermore, the PCA model successfully identified a sample of 100% pork and 100% beef meatball, as well as four of the five commercial samples represented by P (1, 3, 4, 5). P (2) was unlikely 100% beef or 100% pork meatballs, hence, it might have consisted of a variety of meats (Guntarti, 2018).

Based on extensive literature studies, the capability and improvement can be achieved by integrating ATR-FTIR spectroscopic techniques with

isometrics. Since some scientific laboratories in several major cities in Indonesia are currently equipped with ATR-FTIR spectrometers, establishing the combined technique's capabilities is likely to have a significant effect on its use in halal food authentication.

The employed isometric techniques will differ depending on the study's needs or goals. In terms of unsupervised techniques for qualitative purposes, the combination of PCA and chemometrics is commonly used for pig-based derivative authentication. Consequently, PCA with chemometric is used for halal authentication studies (Rohman and Che Man, 2012). The most frequently used quantitative technique for determining the number of adulterants integrated into food is PLS, and the commonly used parameters in food analysis are R^2 and RMSE.

Based on several studies, ATR-FTIR is capable of classifying pig-based derivatives in the form of proteins and fatty acids. In the future, it will be able to analyse particular proteins such as enzymes, keratin, and DNA (Marikkar *et al*., 2002). The analytical method development of enzymes, which are organic biocatalysts produced by organisms is important. Meanwhile, the pig trypsin enzyme is commonly used in the manufacturing of vaccines. ATR-FTIR will help in authenticating halal products in pharmaceutical preparations, to determine the presence of pig enzymes in the product.

Individual DNA is a unique genomic information material that is contained in every human body part, including keratin (hair, fingernails, *etc*.), saliva, and even body fluids. A successful development of ATR-FTIR and chemometric techniques in analysing DNA will aid in the field of forensics by making it easier for investigators to identify crime scene samples. Despite using separate sample processing methods and isometric evaluation techniques, significant correlations were observed in identifying the peaks of equivalent regions of the ATR-FTIR spectrum.

The analysis of DNA in other organisms, such as bacteria, is possible since ATR-FTIR spectroscopy can analyse proteins and fatty acids in pig-based derivatives. Furthermore, ATR-FTIR techniques and isometric analysis with different model variations can be used in a variety of microbiological applications, including detection, differentiation, quantification, and direct classification of bacterial taxonomic levels of food culture. Microorganism analysis development will also be useful, particularly in the field food safety and quality.

Currently, the widespread adoption of ATR-FTIR spectroscopy and isometric models is still a work in progress. Due to the mathematical context required for model creation, chemist experts continue to use and improve isometric models regularly. This situation is predicted to change in the coming years since educational organisations, specifically in the field of statistics and food product review agencies, form more collaborations.

Finally, it is imperative to mention that the potential of forensic science scenarios, including vibration spectroscopy and isometric analysis, are still being created and gaining popularity. The demand for a quick and simple model is also strongly increasing, particularly for the application of food product authentication.

Conclusion

The presence of pig-based derivatives in food products such as lard, gelatine, and pork meat in processed foods can be detected using ATR-FTIR spectroscopy. This analysis can provide the best predictive and descriptive modelling by selecting and combining the optimal frequency region with isometric techniques. ATR-FTIR spectroscopy combined with the isometric PLS and PCA models was also proven to be accurate and precise in determining pig-based derivatives in food products. Conclusively, the analysis of pig-based derivatives in food products using ATR-FTIR and chemometrics can be performed quickly, economically, easily, and does not require complicated sample preparation.

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