MINI REVIEW

The employment of Fourier transform infrared spectroscopy coupled with chemometrics techniques for traceability and authentication of meat and meat products

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ABSTRACT

Meat-based food such as meatball and sausages are important sources of protein needed for the human body. Due to different prices, some unethical producers try to adulterate high-price meat such as beef with lower priced meat like pork and rat meat to gain economical profits, therefore, reliable and fast analytical techniques should be developed, validated, and applied for meat traceability and authenticity. Some instrumental techniques have been applied for the detection of meat adulteration, mainly relied on DNA and protein using polymerase chain reaction and chromatographic methods, respectively. But, this method is time-consuming, needs a sophisticated instrument, involves complex sample preparation which make the method is not suitable for routine analysis. As a consequence, a simpler method based on spectroscopic principles should be continuously developed. Food samples are sometimes complex which resulted in complex chemical responses. Fortunately, a statistical method called with chemometrics could solve the problems related to complex chemical data. This mini-review highlights the application of Fourier-transform infrared spectroscopy coupled with numerous chemometrics techniques for authenticity and traceability of meat and meat-based products.

Introduction

Meat and meat-based products are taken into account as important sources of protein for the human body and have evolved as an essential diet ingredient because of its appreciated taste and flavor and is being widely consumed around the world [1]. Food and Agricultural Organization, the United Nations reported that meat consumption has significantly increased, globally over time [2]. Due to the difference in prices, unethical producers try to blend expensive meat with lower priced meat, such as substitution of beef with pork to get economical profits. The awareness on authenticity and traceability of meat has recently increased because customers are aware of meat they consumed, therefore, the accurate labeling in meat types and meat-based products is needed in order to allow customers to know what they eat [3].

Meat authentication is a part of meat traceability, consisting of identifying meat components in food products in order to verify the accuracy of labeling and to avoid the economic fraud [4]. Meat authenticity has also been of concerned to meat producers that did not wish that their products are exposed to bias competition by unethical producers who intended to gain economic profits from the inaccuracy labeling of meat products [5]. Several cases have been reported regarding the adulteration of meats, including meat origin [3], the replacement of higher quality meats with lower quality ones [6], substitution of meat muscle proteins with vegetable proteins such as soybean [7], and the presence of unsaid meat species and unsaid ingredients in meat-based food products [8]. The meat adulteration practice can cause several problems, namely as follows: (1) health-related problems such as bovine...
spongiform encephalopathy due to bovine consumption [9], (2) allergic reactions because of the use of certain non-meat ingredients [10], and (3) religion and belief issues due to substitution of halal meat such as beef with non-halal meat like pork, wild boar meat, and dog meat (DM) [11,12]. Therefore, scientists have developed some rapid and reliable analytical techniques to identify the adulteration practices of meat-based products.

Several analytical methods have been validated and developed for authentication and traceability of meat and meat-based food either using physicochemical (spectroscopy, electrophoresis, enzyme-linked immunosorbent assay or ELISA, and chromatography), enzymatic, or biological-based techniques (polymerase chain reaction (PCR)) [13], mainly via analysis of lipid, DNA, or protein present in meat products as reviewed by several authors [5,14,15,16,17]. The selection of these methods depends on several variables, including the quantity of analytes, type of analytes target, part of the meat, condition, and processing of meat [18]. Analytical methods based DNA like PCR and its variants have emerged a method of choice for the authentication of meat; however, these methods are complex and need a sophisticated instrument. In addition to the methods based on DNA could be severely influenced by handling and processing of meat such as storage and extensive cooking resulted in DNA degradation. Aslan et al. [19] reported that meat subjected to cooking at very high temperatures resulted in a low amount of DNA. Thus, simple and rapid methods based on spectroscopic methods are developed by analytical chemists for the meat authentication. Fourier-transform infrared (FTIR) spectroscopy is regarded as an ideal analytical technique for fast screening of meat due to its nature of fingerprint [20], in which there are no meats having the same FTIR spectra.

**Methods**

During performing this review, we used several databases including Scopus, PubMed, and Google Scholar to identify and to download the abstracts, reports, and research papers related to meat authentication using FTIR spectroscopy. The keywords used during searching of information was (meat + adulteration + FTIR spectroscopy + chemometrics) and (meat + authentication + FTIR spectroscopy) in the month of June–August 2018.

**FTIR spectroscopy**

Infrared (IR) spectroscopy is an interaction between electromagnetic radiation in the infrared region by investigating the phenomena of scattering, reflection, absorption, or transmission of IR radiation occurring during interaction [21]. The frequencies, wavelengths, or wavenumbers at which samples absorb IR radiation (x-axis) and their corresponding intensities (either transmittance or absorbance) (y-axis) are recorded into IR spectrum [22]. For the sake of analytical purposes, the region of IR is typically divided into three regions, namely, far IR corresponding to wavenumbers (1/λ) of 400–10 cm⁻¹, mid-IR region corresponding to 1/λ 4,000–400 cm⁻¹, and near IR at1/λ 14,285–4,000 cm⁻¹ [23]. However, the differences among these regions vary depending on the instrumentation types applied to measure IR spectra and also depending on the radiation properties [24]. IR spectrum is generally reconsidered as one of the characteristic properties of samples, including meat [25].

There are two types of instruments, namely, dispersive and FTIR instruments. Dispersive instrument has scarcely used in food analyses due to the difficulties in sample handling technique and is not combined with proper spectral scanning and spectral processing in order to give valuable information for quantitative analysis. Consequently, over the last three decades, FTIR spectroscopy has replaced dispersive IR spectroscopy and has appeared to become an emerging technique for confirmation, identification, and quantitative analysis [26]. Instrumentation of FTIR spectrophotometer is based on interferometry, and the most common one is Michelson interferometer. Therefore, FTIR spectrophotometer was fundamentally different from traditional dispersive IR spectroscopy. The Michelson interferometer is employed in most FTIR spectrometers. An interferometer is composed of two perpendicular mirrors, namely, stationary mirror and moving mirror which travels at a constant velocity. Beam splitter, typically made from KBr coated with germanium (Ge), was placed between two mirrors [27]. Beam splitter will divide beam radiation from IR sources into stationary and moving mirrors. The The infrared radiation beams reflected back will recombine at the beam splitter, producing a constructive/destructive interference patterns due to the varying difference between the distance traveled by two components of the beam, and part of the recombined beam subsequently reach to the detector [28].

Compared with dispersive instruments, FTIR spectrophotometers are more preferred for analysis of samples when increased sensitivity is desired [29]. FTIR spectrophotometers have main advantages over dispersive spectrophotometer due to its capability to provide higher speed and sensitivity (Felgett advantage) and increased optical throughput (Jaqinot advantage). In addition, FTIR spectrophotometer instrument enables all frequencies and they are measured simultaneously (the multiplexing advantage), therefore, the entire FTIR spectra of a sample can be collected in a single one-second scan. FTIR spectrophotometers also offer the increased signal-to-noise ratio (S/N) of IR spectrum increases. Another factor which contributes to the success of FTIR spectroscopy
as the powerful analytical method is the capability to be connected to software packages including chemometrics providing a wide variety of data handling systems which facilitate the spectral acquisition and interpretation [30].

**Chemometrics**

The FTIR spectra obtained during meat analysis are commonly complex and difficult to interpret using naked eye, fortunately, some statistical software on chemometrics are available now. Currently, chemometrics has emerged as effective tools for analytical purposes, either qualitative or quantitative [31]. Due to the development of chemometrics software, the chemometrics techniques have been widely used in several fields of chemical analyses including meat authentication analysis based on chemical responses generated with analytical instruments [32,33].

The term chemometrics can be explained as an application of mathematics and statistics in chemical data treatments. Chemometrics was firstly introduced in 1972 by Swante Wold, a scientist from Swedenia, and Bruce R. Kowalski, a scientist from the United States. Chemometrics is intended to: (1) design the procedure for optimal measurement of the assay and (2) collect as much as chemical information by analyzing the data [34]. Chemometrics is an interdisciplinary method involving multivariate statistics, mathematical modeling, computer science, and analytical chemistry. One of the advantages of chemometrics is its ability in the analysis of multivariate data. Multivariate data are data resulted from the measurement of several variables in the same samples [35].

Chemometrics techniques typically intended for making classification among objects studied assisted with either unsupervised or supervised pattern recognition and for assisting quantitative analysis using multivariate regression techniques. Classification and discrimination using pattern recognition techniques is one of the most publicized success stories in chemometrics [36]. The pattern recognition technique is typically grouped into two categories, namely, supervised pattern recognition and unsupervised recognition. Multivariate calibration allows the analyst to analyze one or multiple analytes in a large sample. Multivariate regression builds calibration model using a training dataset with a known concentration of interest. The calibration model is used for predicting the levels of unknown samples. The calibration models have to be evaluated using an appropriate validation dataset before carrying out the analysis of unknown samples [35].

The common chemometrics techniques used in vibrational spectroscopy including mid-IR spectroscopy are: (a) FTIR spectral data treatment intended to increase the quality of FTIR spectra by minimizing the undesired effect based on the mathematical equations and data transformations such as normalizations, derivatization, Savitzky–Golay smoothing, standard normal variate, baseline corrections, and multiplicative corrections; (b) the experiments design which include randomization, factorial design, and response surface methodology; (3) discrimination and classification among objects such as discriminant analysis (DA), partial least square-discriminant analysis (PLS-DA), principal component analysis (PCA), orthogonal projections to latent structures-DA, cluster analysis; and (4) multivariate calibrations such as classical linear regression, multiple linear regression, PCR, and PLS regression [29,35].

The steps of analytical procedures which involved FTIR spectroscopy and chemometrics techniques in meat authentication can be briefly described as (a) definition of authentication problems, (b) sampling process by taking authentic and adulterated meat products, (c) application of FTIR spectrophotometer to measure evaluated samples, (d) evaluation of FTIR spectral data, (e) selection of suitable chemometrics techniques, (f) pre-processing FTIR spectral data, if necessary, (g) choosing training and test sample sets, (h) optimization of models, either in training, validation or test samples, (i) selection of variables (absorbance values at selected wavenumbers), validation of developed model using selected variables, and (j) drawing of conclusion [37].

**Authentication of meat using FTIR spectroscopy**

Generally, the first step of meat authentication using FTIR spectroscopy coupled with multivariate analysis (chemometrics) is the extraction of meat products and meat using different extraction techniques, as shown in Fig. 1. The extracted lipids were further measured using FTIR instruments. FTIR spectra are frequently subjected to numerous spectral treatments including derivatization, smoothing, mean centering, standard normal variate, etc in order to facilitate the best modeling during chemometrics analysis. The optimized spectra and chemometrics models were finally used for the authentication analysis of commercial samples.

Table 1 compiled the application of FTIR spectroscopy coupled with certain chemometrics techniques for authentication and traceability of meat and meat-based products (meatballs and sausages). FTIR spectroscopy in combination with multivariate of PCA and PLS-DA along with the concentrations of certain elements (ash, protein, sodium, chloride, and phosphate) has been used for the authentication of bovine meat (BM) from the addition of non-meat ingredient in natura [38]. This adulteration practice included the injection of non-meat solutions (phosphates, NaCl, carrageenan, and maltodextrin) in beef intended to increase the capacity of water holding. PCA using FTIR spectra at combined wavenumbers region of 3,700–2,400 and 1,800–650 cm\(^{-1}\) was used for the classification of BM and BM added with the non-meat ingredient.
PCA results revealed that separation between adulterated and non-adulterated was more clear using variables of concentrations of ash, protein, sodium, chloride, and phosphate than using FTIR spectra. While, PLS-DA using absorbance values at combined wavenumbers of 3,700–2,400 and 1,800–650 cm\(^{-1}\), previously preprocessed by Savitzky-Golay smoothing, mean standard centering, and class centroid centering, was capable of separating BM and BM adulterated with non-meat ingredients.

Halal meat authentication has emerged an interesting issues, especially in Muslim countries, therefore, analytical methods capable of detecting the presence of non-halal meat such as pork, DM, donkey meat, wild boar meat (WBM), and rat meat (RM) in meat-based food products such as sausages and meatball based on FTIR spectra have been developed [16]. Rohman et al. [12] have developed FTIR spectroscopy coupled with multivariate calibration of partial least square regression (PLSR) for predicting the

**Figure 1.** The sketch of application of FTIR spectroscopy in combination with chemometrics for authentication of meat and meat products.
Table 1. Authentication analysis of meat and meat-based products using FTIR spectroscopy and chemometrics. PCR = polymerase chain reaction; PLSR = partial least square regression; PCA = principal component analysis; DA = Discriminant analysis; PLS-DA = partial least square-discriminant analysis; SIMCA = soft independent modelling class analogy; HCA = hierarchal cluster analysis; ANN = artificial neural network; $R^2$ = coefficient of determination; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction; RMSECV = root mean square error of cross validation.

| Meat adulterant | Meat adulterated | Meat-based products | Chemometrics | Wavenumbers (cm$^{-1}$) | Results | References |
|-----------------|------------------|---------------------|--------------|--------------------------|---------|------------|
| Pork            | Beef             | Beef jerks          | LDA          | Whole mid IR region (4,000–650) | LDA model could classify and predict the adulteration of Beef jerks with pork, allowing 100% accuracy of the sample tested. | [39] |
| Pork offal (PO) | Beef offal (BO)  | Fresh meat          | SIMCA, LDA   | 1,002–1,240, 1,700–1,714, and 1,764–1,795 (BO) and 1,105–1,182 (PO). | SIMCA with mean-centered data could provide best model for the identification of BO, while LDA using non-scaled spectra offered best performance in classifying of PO | [40] |
| Pork            | Beef             | The mixture of beef-pork | PLS-Kernel calibration | Absorbance ratios of $A_{1,654} / A_{1,400}$, $A_{1,745} / A_{1,400}$, and $A_{1,450} / A_{1,745}$ and $A_{1,395} / A_{1,175}$ | PLS-kernel calibration could predict the levels of pork in the mixture of pork-beef | [41] |
| Pork            | Minced beef      | Pork-beef fillet    | PLSR         | 3,200–800 cm$^{-1}$ | PLSR could predict the levels of pork with RMSEC of 4.88%, RMSEP of 9.45% and RMSECV of 10.30%. | [42] |
| Pork            | Beef             | Ham sausages        | PLSDA        | Whole mid IR region (4,000–650) | PLSDA with standard normal variate treatment could classify halal (beef) sausage with sensitivity and specificity of 0.913 and 0.929. | [43] |
| Pork            | Beef             | Beef Meatballs      | PLSR         | 1,200–1,000 cm$^{-1}$ | PLSR could predict pork in beef meatballs with $R^2$ for the correlation between actual value of pork and FTIR predicted values of 0.999 (RMSEC of 0.442%, RMSEP of 0.742%). Using PLSR, the correlation between of actual value and predicted value yielded $R^2$ of 0.9975. PCA could classify beef and pork meatballs through analysis of meatball broth | [12] |
| Pork            | Beef             | Meatball broth      | PLSR (quantitative) PCA (classification) | PLSR (1,128–1,018 cm$^{-1}$) PCA (1,200–1,000 cm$^{-1}$) | Using PLS, the correlation between of actual value and predicted value yielded $R^2$ of 0.9975. PCA could classify beef and pork meatballs through analysis of meatball broth | [44] |
| Pork            | Camel            | Pork-camel mixture  | Ordinary least square | Absorbance ratios of $A_{1,654} / A_{1,294}$ | FTIR spectroscopy-ordinary least square could predict pork levels with $R^2$ of 0.942 FTIR spectroscopy-ordinary least square could predict pork levels in buffalo with $R^2$ of 0.918. | [45] |
| Pork            | Buffalo          | Pork-Buffalo mixture | Ordinary least square | Absorbance ratios of $A_{1,540} / A_{1,294}$ | FTIR spectroscopy-ordinary least square could predict pork levels in buffalo with $R^2$ of 0.918. | [45] |
| Pork            | Mutton and beef  | The mixture of pork with mutton and beef | PLS-DA and support vector machine (SVM) | 4,000–650 cm$^{-1}$ | PLS-DA provided better classification method than SVM | [46] |
| WBM             | Beef             | Beef meatballs      | PLSR and PCA  | 1,250–1,000 cm$^{-1}$ | Equation obtained was $y = 0.9794x+1.4658$ ($R^2$ of 0.988 and RMSEC of 2.0%. PCA was successfully used for classification of WBM meatball and BM meatball | [47] |
levels of pork as an adulterant in BM. Quantitative analysis of pork in BM meatballs was performed using FTIR spectral absorbances at optimized wavenumbers (1/λ), i.e., 1,200–1,000 cm⁻¹, using four factors as a combination of original absorbance values. The linear regression describing the correlation between real values of pork (x-axis) and FTIR predicted values (y-axis) was:

\[ y = 0.999x + 0.004 \]  

\( R^2 \) of 0.999, root mean square error of calibration (RMSEC) of 0.442% and root mean square error of prediction (RMSEP) of 0.742%. Kurniawati et al. [50] have also have also analyzed adulteration practice of pork in BM meatballs through analysis of meatballs broth. Liquid-liquid extraction of lipid components presents in a broth of BM meatballs adulterated with pork was conducted using hexane as solvent. The lipids containing pork fat (lard) extracted were analyzed using FTIR spectrophotometer. Using PLSR, FTIR spectra at wavenumbers of 11,284–1,018 were selected for the quantification of the real value of lard (x-axis) and predicted values (y-axis) with \( R^2 \) and RMSEC values of 0.9975 and 1.34%. Furthermore, PCA at 1/λ 1,200–1,000 cm⁻¹ could classify BM meatballs and pork meatballs through analysis of meatball broth without any misclassification. FTIR spectroscopy combined with PLSR and PCA was used for the analysis of WBM in BM meatballs [47]. The correlation between real value of WBR (x-axis) and FTIR predicted value (y-axis) at 1,250–1,000 cm⁻¹ was:

\[ y = 0.9749x + 1.4658 \]  

\( R^2 \) of 0.988 and RMSEC of 2.0%). PCA was also fruitfully applied for the classification of WBM meatball and BM meatball.

DM is also non-halal meats and its analysis as meat adulterant is very important. Rahayu et al. [48] have analyzed DM in BM meatballs using FTIR spectroscopy. Lipids contained in meatballs were extracted using Folch method and were subjected to scanning with FTIR spectrophotometer at 1/λ 4,000–650 cm⁻¹. PLSR was employed for quantitative analysis of DM in meatball using combined 1/λ regions of 1,782–1,623 and 1,485–659 cm⁻¹. The results revealed that spectral detrending treatment offered an optimum prediction of DM in BM meatballs. The \( R^2 \) values for the relationship between the real values (x-axis) and FTIR predicted values (y-axis) of DM were 0.993 (calibration model) and 0.995 (validation model), respectively. The values of RMSEC and root mean square error of cross-validation (RMSECV) were 1.63% and 2.68%, respectively. Arini et al. [13] also compared two extraction methods, namely, Bligh–Dyer and Folch for lipid extraction contained in BM meatballs adulterated with DM. FTIR spectroscopy in conjunction with PLSR and PCA was exploited for quantitative analysis and classification of DM in BM meatballs, respectively. PCA using absorbances at wavenumber regions of 1,700–700 cm⁻¹ could successfully classify DM in BM meatball. These wavenumbers were also used for quantifying DM in BM meatball using PLSR. With the Folch method, the values of \( R^2 \) and RMSEC obtained were 0.9906% and 1.80%, respectively, while with Bligh–Dyer extraction method, \( R^2 \) of 0.9860 and RMSEC of 2.01% were achieved. Therefore, Folch extraction offered a better prediction model during analysis of DM in BM meatball than Bligh–Dyer.

Another non-halal meat commonly used as meat adulterants in BM is RM. FTIR spectroscopy was also employed for the authentication of BM from RM in a beef meatball. FTIR spectra at selected fingerprint 1/λ of 1,000–750 cm⁻¹ was exploited for the classification between BM meatball and RM meatballs aided with PCA and for quantification aided with PLS regression. PCA could successfully classify BM meatballs and RM meatballs, while PLSR can model the correlation between real values of RM (x-axis) and predicted values using FTIR spectra (y-axis) with the equation of:

\[ y = 0.9417x + 2.8410 \]  

\( R^2 \) of 0.993, RMSEC of 1.79% [49]. RM in beef-based sausage products has been analyzed using FTIR spectroscopy in combination with PLSR and PCA using three different extraction methods (Bligh and Dyer, Folch, and Soxhlet).
The absorbance values at 750–1,800 cm⁻¹ were selected during PCA for the classification between RM and beef sausages. The score plots PCA as expressed with the PC1 or first principle component and PC2 or second principle component revealed good classification between lipids extracted from RM meatballs and those extracted from BM meatballs. The variance percentages of PC1 and PC2 were 97.57% and 1.28%, 85.50%, and 10.64%, as well as 97.86% and 2.02%, of Bligh and Dyer, Folch, and Soxhlet, respectively. The absorbances at 750–1,800 cm⁻¹ were also used for quantification of RM meatballs using PLSR with R² and RMSEC values of 0.945 and 2.73%; 0.991 and 1.73%; 0.992 and 1.69%, using Bligh and Dyer, Folch, and Soxhlet methods, respectively. The validation models yielded R² and RMSEP values for the correlation between real values of RM and FTIR predicted value were 0.458% and 18.90%, and 0.983% and 4.21%, using Bligh and Dyer, Folch, and Soxhlet methods, respectively [51]. PLSR and PCA using absorbance values at 1/\lambda 1,600–750 cm⁻¹ have also been reported for quantification and classification of RM as an adulterant in BM meatballs in Indonesia with acceptable R² (0.994), RMSEC of 1.63%, RMSECV of 1.70%, and RMSEP of 2.60% [52].

FTIR spectroscopy in combination with PLS-DA using spectral treatment of support vector machine (SVM) has been developed and validated for the authentication of mutton and beef from pork. The presence of outlier spectra data was removed based on Mahalanobis distance among FTIR spectral data using absorbance values as variables. FTIR spectral interferences were minimized with spectral treatments including standard normal variate, Savitzky–Golay smoothing, multiple scatter correction, and spectral normalization. PLS-DA and SVM were employed to develop calibration and validation models. After removing the spectral outlier, the performance of PLS-DA for classification models between mutton, beef and that adulterated with pork was enhanced, as indicated by the increased value of R² from 0.93 to 0.99 and decreased values of RMSEC and RMSECV from 0.17 to 0.09 and 0.21 to 0.11, respectively. Using PLS-DA, the R² values obtained were 0.99 either in calibration or validation/prediction sets, with a RMSEC value of 0.06. While using SVM, the classification models were worse than using PLS-DA with R², R² prediction, RMSEC, RMSECV, and RMSEP were of 0.97, 0.96, 0.15, 0.17, and 0.24, respectively. Therefore, it can be concluded that FTIR spectroscopy coupled with PLS-DA provided better classification method than SVM method, which can be used as effective tools to identify beef, mutton and that adulterated with pork [46].

Meat may be subjected to a certain condition (freezing and thawing) which affects the quality and sensory of meat. FTIR spectroscopy in combination with hierarchical cluster analysis (HCA) with the artificial neural network has been developed for the classification between thawed and freezed chicken meat (CM). FTIR spectra of CM which were stored at 4°C and CM which were stored and frozen at −20°C for different days have been analyzed in mid-IR region (4,000–650 cm⁻¹). FTIR spectra at wavenumbers 1,660–1,628 cm⁻¹ combined with HCA could distinguish fresh and frozen CM samples. The frozen samples stored on 15, 30, 75, and 85 days could be clearly discriminated. In addition, FTIR spectra FTIR spectra at wavenumbers 1,660–1,628 cm⁻¹ combined were capable of identification fresh versus frozen meat, even frozen shortly. The results of internal validation revealed that 20 out of 21 samples were accurately grouped either in fresh or in frozen CM [53].

Currently, FTIR spectroscopy coupled with PLS-DA has been employed for authentication of wild fallow deer meat (WFDM) with domestic goat meat (DGM) in different time storage of samples [54]. Using variables of absorbance at combined wavenumbers region of 1,138–1,180, 1,314–1,477, 1,535–1,556, and 1,728–1,759 cm⁻¹, PLS-DA could classify between WFDM and WFDM adulterated with DGM.

### Conclusion

Due to its property of fingerprint spectra, FTIR spectroscopy coupled with chemometrics of classification and multivariate calibration is a powerful analytical technique for authentication analysis of high-priced meat like beef with low-priced meat such as pork, RM, and dog meat. FTIR spectroscopy-chemometrics offered a rapid screening of adulteration practice of meat. Indeed, some confirmation methods like PCR should be used to confirm the presence of meat adulterant.

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### Conflict of Interest

There is no conflict of interest to declare.

### Author Contribution

Abdul Rohman drafted the manuscript, reviewed the manuscript, approved, and submitted the final review.

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