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Original Article

RAPID DETECTION OF RAT MEAT ADULTERATION IN BEEF SAUSAGES USING FTIR-ATR SPECTROSCOPY AND CHEMOMETRICS FOR HALAL AUTHENTICATION

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ABSTRACT

Objective: The objective of this study was to employ Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopy in combination with chemometrics for the analysis of rat meat adulteration in beef sausages.

Methods: Lipid components in sausages were extracted using three extraction methods, namely Bligh and Dyer, Folch, and Soxhlet methods. The lipid components extracted were then analysed using FTIR-ATR spectroscopy , and their spectra obtained were used as variables during chemometrics modeling. Samples were prepared by mixing beef with adulterant of rat meat in the concentration range of 0-100% of rat meat. Each sample was scanned using FTIR-Attenuated Total Reflectance (ATR) spectroscopy in three replicates at 4000-650 cm^{-1} wavenumber region.

Results: The absorbance values at wavenumbers regions of 3100-700 cm^{-1} were used to discriminate lipid components extracted by the Bligh Dyer, Folch, and Soxhlet Method with an accuracy level of 100%. The prediction of rat sausages was successfully determined using multivariate calibrations of Partial Least Square (PLS) and Principle Component Regression (PCR) using optimised conditions.

Conclusion: FTIR-ATR spectroscopy coupled with chemometrics is a rapid and accurate method for detecting and quantifying rat meat in beef sausages for halal authentication.

Keywords: Halal authentication, Beef sausage, Rat sausage, FTIR spectroscopy, Chemometrics

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INTRODUCTION

The problem of halal food is an issue that is often controversial in the community. Adulteration in food products, such as adding or mixing unknown non-halal ingredients, has become a practice that never ceases [1]. Food authenticity has become a primary concern for both food industries and consumers because of halal issues, economic reasons, and assurance of definite quality and safety of the food [2, 3]. Some producers try to profit by adulterating halal meat, such as beef, with non-halal meat, such as canine, pork, and rat meat [4]. In the Indonesian market, one of the popular meat products is beef sausages, and this product is frequently adulterated with rat meat because this product is sold without a registration code and halal certificate. Rat meat can be obtained from local farmers, so rat meat is the best adulterant in beef sausages [5, 6].

Several analytical methods were reported to analysis meat product adulterations based on detecting fat, protein, or DNA biomarkers. Fat detection is a widely used method [7, 8]. The following are examples of ways that have been successfully investigated by using Polymerase Chain Reaction (PCR) [9], Real-time PCR using specific-species primer [10], multiplex PCR [11]. These methods, however, could not be applied in highly processed meat products due to DNA and protein degradation, resulting false negative result. Detection of amino acid using High Perform Liquid Chromatography (HPLC) [12], Liquid Chromatography-Mass Spectroscopy (LC-MC) [13], and detection based on fatty acid composition using Gas Chromatography-Mass Spectroscopy have also been applied [14]. However, sometimes it fails to detect adulteration just by using fatty acid and amino acid profiles in highly processed food products. Food adulteration methods have become more complex and sophisticated, requiring the development of sensitive and rapid methods rather than expensive and time-consuming techniques [15]. Therefore, it is necessary to develop analytical methods capable of detecting lipids in samples because lipids are more stable than DNA and protein in highly processed food products.

Vibration spectroscopy FTIR offers several advantages for food analysis, especially authentication oils and fats, such as reliable, fast, and simple sample analysis. FTIR was used for oils and fats analysis because it can measure the whole part of oils and fats, not only specific compounds, providing a whole pattern of oils and fats, which is beneficial for authentication [16]. FTIR spectroscopy is a fingerprint method where the sample can be directly placed on an ATR crystal without sample preparation steps, and it is less expensive. The Infrared region used FTIR, namely the mid-infrared region (4000-650 cm^{-1}) [17].

Chemometrics is the use of statistical and mathematical tools in the management of chemical problems of various kinds is owing to the simplicity with which large quantities of data can be handled and sophisticated calculations performed with calculators and computers [18]. Chemometrics can make complex data from FTIR measurements more interpretable and intelligible. Halal authentication can be determined by FTIR-based fingerprinting combined with two chemometric techniques, pattern recognition and multivariate calibration [19]. Fourier Transform Infrared (FTIR) spectroscopy is an outstanding tool for qualitative and quantitative analysis when combined with multivariate data analysis or chemometrics [20]. FTIR-ATR spectroscopy in combination with chemometrics of pattern recognition discriminant analysis (DA) and multivariate calibration partial least square (PLS) and principal component regression (PCR) has been used for authentication of beef meatball from rat meat using lipid components from extraction Bligh Dyer, Folch, and Soxhlet methods, and comparing which one the most simple and easiest for extraction lipid methods [21]. The combination of FTIR spectra-chemometrics is reported to authenticate beef sausage from pork [22], Lard [23], dog meat [24]

and rat meat [6]. FTIR-ATR spectroscopy combination with chemometrics for adulteration of rat meat in beef sausages using acid hydrolysis before extraction with three different techniques has never been reported to find non-halal biomarkers for halal authentication.

MATERIALS AND METHODS

Materials

Sample rat meat (*Rattus norvegicus*) was obtained from Godean, Sleman, Yogyakarta. Beef meat, spices, and other sausages additives were obtained from Colombo Market, Kaliurang, Yogyakarta. The entire samples were stored at-18 °C before being used to make reference sausages. HCl (Merck), dichloromethane (Merck), methanol (Merck), petroleum ether (Merck), anhydrous Na_2SO4 (Merck), distilled water, Whatman filter paper, electric stove, analytical scale, vortex, oven, centrifuge, vacuum rotary evaporator, separatory funnel, soxhlet tools and glassware. The reagents and solvents used were of pro-analytical grade.

Preparation of sausages

The sausages reference was prepared by emulsifying 90% of fine ground meat (beef and or rat) with 10% tapioca flour and mixing it with garlic, pepper, and salt. The content of rat meat in the beef sausages was varied from 0, 10, 20, 30, 40, 50, 75, and 100%. The meat and other ingredients were transferred into sausage casings before being boiled in water for 15 min. Before the analysis, the sausages were minced using a commercial blender.

Preparation of calibration and validation samples

Adulteration of beef sausages with rat meat using FTIR-ATR spectroscopy for calibration and validation standards was prepared by reference sausages. The lipid from the sausages was observed in the difference in lipid spectra in triplicate from three different lipid extraction methods the Bligh Dyer, Folch, and Soxhlet.

Acid hydrolysis

Each twenty-gram sample of rat meat in the beef sausages was hydrolyzed using 1 N hydrochloric acid and then the samples were filtered using Whatman filter paper.

Extraction of lipid components using bligh dyer, folch and soxhlet methods

Lipids were obtained using three extraction methods: the Bligh Dyer, the Folch, and the Soxhlet, with slight modifications [25, 26]. The steps in the extraction procedure follow the extraction method in the research that the previous author has done [21].

FTIR-ATR spectroscopy analysis

FTIR spectra acquisition was performed using FTIR-ATR spectroscopy (Thermo Scientific Nicolet iS20). The sampling technique used was attenuated total reflectance (ATR) with deuterated triglycine sulfate (DTGS) detector and diamond crystals (as a sampling accessory). FTIR spectra were scanned in the wavenumber region of 4000–650 cm^{-1} (mid-region) with a resolution of 8 cm-1 and a number scanning of 64. A background spectrum was obtained against the air spectrum as a reference for each sample. FTIR spectra were recorded as absorbance values at each data point in triplicate with OMNIC® software. The chemometric multivariate calibration created the correlation spectra IR and absorbance data using the TQ analyst.

Linear discriminant analysis (LDA)

LDA is a supervised data classification technique that determines linear discriminant functions by maximising and minimising variance between classes. After obtaining a classification model, it is possible to predict if unknown items belong to one of the defined classes [26]. LDA was used for discrimination between rat meat sausages, beef sausages, and the mixture of rat and beef sausages (10–75%). The Coomans plot is built to discriminate between beef and rat meat.

Chemometrics analysis

FTIR lipid spectra were obtained from three extraction methods and subjected to chemometrics analysis of LDA and multivariate calibrations (PLS and PCR) using the software TQ Analyst.

Thermo Scientific TQ Analyst is a chemometric software package that is very flexible and extremely easy to use. Primary chemometric algorithms are divided into Quantitative and Qualitative analysis [27]. LDA assessed discrimination between authentic and adulterated beef sausages by accuracy levels. The PLS and PCR calibration model's precision for quantifying rat meat in sausages was evaluated using the coefficient of determination. In contrast, the precision during calibration and validation models were evaluated using root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), and coefficient of determination (R^2) for accuracy evaluation between actual values and predicted values [28].

RESULTS AND DISCUSSION

Sausage samples were made in several concentrations, namely 0% (100% beef), 10% (mix 90% beef; 10% rat meat), 20% (mix 80% beef; 20% rat meat), 30% (mix 70% beef; 30% rat meat), 40% (mix 60% beef; 40% rat meat), 50% (mix 50% beef; 50% rat meat), 75% (mix 25% beef; 75% rat meat), and 100% rat meat.

Extraction of lipid components using three different extraction methods used in this study shows the Soxhlet method was the simplest, easiest, and used less solvent compared to the Bligh-Dyer and the Folch methods [29-31]. The Soxhlet method produced brown-yellowish oil, while the Bligh-Dyer and the Folch methods produced brown oil. The amount of the lipid produced using Soxhlet was higher than that in Bligh-Dyer and Folch methods due to the high ratio of solvent to sample and differences in triacylglycerol (TAG) fat content. This different fat content is influenced by the extraction method, the part of the animal used, the origin of the animal, and the animal feed [21].

FTIR spectroscopy is a fast method of identifying samples accurately, providing spectra of certain absorption bands associated with functional groups, and reliable [32]. No samples have identical spectra because each is composed of different compounds. Therefore, FTIR spectroscopy is a fingerprint technique [16]. FTIR spectroscopy can be an ideal technique for analyzing fats and oils because they are essentially single-component triglycerides (TGs) systems and can be applied directly in their neat form to ATR crystal or passed through a flow cell [33]. Lipids obtained from the extraction of rats or beef are analyzed using FTIR-ATR spectroscopy in the mid-infrared region (4000–650 cm^{-1}). Each band/peak and shoulder are characteristics of FTIR spectra of triglyceride (TG) because the main components of edible fats are triglyceride [33, 34]. This study uses reference sausage to develop predictive models using FTIR to categorize beef sausages and rat sausages or a mixture of beef and rat sausages [21].

This study uses reference sausages to develop predictive models using FTIR to categorize beef sausages and rat sausages or the mixture of beef and rat sausages. The representative spectra of lipids obtained from the extraction of beef and rat sausages at a concentration of 100% using the Bligh and Dyer, Folch, and Soxhlet methods are demonstrated in fig. 1.

General investigation through our naked eyes showed that FTIR spectra were very similar. However, a deep study of FTIR spectra of beef sausages and rat sausages found some differences, especially in the fingerprint area [16].

Table 1 compiled the functional groups of each peaks and shoulders responsible for infrared absorption in fig. 1. The assignment of prominent peaks and illustrates that the peaks at about 3001–3007 cm^{-1} are due to the C-H strain vibration at = C-H cis. The–CH_2 functional group peaks at 2920–2922 cm^{-1} and 2851–2852 cm^{-1}, respectively, due to asymmetric and symmetrical vibrations. A peak indicates the carbonyl group (C = O) of the triglyceride ester at 1743–1744 cm^{-1}. Due to their bending vibrations, methylene and methyl groups can also be observed in the 1461–1462 cm-1 and

1376–1377 cm^{-1} regions [38]. The absorption of carbonyl (C = O) ester bonds was observed at a frequency of 1743 cm^{-1} with strong intensity due to the large difference in electronegativity of carbon and hydrogen atoms. The bands at 1235, 1158, 1160, and 722 cm^{-1} result from overlapping methylene shake vibrations and out-of-plane bending vibrations of cis-substituted olefins [35].

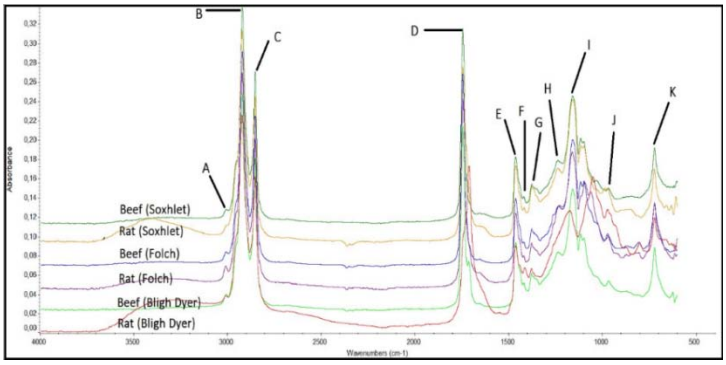

Fig. 1: FTIR spectra of the lipid extracted from beef sausages (Cow) and rat sausages (Rat) at concentration 100%

Table 1: The functional groups and modes of vibration of lipids extracted from 100% rat sausages and 100% beef sausages (explanation from fig. 1) [35-37]

Assignment	Wavenumber (cm^{-1})	Functional group	Intensity
A	3001-3007	Cis C=CH stretching	Medium
B	2920-2922	C-HCH stretching vibration	Very strong
C	2851-2852	C-HCH stretching vibration	Very strong
D	1743-1744	Carbonyl C=O ester	Very strong
E	1461-1462	C-CH_2 scissoring bending	Medium
F	1376-1377	C-CH3 scissoring bending	Medium
G	1173-1235	C-C Alkane	Medium
H	1158-1160	-CH in plane	Medium
I	1048-1198	C-O from ester	Medium
J	965-970	CH=CH (trans)	Medium
K	720-722	-CH=CH-bending (out of plane)	Medium

Linear Discriminant Analysis (LDA) was used to classify beef sausages, rat sausages, and a mixture of beef-rat sausages using FTIR spectra measurements at specific wavenumber regions as variables [39, 26]. The wavenumbers region used for quantitative analysis was used for classification. Absorbances were then converted to Mahalanobis distance and used as variables for grouping beef sausages, rat sausages, and the mixture of beef-rat sausages to form the Cooman plot shown in fig. 2 which shows that both groups are separated without classification objects observed. The absorbance values at wavenumbers regions of 3100-700 cm^{-1} were used to discriminate lipid components extracted by the Bligh Dyer, Folch, and Soxhlet Method with an accuracy level of 100%. LDA successfully distinguished between beef sausages, rat sausages, and a mixture of beef-rat sausages.

Fig. 2: The common plot for discriminant analysis of lipid components extracted from 100% beef sausages (blue), 100% rat sausages (green), and beef-rat sausages mixture at different concentrations (yellow)

This study used both PLS and PCR models to generate calibration. For the PLS and PCR models, out of twenty-four calibration standards, eight of them were used as validation points, and sixteen were used as calibration points in both models. The TQ Analyst software automatically selected the factors used in the PLS model when the method was calibrated [40]. Tables 2, 3, and 4 showed the summary results of PLS and PCR during the analysis of lipid components extracted from beef sausages, rat sausages, and the mixture of beef-rat sausages using three different lipid extraction methods. The statistical parameters used for accuracy evaluation were the coefficient of determination (R^2) between actual values and FTIR predicted values. For precision evaluation, RMSEC and RMSEP were used [15]. The FTIR spectral condition was optimized during PLS and PCR modelling to provide highest R^2 values and low RMSEC and RMSEP values [40].

Table 2: The optimization wavenumbers region of multivariate calibration for beef sausages, rat sausages, and mixed beef-rat sausages using lipid extraction bligh dyer method

Wavenumber (cm^{-1})	Multivariate calibration	Spectra	Calibration		Prediction	
			RMSEC	R^2	RMSEP	R^2
1400-650	PLS	Normal	0.127	0.9150	0.126	0.9158
		1st Derivative	0.164	0.8526	0.166	0.8497
		2nd Derivative	0.00256	0.9999	0.0834	0.9648
	PCR	Normal	0.101	0.9469	0.101	0.9468
		1st Derivative	0.0921	0.9562	0.101	0.9495
		2nd Derivative	0.105	0.9430	0.126	0.9196
1500-600	PLS	Normal	0.126	0.9159	0.126	0.9170
		1st Derivative	0.130	0.9109	0.135	0.9035
		2nd Derivative	0.107	0.9404	0.108	0.9411
	PCR	Normal	0.116	0.9292	0.118	0.9275
		1st Derivative	0.133	0.9064	0.135	0.9041
		2nd Derivative	0.198	0.7761	0.148	0.9087
1800-1000	PLS	Normal	0.124	0.9191	0.124	0.9195
		1st Derivative	0.137	0.9007	0.137	0.9008
		2nd Derivative	0.141	0.8935	0.142	0.8918
	PCR	Normal	0.0932	0.9551	0.0921	0.9562
		1st Derivative	0.0700	0.9747	0.0683	0.9762
		2nd Derivative	0.0706	0.9745	0.0664	0.9777
2800-1800	PLS	Normal	0.102	0.9457	0.119	0.9357
		1st Derivative	0.0421	0.9910	0.103	0.9669
		2nd Derivative	0.0944	0.9540	0.118	0.9473
	PCR	Normal	0.106	0.9410	0.119	0.9356
		1st Derivative	0.110	0.9374	0.138	0.9154
		2nd Derivative	0.139	0.8964	0.158	0.8935
3700-3200	PLS	Normal	0.148	0.8821	0.146	0.8851
		1st Derivative	0.0266	0.9964	0.0838	0.9753
		2nd Derivative	0.0183	0.9983	0.157	0.9375
	PCR	Normal	0.134	0.9042	0.138	0.9000
		1st Derivative	0.106	0.9416	0.118	0.9344
		2nd Derivative	0.157	0.8675	0.209	0.7953
3700-3200 dan 1500-1000	PLS	Normal	0.0653	0.9782	0.0627	0.9800
		1st Derivative	0.165	0.8524	0.165	0.8518
		2nd Derivative	0.138	0.8991	0.142	0.8921
	PCR	Normal	0.0836	0.9640	0.0824	0.9651
		1st Derivative	0.108	0.9389	0.109	0.9386
		2nd Derivative	0.0935	0.9549	0.0883	0.9617

*The selection condition was assigned with bold

Table 3: The optimization wavenumbers region of multivariate calibration for beef sausages, rat sausages, and mixed beef-rat sausages using lipid extraction folch method

Wavenumber (cm^{-1})	Multivariate calibration	Spectra	Calibration		Prediction	
			RMSEC	R^2	RMSEP	R^2
1400-650	PLS	Normal	0.00501	0.9999	0.0145	0.9997
		1st Derivative	0.00118	0.9999	0.0200	0.9986
		2nd Derivative	000174	0.9999	0.0256	0.9975
	PCR	Normal	0,0324	0,9947	0,0192	0,9992
		1st Derivative	0,0180	0,9984	0,0290	0,9977
		2nd Derivative	0,0289	0,9958	0,0406	0,9954
1500-600	PLS	Normal	0.00599	0.9998	0.0262	0.9985
		1st Derivative	0.00430	0.9999	0.0663	0.9924
		2nd Derivative	0.144	0.8896	0.286	0.4969
	PCR	Normal	0.0486	0.9880	0.0366	0.9949
		1st Derivative	0.0526	0.9859	0.0867	0.9670
		2nd Derivative	0.192	0.7932	0.298	0.4253
1800-1000	PLS	Normal	0.0100	0.9995	0.0137	0.9991
		1st Derivative	0.0248	0.9969	0.0290	0.9961
		2nd Derivative	0.0635	0.9795	0.0487	0.9913

Wavenumber (cm^{-1})	Multivariate calibration	Spectra	Calibration		Prediction	
			RMSEC	R^2	RMSEP	R^2
	PCR	Normal	0.0373	0.9929	0.0355	0.9943
		1st Derivative	0.0487	0.9880	0.0423	0.9917
		2nd Derivative	0.0418	0.9911	0.0399	0.9943
2800-1800	PLS	Normal	0.311	0.1491	0.313	0.1048
		1st Derivative	0.00708	0.9997	0.176	0.8855
		2nd Derivative	0.00537	0.9999	0.177	0.8641
	PCR	Normal	0,0892	0,9590	0,142	0,9113
		1st Derivative	0,124	0,9197	0,167	0,8860
		2nd Derivative	0,0938	0,9546	0,164	0,8733
3700-3200	PLS	Normal	0.307	0.2257	0.311	0.1584
		1st Derivative	0.00131	0.9999	0.136	0.9449
		2nd Derivative	0.00281	0.9999	0.129	0.9411
	PCR	Normal	0,113	0,9328	0,182	0,8357
		1st Derivative	0,0532	0,9856	0,138	0,9428
		2nd Derivative	0,167	0,8475	0,176	0,8905
3700-3200 dan 1500-1000	PLS	Normal	0.00600	0.9998	0.00807	0.9997
		1st Derivative	0.0127	0.9992	0.0279	0.9978
		2nd Derivative	0.0179	0.9984	0.0656	0.9839
	PCR	Normal	0,0236	0,9972	0,0206	0,9986
		1st Derivative	0,0202	0,9979	0,0283	0,9977
		2nd Derivative	0,0428	0,9907	0,0725	0,9764

*The selection condition was assigned with bold.

Table 4: The optimization wavenumbers region of multivariate calibration for beef sausages, rat sausages, and mixed beef-rat sausages using lipid extraction soxhlet method

Wavenumber (cm^{-1})	Multivariate calibration	Spectra	Calibration		Prediction	
			RMSEC	R^2	RMSEP	R^2
1400-650	PLS	Normal	0.239	0.6515	0.242	0.6391
		1st Derivative	0.0335	0.9943	0.0538	0.9861
		2nd Derivative	0.0465	0.9890	0.0838	0.9706
	PCR	Normal	0.0638	0.9792	0.0602	0.9824
		1st Derivative	0.0241	0.9971	0.0554	0.9846
		2nd Derivative	0.0743	0.9717	0.123	0.9268
1500-600	PLS	Normal	0.0420	0.9910	0.0590	0.9874
		1st Derivative	0.00348	0.9999	0.116	0.9315
		2nd Derivative	0.0458	0.9893	0.167	0.8668
	PCR	Normal	0.0442	0.9901	0.0638	0.9832
		1st Derivative	0.0487	0.9879	0.126	0.9161
		2nd Derivative	0.116	0.9298	0.252	0.7025
1800-1000	PLS	Normal	0.0607	0.9812	0.0513	0.9867
		1st Derivative	0.175	0.8307	0.175	0.8321
		2nd Derivative	0.172	0.8384	0.171	0.8402
	PCR	Normal	0.0405	0.9917	0.0342	0.9942
		1st Derivative	0.0425	0.9909	0.0530	0.9868
		2nd Derivative	0.0651	0.9784	0.0738	0.9723
2800-1800	PLS	Normal	0.102	0.9462	0.320	0.4783
		1st Derivative	0.0312	0.9951	0.248	0.6468
		2nd Derivative	0.0133	0.9991	0.196	0.8969
	PCR	Normal	0.101	0.9467	0.309	0.5717
		1st Derivative	0.0745	0.9716	0.262	0.5949
		2nd Derivative	0.0980	0.9503	0.219	0.8484
3700-3200	PLS	Normal	0.105	0.9425	0.105	0.9427
		1st Derivative	0.149	0.8810	0.167	0.8507
		2nd Derivative	0.163	0.8546	0.177	0.8536
	PCR	Normal	0.0523	0.9861	0.0550	0.9852
		1st Derivative	0.0864	0.9616	0.135	0.9063
		2nd Derivative	0.155	0.8698	0.187	0.8349
3700-3200 dan 1500-1000	PLS	Normal	0.0635	0.9794	0.0374	0.9936
		1st Derivative	0.0414	0.9913	0.0503	0.9872
		2nd Derivative	0.134	0.9052	0.150	0.8802
	PCR	Normal	0.0537	0.9853	0.0378	0.9928
		1st Derivative	0.0511	0.9867	0.0478	0.9886
		2nd Derivative	0.0476	0.9885	0.0867	0.9623

*The selection condition was assigned with bold.

Based on the optimization, PLS in the Bligh Dyer method using the 2nd derivative spectrum in the wavenumber region 1400-650 cm^{-1} provides the best model, with an R^2 calibration value of 0.9999, RMSEC value of 0.00256, R^2 validation value of 0.9648, and RMSEP value of 0.0834. PLS in the Folch method using the 1st derivative spectrum in the wavenumber region 1400-650 cm^{-1} was also

preferred for quantifying rat meat in sausages with an R^2 calibration value of 0.9999, RMSEC value of 0.00118, R^2 validation value of 0.9986, and RMSEP value 0.0200. The lipid components extracted from sausages using Soxhlet were also quantified using PLS applying normal spectrum in the wavenumber region of 1500-600 cm^{-1} with R^2 calibration value of 0.9999, RMSEC value of 0.00348, R^2 validation value of 0.9315, and RMSEP value of 0.116. Derivatives are used to reveal the fine structure of a band. Shoulders become clear. The second derivative can "act" like a high-resolution signature of a given molecule [38].

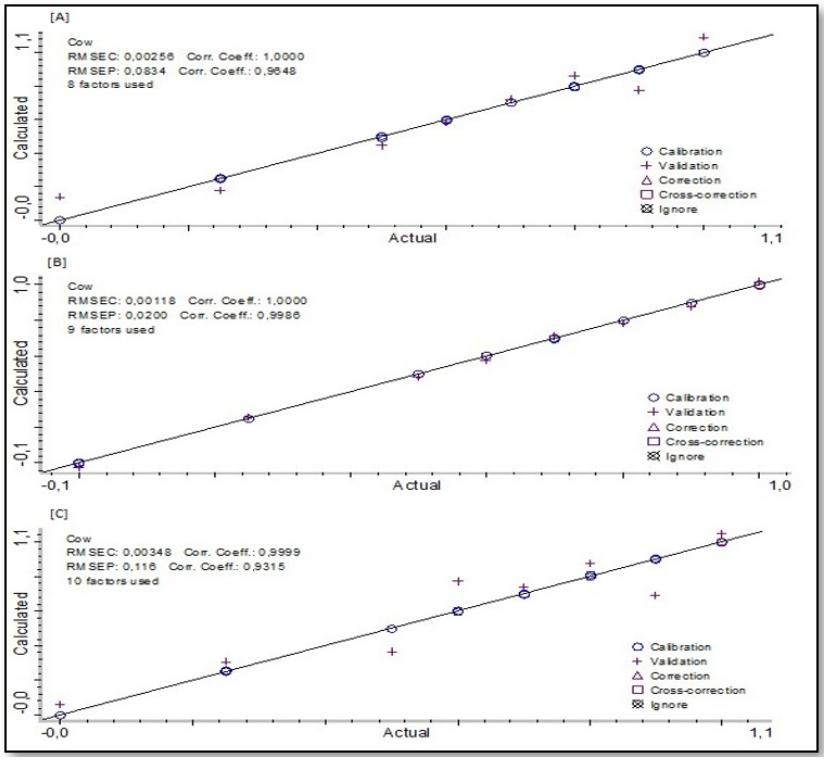

Fig. 3: The correlation between the actual value of lipid components of sausages extracted by bligh dyer method [A], Folch method [B], and soxhlet method [C] with FTIR predicted values facilitated by partial least square calibrations (PLS)

Fig. 3 revealed the correlation between the actual value of lipid components of sausages extracted by the Bligh Dyer Method [A], Folch Method [B], and Soxhlet Method [C] with FTIR predicted values facilitated by PLS. Based on high values of R^2 and low values of RMSEC and RMSEP. The results showed that FTIR-ATR spectroscopy could be used to detect and quantify rats in beef sausages formulation for halal authentication.

CONCLUSION

FTIR-ATR spectroscopy combined with chemometrics of linear discriminant analysis (LDA) was successfully applied to classify beef sausages, rat sausages, and a mixture of beef-rat sausages without any misclassification observed. Multivariate calibrations of PLS and PCR offered a fast and dependable method for authenticating beef sausage from rat meat, applying FTIR spectral absorbances as a variable with acceptable accuracy and precision.

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AUTHORS CONTRIBUTIONS

D. L. performed experiments for this study. D. L. developed techniques and sample preparation for meatballs and sausageSD L. wrote the main manuscript text. A. R. and S. S. investigation, methodology, validation. N. D. Y., K. N. A. B. and S. S. helped prepared fig. and table. W and E. S. S. helped for review and editing. D. H. Designed, supervised, executed, and coordinated this whole study. D. H. wrote and corrected the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest in this research.

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