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IDENTIFICATION OF LARD ON GRILLED BEEF SAUSAGE PRODUCT AND STEAMED BEEF SAUSAGE PRODUCT USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY WITH CHEMOMETRIC COMBINATION

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ABSTRACT

Many issues are spreading about the use of lard in food, one of it is sausage. Sausage is one of the processed foods which are prone of containing pork. In Indonesia, grilled and steamed sausages are popular for children and adults. One of the method which is developed to analyze fat in grilled and steamed sausage products was FTIR spectrophotometry combined with chemometrics. This research aims to develop an analysis method using FTIR spectrophotometry combined with chemometrics to analyze lard content in grilled and steamed beef sausage. Reference sausage made from a mixture of pork and beef. This research was designed by making each 7 concentrations variants of pork and steamed sausage samples (100%, 75%, 65%, 50%, 35%, 25% and 0%). Five samples from market were taken from various beef sausage traders. The fat extraction used n-hexane solvent at the temperature of 70 °C for 5 hours. The extracted fat was analyzed by FTIR spectrophotometry combined with chemometrics. The results of spectrum were analyzed using Horizon MB^{TM} to obtain optimum wave number of steamed sausages in the range of 1000 – 791 cm^{-1} and grilled sausages in the range of 1070 – 796 cm^{-1}. The analysis of steamed sausage with Partial Least Square (PLS) is obtained the equation y = 0.9977x + 0.1166; and the value of R2 0.9977; RMSEC 1.22%; RMSEP 0.22%; and RMSECV 1.26%. The PLS analysis of grilled sausage is obtained the equation y = 0.9972x + 0.1379; and the value of R2 0.9972; RMSEC 1.27%; RMSEP 0.42%; and RMSECV 0.18%. The conclusion of this research is that the FTIR method combined with chemometrics is the proper method for analyzing fat in sausages. The analysis result using *Principle Component Analysis* (PCA) was obtained from 3 of 5 samples in the market which had the similar physicochemical properties of lard sausage.

Keywords: Beef sausage; FTIR; PCA; PLS; pork sausage

INTRODUCTION

Consuming halal food both physically and spiritually is an obligation for every Muslim. Halal and thayyib food are rights for every Muslim consumer **(Regenstein, Chaudry and Regenstein, 2006)**. Nowadays, there are many issues spread about the use of lard in food, one of it is sausage **(Garcia, 2012)**.

The term sausage comes from the Latin word "salsus", which means salt, so sausages can be interpreted as ground meat which is preserved with salt. The grilled sausage is, first, steamed sausage which is, then, grilled and given spices to add its flavor. Generally, the food processing by heating causes oxidation reactions and will cause damaged fat that is contained in these foods. In the process of oxidation, most of the unsaturated fat acids will be damaged and the results of the damage can mostly be evaporated **(Che Man, Syahariza and Rohman, 2010)**. The fat in these food products can be damaged due to the process of steaming (dehydration) and burning (oxidation) **(El-Gindy, Emara and Mostafa, 2006)**. The pork, which the price is relatively cheaper than beef, is often used by the producers for substituting the beef ingredients in producing beef sausages because of its low price **(Guntarti et al., 2015)**.

According to the research result which is conducted by **Jaswir et al. (2003)**, it states that the FTIR method (fourier transform infrared) is potential to be used as a lard detector that is quick and has a consistent result. The FTIR method can give the analysis result of lard acids which mix with the other animal fats consistently, even the very low content **(Jaswir et al., 2003; Rohman and Che Man, 2012)**. In addition, in its development, the FTIR spectroscopic method has succeeded in analysing the difference in profiles and characteristics of animal fats (chicken, beef, and pork) **(Marikkar et al., 2005; Rohman and Che Man, 2010)**. Beside, the FTIR method is also combined with chemometrics for analysing the rat meat (*Rattus diardi*) in beef meatballs **(Guntarti and Prativi, 2017)**. Based on the description above, this research aims to develop the method of analysis using FTIR spectrophotometry which is combined with

chemometrics to analyze lard content in grilled and steamed beef sausage products **(Schieber, 2008)**.

Scientific hypothesis

FTIR Spectrophotometry method can be used to analyze pork in sausage quantitatively as well as for the classification of beef and lard in sausages.

MATERIAL AND METHODOLOGY

Ingredients

Beef, pork and spices which was obtained from Kranggan Market, Yogyakarta. The production sample was some brands of beef sausages that had been circulating in the market, both traditional and modern markets. N-hexane solvent (technical) (Merck), and Na2SO4 anhydrous.

Tools

Tools for sausage production, Soxhlet, ABB Analytical brand of FTIR spectrophotometer: MB3000 (Canada) with DTGS detector (deuterated triglicine sulfate).

The Research Progress

Making reference sausages: Sausages were made by mixing 90% of the ingredients in the blended meat (can be pork, beef or a mixture of pork and beef) with 10% seasoning in sausages production (Table 1).

Fat extraction of the reference sausages and market sausages: some grams of sample (±120 g) sausages in various concentrations (steamed and grilled sausages) and also sausages from the market were mashed using mortars, then in the Soxhlet with n-hexane solvent at the temperature of 70 °C for 5 hours **(Rahmania, Sudjadi and Rohman, 2015)**. The extraction result was added the Na_2SO_4 anhydrous. The fat obtained was weighed and calculated.

Statistic analysis

Then, the fat was analysed by FTIR spectroscopy with the ATR crystals at controlled temperature (20 °C). All spectra were recorded from 4000 – 400 cm^{-1}, it was recorded in the form of absorbance. The data from FTIR analysis were processed using PLS and PCA chemometrics programs with ABB Inc. Horizon MB™ QA software. Microsoft Excel 2007 was used for validation and calibration. The root mean square error of cross validation (RMSECV), root mean square error of prediction (RMSEP) and determination coefficient (R2) value were used as criteria for the calibration and validation model **(Zhao et al., 2014)**.

RESULTS AND DISCUSSION

The fat extraction is done by Soxhlet, n-hexane solvent for 5 hours in the maintained temperature (70 °C). The fat extract which obtained is, then, added with anhydrous Na_2SO_4 sufficiently to attract some water which may still be contained in the n-hexane **(Rowe, Sheskey and Quinn, 2009)**. Then fat extracts are analyzed with FTIR to identification of fungtional groups and analysing of fingerprint field that differentiate between steamed sausage and grilled sausage sample. The reading of IR spectra of extracted fat from steamed sausage and grilled sausage samples is done in the middle wave number area between 4000 – 650 cm^{-1}. The selection of the middle wave region is due to FTIR spectroscopy which will provide information about the types of functional groups in detail which is contained in pork derivatives **(Rohman and Che Man, 2010)**, and fingerprints in the wave numbers of 1500 – 600 cm^{-1} **(Stuart, 2004)**.

The spectra (Figure 1a) are spectra between steamed pork reference sausages of 100% and steamed beef reference sausages of 100%, and the spectra between grilled pork reference sausages of 100% and grilled beef reference sausages of 100% (Figure 1b) which are read by the Horizon MBTM application. In Figure 1 (a) and (b), it shows that between the fat spectra of steamed pork and beef sausages, also pork and beef sausages both have almost the same spectrum pattern due to the main components of the two fats' types are the same, which are triglycerides and both are animal fat **(Rohman and Che Man, 2011)**. The shift of the functional group spectrum of steamed and grilled sausages is presented in Table 2.

In Figure 2, Spectrum A, B, and C are fingerprint areas which become the difference between steamed sausages and grilled sausages. Spectrum A shows a spectral change in the extracted fat that occurs between steamed sausage and grilled sausage 1234 – 1233 cm^{-1} that the C-O Group in the ester has a stretch vibration. Spectrum B was the isolated trans-olefins bending vibration of -CH functional group which gives the m peak in the wave number 964 – 963 cm^{-1}. Spectrum C in the wave number 721 – 719 cm^{-1} is an overlapping vibrations of methylene shake (-CH2) and vibrations out of field by the cis- is substituted. The quantitative analysis in this research was done by Horizon MB™ chemometric application with 2 methods, which are Partial Least Square (PLS) and Principle Component Analysis (PCA). The optimal wave number for steamed sausage is 1000 – 791 cm^{-1} and the grilled sausage is 1076 – 796 cm^{-1}. In the research which was conducted by **Sari and Guntarti (2018)**, the wild boar fat wave number optimization of steamed sausages at 1250 – 900 cm^{-1}. There is a shift in the wave number because of the different types of animal fat (Figure 3).

Based on Figure 3, the R2 value is close to 1. Beside the R2, PLS modelling can use the RMSEC calibration (root mean square of error calibration), RMSEP (root mean square error of prediction), and RMSECV (root mean square error of cross validation), which is a precision parameter that is used to evaluate errors in the calibration model. Table 3 presents the PLS calibration and validation parameters.

When the R2 value is close to 1 the more linear the relation between the actual value (x axis) and the predicted value (y axis). The low RMSEC, RMSEP, and RMSECV values indicate the errors that occur in the analysis are lower or smaller, so the method is more valid **(Zhao et al., 2014)**. Steamed and grilled sausages are also given the same treatment and the same modeling that is done as the original sausage, because the modeling which is done on the original sausages is successfully separated lard and cow fat in the different quadrants. The number of samples that is analyzed consists of 5 products. Next, it is done the analysis by the PCA chemometrics method for grouping for the aim of finding out the existence of pork in steamed

and roasted sausage samples. The sample grouping is done at the wave number area of 1000 – 791 cm^{-1} (Figure 4). The PCA analysis of steamed production sausage samples and grilled production sausages shows the separation or grouping of lard and beef fat in the two different quadrants, which are quadrant A and quadrant B (Figure 5).

In Figure 4, the pork and beef quadrants is far apart, it means the grouping lard and beef fat in steamed sausages (a) and grilled sausages (b) works well. Then, the products on the market (A, B, C, D and E) are taken. In Figure 5, the products on the market C, D, E both on the steamed and grilled sausages are located in the quadrant of lard sausage. The products on the market are steamed and grilled sausages of A, B located in the quadrant of beef fat. It means that the products of steamed and grilled sausage C, D, E have the same physicochemical properties as pork sausage, while the products of steamed and grilled sausage of A, B have the same physicochemical properties as beef sausage. Steamed sausage which will be grilled, based on PCA chemometrics grouping, gives the same results. To ensure the content of pork, it is needed a more sensitive and specific method.

Table 1 The Formula of reference grilled and steamed beef sausages in various concentration.

Concentration (%)	Pork (g)	Beef (g)	Spices (g)
Beef 100	-	225.00	25.00
Pork 100	225.00	-	25.00
(B-S) 25	56.25	168.75	25.00
(B-S) 35	78.75	146.25	25.00
(B-S)50	112.50	112.50	25.00
(B-S) 65	146.25	78.75	25.00
(B-S) 75	168.75	56.25	25.00

Table 2 The Shift of the Functional Group and Vibration Model of Lard and Beef Fat in the Steamed and Grilled Sausage.

Wave Number of Steamed Sausage (cm^{-1})	Wave Number of Grilled Sausage (cm^{-1})	Functional Group Vibration	Intensity	Desc
3003	3010	Stretched *Cis* C=CH	Weak	Shift to right
2951	2956	Methylene group of asymmetrical stretching vibration (-CH3)	Average	Shift to right
2920 and 2850	2918 and 2852	Vibration Asymmetrical and symmetrical stretch of methylene group (-CH2)	Strong	Shift to left, Shift to right
1744	1745	The carbonyl (C = O) function group of triacylglycerol ester bonds	Strong	Shift to right
1655	1653	*Cis* C=C	Weak	Shift to left
1465	1463	Bending vibration of the aliphatic CH2 group	Strong	Shift to left
1374	1377	Symmetrical bend vibration of the CH3 group	Average	Shift to right
1234 and 1156	1233 and 1156	Vibration stretch of the C-O group in ester	Strong	Shift to left, No shifting
1113 and 1096	1112 and 1095	Bended -CH vibration and change -CH form of fat acid	Weak	Shift to left
964	963	The isolated *trans*-olefins bending vibration of –CH functional group	Weak	Shift to left
719	721	Overlapping vibrations of methylene shaking (-CH2) and vibrations out of the cis- dis-substituted field	Strong	Shift to right

able 3 The PLS calibration result on steamed and grilled sausage.

Parameters Value	Steamed Sausage	Grilled Sausage
Linear Similarity	y = 0.9977x + 0.1166	y = 0.9972x + 0.1379
R^2	0.99776	0.99772
RMSEC	0.12	1.27
RMSEP	0.22	0.42
RMSECV	1.26	0.18

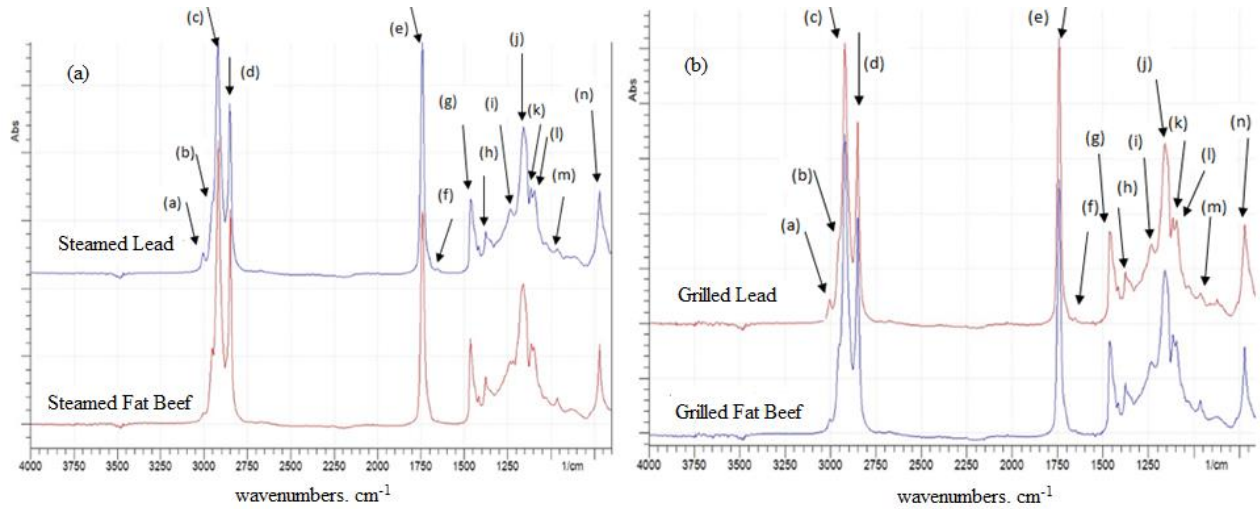

Figure 1 (a) Spectra of Steamed Pork Sausage Fat of 100% and Steamed Beef Sausage of 100 %. (b) Spectra of Grilled Beef Sausage Fat of 100% and Grilled Pork Sausage Fat of 100%

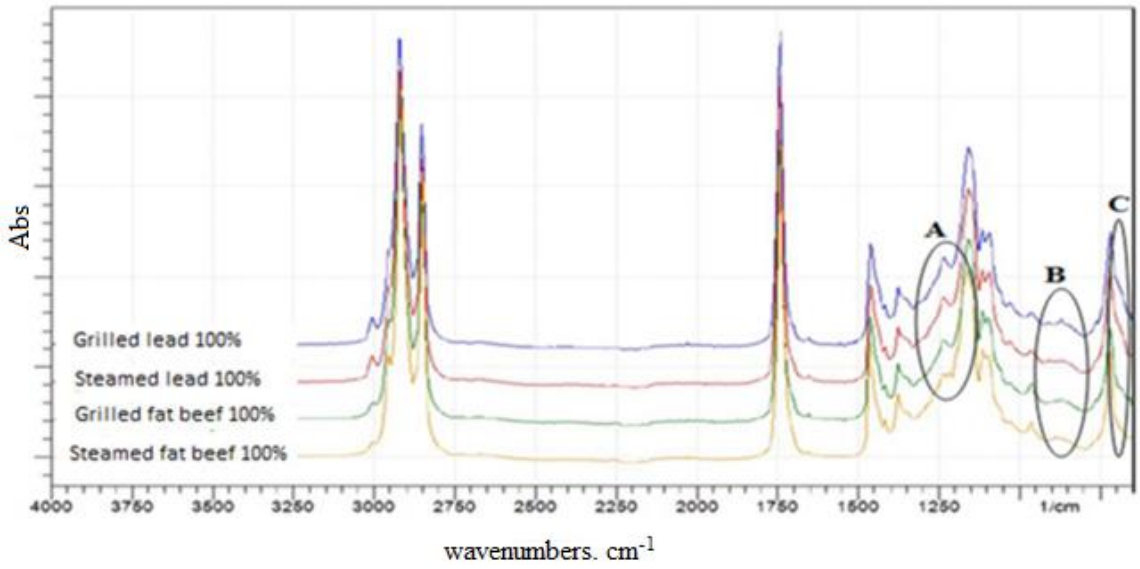

Figure 2 The infrared spectrum of Steamed and Grilled Pork Sausage.

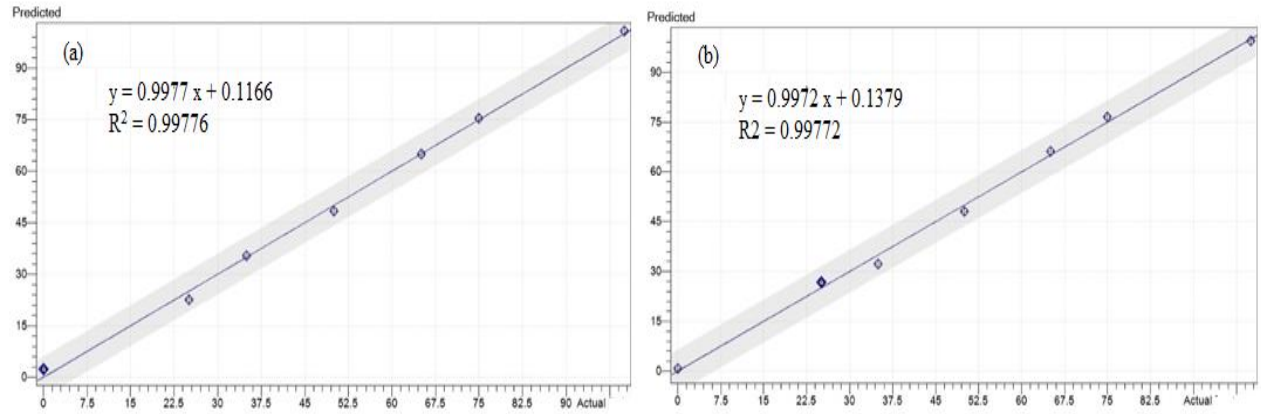

Figure 3 The Result of PLS Curve Relation between The Actual Value (x Axis) and Prediction Value (y Axis) on the Steamed Sausage Reference (a) and Grilled Sausage Reference (b) with Levelled Concentration.

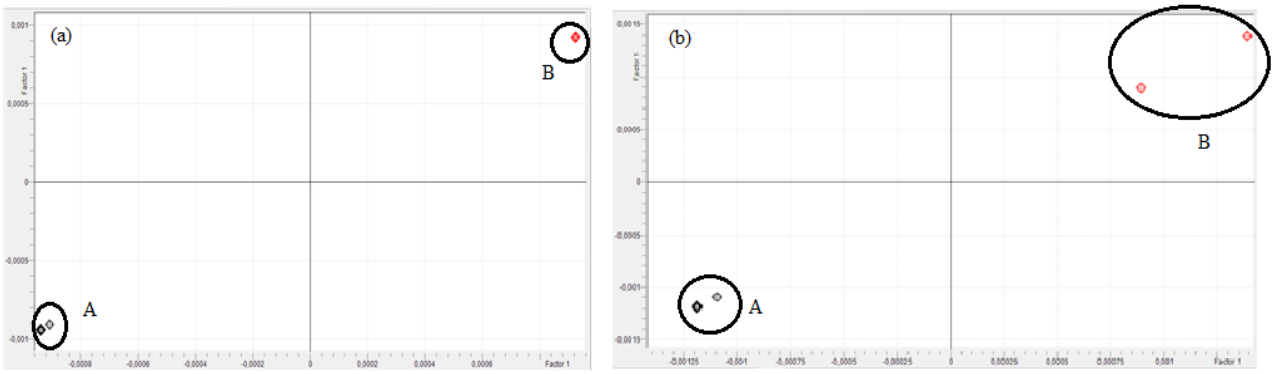

Figure 4 Plot score PCA of Beef Fat (A) and Lard (B) from steamed sausage (a), and grilled sausage (b).

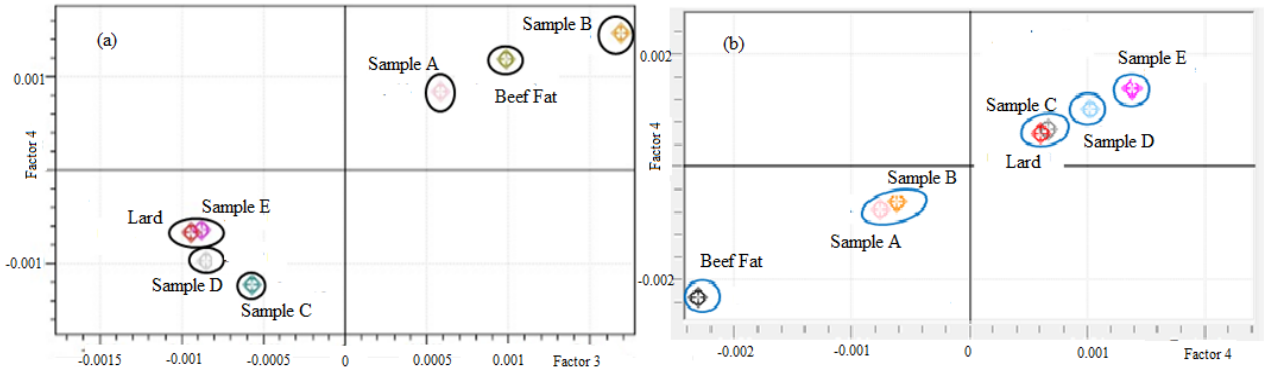

Figure 5 The PCA Result of Products in the market of steamed sausage (a), and Product on the market of grilled sausage (b).

CONCLUSION

Partial Least Square (PLS) chemometrics can be used for quantitative analysis of lard in steamed beef sausages and grilled sausages products. The combination with PCA chemometrics can grouped the lards in the steamed beef sausages and grilled beef sausages products in the market.

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