Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage : www.journal.uii.ac.id/index.php/JKKI

Wild boar fat analysis in beef sausage using Fourier Transform Infrared method (FTIR) combined with chemometrics

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Original Article

ARTICLE INFO

Keywords:
beef sausage,
wild boar fat,
FTIR,
chemometrics

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DOI : 10.20885/JKKI.Vol9.Iss1.art4

History:
Received: August 4, 2017
Accepted: April 24, 2018
Online: April 30, 2018

ABSTRACT

Background: Sausage is ready to eat meals for children, adolescents, and older adults. The meat contained in a beef sausage might not be consistent to the one listed on the label. Fourier Transform Infrared (FTIR) spectroscopy combination with a chemometric method is one of the most used methods to detect wild boar fat with rapid and consistent results. Results of analysis can classify fatty acid composition contained.
Objective: The aim of this study is to determine the fat profile in sausage samples and differentiate the grouping of wild boar fat and beef fat in sausage preparation.
Methods: The study was designed by making 7 different variations of wild boar reference sample concentrations, which were 100%, 75%, 65%, 50%, 35%, 25% and 100% beef. Six other samples were gathered from various street vendors. The results were analyzed using FTIR spectroscopy combined with chemometric with Partial Least Square (PLS) and Principal Component Analysis (PCA).
Result: The results of analysis using Horizon MB™ apps showed optimal wave number within 1250-900 cm^{-1}. The results of calibration with equation y= 0.994x + 0.334 and the value of (R^2) determination was 0.998, and root mean square error of calibration (RMSEC) was 1.22%. The results of validation using parameter value of root mean square error of cross validation (RMSECV) 2.68%, and root mean square error of prediction (RMSEP) was 0.11%.
Conclusion: PCA result showed that five from six samples possibly have same physical and chemical properties similar to fatty beef sausage.

Latar Belakang: *Sosis merupakan makanan siap saji dan diminati dari banyak kalangan baik dari anak-anak, remaja, dewasa hingga tua. Kandungan daging yang terdapat didalam sosis tersebut dapat berasal dari daging non-halal. Salah satu metode yang dapat digunakan untuk mendeteksi lemak secara cepat dan hasilnya konsisten dapat digunakan Spektrofotometer Fourier Trasform Infra-Red dikombinasikan dengan kemometrika.*
Tujuan: *Mengetahui profil spektra lemak celeng dan lemak sapi, serta pengelompokkan lemak celeng dan lemak sapi pada sediaan sosis.*
Metode: *Penelitian dirancang dengan membuat 7 variasi konsentrasi sampel referensi daging celeng, yaitu 100%, 75%, 65%, 50%, 35%, 25%, dan 100% daging sapi. Sampel di pasaran diambil enam sampel dari berbagai pedagang sosis sapi. Hasil analisis dengan instrumen FTIR dikombinasi kemometrika Partial Least Square dan Principal Component Analysis.*
Hasil: *Hasil analisis menggunakan aplikasi Horizon MB™ diperoleh bilangan gelombang optimal 1250-900* cm^{-1}*. Persamaan kalibrasi y= 0,994x + 0,334, nilai koefisien determinasi (*R^2*) 0,998, dan nilai* root mean square error of calibration *(RMSEC) sebesar 1,22%. Hasil validasi dengan parameter nilai* root

mean square error of cross validation *(RMSECV) sebesar 2,68% dan* nilai root mean square error of prediction *sebesar 0,11%.*
Kesimpulan: *Optimasi bilangan gelombang pada 1250-900 cm^{-1} dan analisis multivariat* Principal Component Analysis *mampu mengelompokkan lemak sosis sapi dan lemak sosis celeng.*

INTRODUCTION

Meat is one of the most sought after dietary needs. It could be made into various kinds of dishes, such as sausage, meatballs, nuggets, corned beef, and canned foods. An independent survey conducted by a private company in 2010 showed that the consumption of processed meat like nugget and sausage in Indonesia is steadily growing. The consumption of sausage in Indonesia is growing 4.46% each year. In April 2014, meatballs containing wild boar meat were discovered in kecamatan Tambora Jakarta Barat.[1] The high potentials of forgery especially those regarding processed meat are unsettling, especially among moslem population.[2,3] Nonhalal ingredients are prohibited for consumption according to Islamic laws (haram).[4] Mixing beef with a chicken mat or other kinds of meat is thought to be an effective solution to decrease the cost of sausage production.[5] The most recent case of wild boar meat distribution from Lampung to Solo, in an astonishing amount of 1.5 ton, induced the suspicion of beef forgery among the public.[6]

Nowadays FTIR spectophotometry has been widely used to analyze pork derivates, non-halal ingredients within various food products like cake and chocolate, analysis of beef meatballs forgery and wild boar meat content in meatballs.[7-12]

This study aims to determine spectra profiles and classifying wild boar fat with cow meat fat using FTIR. It is also to determine the presence of wild boar meat in beef sausage products circulating in the market using FTIR instrument combined with chemometrics. Chemometrics is a mathematical and statistical science used to extract chemical information from data analysis.[13] Obtained data are extracted, for instance, spectrum data in infrared spectophotometry.[14]

METHODS

Ingredients

Sausage samples were obtained from supermarkets (sample I and VI), sample II and III were obtained from street vendors in Alun-alun Kidul, sample IV was obtained from sunday morning vendor, and sample V was obtained from Bringharjo traditional market Yogyakarta. Sampling was done in August 2015.

Tools

Tools used in this study were FTIR ABB MB 3000 (Canada) spectrophotometer with detector deuterated triglycine sulfate (DTGS), glassware, soxhlet, n-hexane (technical), Na_2SO_4 anhydrate, p.a.

Research Prosedure

Meat Supply

The meat was obtained from wild boar hunter from kabupaten Kotawaringin Lama, Kalimantan Tengah and beef was obtained from Sentul traditional marker, D.I. Yogyakarta which was processed into sausage. Aside from that, sausage products were also obtained from street vendors and traditional markets in Yogyakarta.

Sausage production

Ground beef was measured according to the formula, and then it was mashed with a blender. After that, excipient was added, including flour, garlic, and eggs.

Fat extraction

The beef and wild boar meat were measured according to the formulation, and then it was chopped and mashed, and it was strained and put in soxhlet. Solvent n-hexane was added twice. Extraction using soxhlet was done for 5-7 hours and the temperature was maintained in 70^{0}C. The finished results were fat containing n-hexane solution. Then, Na_2SO_4 anhydrate was added and filtered using strain paper. Obtained fat was moved into effendorf to be further analyzed with FTIR.

Table 1. Sausage formula mixtre of beef and wild boar meat

Concentration	Beef(Gram)	Wild Boar meat (Gram)	Excipient (Gram)
Beef 100%	45,00	-	5,00
Wild Boar meat 100%	-	45,00	5,00
Wild Boar meat 25%	33,75	11,25	5,00
Wild Boar meat 35%	29,25	15,75	5,00
Wild Boar meat 50%	22,50	22,50	5,00
Wild Boar meat 65%	15,75	29,25	5,00
Wild Boar meat 75%	11,25	33,75	5,00

Data analysis

The results of FTIR spectrum was processed using chemometric analysis program software HorizonMBTM. Sample spectrum was analyzed in wave number 4000-400 cm^{-1}. The multivariate analysis includes calibration with PLS and PCA using software Horizon MB™.

RESULTS

Fat extraction from Sausage samples

This research was done by extracting lipid from sausage prepared using soxhlet. This method was based on solid-liquid extraction. We used n-hexane solvent because it has a non-polar solubility, low boiling point (easy to separate using evaporation), economical. N-hexane solvent is also stable and rigid.

Extraction using soxhlet was done for 6-7 hours in 69-70°C temperature.[15] The obtained lipid extract was added with Na_2SO_4 anhydrate to remove water content.[16] The water content in oil can alter the results of the spectrum, thus can alter the chemometric analysis.

Identification of the Functional Groups of Beef Sausage Fat and Wild Boar meat Sausage Fat

IR spectrum analysis is unique, thus it is usually called fingerprint analysis. Different kind of substance will create different intensity, peak value, or wave number of extract for every peak. The reading of IR spectrum was done by mid-wave number, which was 4000-400 cm^{-1}. Mid-wave number was chosen because it can give sufficient information of the functional groups within the lipid. The description of lipid spectrum of beef sausage (BSL) and Wild boar meat sausage (WSL) was displayed in Figure 1.

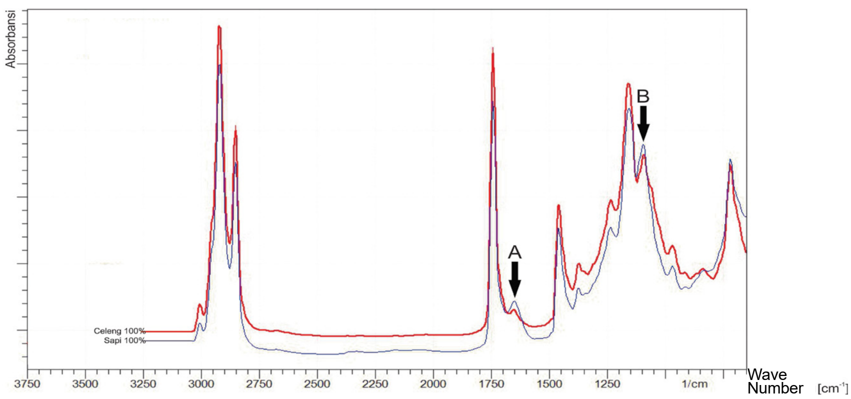

Figure 1. The difference between beef sausage lipid spectrum (BSL) 100 % and wild boar sausage lipid spectrum (WSL) 100%

At a glance, there is no significant difference between the lipid spectrum of beef sausage and wild boar sausage because both have similar main structure, which is triglycerides and a few other small components. Both peaks at 3009 cm^{-1} which happened due to the stretching of double bond CH. *Cis*–C=CH stretch vibration in wave number 3005 cm^{-1} and stretch *cis*–C=C- in wave number 1651 cm^{-1} in WSL spectrum identified the presence of unsaturated fatty acid. In wave number 2924 cm^{-1} and 2854 cm^{-1}, there was peak similarity in BSL and WSL, in which asymmetric and symmetric stretch vibration happened in methylene group (-CH_2-). Each methylene and methyl groups could also be observed in wave number 1373 cm^{-1} due to their vibration bend. In wave number 1744 cm^{-1} there was a peak that suggested the presence of carbonyl group (C=O). This peak was found both in BSL and WSL. The peak in wave number 1243 cm^{-1} showed the presence of C-O esther group. The results of various vibration types and functional groups identification can be seen in Table 2.

Tabel 2. The analysis of functional group of BSL and WSL as well as IR vibration

No	Wave number (cm^{-1})	Functional group vibration	Intensity
1.	3009 and 3005	*Cis* C=CH stretching	Weak
2.	2920 and 2924	Asymetric and symetric stretching vibration of methylen group (-CH_2)	Strong
3.	1744	Functional carbonyl group (C=O) of triacylglycerol esther bond	Strong
4.	1651 and 1655	*Cis* C=C stretch	Strong
5.	1458 and 1462	Bending vibration of aliphatic CH_2 and CH_3 group	Medium
6.	1373	Symetric bending vibration of CH_3 group	Medium
7.	1234	Stretching vibration of C-O group in esther	Strong
8.	1157 and 1161	Bendring vibration of –CH in plane group	Strong
9.	1095 and 1092	Stretching vibration of C-O from esther bond	Medium
10.	968	Bendingvibration of CH functional group of isolated trans-olefin	Medium
11.	717	Overlapping vibration of methylen (-CH_2) and out of plane vibration by *cis*-distribution	Strong

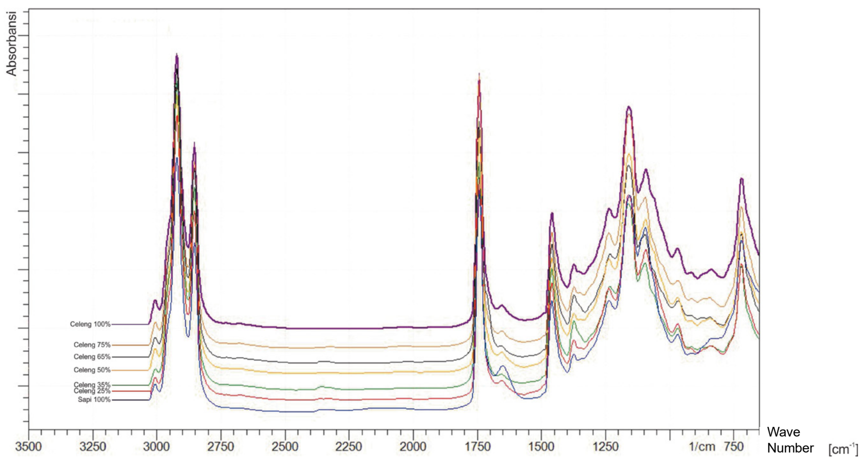

Figure 2. FTIR spectrum of BSL and WSL in gradual concentration in the middle infrared area (3100-650cm^{-1})

Quantitative analysis using PLS

Results of wavenumber optimization in area 1250-900 cm^{-1}. This wavenumber has an unique functional group of wild boar meat. The generation of calibration models was done by measuring the intensity produced from seven different sausage concentration in wave number 1250-900 cm^{-1}. The results of FTIR spectrum concentration was shown in Figure 2.

As seen in Figure 2 the results of spectrum formulation reading of beef sausage and wild boar meat in gradual concentration showed similar spectrum results, this indicated that refference sample had the same fatty acid compound.[17] The next process was data analysis using PLS. The results of optimization showed that wave number 1250-900cm^{-1} generated value R^2 = 0,988 and RMSEC = 1,22%.

The formulation of calibration model in Figure 3 showed the association value between actual WSL in X-axis and FTIR calculation value in Y-axis.

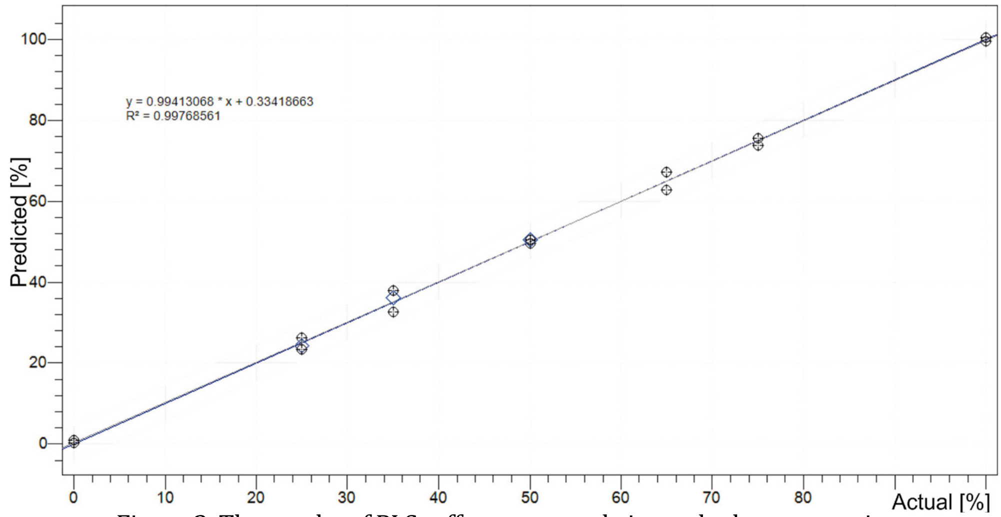

Figure 3. The results of PLS refference sample in gradual concentration

Determination coefficient showed the capability of the independent variable (X-axis) on its dependent variable (Y-axis). The closer R^2 value to 1, then the correlation coefficiency was also more linear between actual variable and predictive variable.[18]

Classification of fatty acid using PCA

Principal component analysis is a data interpretation method done by data prediction, the amount of variable in a matrix is reduced to generate a new variable by maintaining the information of the data. The new variable is in the form of scores or main component. This technique can decrease the effect of noise and utilize the slight differences of IR spectrum.[19]

To analyze PCA, BSL 100% samples and WSL 100 samples from sausage extraction were used. BSL and WSL samples were read in an optimized wave number between 1250-900 cm^{-1}, as shown in Figure 4.

Figure 4 showed a significant distance difference between beef lipid and wild boar meat lipid. PCA chemometric can be used to clasified beef lipid and wild boar meat lipid.

Analysis of sausage products circulating in the market

Sausage samples in the general market was obtained from traditional market vendors. Spectrum was measured in an optimized wavenumber, which was 1250-900 cm^{-1}. The results of chemometric analysis of these samples was shown in Figure 5.

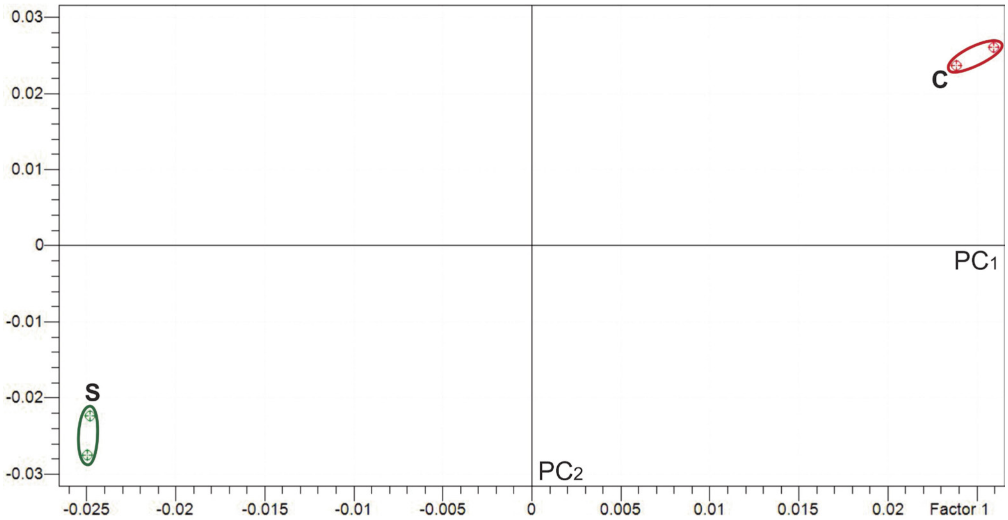

Figure 4. Score plot graphic of PC1 and PC2 of beef lipid and wild boar meat lipid.
Note : S (beef), C (wild boar meat)

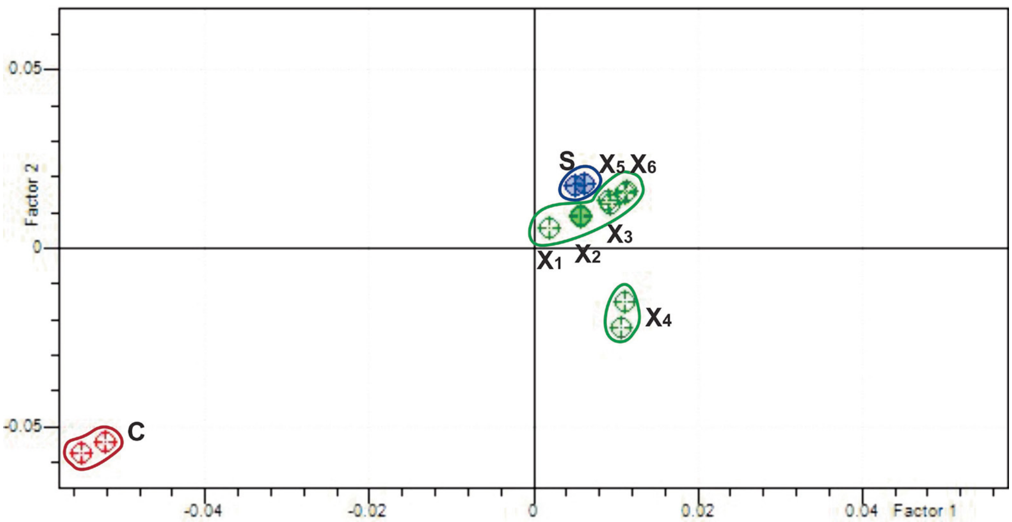

Figure 5. The results of PCA analysis in a score plot. Note C (Wild Boar meat lipid) and S (beef lipid).

Figure 5 showed that sample X 1, 2, 3, 5 and 6 was similar to the beef lipid group, thus it can be concluded that the sausage circulating in the market has similar physical-mechanical compund with beef lipid.

DISCUSSION

The spectrum difference between wild boar meat and beef meat in wavenumber 1750-1650 cm^{-1}, the intensity of BSL was found higher than WSL. This area is a carbonyl area which shows unique traits of triglyceride. Another difference was found in wavenumber 1050-1100 cm^{-1}, BSL had a higher intensity than WSL. The BSL peaked in wavenumber 1095 cm^{-1}, this showed the presence of stretching vibration C-O from esther group.

The results of wave number optimization in area 1250-900 cm^{-1} showed unique functional groups of Wild Boar meat lipid. The manufacturing of calibration model was done by measuring the intensity produced by 7 different sausages concentration in wavenumber 1250-900 cm^{-1}.

Validation model built by parameter RMSEP, RMSECV and RMSEC gave a lower value, which proved that spectophotometry validation model of FTIR was improving.[20] Samples circulating in the market had the similar physical-chemical character of beef lipid. This indicates that the sausage samples most likely contained beef.

CONCLUSION

Spectophotometry FTIR combined with chemometric PLS in wave number area 1250-900 cm^{-1}. Calibration model with value R^2 0.998 and RMSEC 1.22%. The results of validation model were obtained from RMSEP value 0.11% and RMSECV value 2.68%. PCA multivariate analysis could classified beef sausage lipid and wild boar meat sausage lipid. The analysis results of sausage samples circulating in the market showed no wild boar meat content.

CONFLICT OF INTEREST

We declare there is no conflict of interest

ACKNOWLEDGEMENT

Non declare

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