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Research Article

The use of Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography Mass Spectroscopy (GCMS) for Halal Authentication in Imported Chocolate with Various Variants

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ABSTRACT

The analysis using FTIR and GCMS spectrophotometry for halal authentication on several variants of imported chocolate products circulating on the market has been performed. FTIR spectra analysis result of lard and chocolate in the wave number region of 4000-650 cm^{-1} shows a typical lard-specific difference at wavenumber region 3006.8 cm^{-1}; 1118.84 cm^{-1}; 1097.42 cm^{-1}. Analysis of PCA and PLS calibration models in the fingerprint region 999.053 - 1190.638cm^{-1} can be used for lard identification in chocolate fat. The relationship between the actual value and the predicted value of lard in chocolate yields the equation Y= 1,000x-0,0378, (R^2 0,997 and RMSEC 1,563) with a minimum limit of detection at a concentration of 4%. Based lard chromatogram, it shows the peak appeared at a retention time of 38,8 minutes. After being compared with library WILLEY7, it shows eikosadienoat 11.14 acidic compounds. Eikosadienoat 11.14 acid is a marker of the presence of lard appearing with a mixture of lard concentration at ≥ 10%. FTIR spectra and analysis results using PCA and PLS on samples of 6 imported chocolate variants show a lard content which is marked by the appearance of eikosadienoat 11.14 acid at a retention time of 38,8 minutes in the chromatogram. PLS quantitative analysis shows lard content in the sample is 43.6%; 73.5%; 61.7%; 63.0%; 37.0%; and 30.4%.

Keywords: FTIR, GCMS, Eikosadienoat, Chocolate

1. Introduction

Indonesia is a country of which majority population is Muslim, thus it is important to ensure the halalness of food products. This non-halalnes may be resulted from a mixture of pork and its derivatives (Riaz & Chaundry, 2004). Lard is pig derivatives which is commonly mixed with other food ingredients with the aim of lowering production costs which tend to be high (Marikkar *et al.,* 2005; Che Man & Sazili, 2010).

One of food products suspected containing lard is chocolate, especially imported chocolate products without halal label. In the manufacture of chocolate, materials suspected of containing lard are emulsifiers (lecithin) (Surya, 2008) and cocoa butter (Che Man *et al.,* 2005). Lard is widely used because of its good quality, i.e. produces savory and delicious taste, soft and malleable texture (Dana, 2011).

One of lard analysis methods in food is by observing the spectrum pattern using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography Mass Spectroscopy (GCMS). FTIR method is fast and consistent method, even in low analyte concentration (Hermanto, et al., 2008), and can be used to detect the lard in a variety of mixtures with different concentrations (Siti et al., 2009), non-destructive, sensitive, and does not require complicated sample

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Table 1. Differences of FTIR Spectrum in lard compared to chocolate fat Che Man *et al.*, 2005; Guillen and Cabo, 1997; Vlachos et al, 2006).

	Wave number region (cm^{-1})	Vibration type
(a)	3006,8	Stretching vibration *cis* C=C
(b)	2961,19	Asymmetric stretching vibration of methyl groups ($-CH_3$)
(c)	2921,94	Symmetric stretching vibration of methylene groups ($-CH_2-$)
(d)	2852,51	Asymmetric stretching of methylene groups ($-CH_2-$)
(e)	1743,52	Stretching vibration of carbonyl groups (C=O) from esters of triglycerides
(f)	1629,73	*Cis* C=C
(g)	1458,08	Bending vibration of CH_2 and CH_3 aliphatic groups
(h)	1421,50	Vibration of CH bonds oscillation of substituted alkenes –*cis*
(i)	1377,07	Symmetric bending vibrations of CH3 (methyl) of symmetric stretching
(j)	1234,35	C-O stretching vibration in ester
(k)	1159,13	C-O stretching vibration in ester
(l)	1118,84	Bending vibration – CH and – CH changes of fatty acid
(m)	1097,42	
(n)	1031,84	C-O Stretching vibration
(o)	964,34	CH bending vibration of isolated trans-olefin
(p)	721,32	Overlapping vibration of methylene oscillation (-CH2) and vibration outside *cis*- substituted olefins field

preparation (Rohman, 2011), thus the lard using FTIR analysis on the wave number region of 4000-400cm^{-1} provide information regarding detailed molecular bonds and other types of functional group contained in pig derivatives (Rohman, 2011). However, the FTIR method has limitations, i.e. it cannot identify the type of content of each fatty acid component of a sample with certainty (Herman and Anna, 2008).

Analysis using GCMS can show specific swine fatty acids, such as trans-9,12,15-oktadeka trieonat (C18: 3 N3T), 11,14,17-eikosatrienoat acid (C20: 3 N3T), and acid-eikosadienoat 11.14 (C20: 2 rt6) (Chin et al, 2009). Furthermore, GCMS method has the advantage of not requiring standard of samples to be analyzed, more sensitive, can be used to identify a compound, and if there is noise in the analysis, it will not complicate the reading of analysis results (Sumarno, 1995).

This research was designed to determine the ability of Fourier transform infrared spectroscopy (FTIR) and Gas Chromatography Mass Spectrometry (GCMS) for halal authentication in chocolate products especially imported chocolate products circulating in the market.

2. Material and Methods

2.1. Preparation of Lard

Pig adipose tissues were filleted and were then roasted at 90-100 ° C for ± 6 hours. Na_2SO_4 was added to the melted fat and centrifuged at the speed of 3000 rpm for 20 minutes. Then the oil layer was taken and stirred back and centrifuged and subsequently filtered with Whatman filter paper.

2.2. Extraction of Comparative Chocolate and Samples

A total of 100 g of halal-certified chocolate and each 25 g of chocolate samples were extracted by Soxhlet tool using n-hexane solvent for 6 hours. The extracts obtained were later evaporated using a rotary evaporator, then the resultant fat stored in flacon.

2.3. Fat Analysis Using FTIR

Concentration series of 0-10% in lard and fat from each sample were dropped on the ATR crystal placed in a controlled temperature (20°C) as much as 1 drop. Then they were scanned for 32 times the wave number 4000-650 cm^{-1} with a resolution of 4 cm^{-1} and were recorded in the form of absorbance. FTIR spectra were analyzed using kemometric in the form of PLS and PCA form using Horizon MB software.

2.4. Fatty Acid Analysis using GCMS

Sodium methoxide was added into the lard, and then heated in a water bath at 70ºC for 15 minutes and was stirred every 3 minutes. boron tri fluoride methanolic at 20% was added once it became cold. It was then heated in a water bath at 70ºC for 15 minutes and was stirred every 3 minutes. It was cooled back and n-heptane and saturated NaCl, vortex was added for while, subsequently it would form two layers, the top layer was taken and injected into GCMS system.

3. Results and Discussions

3.1 Lard and Chocolate Profile in FTIR Analysis Result

FTIR analysis was carried out based on the differences between the functional groups of lard and chocolate fat measured at wave number 4000-650 cm^{-1}. FTIR spectra of lard has a specific area that does not appear in the FTIR spectrum of other fats, the specific area that is typical of a relatively high peak at wave number 3000-3010 cm^{-1}, then at 1120-1095 cm^{-1}, lard shows an overlapping of the two peaks with maximum absorbance at number 1118 and 1098 cm^{-1}. The third point of difference is in the region 966-967cm^{-1} ([b]Rohman & Che Man, 2010).

FTIR spectra of lard and chocolate (Figure 1) when visually observed would look similar, but still there

are differences in the intensity of the bands produced as well as the maximum absorbance frequencies which were different from one another. It was caused by the difference between the fatty acid composition of lard and chocolate fat. The difference in peak intensity lard of FTIR analysis result shows the kind of molecular vibration of lard that does not appear in chocolate fat (Table 1).

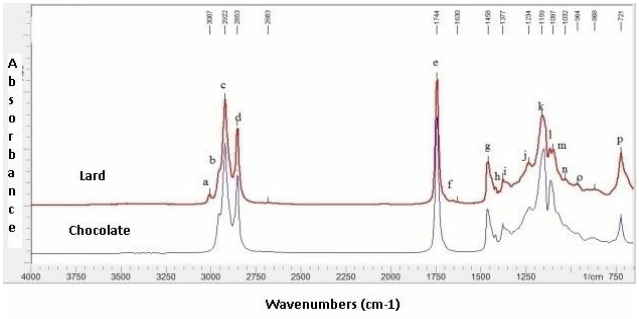

Figure 1. FTIR spectra of lard and chocolate at wave number 4000-650 cm^{-1}. Typical areas of swine in the spectra appear at point A (3006,8 cm^{-1}); point l (1118,84 cm^{-1}); point m (1097,42 cm^{-1}) and point o (964,34 cm^{-1}).

Chemometric analysis of FTIR spectra on the Principle Component Analysis (PCA) on the wave number 999.053 to 1190.638 cm^{-1} in lard 100%, 50% in chocolate fat and chocolate fat 100% respectively were replicated 5 times indicating grouping formed, i.e. each series of concentration forming a group and separately from other groups. Analysis with Partial Least Square (PLS) shows PLS calibration curve formed by making a mixture of lard in chocolate fat with serial concentrations series of 0-100% (% v/v), a peak intensity of the typical swain looks gradually decrease with decreasing concentration. It shows that visually, FTIR spectra of lard with a low concentration in the mixture are hard to interpret (Figure 2). Determination of the smallest concentration that can still be detected shows that the detectable concentration and the value is not far from the actual value, i.e. at concentration of 4%.

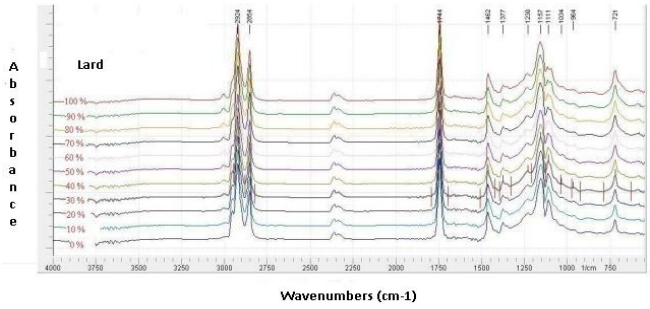

Figure 3. Spektra FTIR Concentration Series 0-100% lard in chocolate fat on Wave Numbers 4000-650 cm^{-1}.

The FTIR readings of concentration series were then analyzed using PLS calibration in the range of wave numbers 999,053 to 1190,638 cm^{-1}. This area was chosen because it produces a high R^2 value and small Root Mean Square Error of Calibration value (RMSEC), thus it shows a good calibration model which is proportional to the actual value. PLS calibration of the relationship between the actual value of the predictive value of FTIR using PLS generates equation Y = 1,000x - 0.0378, with R^2 value of 0.997 and RMSEC value of 1.563. Value (Root Mean Square Error of Prediction) RSMEP and R^2 were calculated to evaluate whether the data validation good or not.

The high value of R^2 and the low value of RMSEP indicate the calibration model to determine the lard in the chocolate fat mixture. RMSEP value result and R^2 calculation result is are 1,650 and R^2 of 0.997.

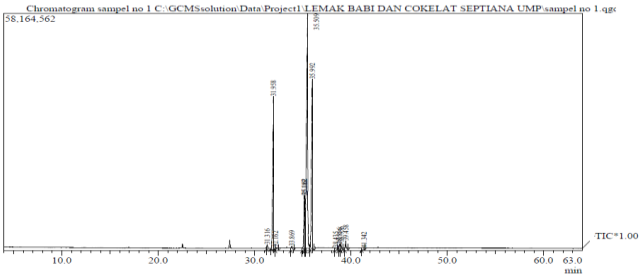

Figure 4. 100% Pork Oil Chromatogram. In both chromatograms, eikosadienoat 11.14 acid appears with a retention time of 38,850 minutes.

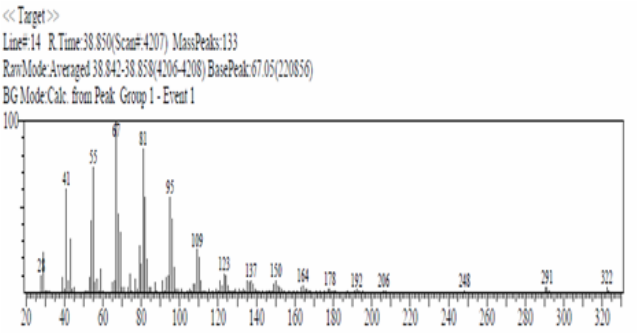

Figure 5. Eikosadienoat 11.14 Acid mass spectrum

3.2. Lard and Chocolate Profile in GCMS Analysis Results

Lard contains specific fatty acids that distinguish lard and other fats. The analysis result using GCxGC-TOF-MS in lard shows 3 fatty acids specifically of swain, i.e. acid trans-9,12,15-oktadeka trieonat (C18: 3 N3T), eikosatrienoat 11,14,17 acid (C20: 3 N3T), and eikosadienoat 11.14 acid (C20: 2 rt6) (Chin et al, 2009). Specific fatty acids in lard cause the appearance of a specific area in the FTIR spectrum. Analysis result of lard using GCMS produces a chromatogram in Figure 4.

Based on the standard chromatogram formulation, 100% pork oil shows the presence of eikosadienoat 11.14 acid compounds. Eikosadienoat 11.14 acid compounds also appear in other formulations added into lard. At a 100% lard concentration, three markers appears specifically in swine, whereas when less than 100% pork oil concentration, only eikosadienoat 11.14 acid appears as a marker. Therefore, eikosadienoat 11.14 sour is a marker that will be used in this research.

In GCMS, besides the chromatogram, mass spectrum data was also obtained. In each compound, it will have a fragmentation pattern in the different mass spectrum. Of the fragmentation pattern above, it can be ascertained that the acid compounds eikosadienoat 11, 14 evidenced by the presence of m/ z 322 which are BM from eikosadienoat 11.14 acidic compounds. Additionally, eikosadienoat 11.14 acid has the same structure with the fragments in the target compound. If the compound is incorporated, it will become eikosadienoat 11.14 acidic compounds.

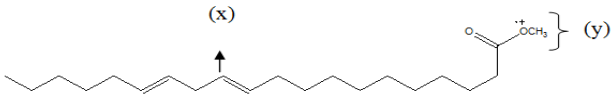

Figure 6. Eikosadienoat 11.14 Acidic Structure

The analysis result of lard in imported chocolate use GCMS with the emergence of specific pork fatty acids, i.e. eikosadienoat 11.14 acid (C20: 2 rt6) with the following chemical structure (Figure 6).

Thus, the specific peak of pork in FTIR analysis results in wave numbers 3006.8 cm^{-1} which is the stretching vibration of cis C= C is shown at point (x), the peak at wave number 1743.52 cm^{-1} which is the stretching vibration of the carbonyl group (C = O) of triglyceride ester (y), bending vibration –CH and changes of fatty acids at wave number 1118.84 and 1097.42 cm^{-1} is the vibration of the carboxylic acid group (y) in eikosadienoat 11.14 acid, meanwhile the CH bending vibration of isolated trans-olefin (isolated alkenes) at wave number 964,34 cm^{-1} is the vibration of the point (y) on the eikosadienoat 11.14 structure.

3.2 Analysis of Lard in Imported Chocolate

The spectra result of FTIR samples (Figure 7) shows the presence of specific peak for pork that appear and can be seen very clearly from all the FTIR spectra of samples in the area of wave number 3006 cm^{-1} (a), 1118 cm^{-1} (l) and 1097 cm^{-1} (m). The analysis result of the qualitative analysis using PCA and PLS at wave number 999,053 cm^{-1} -1190.638 cm^{-1} indicates that all positive samples containing lard because of input samples spots in pork area and the relatively high fat content in each sample (Table 2).

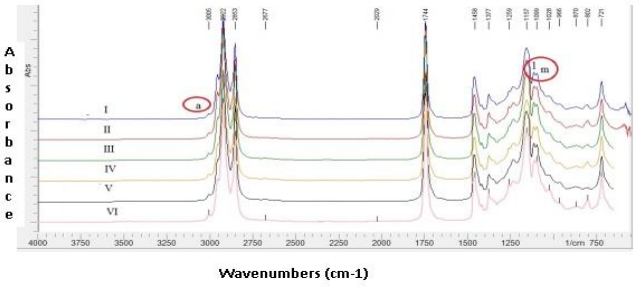

Figure 7. FTIR spectra of Imported Chocolate Samples in Wave Numbers 4000-650 cm^{-1}. The spectra results show the presence of pork-specifically peak in point (a), (l) and (m)

The analysis result with GCMS shows a positive result because of the six samples, there is compound appearing at a retention time of 38.8 minutes. In which the retention time at 38.8 minutes is the retention time of eikosadienoat 11, 14 acidic compounds, a marker of lard presence (Table 3).

Table 2. Content of Lard in Chocolate Sample of Quantitative Analysis result using PLS (% v/v) and in 25 g of sample (% v/w).

Samples	PLS analysis on Lard Content (% v/v)	Lard content in 25g samples (% v/b)
I	43,6	0,200
II	73,5	0,299
III	61,7	0,234
IV	63,0	0,241
V	37,0	0,129
VI	30,4	0,098

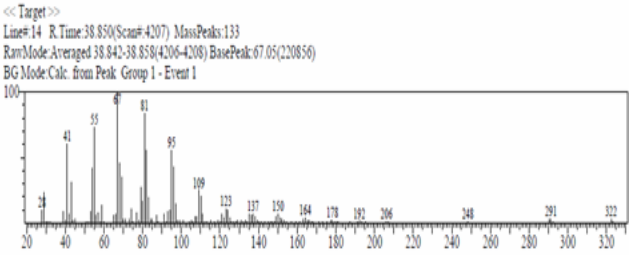

Figure 8. The fragmentation pattern of peak at a retention time at 38.8 minutes.

Table 3. Retention Time of Eikosadienoat 11.14 acids Appearing On Samples Samples

Samples	Peak Number	Retention Time (minutes)	Area (%)
I	10	38,816	0,85
II	8	38,797	0,18
III	14	38,796	0,44
IV	7	38,806	0,80
V	7	38,807	0,73
VI	10	38,811	0,28

4. Conclusions

The variant sample is of one imported chocolate brands circulating in the market which is positively containing lard. FTIR and GCMS spectroscopy methods can be used as fast and accurate methods in detecting lard content in chocolate products.

5. References

Adahchour, M., Beens, J., and Brinkman, U.A. 2008. Recent development in the application of comprehensive two-dimensional gas chromatography, Journal of Chromatography A. 1186 : 67-108.

[a]Rohman,A and Che Man. 2010. Potential of FTIR-ATR Spectroscopic Method for Determination of Virgin Coconut Oil and Extra Virgin Olive Oil in Ternary Mixture Systems. Food Anal,Method. 4 (2): 155-162.

[b]Rohman,A and Che Man. 2010. FTIR Spectroscopy Combined With Chemometrics For Analysis Of Lard In The Mixtures With Body Fats Of Lamb, Cow, And Chicken. International Food Research Journal 17: 519-526.

Che Man, Y.B and Sazili, A. Q. 2010, Food production from the halal perspective. In : Isabel Guerrero-Legarreta and YH Hui (Ed.), Handbook of Poultry Science and Technology. Volume 1, Primary processing, Wiley, New york, USA, pp.183-215.

Che Man, Y.B. *et al.,* 2005. Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy, Food Chemistry 90: 815–819.

Che Man, YB., Z.A. Syahariza, M.E.S.Mirghani, S. Jinap and J. Bakar. 2005. Analysis of Potential Lard Adulteration in Chocolate and Chocolate Products Using Fourier Transform Infrared Spectroscopy. Food Chemistry 90: 815–819.

Chin, Che Man, Tan, and Hashim, 2009. Rapid Profiling of Animal-Derived Fatty Acids using Fast GC 3 GC Coupled to Time-of-Flight Mass Spectrometry. Journal of the American Oil Chemist'Society 86: 949-958.

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Danar, 2011. Beware! Haram Food encircled us. http: //penapenakecil. wordpress.com, (12 September 2012). (In Indonesian)

Douglas *et al*., 1998. Principles of Instrumental Analysis Fifth edition. Philadelphia: Saunders Golden Sunburst Series.

Gandjar, I.G., & Rohman, A., 2007, Pharmaceutical Analysis, Yogyakarta, *Yogyakarta*, Pustaka Pelajar. (In Indonesian)

Guille and Nerea Cabo,1997. Infrared Spectroscopy in the Study of Edible Oils and Fats. J Sci Food Agric, 75: 1È11

Hermanto, S dan Anna Muawanah. 2008. Profile and Characteristics of Animal Fat (Chicken, Beef, Pork).The results of FTIR and GCMS analysis. Jakarta: Universitas Islam Negeri Syarif Hidayatullah. (In Indonesian)

Indrasti, D., Che Man, Y.B., Mustafa, S. and Hashim, D.M. 2010. Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry, Food Chemistry 122: 1273-1277.

Johnson, S, Salkia, N. 2009, Fatty acids profile of Edible Oils and Fats in India, Centre For Science and Environment, New Delhi.

Langkong,J., Elly Ishak, Maryati Bilang dan Junaedi Muhidong. 2012. Mapping of Seed Fat from cacao grain (Theobroma cocoa L) in South Sulawesi. (In Indonesian)

Marikkar, J.M.N., Ghazali, H.M., Che Man, Y.B., Peiris, T.S.G. and Lai, O.M. 2005. Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. Food Chemistry. 91: 5–14.

Miller and Miller, 2005. Statistics and Chemometrics for Analytical Chemistry Fifth edition. Harlow: Pearson Education.

Riaz, M. N., & Chaudry, M.M., 2004, Halal food production, New York, CRC Press .

Richard, A and Brian Wailes. 2012. Estimation of Fat-Free Cocoa solids in Chocolate and Cocoa Products-Global Survey of Typical Concentration of Theobromine and Caffeine determination by HPLC. Journal of the association of Public Analysis. 40: 01-12.

Rohman, A. 2009. Monitoring the Presence of Lard in Virgin Coconut Oil (VCO) using Fourier Transform Infrared (FTIR) Spectroscopy for Halal Authentication Study. Halal Products Research Institute, University Putra Malaysia.

Rohman, A., 2011, Analysis of Food Ingredients for Practice Approach, Yogyakarta, Pustaka Pelajar. (In Indonesian)

Rohman, A., Che Man, Ismail, and Puriziah. 2011. FTIR Spectroscopy Combined With Multivariate Calibration for Analysis of Cod Liver Oil in Binary Mixture with Corn Oil. International Food Research Journal. 18: 757-761.

Rohman, A., Triyana, K., Sismindari and Erwanto,Y., 2012. Differentiation of Lard and Other animal Fats Based on Triacyglycerols composition and Principal component analysis. International Food Research Journal. 19(2): 475-479

Rohman, A., 2011. Analysis of Food Ingredients for Practice Approach. Yogyakarta: Pustaka Pelajar. (In Indonesian).

Wan, S., Che Man, Y.B., Ismail, A., and Hasyim, P. 2009. Fourier Transform Infrared (FTIR) Spectroscopy Differentiation of Lard and Other Shortening in Puff Pastry. Halal Products Research Institute, University Putra Malaysia.

Smith, B.C., 1996. Fundamental of Fourier Transform Infrared Spectroscopy. New York : CRC Press.

Stuart, B. 2004. Infrared Spectroscopy : Fundamentals and Application. John Wiley & Sons,Ltd. New York.

Varmuza, 2002. Applied Chemometrics: From Chemical Data To Relevant Information. 1st Converence on Chemistry. Cairo, Egypt.

Vlachos, N., Y. Skopelitis, M. Psaroudaki, V. Konstantinidou, A. Chatzilazarou, E. Tegou. 2006. Applications of Fourier Transform-Infrared Spectroscopy to Edible Oils. Analytica Chimica Acta. 573–574: 459–465.