# APPLICATION OF HIGH-PRESSURE LIQUID CHROMATOGRAPHY FOR ANALYSIS OF LARD IN THE MEATBALL PRODUCT COMBINED WITH PRINCIPAL COMPONENT ANALYSIS

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## ABSTRACT

**Objective:** The aim of this research to design the discriminant method of pork and beef meatball using high-pressure liquid chromatography-ultraviolet (HPLC-UV) combined with principal component analysis.

**Methods:** The 100% pork and 100% beef meatball was analyzed using HPLC-UV. The all chromatogram data from HPLC were processed using chemometrics method especially principal component analysis for discriminant the pork and beef meatball.

**Results:** The results showed that the beef and pork meatball can be distinguished using HPLC which was combined principal component analysis. However, the application of HPLC has a problem for lard detection in meatball because cannot to control the hydrolysis of triglyceride (TGA). Hydrolysis of TGA can also be detected using HPLC-UV and giving the interference for halal authentication.

**Conclusion:** The discriminant process of pork and beef meatball using HPLC-UV has a problem in TGA analysis. The TGA will be hydrolyzed to diglycerides, monoglyseride, and fatty acid form. All products from hydrolysis of TGA will give the interference in the discriminant process.

**Keywords:** High-pressure liquid chromatography, Principal component analysis, Halal authentication, Hydrolysis of triglyceride.

## INTRODUCTION

Development of an analysis in the detection process of the presence of lard is something very important for Moeslems. This is one of the consumer's rights to get a good and halal product. Besides, the condition global for marketing the halal food has the improvement and increased by 12.6% in 2004 to 2010 years [1]. Therefore, halal products ware not only interesting for moeslem people but nonmoeslem also supports to development the halal food. Fadzlillah *et al*. [2] reported that the use of lard in food because it is cheaper and easily. In general, the non-halal products from pig derivatives example pork, lard, and gelatin from pigs.

Recently, the developing methods for analyze the lard in the foods using Fourier transform infrared (FTIR) [3]. Rohman and Che Man [4] was successfully to classify the cream containing lard using FTIR method combined with partial least square. The halal authentication process can also be analyzed using PCR instrument [5,6]. Meanwhile, Mottram *et al*. [7] analyzed the fatty acid by high pressure liquid chromatography (HPLC). Thus, the development of the analysis method is very important. Mursyidi [8] reported that development of analytical methods for the authentication process and halal products because it has the complexity of products.

The analysis of fat or oil can be determined from the triglyceride (TGA) and fatty acids composition. Determination of TGA in the sample can be performed using instruments HPLC [9-11]. Whereas the determination of fatty acid content can be analyzed using the instrument TLC [12], GC-MS [13-15]. Therefore, this study was focused on lard detection in meatball product using HPLC. The detection and authentication process of lard using HPLC combined with chemometrics for lard analysis in meatball products.

## METHODS

### Extraction process of TGA from beef meatball and pork meatball products[16]
About 200 g meatball samples ware extracted with n-hexane using Soxhlet for 6 hrs. The result of extract then added with $Na_2SO_4$, then filtered and evaporated to obtain the oil. The oil is stored in a temperature of 20°C.

### Analysis of TGA on beef and pork meatball
About 10 mg fat from meatball samples and are placed on the tube then are homogenized using a vortex. After that, the solution is diluted in a 50 ml flask with methanol. Finally, the solution was analyzed by HPLC (Shimadzu, LC 20 AT, Jepang) at a wavelength of 203 nm using a mobile phase composition of acetonitrile:methanol = 9: 1 and 8: 2.

### Data analysis
The analysis process for authentication halal was performed with processing the data of chromatogram of TGA from HPLC using chemometrics, especially principal component analysis (PCA) using software The Unscrambler 10 (Camo software).

## RESULT AND DISCUSSION

The detection process of lard contamination in beef meatball products using HPLC from fingerprints data which were resulted from chromatogram of HPLC. The result of chromatogram is influenced by various factors. The ionic species can influence the retention time on HPLC. Rosés *et al*. [17] reported that retention time on HPLC was influenced some factors the ionic species from properties mobile phase such as pH, ionic strength, and composition of the mobile phase. Therefore, this study was focused on the influence of mobile phase composition of acetonitrile:methanol = 90:10 and 80:20, respectively,

in the TGA analysis and discriminant analysis in the beef and pork meatball products. Rohman *et al.* [18] showed that the TGA composition of pork more than beef which the main TGA are palmitooleoolein (POO), palmitooleostearin (POS), and palmitooleopalmitin (POP) with high levels of 21.55±0.08%, 14.08±0.04%, and 5.10±0.04%. The same result was reported by Scrimgeour [19] that the TGA composition the largest of pork are POO, POS, OOO, POP and POL with content are 18 %, 13%, 12%, 8% and 7%, respectively.

Halal authentication in this study was focused on the TGA composition in the meatball. The discriminant analysis using PCA to classify the beef and pork meatball base on TGA compotision. TGA analysis results in a 100 % beef and 100% pork meatball clearly distinguishable with a maximum difference (Fig. 1).

The mobile phase composition of acetonitrile: methanol = 9:1 gives good result in discriminant process. Besides, this research also analyze for the mobile phase composition of acetonitrile:methanol = 8:2. The results of data processing using PCA showed that the use of mobile

phase composition acetonitrile:methanol = 8:2 may also be used in the discriminant process (Fig. 2). The results showed that the mobile phase composition of acetonitrile:methanol = 8:2 has a score plot better than the mobile phase composition acetonitrile:methanol = 9:1. It was seen by far distances which show the difference has increasing. The same evidence also was seen the result from residual variances using mobile phase composition of acetonitrile:methanol = 8:2 smaller than the mobile phase composition of acetonitrile:methanol = 9:1 (Figs. 3 and 4). The result of Figs. 3 and 4 showed that the beef meatball has different characteristics from pork meatball because beef meatball has different colors if was compared by pork meatball.

This study shows that halal authentication process should be done in the process with the same condition. The same conditions will indicate that the discriminant happens if differences in the chemical composition of TGA in the Meatball. The chemometrics analysis also showed that the mobile phase composition in TGA analysis of meatball showed a difference quadrant between pork and beef meatball (Figs. 1 and 2).
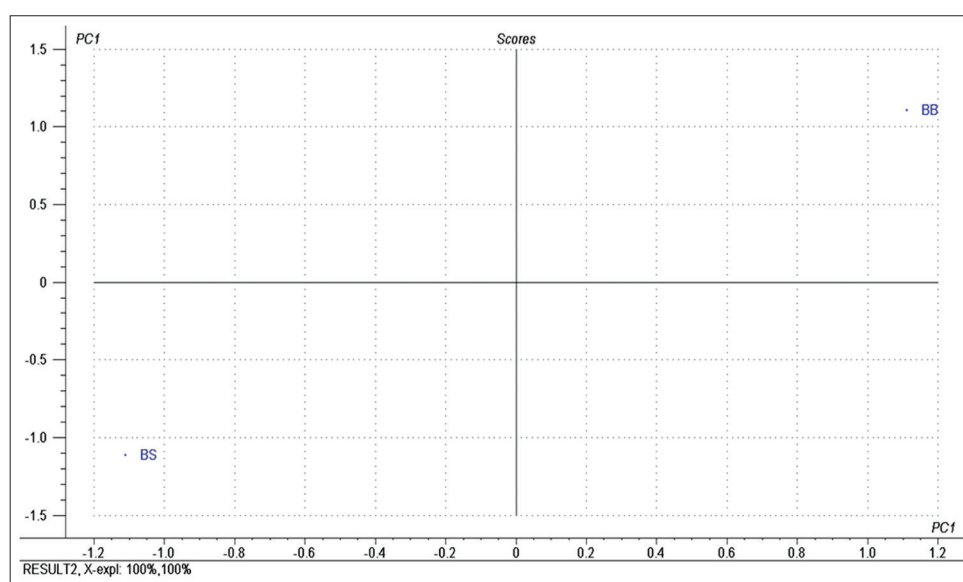


**Fig. 1: Results of PCA analysis of chromatograms the TGA from beef meatball (BS) and pork meatball (BB) were analyzed using HPLC UV with the mobile phase composition acetonitrile:methanol = 9:1**
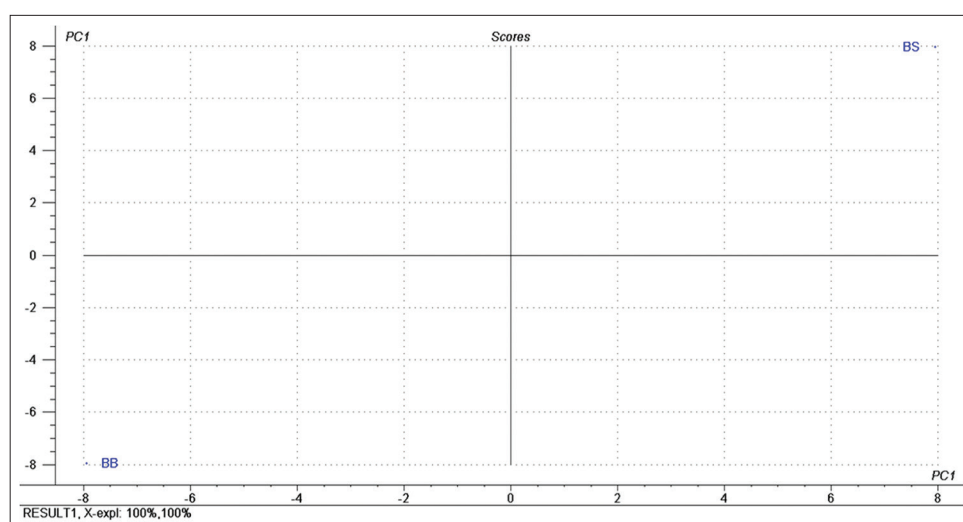


**Fig. 2: Results of PCA analysis of chromatograms the TGA from beef meatball (BS) and pork meatball (BB) were analyzed using HPLC with mobile phase composition of acetonitrile:methanol = 8:2**
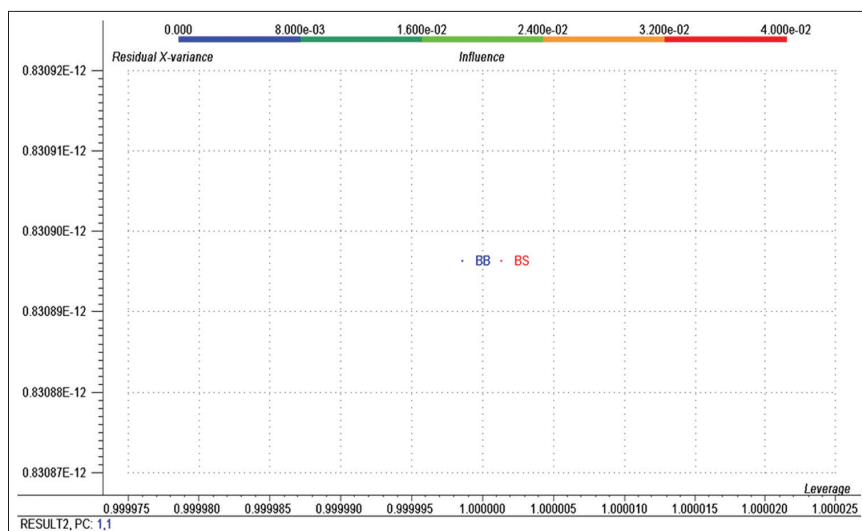
**Fig. 3: Results of residual analysis of chromatograms the TGA from beef meatball (BS) and pork meatball (BB) were analyzed using HPLC UV with the mobile phase composition acetonitrile:methanol= 9:1**
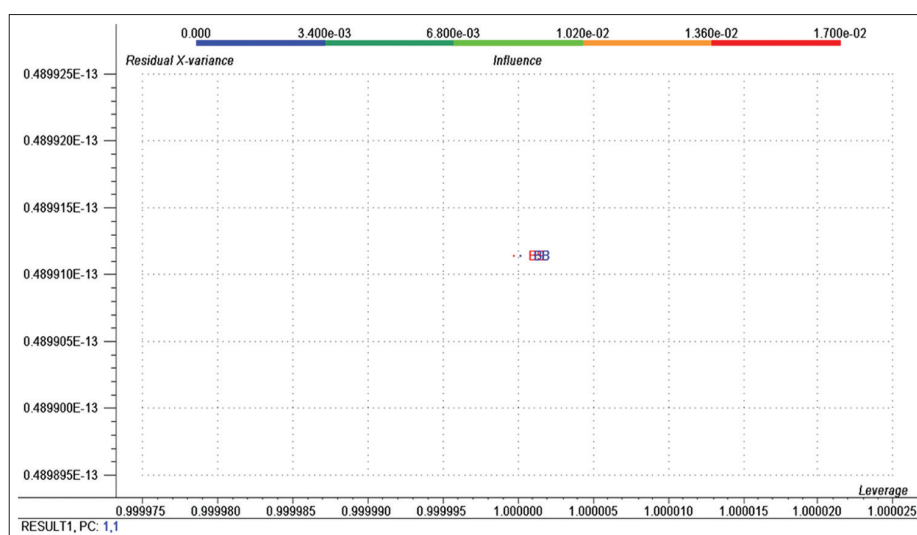


**Fig. 4: Results of residual analysis of chromatograms the TGA from beef meatball (BS) and pork meatball (BB) were analyzed using HPLC with mobile phase composition of acetonitrile:methanol = 8:2**
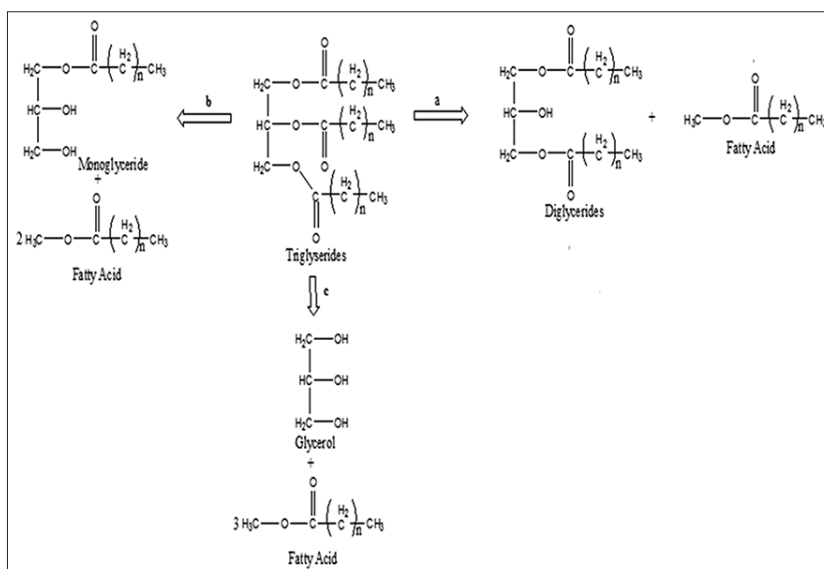


**Fig. 5: The Pathway of triglyceride hydrolysis process. (a) 1st Pathway, (b) 2nd pathway and, (c) 3rd pathway**

This problem can occur due to hydrolysis of TGA. TGA will be hydrolyzed into fatty acids and glycerol form. The hydrolysis process also makes to be another form such as monoglycerides diglycerides from the meatball product (Fig. 5). This result showed that the analysis of halal authentication should be performed under controlled conditions. Hence, that is a problem in the analysis of pork meatball because the sample cannot be controlled. Many product hydrolyses will detect in analysis TGA using HPLC-UV. This result was reported by Carvalho *et al.*[20] that the HPLC-UV can analyze the methyl ester, free fatty acid, monoglycerides, diglycerides, and TGA with different retention times. Hence, this is evidence to controlling the process of making the meatball.

**CONCLUSION**

The influence of mobile phase composition of acetonitrile: methanol = 9:1 and 8:2 caused the difference in the resulting chromatograms of HPLC. The results showed that the chromatogram from beef and pork meatball have different which it's characterized for TGA composition. The results of discriminant showed that beef and pork meatball can be distinguishing using PCA. However, this detection for sample and discriminant process has a problem because we can't control the hydrolysis of TGA during the process of making the meatball.

**ACKNOWLEDGMENTS**

**REFERENCES**

1. Berry B. Global Halal Food Market. Canada: Agriculture and Agri-Food Canada; 2011.
2. Fadzlillah NA, Che Man YB, Jamaludin MA. Halal Food Issue from Islamic and Modern Science Prespective. 2nd International Conference on Humanities, Historical and Social Sciences. Vol. 17. Singapore: IACSIT Press; 2011. p. 159-63.
3. Kurniawati E, Rohman A, Triyana K. Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics. Meat Sci 2014;96(1):94-8.
4. Rohman A, Che Man YB. Analysis of lard in cream cosmetics formulations using FT-IR spectroscopy and chemometrics. Middle East J Sci Res 2011;7(5):726-32.
5. Jonker KM, Tilburg JJ, Hagele GH, de Boer E. Species identification in meat products using real-time PCR. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008;25(5):527-33.
6. Sakalar E, Abasiyanik MF. Qualitative analysis of meat and meat products by multiplex polymerase chain reaction (PCR) technique. Afr J Biotechnol 2013;10(46):9379-86.
7. Mottram HR, Woodbury SE, Richard P. Evershed identification of triacylglycerol positional isomers present in vegetable oils by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 1997;11:1240-52.
8. Mursyidi A. The role of chemical analysis in the halal authentication of food and pharmaceutical products. J Food Pharm Sci 2013;1:1-4.
9. Nájera AI, Perea S, Barcina Y, de Renobales M, Barron LJ. A reverse-phase high-performance liquid chromatographic method for analyzing complex mixtures of triglycerides. Application to the fat fraction of an aged cheese. JAOCS 1999;76(3):399-407.
10. Gutierrez VR, Perona JS. Liquid Chromatography: Trigliseride, *in situ* to de la Grasa (CSIC). Spain: Academic Press; 2000.
11. Bullock S. High Resolution Analysis of Triglycerides in Vegetable Oils by HPLC with ELSD. Application Note SI-01231/TB1066; 2010.
12. Barma KS, Goswami BC. Identification and estimation of fatty acids in fresh water fish *Anabus testudineous*. Ann Biol Res 2013;4(11):18-24.
13. Ismiyarto, Halim SA, Wibawa PJ. Identification of fatty acid compotition in turi seed oil (*Sesbania grandiflora* (L) Pers). JSKA 2006;IX(1):1-3.
14. de Almeida JC, Perassolo MS, Camargo JL, Bragagnolo N, Gross JL. Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. Braz J Pharm Sci 2006;42(1):109-17.
15. El-Ghorab AH, El-Massry KF, El-Hadadd AF, Shedied SA, Shaheen MS, Gad AM. Chemical composition and antiradicals activity of the volatile compounds from reaction of cysteine/ribose and beef fat. World J Dairy Food Sci 2009;4(2):164-75.
16. Ahda M, Guntarti A, Kusbandari A, 2016, The discriminant method between lard, virgin coconut oil (VCO), chicken and beef fat based on triglycerides composition using HPLC-UV. JOCPR 2016;8(5):555-8.
17. Rosés M, Canals I, Allemann H, Siigur K, Bosch E. Retention of ionizable compounds on HPLC 2. Effect of pH, ionic strength, and mobile phase composition on the retention of weak acids. Anal Chem 1996;68(23):4094-100.
18. Rohman A, Triyana K, Sismindari, Erwanto Y. Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis. Int Food Res J 2012;19(2):475-9.
19. Scrimgeour C. In: Shahidi F, editor. Bailey's Industrial Oil and Fat Products: Chemistry of Fatty Acids. Vol. 6. New York: John Wiley & Sons, Inc.; 2005.
20. Carvalho MS, Mendonça MA, Pinho DM, Resckc IS, Suarez PA. Chromatographic analyses of fatty acid methyl esters by HPLC-UV and GC-FID. J Braz Chem Soc 2012;23(4):763-9.