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Untargeted lipidomics approach using LC-Orbitrap HRMS to discriminate lard from beef tallow and chicken fat for the authentification of halal

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SUMMARY: This research aimed to perform a lipidomics study using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to identify lard, beef tallow and chicken fat. A total of 292, 345, and 403 lipid compounds were observed in lard, beef tallow, and chicken fat, respectively. The lipid groups of AcHexStE (acyl hexosyl stigmasterol ester), biotinylPE (biotinylphosphoetanolamine), LPC (lysophosphatidylcholine), MePC (monoetherphosphatidylcholine), PC (phosphatidylcholine) and PI (phosphoinocitol) were found to be specific for lard. The principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) successfully differentiated lard from beef tallow and chicken fat. This research suggested that the untargeted lipidomics technique using LC-HRMS combined with chemometrics could be used to discriminate lard from beef tallow and chicken fat. This method is a promising technique for the detection of lard adulteration in beef tallow and chicken fat for halal authentication purposes.

KEYWORDS: *Chemometrics; Halal Authentication; Lard; LC-HRMS; Metabolomics; Untargeted*

RESUMEN: ***Enfoque de lipidómica no dirigida utilizando LC-Orbitrap HRMS para discriminar manteca de cerdo, sebo de res y grasa de pollo para la autenticación halal.*** Esta investigación tuvo como objetivo realizar un estudio de lipidómica utilizando cromatografía líquida-espectrometría de masas de alta resolución (LC-HRMS) para discriminar manteca de cerdo, sebo de res y grasa de pollo. Se pudo observar un total de 292, 345 y 403 compuestos lipídicos en manteca de cerdo, sebo de res y grasa de pollo, respectivamente. Se encontró que los grupos lipídicos de AcHexStE (éster de acil hexosil estigmasterol), biotinilPE (biotinilfosfoetanolamina), LPC (lisofosfatidilcolina), MePC (monoéterfosfatidilcolina), PC (fosfatidilcolina) y PI (fosfoinocitol) son específicos para la manteca de cerdo. El análisis de componentes principales (PCA) y el análisis discriminante de mínimos cuadrados parciales (PLS-DA) diferenciaron con éxito la manteca de cerdo del sebo de res y la grasa de pollo. Esta investigación sugirió que la técnica de lipidómica no dirigida que usa LC-HRMS combinada con quimiometría podría usarse para discriminar la manteca de cerdo del sebo de res y la grasa de pollo. Este método es una técnica prometedora para la detección de la adulteración de manteca de cerdo en sebo de res y grasa de pollo con fines de autenticación halal.

PALABRAS CLAVE: *Autenticación Halal; LC-HRMS; Manteca de cerdo; Metabolómica no dirigida; Quimiometría.*

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1. INTRODUCTION

Food authentication has become the main important issue in the world recently because it is associated with many aspects of food, such as quality, safety, and the halal status of food products (Balkir *et al*., 2021). The adulteration and mislabelling of food products are often carried out in high quality food products by unethical players. The main reason for such adulteration is related to the aim of obtaining higher profits (Danezis *et al*., 2016). Edible fat is one of the food products which is prone to adulteration and mislabelling because it is easy to mix a fat with other types of fats. High quality fats such as beef tallow and chicken fats have many functions in various food products, for instance to obtain a desired flavour, typically in breads, baked goods, meat products, and many more. Thus, it is susceptible to adulteration with lower quality fats such as lard (Lee *et al*., 2018). Lard, a type of fat obtained from pork, is known as the cheapest fats. It spreads widely in the markets and has been utilized in numerous food products (Taylan *et al*., 2020). However, the consumption of lard is prohibited by certain religions such as Muslim and Jewish (Hossain *et al*., 2020). Lard is categorized as containing non-halal lipids which are not allowed to be consumed according to Shariah law. Differentiating lard from beef tallow and chicken fat is obviously difficult due to their similar appearance and characteristics (Rohman and Windarsih, 2020). Thus, analytical methods capable of discriminating lard from other fats such as beef tallow and chicken fat are required.

Various analytical techniques have been developed and validated for the analysis of fats including spectroscopy and chromatography, especially in combination with multivariate data analysis or chemometrics (Valdés *et al*., 2018). Gas chromatography using a flame ionization detector (GC-FID) and mass spectrometer (GC-MS) have evolved as the most common methods used for fat analysis (Guntarti *et al*., 2020). Both GC-FID and GC-MS analyse fats through the fatty acid compositions. GC-FID has been used for the analysis of lard, chicken fat, and beef tallow based on their fatty acid compositions. The results showed that the fatty acid of *cis* C18: 2 become the major fatty acid found in lard (Dahimi *et al*., 2014). Apart from GC-FID, GC-MS has been more widely utilized in the analysis of fats due to its high specificity and sensitivity. GC-MS combined with PCA has been used for the analysis of dog fats in beef meatballs (Guntarti, 2018). However, GC-based methods require complex preparation steps including the derivatization of fatty acids, which becomes time consuming. Vibrational spectroscopy such as Fourier transform infrared (FTIR) spectroscopy has been widely used for the análisis of fats and oils. FTIR spectroscopy is known as the most rapid screening method for the analysis of fats and oils (Li *et al*., 2019). Combined with chemometrics, FTIR spectroscopy has been successfully used to identify, differentiate, and classify fat samples (Jiménez-Sotelo *et al*., 2016; Jamwal *et al*., 2021). However, FTIR spectroscopy is not a confirmatory method, and could not be used to identify unknown samples.

The emerging of omics-based techniques such as metabolomics, proteomics, genomics, and transcriptomics have boosted research in food authentication (Böhme *et al*., 2019). Metabolomics is the comprehensive study of metabolites, including amino acids, lipids, organic acids, nucleosides, phenolic compounds, alkaloids, flavonoids, sugars and many more in biological samples under particular conditions (Castro-Puyana *et al*., 2017). Lipidomics, a subsection of metabolomics, focuses on the study of lipid metabolites. Lipidomics provides a comprehensive lipid analysis to identify as many lipid compounds as possible in food samples (Sun *et al*., 2020). Untargeted lipidomics has advantages in the global screening of lipids in samples. Therefore, we can identify a global lipid overview in samples. It does not only analyzing one or few lipids as in a targeted approach. Moreover, the identification of discriminating lipids can be further used as potential biomarkers to differentiate samples through chemometrics análisis. Recently, the use of untargeted lipidomics in food analysis has become more widespread due to its ability to identify lipid compositions from different types of food samples (Wu *et al*., 2021). It could be used to analyze not only fatty acids but also other types of lipids such as phospholipids, glycolipids, ceramides, sphingolipids and many more (Lee and Yokomizo, 2018; Song *et al*., 2022). Thus, it offers potential advantages for the comprehensive identification of lard, chicken fat, and beef tallow to identify the potential biomarkers of each fat.

Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) techniques are the most common methods used for lipidomic analysis due to

their applications in throughput analysis (Balkir *et al*., 2021). NMR offers minimum sample preparation, rapid analysis, and minimum use of solvent. However, it has lower sensitivity and lower resolution compared to MS-based techniques (Li *et al*., 2017). MS-based techniques coupled with a chromatography technique such as liquid chromatography offer potential advantages for lipid separation, thus enhancing the resolution, and obtaining greater lipid metabolites. (Sun *et al*., 2020). Liquid chromatography-high resolution mass spectrometry (LC-HRMS) could be used for throughput screening of metabolites including lipids with high sensitivity and high specificity. The utilization of an Orbitrap mass analyzer has advantages in resolving complex samples with high resolution due to its high resolving power (Zeki *et al*., 2020). Combinations with advanced statistical tools such as chemometrics are required to process the huge amount of data on lipids which is obtained from the measurement of LC-HRMS. Pattern recognition chemometrics such as principal component analysis (PCA), partial least square-discriminant analysis (PLS-DA), orthogonal projections to latent structures-discriminant analysis (OPLS-DA) and soft independent modelling class analogy (SIMCA) have been widely used in metabolomic and lipidomic analyses (Jia *et al*., 2022; Mi *et al*., 2018).

The lipidomic approach has been successfully used to differentiate beef and pork as well as to detect pork adulteration in beef meat. Analysis was carried out using LC-MS LTQ-Orbitrap combined with PLS-DA (Trivedi *et al*., 2016). A lipidomic study using liquid chromatography-Quadrupole time of flight mass spectrometry (LC-QTOF-MS) has also been used for the characterization and discrimination of China´s selected domestic pork. PCA and PLS-DA were successfully used to differentiate and classify different samples of China's domestic pork. One hundred variables consisted of glycerolipids, glycerophospolipids, sterol lipids, phospholipids, polyketides, fatty acids and prenol lipids were found as potential biomarkers to differentiate among samples (Mi *et al*., 2019). In addition, lipidomic analysis has been applied for the analysis of phospholipids in Tan sheep meat subjected to thermal processing. The quantification of ninety lipids from six subclasses, namely ceramide, triacylglycerol, phosphatidylcholine, lysophosphatidylcholine, phosphatidyletanolamine and sphingomyelin, was performed with Tan sheep meat with and without thermal processing (Jia *et al*., 2021).

To the best of our knowledge, studies on the discrimination of non-halal fats such as lard from beef tallow and chicken fat using a lipidomic approach employing LC-Orbitrap HRMS are still limited. Therefore, the aim of this research was to develop an untargeted lipidomic approach using LC-Orbitrap HRMS and chemometrics to identify lipid compositions for the discrimination of lard, beef tallow, and chicken meat as well as to detect lard adulteration in beef tallow and chicken fat based on their lipid compositions.

2. MATERIALS AND METHODS

2.1. Materials

Methanol, acetonitrile, water and isopropanol were all LC-MS grade and obtained from Thermo Fisher Scientific (Fairlawn, NJ, USA). Ammonium format, formic acid and HPLC-grade methanol were purchased from E. Merck (Darmstadt, Germany). A calibrant solution of Pierce LTQ Velos positive and Pierce negative was obtained from Thermo Fisher Scientific (Rockford, IL, USA).

2.2. Sample preparation

Lard, chicken fat, and beef tallow were obtained from the rendering of corresponding animals' adipose tissues according to Rohman & Che Man (2010). An amount of 20 mg fat sample was weighed and placed in a 2-mL microcentrifuge tube. Samples of pure lard, pure chicken fat, and pure beef tallow were prepared. The adulterated beef tallow and chicken fat with lard were prepared by mixing beef tallow and chicken fat with lard using a ratio of 50:50 (% w/w). It was aimed to observe the profile of beef tallow and chicken fat when the adulteration was present. The ratio (50:50) was chosen because adulteration is usually performed in high concentrations. Each sample was dissolved in 1 mL isopropanol, then vortexed for 1 min at room temperature. Subsequently, the sample was ultrasonicated at room temperature for 30 min. After sonication finished, the sample was then centrifuged at 12,000 x *g* for 10 min at 4 °C. The supernatant was collected and filtered using PTFE filter 0.22 µm and placed into a clear HPLC vial for lipidomic analysis using LC-HRMS. Each sample was prepared in three replicates.

2.3. Lipidomics analysis using LC-HRMS

The lipidomic analysis was performed using an ultra-high-performance liquid chromatography (Thermo Scientific™ Vanquish™ UHPLC binary pump) and high-resolution mass spectrometry-Orbitrap (Thermo Scientific™ Q-Exactive™ Hybrid Quadrupole-Orbitrap™ High Resolution Mass Spectrometer). The separation of analyte was carried out using an analytical column of Thermo Scientific™ Accucore™ C-18 (100 mm x 2.1 mm ID x 2.6 µm). Analysis was performed according to Jia *et al*. (2022) with modifications. Lipidomic analysis was performed using a mobile phase of water:acetonitrile (40:60 v/v) containing 40 mM ammonium format and 0.1% formic acid as the mobile phase A and isopropanol:acetonitrile (90:10 v/v) containing 40 mM ammonium format and 0.1% formic acid as the mobile phase B. The gradient mode was applied as follows: initially, the mobile phase B was set at 32% B for 1.5 min, then increased to 45% B until reaching a minimum of 4.0. After that, it was increased to 54% B (4.01-5.0 min), 58% B (5.01-8.0 min), 66% B (8.01-11 min), 70% B (11.01-14.00 min), 75% B (14.01-18.00 min), 97% B (18.01-21.00 min), then held at 97% B for 25 min. At the end, the process was returned to its initial condition (32% B) for 25.01–30.00 min. The flow rate of the mobile phase was 0.260 mL/min with a sample injection volume of 5 µL. The temperature of the sampler was set at 25 °C, while the column temperature was maintained at 40 °C. The mass spectrometry condition for untargeted lipidomic screening was carried out using full MS/dd-MS2 acquisition mode. Lipid analysis was performed both in positive and negative ionization modes. The sheath gas flow rate, auxiliary gas flow rate, and sweep gas flow rate applied in this research were set at 32, 8, and 4 arbitrary unit (AU), respectively. The electrospray ionization used spray voltage of 3.30 kV with capillary temperature set at 320 °C. The auxiliary gas heater temperature was set at 30 °C. The analysis was performed using a scan range of 100-1500 m/z and a resolution of 70,000 for full MS and 17,500 for dd-MS2. The mass spectrometer instrument was weekly calibrated using Thermo Scientific Pierce ESI calibration solutions both in positive and negative modes to warrant the mass accuracy.

2.4. Data processing and identification of lipids

The raw data of the total ion chromatogram (TIC) obtained from the LC-HRMS measurement both in positive and negative ionization modes were analyzed using Lipid Search 4.2 software (Thermo Scientific, USA) for peak alignment, baseline correction, background correction, retention time alignment (0.2 min tolerance) and mass tolerance (5 ppm). The identification of the lipid compositions was compared to the predicted in silico spectra from various lipid compounds. The results of lipid metabolomes were classified according to their lipid groups and lipid ions. Data were filtered using RSD (relative standard deviation) < 20 and S/N ratio > 10. The molecules with RSD at more than 30% and missing values exceding 50% were deleted.

2.5. Chemometrics analysis

Chemometrics was carried out using variables of lipid ions and the relative areas. Analysis was performed using SIMCA 14.0 software (Umetrics, Sweden). Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were used in this study. The PCA model was evaluated using PCA score plot, R^2 value and Q^2 value. In addition, the PLS-DA model was evaluated using PLS-DA score plot, R^2X, R^2Y, and Q^2 values. The permutation test using 999 permutations and receiver operating characteristics (ROC) value were used to validate the PLS-DA model. The identification of potential biomarkers which are important for sample discrimination was performed using the variable importance for projections (VIP) value in the PLS-DA analysis. Variables with a VIP value higher than 1 were considered as discriminating metabolites which are potential for biomarkers.

3. RESULTS AND DISCUSSION

3.1. Lipid compositions of pure lard, beef tallow, and chicken fat

The physical appearance of lard, beef tallow (BT), and chicken fat (CF) are similar, thus making them vulnerable for adulteration and mislabelling. Figure 1 shows the total ion chromatogram (TIC) of lard, BT, and CF obtained from the LC-Orbitrap HRMS measurement. The TIC of those three samples were very similar, thus it is very difficult to dif-

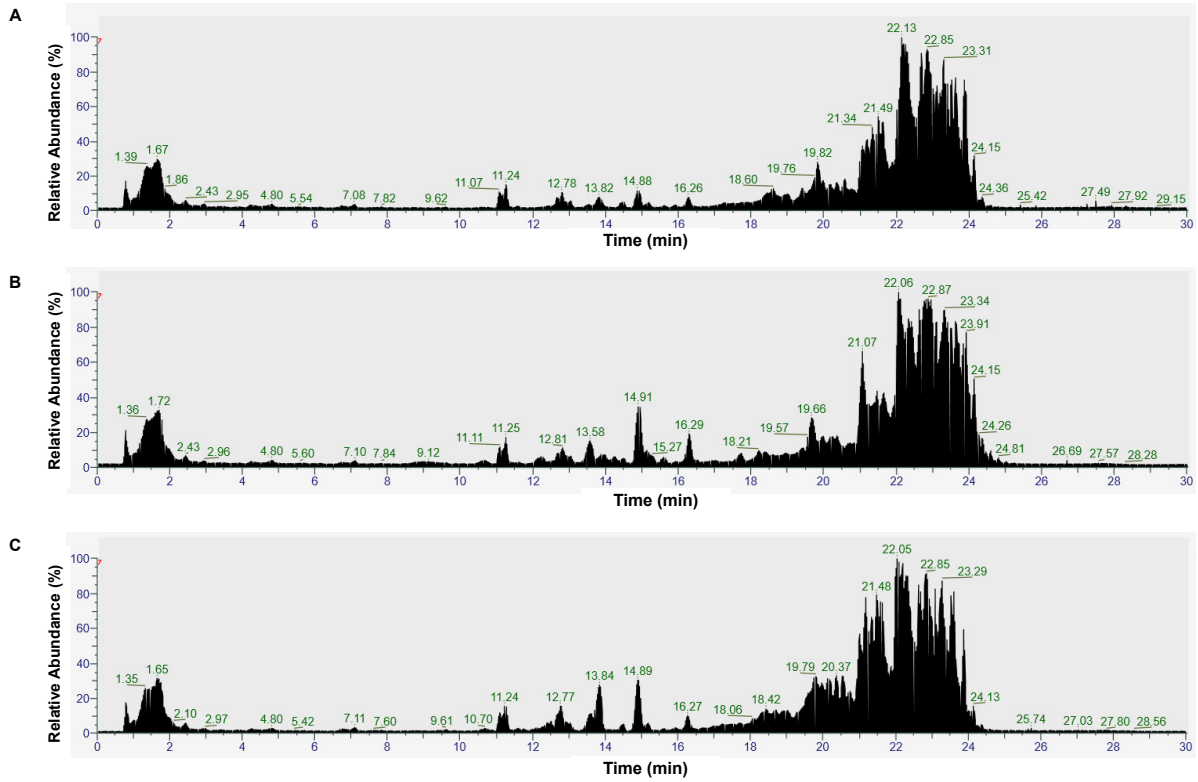

Figure 1. Total ion chromatogram (TIC) of lard (A), beef tallow (B), and chicken fat (C).

ferentiate lard, BT, and CF only by using visual observation on the TIC. The lipid compositions of lard, BT, and CF were successfully identified using Lipid Search software by extracting the raw TIC data. A total of 281 lipid ions from 18 lipid groups was obtained in lard using the positive ionization mode and 11 lipid ions from two lipid groups were observed using the negative ionization mode. The main lipid composition of lard was triglycerides (TG = 51.03%) followed by diglycerides (DG = 19.52%) and ceramides (Cer = 8.90%). BT contained 339 lipid compounds from 12 lipid groups observed in the positive ionization mode as well as 6 lipid compounds from the negative ionization mode. The mos abundant lipid compositions in BT were TG (53.33%), DG (25.80%), and cer (8.99%), respectively. In addition, the main compositions of lipids in CF were also the same as lard and BT, which were TG (58.31%), DG (25.31%), and cer (5.21%), respectively. The total lipid compounds observed in CF were 395 compounds from the positive ionization mode and 8 compounds from the negative ionization mode.

Many lipid compounds in lard, BT, and CF could be found from various lipid groups. Figure 2 illustrates the Venn diagram of the lipid metabolites contained in the three types of fats. According to the diagram, it could be observed that 117 lipids were found only in lard while 172 and 210 lipids were only found in BT, and CF, respectively. On the other hand, 82 lipid compounds were identified in all three types of fats (lard, CF and BT). Further investiga-

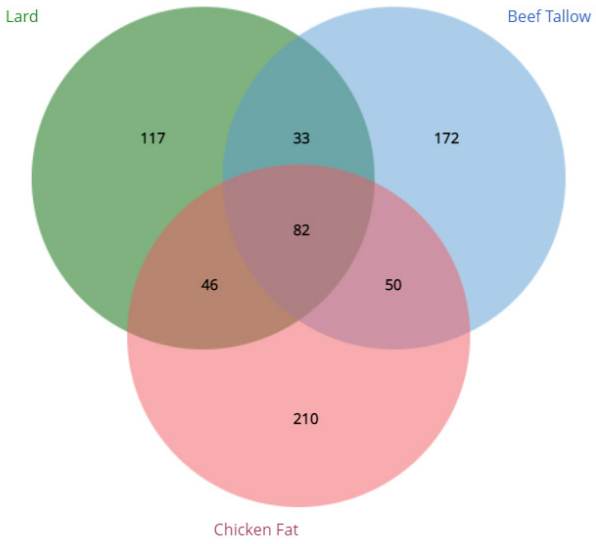

Figure 2. Venn diagram of lipid compositions in lard, beef tallow, and chicken fat

tion detected specific lipid groups only found in lard such as AcHexStE (acyl hexosyl stigmasterol ester), BiotinylPE (biotinyl phosphoetanolamine), LPC (lysophosphatidylcholine), MePC (monoether phosphatidylcholine), PC (phosphatidylcholine) and PI (phosphoinocitides). These lipid groups were absent from CF and BT. Lipid groups of SPH (sphingomyelin) and WE (wax esters) were found to be specific to CF, whereas lipid groups of MG (monoglyceride) and SiE (silyl ether) were observed only in BT. This information is very useful for the differentiation of lard, BT, and CF in order to avoid adulteration and mislabelling. The details of the specific lipid groups found in lard, CF, and BT with their lipid compounds for each group are presented in Table 1. Previous research on the discrimination of lard from other fats such as chicken fat, goat fat, and cattle fat has been performed based on fatty acid profiles using GC-TOF-MS. It was found that three fatty acid methyl esters of methyl trans-9,12,15-octadecatrienoate (C18:3 n3t), methyl 11,14,17-eicosatrienoate (C20:3 n3t) and methyl 11,14-eicosadienoate (C20:2 n6) could be used as potential discriminating lipids of lard from other animal fat samples (Indrasti *et al*., 2010). However, it is only capable of identifying fatty acids, not the comprehensive types of lipids. Another study aimed to apply different analytical approaches such as gas liquid chromatography

Table 1. Specific lipid compounds in lard, beef tallow, and chicken fat observed in untargeted lipidomics using LC-Orbitrap HRMS

Types	Lipid Groups	Compounds	Ionization mode
Lard	BiotinylPE	BiotinylPE(31:0)	Positive
	LPC	LPC(18:0)	Positive
	MePC	MePC(33:0)	Positive
		MePC(33:0e)	Positive
		MePC(33:1)	Positive
		MePC(35:0)	Positive
		MePC(35:1)	Positive
		MePC(35:2)	Positive
	PC	PC(16:0_18:1)	Negative
		PC(16:0_18:2)	Negative
		PC(18:0_18:1)	Negative
		PC(18:0_18:2)	Negative
		PC(34:2)	Positive
		PC(36:1)	Positive
		PC(36:2)	Positive
		PC(36:3)	Positive
		PC(36:4)	Positive
		PC(37:3e)	Positive
		PC(39:4e)	Positive
	PI	PI(18:0_20:4)	Positive
Beef Tallow	MG	MG(34:0)	Positive
		MG(34:1)	Positive
	SiE	SiE(28:0)	Positive
Chicken Fat	SPH	SPH(d22:1)	Positive
	WE	WE(6:0_16:3)	Positive
	LPA	LPA(15:0)	Negative

BiotinylPE = biotinyl phosphoetanolamine, LPC = lysophosphatidylcholine, MePC = monoetherglycerophosphocoline, PC = phosphatidylcholine, PI = phosphatidylinositol, MG = monoglycerides, SiE = SPH = sphingomyelin, WE = wax esters, LPA = lysophospatidic acid

(GLC), HPLC, and differential scanning calorimetry (DSC) to discriminate lard from beef tallow, mutton tallow, and chicken fat. The GLC method was not suitable for discriminating lard from the others by using overall fatty acid compositions. Triacylglycerol (TAG) análisis using HPLC showed a TAG profile for lard that differs from beef tallow and mutton tallow, but similar to chicken fat. The analysis of lard using DSC showed a different melting temperature for lard compared to other animal fats, although further analysis is still required in order to be more specific (Marikkar *et al*., 2021).

Overall, liquid chromatography-high resolution mass spectrometry using the Orbitrap mass analyzer could be used for the comprehensive identification of lipid compositions in lard, BT, and CF. Some differences in the lipid groups were detected, which is important for the differentiation of lard from BT and CF. The chemometric analysis could be used to identify the metabolite pattern, in this case lipids, to differentiate and classify lard, beef tallow, and chicken fat.

3.2. Lipidomics using LC-HRMS and chemometrics to detect lard adulteration in BT and CF

Lipidomic analysis using LC-HRMS could be used to detect the presence of lard adulteration both in BT and CF at a ratio of 50% adulteration. The TIC of adulterated CF and BT with 50% lard was still similar to samples of pure BT and pure CF (data not shown). The main composition of lipid groups in adulterated BT and CF with lard, such as triglycerides, followed by diglycerides and ceramides, was similar to pure samples. Investigations using lipid compositions showed that BT and CF adulterated with lard could be differentiated from pure BT and CF samples. The specific lipid groups in lard could be detected in adulterated imples of BT, namely LPC (lysophosphatidylcholine), MePC (monoetherglycerophosphocoline), PC (phosphatidylcholine), and PI (phosphatidylinositol). These lipid groups were absent from pure BT. Therefore, it can be used to indicate the presence of lard in BT. At the same time, in adulterated CF with 50% lard, the specific lipids of lard which were absent from CF such as biotinylPE (biotinylphosphoetanolamine), PC, LPC, MePC and DG were detected.

Figure 3A shows the Venn diagram of lipid metabolites between pure lard, pure BT and adulterated BT with 50% lard. The results showed that 99 lipid compounds were present in lard, BT and adulterated BT. 161, 119, and 85 lipid compounds were found specific to BT, lard, and adulterated BT, respectively.

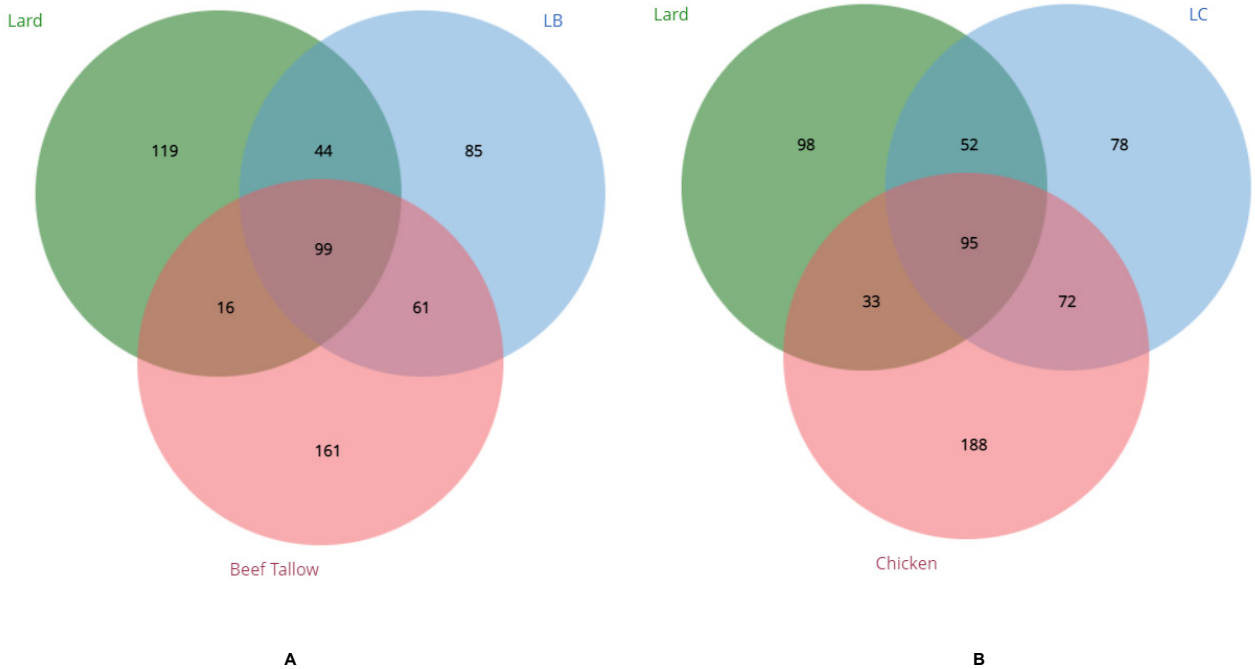

Figure 3. Venn diagrams of lipid compositions in lard, beef tallow, and beef tallow adulterated with 50% lard (LB) (A) and lard, chicken fat and chicken fat adulterated with 50% lard (LC) (B)

On the other hand, the results of the Venn diagram from pure lard, pure CF and adulterated CF with 50% lard as depicted in Figure 3B show that 98 lipid compounds were found only in lard, 188 lipids were specific to CF, and 78 lipids were observed only in adulterated CF. These lipids could be used to identify the authentication purposes of CF from lard. Meanwhile a number of 95 lipid compounds were found in lard, CF, and CF adulterated with lard.

The chemometric analysis using PCA successfully differentiated between pure samples of BT and CF and the adulterated ones using lard as shown in the PCA score plot in Figure 4A. The PCA performed with six principal components successfully differentiated adulterated samples from pure samples with R^2 = 0.999 and Q^2 = 0.996. A high R^2 value indicated high model accuracy, whereas a high value for Q^2 (> 0.500) showed good model predictability. (Bevilacqua *et al*., 2017). All adulterated samples of BT and CF with 50% lard appeared around the score plot for lard. PLS-DA using three components was successfully used for discrimination and classification between pure and adulterated samples of BT and CF with lard as depicted in the PLS-DA score plot in Figure 3B. The goodness of fit of the PLS-DA model was shown by R^2X (0.706) and R^2Y (0.988) values. Meanwhile, the Q^2 value (0.976) demonstrated the good predictability of the model. In addition, all the adulterated samples of BT and CF could be correctly classified as adulterated samples with 100% accuracy. The PLS-

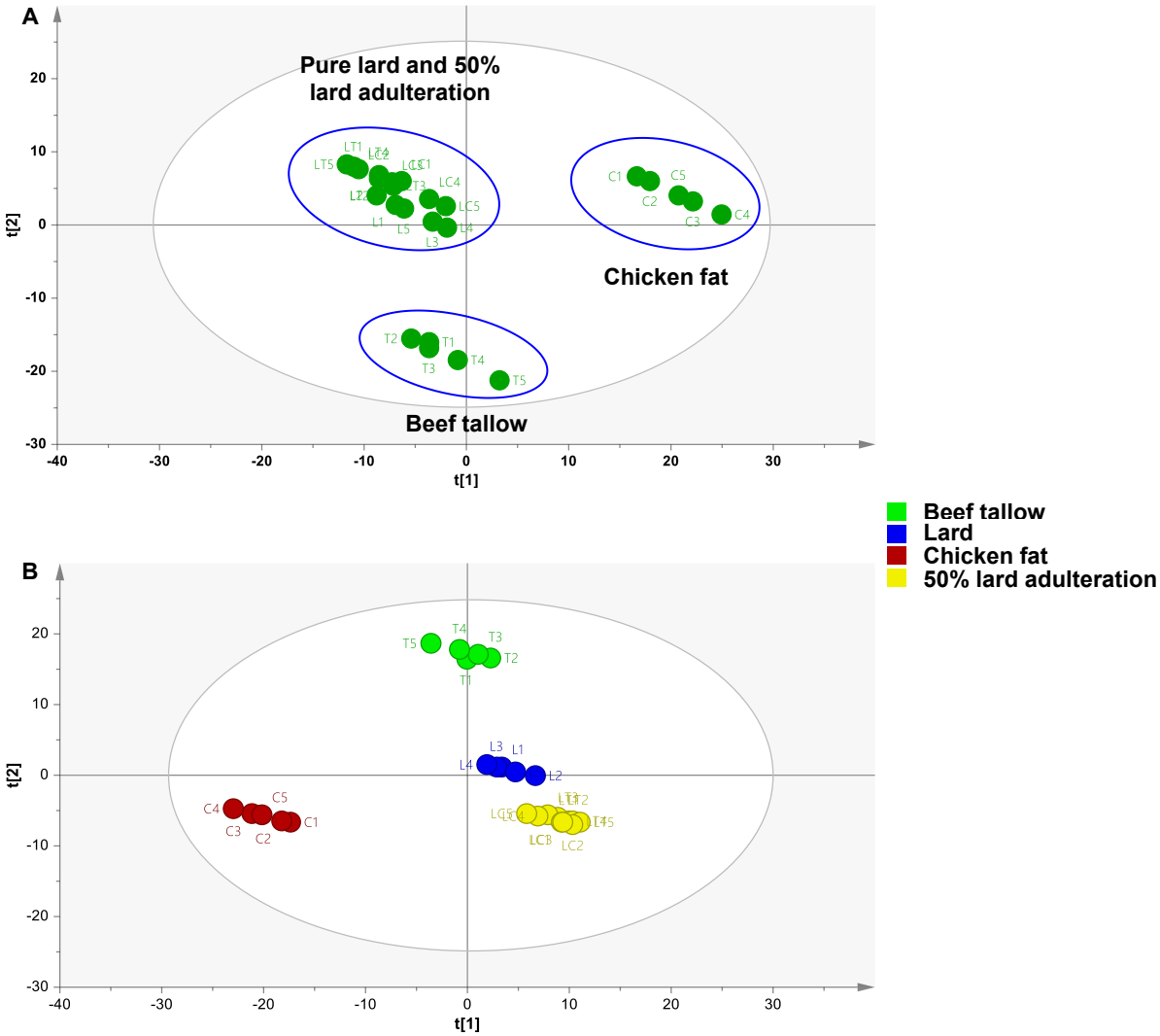

Figure 4. PCA score plot (A) and PLS-DA score plot (B) for differentiation of lard, beef tallow, chicken fat, and adulterated beef tallow and chicken fat with 50% lard [L1-L5= lard (n=5), T1-T5 = beef tallow (n=5), C1-C5 = chicken fat (n=5), LT1-LT5 = mixture of 50% lard and 50% beef tallow (n=5), LC1-LC5 = mixtures of 50% lard and 50% chicken fat (n=5)]

DA model was evaluated by means of a permutation test and ROC value to validate the PLS-DA model as shown in Figure 5. The permutation test used 999 permutations to confirm the validity of the PLS-DA model. All permutated models on the left side were lower than the original models on the right side (Figure 5A). In addition, the intercept of Q2 was zero and lower than zero (0.0, -0.43), thus indicating good model validity. The analysis of ROC was evaluated using the area under the curve (AUC) value. The resulting AUC value was 1 for each class (Figure 5B), which confirmed the validity of the model (Rivera-Pérez *et al*., 2021). In addition, the analysis of variable importance projections (VIP) value in PLS was used to identify potential lipids which play important roles in discriminating between pure fat samples (BT and CF) and those adulterated with lard. Table 2 shows the potential lipid biomarkers obtained from the VIP analysis. Variables with a VIP value greater than 1 are considered important variables as potential biomarkers for sample discrimination. Most of them were glycerolipids (DG and TG).

Previous research on lipidomic analysis using DART-TOF-MS (direct analysis in real time-time of flight-mass spectrometry) has been successfully used for the authentication of beef tallow. This research focused on triacylglycerol (TAG) compositions. A chemometric linear discriminant analysis (LDA) using TAG compositions was performed to successfully discriminate between pure and adulterated beef tallow

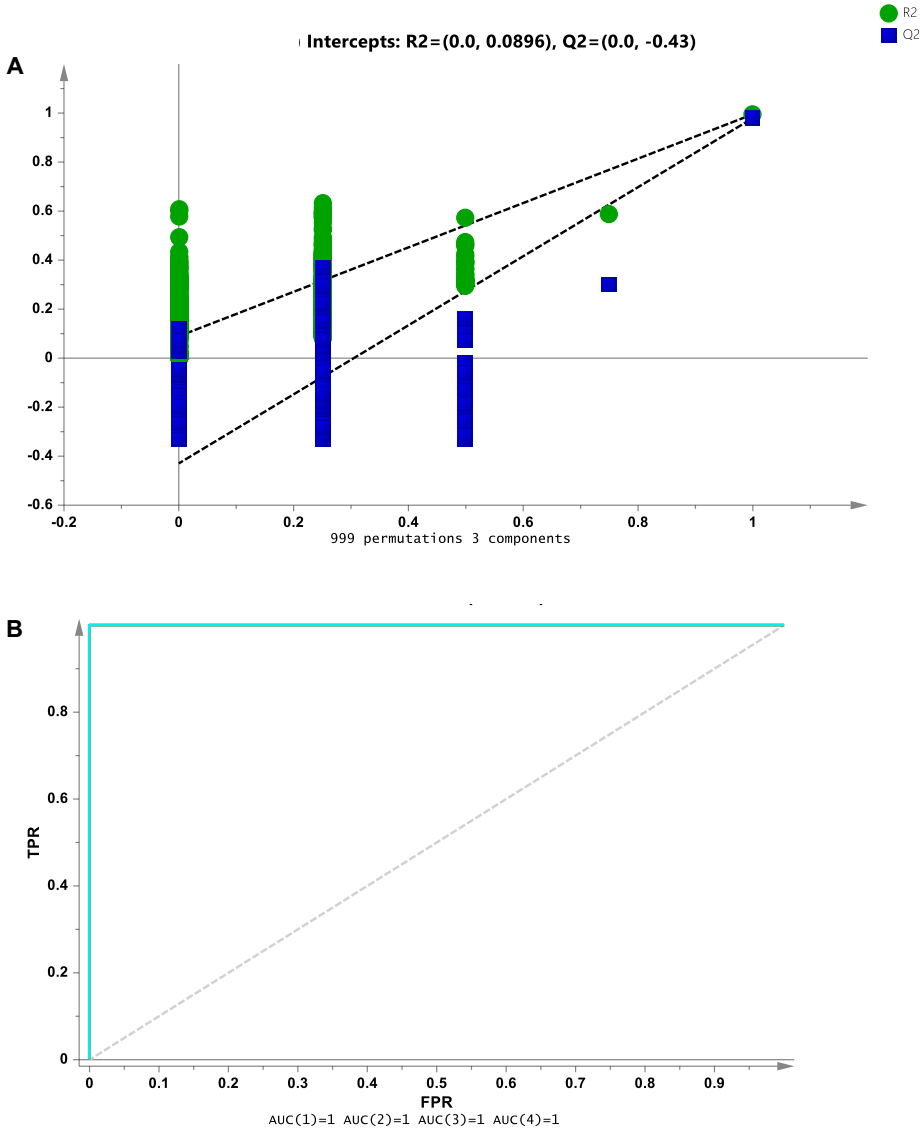

FIGURE 5. Permutation test (A) and receiver operating characteristic value (B) of PLS-DA model.

Table 2. Potential lipid biomarkers for discrimination between pure beef and adulterated beef tallow and chicken fat with 50% lard obtained from PLS-DA

No.	Lipids	VIP Value
1	TG(16:0_14:0_18:3)	1.31
2	TG(16:1_18:1_18:3)	1.31
3	TG(16:0_10:0_18:2)	1.31
4	TG(18:1e_16:0_18:2)	1.31
5	TG(16:0_18:1_18:2)	1.31
6	TG(16:0_14:4_16:0)	1.30
7	TG(18:0_16:0_18:3)	1.30
8	TG(8:0_14:1_18:2)	1.30
9	TG(16:0_17:1_18:3)	1.30
10	TG(15:0_18:1_18:2)	1.30
11	DG(32:3e)	1.30
12	DG(18:0_18:1)	1.30
13	DG(18:0_16:0)	1.30
14	DG(34:3e)	1.30
15	DG(34:4e)	1.30
16	TG(16:1_16:1_18:2)	1.30
17	TG(18:2_18:2_18:2)	1.30
18	TG(18:1_10:1_18:2)	1.30
19	TG(18:0e_18:1_18:2)	1.30
20	TG(17:0_18:1_18:2)	1.30
21	DG(18:0_17:0)	1.29
22	DG(54:3)	1.29
23	DG(17:0_18:2)	1.29
24	DG(19:1_18:1)	1.29
25	DG(20:3_18:2)	1.29
26	TG(18:1_14:4_18:1)	1.29
27	TG(18:3_18:2_18:2)	1.29
28	TG(16:0_12:3_18:1)	1.29
29	TG(18:1_18:1_18:2)	1.29
30	TG(18:0_18:0_18:0)	1.29
31	TG(18:0_18:0_18:1)	1.29
32	ZyE(35:6)	1.29
33	TG(16:0_16:0_18:1)	1.29
34	TG(70:2)	1.29
35	TG(18:1_18:2_22:3)	1.29
36	TG(16:1_14:1_14:2)	1.29
37	TG(16:0_17:0_18:1)	1.29
38	TG(4:0_16:0_18:1)	1.29
39	TG(16:0_16:0_18:2)	1.29
40	TG(16:0_16:1_18:2)	1.29

VIP = variable importance for projections, TG = triglycerides, DG = diglycerides, ZyE = zymosteryl ester

samples with lard (Vaclavik *et al*., 2011). Our study provided more comprehensive lipid compounds because it is focused not only on the TAG compositions but a wider range of lipid compounds as well.

4. CONCLUSIONS

In the current study, liquid chromatography-Orbitrap high resolution mass spectrometry provided high throughput screening for the lipidomic analysis of lard, beef tallow, and chicken fat. The identification of lipid composition could be used to differentiate lard, beef tallow, and chicken fat. A combination with chemometrics such as PCA and PLS-DA could be used to detect the adulteration of chicken fat and beef tallow with 50% lard. Some potential lipid markers could be identified to detect and discriminate lard in beef tallow and chicken fat. This method is promising as a feasible strategy to discriminate lard from beef tallow and chicken fat for food authentication purposes. This research also supports the authorities responsible for halal authentication testing by providing effective and powerful analytical techniques for halal authentication of fat products. Future research using larger samples is required to validate the lipid markers of lard and to ensure the consistency of the results.

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