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Original Research Article

Forensic Feed Strategy: Incorporation of Multivariate and Instrumental Analyses for Authentication of Fish Feed Sources

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Abstract: This study authenticated fish feed sources and determined lard adulteration using dataset pre-processing, principal component analysis (PCA), discriminant analysis (DA) and partial least square regression (PLSR) on 19 triacylglycerols (TAGs) and 16 thermal properties (TPs). At cumulative variability (90.625%) and Keiser-Meyer Olkin (KMO) value (0.811), the PCA identified 10 TAGs and 3 TPs with strong factor loading. The dioleoyl-1-palmitoyl glycerol (POO),

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dipalmitoyl-3-oleoyl glycerol (PPO) and dipalmitoyl-1-linoleoyl (PPL) characterized fish feeds containing palm oil while dilinoleoyl-1-oleoyl glycerol (OLL), dilinoleoyl-1-palmitoyl (PLL), dioleoyl-3-linoleoyl glycerol (OOL), initial cooling temperature (ICT), palmitoyl-oleoyl-linoleoyl glycerol (POL), palmitoyl-stearoyl-oleoyl glycerol (PSO) and final heating temperature (FHT) characterized lard-containing fish feeds. The DA had successfully classified the fish feed sources and selected the PPL, POL, PPO, OOL, ICT, PLL, FHT, POO and OLL as the most influential biomarkers for the authentication purpose. The T-test result ($p > 0.05$) indicated that the PLSR could determine the percentage of lard adulteration in fish feed. Hence, incorporating multivariate and instrumental analyses could authenticate the fish feed sources.

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1. Introduction

The fish feed is formulated to effectively supply nutritional requirements and maintain physiological functions such as effective growth, reproduction and immune systems. For this purpose, a wide range of additives in fish feed formulation has been introduced. Besides antioxidants, enzymes and feed preservatives, phytogenic feed additives (PFA) from roots, leaves and fruits in solid and liquid forms have received interest within the aquaculture industry (Encarnação, 2015). Among the PFAs, palm and seed oils are often used in fish formulation due to their nutritional quality, cheap source, generally recognized as safe and improving feed digestibility and intake (Tyapkova *et al.*, 2016). Because of these properties, there is an increasing interest in substituting these oils with lard. Although the addition of lard into fish feed has rendered a greater fish growth than other PFAs (Glencross, 2015), this substitution has raised concern among consumers, especially vegetarians (Mutalib *et al.*, 2015), Jews (Mukherjee, 2014) and Muslim consumers (Department of Standards Malaysia, 2019) when the fish feed manufacturers issue a false claim and taint the feed integrity. Muflih *et al.* (2017) reported that such activity became a national issue in Malaysia, where fish farmers fed their catfish with pig derivatives in several ponds at Batu Gajah, Tronoh and Papan, Perak. Hence, authentication of the fish feed source via analytical method is in dire need to address this issue.

Various authentication methods have been utilized to address the issue of lard adulteration in products. Most testing use polymerase-chain-reaction (PCR) method, competitive indirect enzyme-linked immunosorbent assay (ELISA) and liquid chromatography methods, e.g. liquid chromatography time of flight mass spectrometer (LC-QTOF/MS) and liquid chromatography-mass spectrometer (LC/MS) to identify the presence of pork-originated adulterants. However, these methods are protein-targeted, complex, and prone to contamination (Yap & Gam, 2019). Additionally, these methods are costly for maintenance in testing laboratories (Abbas *et al.*, 2018). Due to these disadvantages, affordable analytical methods using high-performance liquid chromatography refractive index detector (HPLC-RI) and differential scanning calorimeter (DSC) (Naquiah *et al.*, 2017) have successfully identified lard adulteration by analysing the triacylglycerols (TAGs) and thermal properties (TPs) of the samples (Azir *et al.*, 2017; Noorzyanna *et al.*, 2017; Yanty *et al.*, 2017). Nonetheless, these identification methods were inadequate for authentication since the lard adulteration was identified using sample profiling only. Moreover, these methods were applied to food products and have never been tested in fish feed. Hence, our study developed the authentication method for that purpose.

The comparison of sample profiles was insufficient to authenticate fish feed sources. Although the HPLC-RI may render lower TAG detection sensitivity, it could not differentiate the fish feed source via comparing the individual TAG in the sample with the TAG standard since the TAG of plant and animal origins possess almost similar characteristics distribution of TAG. Likewise, the TP of plant and animal oils have a similar profile pattern. These claims were evident from comparing TAG and TP profiles between palm oil and lard (Noorzyanna *et al.*, 2017), although a thorough comparison was made via ANOVA. Naquiah *et al.* (2017) extended the ANOVA of the TP to multivariate data analysis (MDA) using principal component analysis (PCA); however, the research did not identify the potential biomarkers to discriminate the lard, authenticate the sample source and determine the composition of the lard adulteration in the sample.

The MDA, including PCA, discriminant analysis (DA) and partial least square regression (PLSR), needs pre-requisite analyses. These analyses entail outlier treatment (Bower, 2013a), dataset transformation for normal distribution (Granato, de Araújo Calado, *et al.*, 2014) and test of dataset adequacy (Williams & Brown, 2012), which were absent in previous PCA of feed. Without fulfilling these pre-requisite analyses, the MDA may lead to erroneous results and interpretation. Previous research has neglected the qualitative

authentication of fish feed analysis via DA and determining lard adulteration levels via PLSR. The MDA should test the training, validation and testing datasets of the fish feed before confirming the authentication ability of the analytical method and MDA models. Hence, this study outlined a guideline to explore the fish feed dataset, identify the significant biomarkers, and determine the level of lard adulteration in the fish feed. Subsequently, this study anticipated an adaptation of this guideline by the certification or regulatory bodies in developing fish feed guidelines or standards for research and testing laboratories.

2. Materials and Methods

2.1 Formulation of Fish Feed

The ingredient percentages in the fish feed were calculated using WinFeed 2.8 software (Cambridge, UK). The fish feed was isonitrogenous at 30% crude protein as feed basis, which contained 6% of total oil, 0.5% of vitamin, 0.5% of mineral and 1% of yeast. The oil in the fish feeds was prepared at 0:100, 25:75, 50:50, 75:25, and 100:0 of the L-PO ratio.

2.2 Preparation of Fish Feed

The ingredients of each diet formulation were weighed as determined by Winfeed 2.8 Software and mixed with 60 ml of pre-heated distilled water at 70ºC using a food mixer (Giselle, Malaysia) for 3 min. The mixture was transferred into a dough-making machine (Hengfeng, China) and mixed at 75 rpm for 5 min. The dough was kept at 25°C for 2 h to initiate the fermentation process, extruded at 7 mm diameter using a manual meat mincer and cut uniformly to produce consistent pellet length. The pellets were steamed (Little Homes, Malaysia) for 5 min and dried at 60°C for 3 h in a pre-heated electric oven (Memmert GmbH, Germany). The dried pellets were then kept in a desiccator for 15 min and stored in a dry and closed container.

2.3 Extraction of Fish Feed

Before extraction, the dried pellets were ground for 5 min in a 240 W electrical blender (Panasonic MX-337, Malaysia). The oil from 2 g dried fish feed was extracted with 150 mL petroleum ether at 60°C for 8 h in the Soxhlet apparatus (Khallouki *et al.*, 2018). The extract was transferred into a pre-weighed flat bottom flask, concentrated using a rotary vacuum evaporator (Eyela N-1001, Japan) at 40°C and frozen at -20°C in a glass container.

2.4 Triacylglycerol Analysis of Fish Feed

The frozen extract was thawed at 25°C for 1 h and warmed in a water bath at 60°C until completely melted. A concentration of 5% extract in chloroform (w/w) was subjected to TAG separation by high-performance liquid chromatography (HPLC) of 510 model (Waters, USA) and TAG detection by differential refractometer (RID) of 410 Waters model. A volume of 10 µL of the extract was injected into the HPLC-RID and eluted by a pre-filtered eluent mixture of acetone: acetonitrile (63.5:36.5) at a 1.5 mL/min flow rate. The TAG separation was separated by 12.5 cm × 4 mm i.d. Lichrosphere RP-18 column (Merck, Germany) at 30°C. The detected peak was confirmed with a mixture of 19 TAG standards (Waters, USA) containing dilinoleoyl-3-linolenileoyl glycerol (LLLn), trilinoleoyl glycerol (LLL), trimyristoyl glycerol (MMM), dilinoleoyl-1-oleoyl glycerol (OLL), dilinoleoyl-1-palmitoyl glycerol (PLL), myristoyl- palmitoyl-linoleoyl glycerol (MPL), dioleoyl-3-linoleoyl glycerol (OOL), palmitoyl-oleoyl-linoleoyl glycerol (POL), dipalmitoyl-1-linoleoyl glycerol (PPL), trioleoyl glycerol (OOO), dioleoyl-1-palmitoyl glycerol (POO), dipalmitoyl-3-oleoyl glycerol (PPO), tripalmitoyl glycerol (PPP), dioleoyl-1-stearoyl glycerol (SOO), palmitoyl-stearoyl-oleoyl glycerol (PSO), dipalmitoyl-3-stearoyl glycerol (PPS) and tristearoyl glycerol (SSS), 1,3-distearoyl-2-oleoyl (SOS) and 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol (SPO). The confirmed peaks were reported as peak area percentages. The TAG analysis was performed in triplicates (Yanty *et al.*, 2017).

2.5 Thermal Analysis of Fish Feed Samples

Thermal analysis was carried out using a differential scanning calorimeter (DSC) of the 823 models equipped with a station of thermal data analysis (Mettler Toledo, Switzerland). Approximate 4–8 mg of the extract was placed in an aluminium pan, hermetically sealed and analyzed according to continual temperature setting: heated at 70°C for 1 min, cooled at 5°C/min to -70°C, held at -70°C for 1 min and heated at 5°C/min to 70°C. The cooling procedure was executed with nitrogen (99.999% purity) at ~20 mL/min. An empty, hermetically sealed aluminium pan was used as the reference. Thermal properties (TPs) of cooling and heating activities, including cooling and heating temperatures of the fish feed, were recorded. Thermograms of the fish feeds were compared to identify the numbers of cooling and heating temperatures.

2.6 Dataset Pre-processing

The percentage of TAG and TP peak area was imported to the dataset table in XLSTAT 2017 software (Addinsoft, Paris, France). About 45 fish feeds, entailing 19 TAGs and 16 TPs, were pre-processed to reduce the variation of the TAGs and TPs in the dataset. The dataset was analyzed via box-and-whisker plot (BWP) to treat outlier, ANOVA test, dataset transformation, KMO test and PCA.

2.6.1 Outlier treatment

The individual TAG and TP were subjected to outlier treatment using the BWP method from a standardized dataset before the ANOVA test and PCA. The confidence interval of the BWP was set at 95%. The skewness of the BWP was examined to confirm the need for dataset transformation. The dataset, which showed different patterns within the individual TAG and TP, was discriminated and shown in the BWP as an outlier. Outlier value exceeded three times the box's height was signed with a dot, star or asterisk (Saiful *et al.*, 2019) and replaced with the variable's mean value.

2.6.2 Analysis of variance

Results were expressed as mean and standard deviation of triplicate analyses for the distribution of TAGs and TPs of the fish feed. The ANOVA test of Tukey's test was applied to determine the significant difference between means of the TAGs and TPs at a 95% confidence level ($p < 0.05$).

2.6.3 Dataset transformation

To ensure the dataset following normal distribution prior to the PCA, the dataset normality was tested using Shapiro-Wilk (SWT), Anderson-Darling (ADT), and Lilliefors (LT) tests at α of 0.05. The dataset was transformed using standardize n-1, standardize (n), centre, standard deviation^{-1} (n-1), standard deviation^{-1} (n), rescale from 0 to 1, rescale from 0 to 100, and Pareto transformation methods. The undetected thermal properties were acknowledged as missing data and subjected to removal before the dataset transformation. The transformation of each TAG and TP was employed to ensure the transformed dataset remained closer to the original dataset (Ismail *et al.*, 2021). The normal distribution of the

transformed dataset was tested, and the best transformation method and normality test were selected from the result.

2.7 Dataset Exploratory by Principal Component Analysis

The PCA of Pearson correlation was employed to examine the dataset pattern, explore the TAGs and TPs contributions to the fish feeds, find and explain the TAGs and TPs correlation and reduce the dataset significantly (p<0.05) into smaller sets of new independent variables, which were called as principal components (PCs) via the following formulae:

$$S_{pq} = f_{p1}v_{1q} + f_{p2}v_{2q} + f_{p3}v_{3q} + \cdots + f_{pn}v_{nq}$$

Where S is the component score, f is the FL, v is the concentration of TAG and TP, p is the PC number, q is the sample number, and n is the total TAG and TP.

In this study, two PCAs were executed. The first PCA was executed, and cumulative variability (CV), eigenvalue (EV) and KMO and FL values were evaluated at a significant level (α) of 0.05.

The second PCA was executed using TAGs and TPs with strong FL to produce a new TAG and TP plot and a biplot of TAG, TP, and fish feed. The cumulative variability, eigenvalue, KMO and FL value were evaluated and compared to the first PCA. The FL and correlation of TAGs and TPs were assessed, and the apportionment of TAGs and TPs to the fish feed clusters was examined. From the second PCA, the significant TAGs and TPs contributed to the fish feed clusters were proposed as the biomarkers (Idris *et al.*, 2021).

2.8 Authentication of Fish Feed Source by Discriminant Analysis

In this study, DA was employed to authenticate fish feed. The same fish feed dataset was acknowledged as training and validation datasets, while the testing dataset consisting of 25 known fish feeds was prepared for authentication purposes.

A new column labelled 'cluster' was added to the dataset, and the fish feeds were assigned as 'palm oil' for 0:100 L-PO, 'lard + palm oil' for 25:75 L-PO, 50-50 LPO and 75:25 L-PO and 'lard' for 100:0 LPO clusters. The DA model was executed at set α of 0.05. The function of the DA model is as follows:

$$f(C_a) = k_a + \sum_{a=1}^{n} w_a T_a$$

Where *a* was the number of fish feed clusters (*C*), *k* was the constant for each cluster, *n* is the number of TAG and TP denoted as *T* that was used to classify the training dataset into the cluster, and *w* is the weight coefficient which was assigned by the DA to the selected *T*. The DA model was developed for fish feed, the dissimilarity of palm oil, L-PO and lard clusters was explored, and 25 known fish feeds were authenticated. The significant TAGs and TPs that caused the dissimilarity between the clusters were identified (Sharin *et al.*, 2021).

2.9 Determination of Lard Adulteration Percentage by Partial Least Square Regression

The PLSR is employed to develop the PLSR model and determine the L-PO composition in fish feed. The training, validation and training datasets were utilized in this study. A new column labelled as 'lard adulteration percentage' was to the dataset, and the fish feeds were assigned as '0%', '25%, '50%', '75%' and '100%' lards according to the percentage of adulterated lard in the fish feeds. The PLSR model was executed at set α of 0.05. The function of the DA model is as follows:

$$Y = X \times V$$

where a mathematical relation was created between the matrix of the fish feed training dataset (*X*) and the regression coefficient vector (*V*). A principal component of PLSR was also developed in this stage. Then, the validation dataset was employed to optimize this mathematical relation to set up calibration. Then the lard adulteration percentage (*Y*) of the 25 known fish feeds was determined based on this calibration (Alexandre *et al.*, 2010).

The PLSR model was developed, and the model characteristics such as determination coefficient (R^2), low mean square error (MSE) and root mean square error (RMSE) were evaluated. The list of VIPs was assessed to determine the model's significant TAGs and TPs (biomarkers). The T-test value of the predicted and actual percentage of lard adulteration was calculated. The determined percentage of lard adulteration in the fish feed was examined and evaluated to confirm the ability of the PLSR model (Abdullah Sani *et al.*, 2021).

3. Results

3.1 Outlier Treatment

Table 1 shows the detected outliers from the BWP of the fish feed. The fish feeds with 0:100, 25:75, 50:50, 75:25, and 100:0 L-PO mixtures present 1, 3, 10, 2, and 5 outliers, respectively, whereas the fish feed with 50:50 LPO mixture had the highest number of outliers. The outlier treatment that replaced the outliers with the mean of each parameter (Berg *et al.*, 2006) decreased the PCA's dataset distortion. However, previous research had never performed the outlier treatment before the PCA, which analyzed TAGs and thermal properties (Golijan *et al.*, 2019). Post outlier treatment of the BWP, the new dataset was also subjected to dataset normalization prior to the PCA.

Table 1. The detected outliers and mean of triacylglycerols and thermal properties of fish feed

Variable, (unit)[1,2]	Fish feed with 0:100 lard-palm-oil mixture[3]		Fish feed with 25:75 lard-palm-oil mixture[3]		Fish feed with 50:50 lard-palm-oil mixture[3]		Fish feed with 75:25 lard-palm-oil mixture[3]		Fish feed with 100:0 lard-palm-oil mixture[3]	
	Number of outliers	Mean	Number of outliers	Mean	Number of outliers	Mean	Number of outliers	Mean	Number of outliers	Mean
Triacylglycerol (TAG)[1]										
LLLn (%)	0	1.46 ± 1.06^{a}	0	1.96 ± 1.28^{a}	0	2.10 ± 1.38^{a}	0	2.47 ± 1.35^{a}	0	2.72 ± 1.59^{a}
LLL (%)	0	0.29 ± 0.14^{c}	0	0.52 ± 0.22^{bc}	1 (LP3-1)	0.55 ± 0.18^{bc}	0	0.70 ± 0.38^{ab}	0	0.98 ± 0.31^{a}
OLL (%)	0	2.09 ± 1.00^{d}	0	3.21 ± 1.03^{cd}	0	4.12 ± 1.08^{bc}	0	4.98 ± 0.98^{ab}	0	6.10 ± 0.95^{a}
PLL (%)	0	4.31 ± 0.70^{d}	0	5.57 ± 0.59^{c}	1 (LP3-6)	6.25 ± 0.68^{c}	0	7.33 ± 0.65^{b}	1 (L5-1)	8.73 ± 0.44^{a}
MPL (%)	0	0.55 ± 0.25^{a}	0	0.47 ± 0.28^{a}	0	0.55 ± 1.54^{a}	0	0.03 ± 0.04^{a}	0	0.32 ± 0.31^{a}
OOL (%)	0	3.09 ± 0.56^{e}	0	4.31 ± 0.59^{d}	1 (LP3-6)	5.35 ± 0.49^{c}	0	6.73 ± 0.50^{b}	0	7.97 ± 0.36^{a}
POL (%)	0	13.55 ± 0.34^{e}	0	15.77 ± 0.41^{d}	0	17.88 ± 0.82^{c}	0	20.03 ± 0.64^{b}	1 (L5-2)	22.32 ± 0.82^{a}
PPL (%)	0	11.20 ± 0.82^{a}	0	9.05 ± 0.59^{b}	0	7.12 ± 0.53^{c}	0	4.92 ± 0.31^{d}	0	2.61 ± 0.19^{e}
OOO (%)	0	5.12 ± 0.30^{a}	0	5.10 ± 0.31^{a}	0	5.07 ± 0.31^{a}	0	5.04 ± 0.26^{a}	0	5.20 ± 0.49^{a}
POO (%)	0	28.65 ± 1.56^{a}	0	26.44 ± 1.46^{b}	0	25.01 ± 1.40^{b}	0	22.93 ± 1.17^{c}	0	20.93 ± 1.14^{d}
PPO (%)	0	22.52 ± 1.54^{a}	0	18.77 ± 1.61^{b}	0	15.98 ± 1.16^{c}	0	12.37 ± 0.86^{d}	2 (L5-1, L5-7)	8.88 ± 0.30^{e}
PPP (%)	0	0.00 ± 0.00^{b}	0	0.00 ± 0.00^{b}	0	0.00 ± 0.00^{b}	2 (LP4-2, LP4-3)	0.24 ± 0.09^{a}	0	0.18 ± 0.17^{a}
SOO (%)	0	1.30 ± 1.41^{ab}	0	1.38 ± 1.37^{ab}	1 (LP3-4)	0.50 ± 0.48^{b}	0	1.39 ± 1.72^{ab}	0	2.56 ± 1.34^{a}
SPO (%)	0	2.40 ± 1.95^{a}	0	1.89 ± 2.83^{a}	0	0.00 ± 0.00^{a}	0	2.30 ± 3.45^{a}	0	0.00 ± 0.00^{a}
PPS (%)	0	2.45 ± 1.85^{b}	0	3.22 ± 2.43^{ab}	1 (LP3-4)	6.36 ± 0.69^{a}	0	2.71 ± 3.92^{ab}	0	2.89 ± 3.84^{ab}
SOS (%)	0	0.00 ± 0.00^{b}	0	0.23 ± 0.25^{ab}	0	0.12 ± 0.20^{ab}	0	0.26 ± 0.26^{ab}	0	0.33 ± 0.30^{a}

SSS (%)	0	0.00 ± 0.00[b]	1 (LP2-5)	0.04 ± 0.07[ab]	0	0.00 ± 0.00[ab]	0	0.16 ± 0.17[a]	0	0.12 ± 0.14[ab]
MMM (%)	0	0.10 ± 0.15[a]	2 (LP2-1, LP2-3)	0.03 ± 0.05[a]	2 (LP3-1, LP3-3)	0.03 ± 0.05[a]	0	0.21 ± 0.35[a]	1 (L5-1)	0.12 ± 0.15[a]
PSO (%)	0	0.92 ± 1.39[c]	0	1.90 ± 1.52[c]	2 (LP3-1, LP3-4)	3.35 ± 0.32[bc]	0	5.07 ± 2.64[ab]	0	7.10 ± 3.23[a]
Thermal properties (TP)[2]										
CST (°C)	0	4.68 ± 0.47[d]	0	8.37 ± 1.46[c]	0	10.34 ± 0.45[b]	0	12.51 ± 0.62[a]	0	13.53 ± 0.65[a]
CPT1 (°C)	0	nd	0	2.23 ± 3.35[c]	0	8.21 ± 0.92[b]	0	10.24 ± 1.08[ab]	0	11.16 ± 1.49[a]
CPT2 (°C)	0	nd	0	4.19 ± 1.26[a]	0	4.57 ± 1.08[a]	0	4.87 ± 1.31[a]	0	5.51 ± 1.43[a]
CPT3 (°C)	0	0.45 ± 0.60[d]	0	1.28 ± 0.52[cd]	0	2.18 ± 0.84[bc]	0	2.70 ± 1.06[ab]	0	3.73 ± 1.68[a]
CPT4 (°C)	0	-3.43 ± 0.42[a]	0	-2.95 ± 1.01[a]	1 (LP3-1)	-7.67 ± 16.22[a]	0	-1.69 ± 2.20[a]	0	-1.81 ± 2.17[a]
CPT5 (°C)	0	-19.28 ± 0.71[a]	0	-18.61 ± 0.96[a]	0	-16.82 ± 1.29[a]	0	-14.79 ± 0.62[a]	0	-18.63 ± 17.09[a]
CPT6 (°C)	0	nd	0	-13.69 ± 20.53[ab]	0	-26.00 ± 19.79[bc]	0	-43.37 ± 8.01[c]	0	-39.28 ± 12.05[c]
CET (°C)	0	-29.92 ± 5.51[a]	0	-39.51 ± 8.57[b]	0	-46.66 ± 4.97[bc]	0	-51.54 ± 5.24[c]	0	-49.13 ± 7.55[c]
HST (°C)	0	-28.15 ± 4.52[a]	0	-26.37 ± 4.03[a]	0	-25.67 ± 3.01[a]	0	-27.06 ± 4.52[a]	0	-33.46 ± 1.25[b]
HPT1 (°C)	1 (P1-8)	-21.37 ± 3.76[b]	0	-20.31 ± 3.24[b]	0	-18.82 ± 3.81[ab]	0	-18.92 ± 1.40[ab]	0	-13.01 ± 9.77[a]
HPT2 (°C)	0	-6.49 ± 7.95[a]	0	-5.83 ± 7.83[a]	0	-4.68 ± 6.94[a]	0	-3.86 ± 5.79[a]	0	-4.83 ± 7.25[a]
HPT3 (°C)	0	0.40 ± 2.01[a]	0	0.60 ± 1.29[a]	0	1.84 ± 2.77[a]	0	0.70 ± 1.04[a]	0	0.00 ± 0.00[a]
HPT4 (°C)	0	3.97 ± 2.58[a]	0	4.40 ± 2.91[a]	0	5.38 ± 2.90[a]	0	2.61 ± 2.55[a]	0	-2.76 ± 0.89[b]
HPT5 (°C)	0	nd	0	4.07 ± 6.10[b]	0	11.63 ± 8.87[a]	0	15.44 ± 1.09[a]	0	16.74 ± 0.54[a]
HPT6 (°C)	0	nd	0	5.77 ± 8.66[b]	0	19.99 ± 2.27[a]	0	21.22 ± 1.52[a]	0	23.14 ± 1.71[a]
HET (°C)	0	15.26 ± 0.14[d]	0	19.19 ± 2.28[c]	0	22.55 ± 4.28[b]	0	27.29 ± 1.20[a]	0	29.18 ± 1.85[a]

Note:

[1]LLLn = dilinoleoyl-3-linolenileoyl glycerol, LLL = trilinoleoyl glycerol, MMM = trimyristoyl glycerol, OLL = dilinoleoyl-1-oleoyl glycerol, PLL = dilinoleoyl-1-palmitoyl glycerol, MPL = myristoyl palmitoyl-linoleoyl glycerol, OOL = dioleoyl-3-linoleoyl glycerol, POL = palmitoyl-oleoyl-linoleoyl glycerol, PPL = dipalmitoyl-1-linoleoyl glycerol, OOO = trioleoyl glycerol, POO = dioleoyl-1-palmitoyl glycerol, PPO = dipalmitoyl-3-oleoyl glycerol, PPP = tripalmitoyl glycerol, SOO = dioleoyl-1-stearoyl glycerol, PSO = palmitoyl-stearoyl-oleoyl glycerol, PPS = dipalmitoyl-3-stearoyl glycerol, SSS = tristearoyl glycerol, SOS = 1,3-distearoyl-2-oleoyl and SPO = 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol.

[2]ICT = initial cooling temperature, CT = cooling temperature, FCT = final cooling temperature, IHT = initial heating temperature, HT = heating temperature and FHT = final heating temperature.

[3]Means with different superscript letter are significantly different ($p < 0.05$).

3.2 Triacylglycerol of Fish Feed

The TAG percentage in the fish feeds is presented in Table 1. The TAGs of fish feed containing 0:100 L-PO, 25:75 L-PO, 50:50 L-PO, 75:25 L-PO and 100:0 L-PO mixtures from 0.00 ± 0.00% (PPP, SSS and SOS) to 5.12 ± 0.30% (POO), 0.00 ± 0.00% (PPP) to 9.05 ± 0.59% (POO), 0.00 ± 0.00% (SPO, SSS and PPP) to 25.01 ± 1.40% (POO), 0.03 ± 0.04% (MPL) to 22.93 ± 1.17% (POO), 0.00 ± 0.00% (SPO) to 22.32 ± 0.82% (POL), respectively. The POO had the highest percentage in all fish feed formulations, while POL had the highest percentage in fish feed with 100:0 L-PO. The high content of POO in fish feed containing 0:100 L-PO was in line with Indelicato *et al.* (2017), except for the latter reported 1,3-dipalmitoyl-2-oleoyl glycerol (POP) presence. Likewise, the highest content of POL in fish feed containing 100:0 L-PO was comparable to the POL determined by Yanty *et al.* (2011).

For the fish feed with 100% palm oil, the ranking of TAG percentage as follows: PPP < SOS < SSS < MMM < LLL < MPL < PSO < SOO < LLLn < OLL < SPO < PPS < OOL < PLL < OOO < PPL < POL < PPO < POO. In contrast, the fish feed of 100% lard exhibited different ranking of TAG percentage as follows: SPO < SSS < MMM < PPP < MPL < SOS < LLL < SOO < PPL < LLLn < PPS < OOO < OLL < PSO < OOL < PLL < PPO < POO < POL. It was evident that the ranking of TAGs in 25:75 L-PO, 50:50 L-PO, and 75:25 L-PO mixtures differed. The different rankings indicated that adding lard into fish feed containing palm oil affected the distribution of the TAGs.

All fish feeds exhibited a significant difference in OOL, POL, PPL, and PPO percentages (p< 0.05). Of these TAGs, fish feed with 100:0 L-PO possessed the highest percentage of OOL (7.97 ± 0.36%) and POL (22.32 ± 0.82%), while fish feed with 0:100 L-PO showed the highest percentage of PPL (11.20 ± 0.82%) and PPO (22.52 ± 1.54%), and vice versa. The 25:75 L-PO, 50:50 L-PO and 75:25 L-PO mixtures had moderate percentage of OOL (4.31 ± 0.59% to 6.73 ± 0.50%), POL (15.77 ± 0.41% to 20.03 ± 0.64%), PPL (4.92 ± 0.31% to 9.05 ± 0.59%) and PPO (12.37 ± 0.86% to 18.77 ± 1.61%) with significant differences ($p<0.05$). From the ANOVA result, OOL, POL, PPL, and PPO could become potential biomarkers to discriminate fish feed sources.

3.3 Thermal Properties of Fish Feed

The thermal properties (TP) of the fish feeds are presented in Table 2. The thermal analysis of the fish feeds yielded one initial cooling temperature (4.68 ± 0.47°C to 13.53 ± 0.65°C) and one final cooling temperature (-29.92 ± 5.51°C to -49.13 ± 7.55°C). Yanty *et al.* (2014) analyzed palm oil and recorded cooling temperatures at 20.1°C and 3.05°C as compared to 0.45°C, -3.43°C, and -19.28°C in our study due to the former analyzed palm oil only while our study analyzed the fish feed extract containing the palm oil. The fish feed containing 100:0 L-PO showed the cooling temperature at 10.24°C, 4.87°C, 2.70°C, -1.69°C, -14.79°C, and -43.37°C, which contradicted the observed cooling temperature at 17.99°C, and 11.98°C by Azir *et al.*, 2017 and 9°C and -19°C by Naquiah *et al.* (2017). Among the fish feeds in Table 1, all fish feeds recorded six cooling temperatures, while those with 0:100 L-PO recorded three cooling temperatures. Although the absence of three cooling temperatures may distinguish the fish feed containing 0:100 L-PO from other fish feed, no significant difference in temperature ($p < 0.05$) was observed at initial cooling, cooling, and final cooling temperatures in all fish feeds.

The thermal analysis of the fish feeds (Table 1) yielded one initial heating temperature (-25.67 ± 3.01°C to -33.46 ± 1.25°C) and one final heating temperature (15.26 ± 0.14°C to 29.18 ± 1.85°C). The fish feed with 0:100 L-PO and 100:0 L-PO exhibited four and five heating temperatures, respectively, compared to six heating temperatures in other fish feeds. These results were entirely contrary to those shown in the DSC analysis of palm oil by Yanty *et al.* (2014b), which exhibited five heating temperatures at 14.00°C, 10.00°C, 8.00°C, 6.00°C, and -4.00°C. These results also contradicted three recorded heating temperatures at -4.00°C, 28.78°C, and 34.09°C in lard (Azir *et al.*, 2017). The absence of these heating temperatures may facilitate the identification of the fish feed containing 0:100 L-PO and 100:0 L-PO. Similar to the result of cooling activity, all fish feeds did not exhibit a significant difference in all heating temperatures. Therefore, the lack of an insignificant difference in the temperatures could not differentiate the fish feeds containing lard-palm-oil mixtures.

This ANOVA result considered only the significant difference between the TAGs and TPs and was subjected to further improvement by applying PCA to the multivariate dataset (Jasour *et al.*, 2018). Although little research has adopted the application of PCA to explore the dataset entailing thermal properties, Green *et al.* (2020) recommended employing TAGs and PCA to investigate the source of edible oils. This approach is practical to uphold the fish feed integrity claimed by manufacturers and bring consumer confidence.

3.4 Dataset Transformation

This study investigated issues of (1) which dataset transformation was suitable for TAGs and TPs analysis in fish feed and (2) which normality test was the best to examine the dataset normality.

The most common dataset transformation was the standardize (n-1), followed by other dataset transformations deemed suitable according to sample type. Supplementary data 1, 2 and 3 show the p-value of the normality test for SWT, ADT and LT, respectively. After dataset transformations, not all TAGs and TPs of the transformed dataset demonstrated normal distribution.

Before dataset transformation, the normality test of SWT exhibited 13 TAGs and 15 TPs that followed a normal distribution (Supplementary 1). Post transformation via standardize (n-1), OOL showed normal distribution with 0.0491 p-value. The SWT identified 14 TAGs and 15 TPs that followed normal distribution after the dataset transformations. The ADT exhibited 10 TAGs, and 14 TPs followed a normal distribution before the dataset transformation (Supplementary 2). The PPL and PPO followed the normal distribution after standardize (n-1) transformation. Hence, the ADT resulted in 16 TAGs and 14 TPs that followed normal distribution after the dataset transformations. The LT showed 10 TAGs, and 14 TPs followed the normal distribution before the dataset transformation (Supplementary 3). After the dataset underwent all transformations, no TAGs and TPs followed a normal distribution. From these findings, the ADT was recommended as the best normality test to investigate the dataset normality of TAGs and TPs in the fish feed, which was in agreement with the study of Razali *et al.* (2011), who found that the normality test of ADT was effective in low sample size ($n < 10000$). Besides, Bower (2013b) recommended the acceptance of the non-normal distribution of the dataset because the instrumental or continuous measurement of the sample was principally following the normal distribution.

Several studies have investigated the suitability of different dataset transformations for specific matrices. For instance, the standardize (n), 0 to 100 rescaling and Pareto transformations were corroborated as suitable for sugarcane spirits (Granato *et al.*, 2014), water quality (Juahir *et al.*, 2011) and plant volatiles (Gogna *et al.*, 2015) matrices, respectively. Bloomfield et al. (2011) did not transform the fish feed dataset for the fish feed matrix, possibly due to the principle that the dataset distribution will never achieve normal distribution (Rodriguez, 2020). However, our study proved that the standardize (n-1) transformation was suitable for the fish feed matrix. This study proposed testing the fish feed

dataset with various transformations to confirm the most suitable transformation. Hence, 10 TAGs and 14 TPs were not transformed, and LLL, PPL and PPO were transformed using standardize (n-1). Although OLL, PLL, OOL, POL, OOO, POO, CT2 and HT4 remained with non-normal distribution after the transformations, these variables were also transformed using standardize (n-1) prior to PCA.

3.5 Dataset Exploratory by Principal Component Analysis

The exploratory of the dataset via the first PCA demonstrated PC1 and PC2 with eigenvalue (EV) > 1 (Falcó *et al.*, 2019), which explained the 57.766% cumulative variability (CV) of the dataset (Table 2). The EV and percentage variability (PV) reflect the size and significant PC ($p < 0.05$), whereby PC1 has a larger EV than PC2. The EV information supported or our result that the EVs decreased as the PC number increased, i.e. PC1 (EV = 13.318, PV = 38.050) > PC2 (EV = 6.900, PV = 19.715). Although there is no cut-off value of EV or PV, our study adopts the suggested EV > 1 as a principal guideline for feed composition study (Falcó *et al.*, 2019). The KMO test calculated the value of 0.513 for the first PCA. Although no fish feed study has determined the KMO value and provided the acceptance limit specifically for the fish feed matrix, KMO > 0.5 indicated compliance with PCA's dataset adequacy (Kaiser, 1974; Williams & Brown, 2012).

Table 2. Factor loading of triacylglycerols and thermal properties of fish feed

Variable[1,2]	**Factor loading (FL) of first PCA**[3,4]		**Factor loading (FL) of second PCA**[3,4,5]	
	PC1	**PC2**	**PC1**	**PC2**
Triacylglycerol (TAG)[1]				
LLLn	-0.3693[c]	**-0.8583**[a]	-0.4737[c]	**-0.8067**[a]
LLL	-0.6832[b]	-0.4222[c]	nt	Nt
OLL	**-0.8417**[a]	-0.5081[b]	**-0.9095**[a]	-0.3906[c]
PLL	**-0.9392**[a]	-0.2783[c]	**-0.9768**[a]	-0.1464[c]
MPL	0.1756[c]	0.1404[c]	nt	Nt
OOL	**-0.9676**[a]	-0.2034[c]	**-0.9912**[a]	-0.0718[c]
POL	**-0.9554**[a]	0.1289[c]	**-0.9431**[a]	0.2615[c]
PPL	**0.9850**[a]	0.0934[c]	**0.9959**[a]	-0.0395[c]
OOO	-0.0839[c]	**-0.7550**[a]	-0.1840[c]	**-0.7549**[a]
POO	**0.9189**[a]	0.3358[c]	**0.9608**[a]	0.2135[c]
PPO	**0.9786**[a]	0.1462[c]	**0.9964**[a]	0.0155[c]
PPP	-0.7287[b]	0.2232[c]	nt	Nt
SOO	-0.2593[c]	0.5575[b]	nt	Nt

Variable[1,2]	Factor loading (FL) of first PCA[3,4]		Factor loading (FL) of second PCA[3,4,5]	
	PC1	**PC2**	**PC1**	**PC2**
SPO	0.2491[c]	-0.0246[c]	nt	Nt
PPS	0.0373[c]	-0.4104[c]	nt	Nt
SOS	-0.3788[c]	0.6312[b]	nt	Nt
SSS	-0.4686[c]	0.4293[c]	nt	Nt
MMM	-0.2644[c]	-0.4596[c]	nt	Nt
PSO	**-0.7682**[a]	0.3448[c]	**-0.7236**[b]	0.4748[c]
Thermal property (TP)[2]				
ICT	**-0.9202**[a]	0.0646[c]	**-0.9359**[a]	0.1833[c]
CT1	-0.6640[b]	0.1120[c]	nt	Nt
CT2	-0.2699[c]	**0.8698**[a]	-0.1259[c]	**0.9198**[a]
CT3	-0.7371[b]	0.6247[b]	nt	Nt
CT4	-0.1084[c]	0.3735[c]	nt	Nt
CT5	-0.1564[c]	0.5707[b]	nt	Nt
CT6	0.3836[c]	-0.1570[c]	nt	Nt
FCT	0.6722[b]	0.1775[c]	nt	Nt
IHT	0.4695[c]	0.6305[b]	nt	Nt
HT1	-0.4618[c]	0.6203[b]	nt	Nt
HT2	-0.1364[c]	-0.1235[c]	nt	Nt
HT3	0.1405[c]	0.5329[b]	nt	Nt
HT4	0.6772[b]	0.5265[b]	nt	Nt
HT5	-0.4831[c]	0.4503[c]	nt	Nt
HT6	-0.5425[b]	0.2279[c]	nt	Nt
FHT	**-0.8845**[a]	0.3438[c]	**-0.8404**[a]	0.4689[c]
Eigenvalue	13.3176	6.9003	8.9409	2.8404
Variability (%)	38.0503	19.7152	68.7759	21.8493
Cumulative explained variability (%)	38.0503	57.7655	68.7759	90.6252
Kaiser-Meyer-Olkin value	0.5127		0.8109	

Note:
[1]LLLn = dilinoleoyl-3-linolenileoyl glycerol, LLL = trilinoleoyl glycerol, MMM = trimyristoyl glycerol, OLL = dilinoleoyl-1-oleoyl glycerol, PLL = dilinoleoyl-1-palmitoyl glycerol, MPL = myristoyl palmitoyl-linoleoyl glycerol, OOL = dioleoyl-3-linoleoyl glycerol, POL = palmitoyl-oleoyl-linoleoyl glycerol, PPL = dipalmitoyl-1-linoleoyl glycerol, OOO = trioleoyl glycerol, POO = dioleoyl-1-palmitoyl glycerol, PPO = dipalmitoyl-3-oleoyl glycerol, PPP = tripalmitoyl glycerol, SOO = dioleoyl-1-stearoyl glycerol, PSO = palmitoyl-stearoyl-oleoyl glycerol, PPS = dipalmitoyl-3-stearoyl glycerol, SSS = tristearoyl glycerol, SOS = 1,3-distearoyl-2-oleoyl and SPO = 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol.
[2]ICT = initial cooling temperature, CT = cooling temperature, FCT = final cooling temperature, IHT = initial heating temperature, HT = heating temperature and FHT = final heating temperature.
[3a]FL ≥ |0.750| = strong factor loading, [b]|0.500| < FL < |0.749| = moderate factor loading and

[c]FL ≤ |0.499| = weak factor loading in the principal component
[4]Bold factor loading indicated strong factor loading in the principal component.
[5]nt = the TAGs and TPs were not tested in the second PCA.

The first PCA generated TAGs and TPs with strong FL (FL ≥ |0.75|): OLL, PLL, OOL, POL, PPL, POO, PPO, PSO, ICT and FHT in PC1 and LLLn, OOO and CT2 in PC2. The TAGs and TPs with moderate FL (|0.500| < FL < |0.749|) were LLL, PPP, CT1, CT3, FCT, HT4 and HT6 in PC1 and SOO, SOS, CT3, CT5, IHT, HT1, HT3 and HT4 in PC2. The PCA also exhibited weak FL (FL ≤ |0.499|) of MPL, SPO, SSS, CT6, HT2, and HT5 in PC1 and PPS, MMM and CT4 in PC2.

By executing the second PCA using TAGs and TPs with strong FL from the first PCA, the second PCA demonstrated PC1 and PC2 with higher CV (90.625%) and KMO (0.811) than the first PCA (Table 2), while the TAGs and TPs with strong FL remained in the same criterion. This result confirmed that 10 TAGs and 3 TPs with strong FL adequately explained the 90.625% variation of the fish feed dataset. Our study also found that the TAGs with strong FL were oleic and palmitic acids in their chemical structure. The ICT and CT2 were attributed to the amount of saturated and unsaturated TAGs, and FHT was associated with the melting of crystallized and polymorphic transitions of fat (Azir *et al.*, 2017).

The positive and negative signs of the FL in the second PCA (Table 2) explained the correlation between the TAGs and TPs. The TAGs and TPs that shared the same FL sign were positively correlated, indicating that a positive change of a TAG or TP would positively change other TAGs and TPs and vice versa. Based on this principle, two groups had their correlations in PC1: (1) PPL, PPO and PPO in group 1 were positively correlated, and (2) OLL, PLL, OOL, POL, PSO, ICT and FHT in group 2 were positively correlated, and (3) TAGs and TPs in group 1 and 2 were negatively correlated. Likewise, CT2 was negatively correlated with LLLn and OOO in PC2. The TAG and TP plots of Figure 1 (a) confirmed these correlations. This plot also rendered additional information regarding TAGs and TPs with weak or without correlations when the directions of their FL were ~ 90° (Hair *et al.*, 2014), i.e. POO, PPO, and PPL against CT2, OOO, and LLLn.

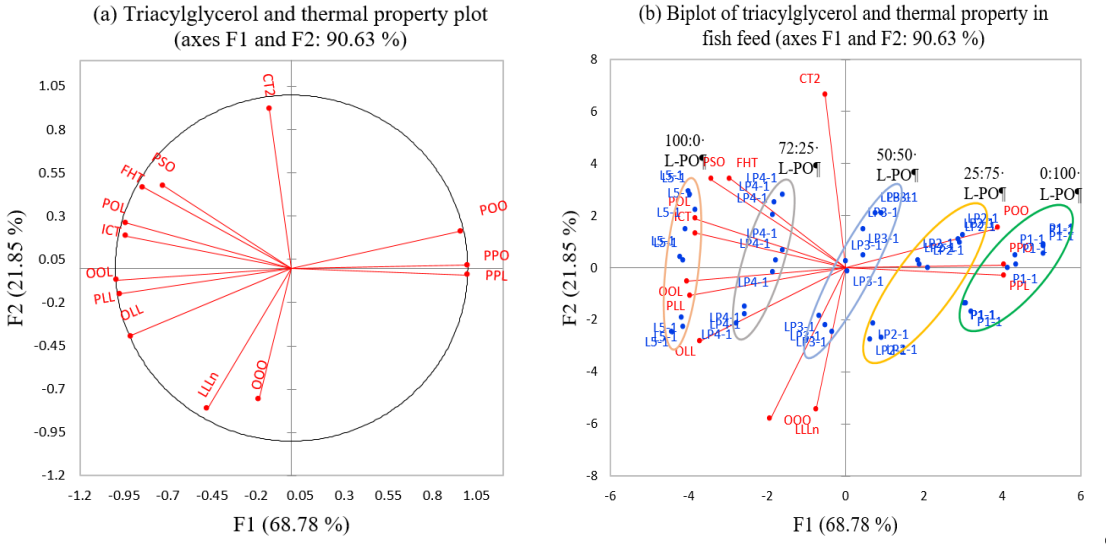

Figure 1. (a) Triacylglycerol and thermal properties plot and (b) biplot of triacylglycerol, thermal property, and fish feed

Figure 1 (b) depicts the biplot of TAG and TP in fish feed. The biplot generated five clusters that corresponded to each L-PO mixture. The biplot showed the TAGs and TPs that significantly contributed ($p < 0.05$) to each L-PO cluster: (1) high loading of POO, PPO and PPL and low loading of OLL, PLL, OOL, ICT, POL, PSO and FHT in 0:100 and 25:75 L-PO clusters, (2) high loading of CT2, OOO and LLLn in 50:50 L-PO cluster and (3) high loading of OLL, PLL, OOL, ICT, POL, PSO and FHT and low loading of POO, PPO and PPL in 72:25 and 100:0 L-PO clusters. The POO, PPO and PPL were of high loading in 0:100 and 25:75 L-PO clusters due to high concentration of oleic acid and palmitoleic acids, while high loading of OLL, PLL, OOL, ICT, POL, PSO in 72:25 and 100:0 L-PO clusters corresponded to a high concentration of oleic and linoleic acids (Indrasti et al., 2010). The high loading of ICT and FHT in 72:25 and 100:0 L-PO clusters was attributed to the polymorphic transition of high concentrations of saturated and unsaturated TAGs (Azir *et al.*, 2017). The PCA had selected the OLL, PLL, OOL, POL, PPL, POO, PPO, PSO, ICT, FHT, LLLn, OOO and CT2 as the biomarkers to identify the fish feed source. Nonetheless, the PCA is an unsupervised MDA which is more suitable for dataset exploratory; hence this study employed these biomarkers to authenticate fish feed source via DA and determine the percentage of lard adulteration via PLSR.

3.6 Authentication of Fish Feed Source by Discriminant Analysis

The DA was performed on the training and validation datasets to (1) develop a DA model for fish feed, (2) explore the dissimilarity of palm oil, L-PO and lard clusters, (3) authenticate 25 known fish feed in the testing dataset and (4) identify the significant TAGs and TPs that caused the dissimilarity between the clusters. Table 3 shows the classification matrix of training, validation, and testing datasets by the DA model. The p-value of Wilks' lambda for the DA model ($p < 0.0001$) indicated that the three clusters were significantly different from each other. This result was confirmed by the calculated p-value of Fisher distance ($p < 0.0001$) between the two clusters. The training and validation datasets had 100% and 84.44% correct classification values, respectively. Although the correct classification of the latter was lower than the former, 100% correct classification was recorded for the testing dataset, thus proving the DA model was reliable in authenticating 25 known fish feeds (Figure 2). The DA model selected PLL, OOL, POL, PPL, PSO, ICT and FHT as the significant biomarkers ($p < 0.05$) from the 10 TAGs and 3 TPs proposed by the PCA.

Table 3. Classification matrix of training, validation, and testing datasets by discriminant analysis.

Dataset	**Correct classification, %**	**Number of fish feed and p-values of Fisher distance**			**Total fish feed**
		Palm oil	**Lard + palm oil**	**Lard**	
Training dataset					
Palm oil	100.00	9 (1)	0 (< 0.0001)	0 (< 0.0001)	9
Lard + palm oil	100.00	0 (< 0.0001)	27 (1)	0 (< 0.0001)	27
Lard	100.00	0 (< 0.0001)	0 (< 0.0001)	9 (1)	9
Total	100.00				
Validation dataset					
Palm oil	100.00	0 (1)	0 (< 0.0001)	9 (< 0.0001)	9
Lard + palm oil	81.48	2 (< 0.0001)	22 (1)	3 (< 0.0001)	27
Lard	77.78	7 (< 0.0001)	2 (< 0.0001)	0 (1)	9
Total	84.44				
Testing dataset					
Palm oil	100.00	5 (1)	0 (< 0.0001)	0 (< 0.0001)	5
Lard + palm oil	100.00	0 (< 0.0001)	15 (1)	0 (< 0.0001)	15
Lard	100.00	0 (< 0.0001)	0 (< 0.0001)	5 (1)	5
Total	100.00				

Note:

[1]Wilks' lambda calculated p-value < 0.0001 at significant level (α) of 0.05.

[2]Seven (PLL, OOL, POL, PPL, PSO, ICT and FHT) out of 10 TAGs and 3 TPs parameters were the significant variables (p < 0.05).

[3]Calculated p-value of Fisher distance < 0.05 indicated two clusters were significantly difference.

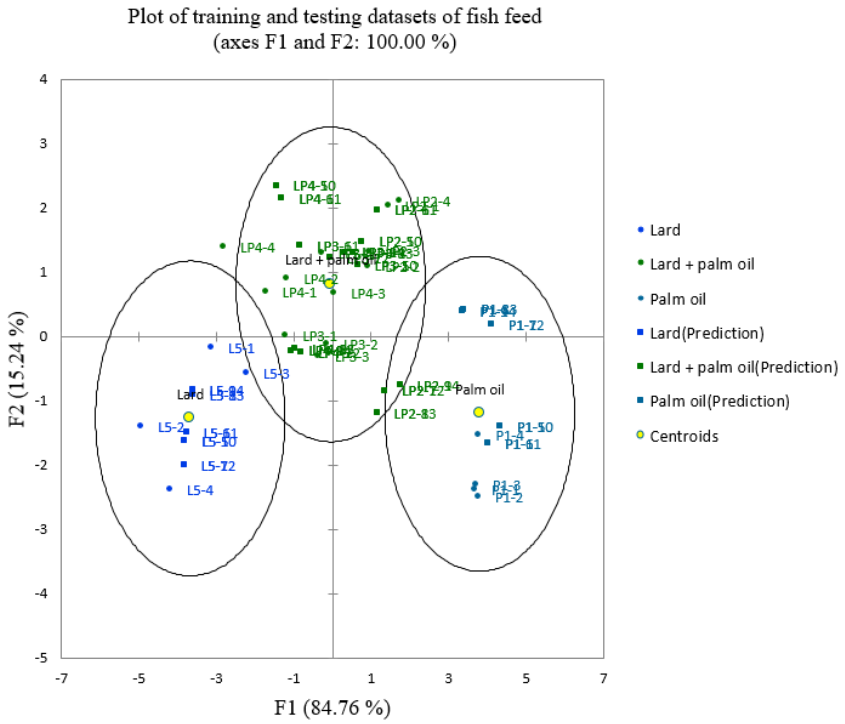

Figure 2. Plot of training and testing datasets of fish feed by discriminant analysis.

3.7 Determination of Lard Adulteration Percentage by Partial Least Square Regression

The PLSR result in Table 4 shows the determination for the testing dataset consisting of the known percentage of lard adulteration in the fish feeds. The PLSR had developed a model equation as follows:

Lard adulteration, % = 18.082 + 1.165ICT + 1.047CT2 + 0.648FHT + 0.951LLLn + 3.313OLL + 3.731PLL + 3.875OOL + 3.949POL - 3.967PPL + 0.199OOO - 3.626POO - 3.913PPO + 0.997PSO.

Table 4. Determination for the testing dataset of known lard adulteration percentage in fish feed.

Fish feed	**Actual lard adulteration[1,2], %**	**Determined lard adulteration, %**	**95% lower and upper bounds of determined lard adulteration**	**Relative error, %**	**T-test value [3]**
P1(10) - P1(14)	0.00	-4.99 ± 4.95	-8.55 - 4.45	0.00	0.054
LP2(10) - LP2(14)	25.00	21.50 ± 6.27	19.09 - 30.92	14.01	0.219
LP3(10) - LP3(14)	50.00	45.42 ± 3.27	43.49 - 52.06	9.16	0.057
LP4(10) - LP4(14)	75.00	72.50 ± 1.23	70.23 - 76.14	3.33	0.060
L5(10) - L5(14)	100.00	99.18 ± 1.24	95.88 - 103.50	0.82	0.139

Note:

[1]Determination coefficient (R^2) = 0.9693, mean square error (*MSE*) = 38.382 and root mean square error (*RMSE*) = 6.195.

[2]Ranking of variable importance in the projection (VIP): PPL > POL > PPO > OOL > ICT > PLL > FHT > POO > OLL > PSO > LLLn > CT2 > OOO.

[3]*p*-value > 0.05 indicated that this study should accept the null hypothesis (H_o)

This model had a strong R^2 of 0.9693 and low MSE and RMSE of 38.382 and 6.195, respectively. Also, analysis of VIP rendered the ranking of TAGs and TPs according to their influence on the model. The developed ranking was as follows: PPL (1.211) > POL (1.206) > PPO (1.195) > OOL (1.183) > ICT (1.168) > PLL (1.139) > FHT (1.118) > POO (1.107) > OLL (1.012) > PSO (0.911) > LLLn (0.393) > CT2 (0.320) > OOO (0.061). According to Mercader *et al.* (2016), a variable with VIP > 1 strongly impacts the PLSR model; thus, all TAGs and TPs except PSO, LLLn, CT2 and OOO became the most significant biomarkers in this model. Additionally, PSO was the TAGs with the lowest FL determined in the PCA, while LLLn, OOO and CT2 were the TAGs and TP of the PC2 in the PCA. Hence, the PLSR model only selected the TAGs and TPs with strong FL in the PC1 of the PCA as the most

significant biomarkers. On the contrary, the PSO was included in the list of significant biomarkers by the DA model to authenticate the fish feed source qualitatively.

Table 4 also exhibited the determination of lard adulteration percentage for five clusters of fish feed. Based on the mean and standard deviation, the determined value for each cluster was within the actual value. The determined value also fell between the 95% lower and upper bounds of the determined value. Nonetheless, the relative error of the determined value decreased as the lard adulteration percentage increased, signifying that the PSLR model was more sensitive to a higher percentage of lard adulteration. Since the T-test value showed a *p*-value > 0.05, the null hypothesis (H_o) was accepted, indicated the percentage means of the determined and actual lard adulteration were not significantly different. This result proved that the PLSR model could determine the percentage of lard adulteration in the fish feed.

4. Conclusion

The demand to produce fish feed for aquaculture fields has shown exponential growth, which requires the testing laboratory's authentication as a feed forensic tool. The TAG and TP testing analyses by HPLC-RID and DSC incorporated with PCA, DA and PLSR are imperative to address the false claim and ensure feed integrity from the manufacturers. The analysis undergoes fish feed extraction, TAG and TP analyses, dataset pre-processing, exploration of the dataset via PCA, authentication of the fish feed by DA and determination of lard adulteration percentage in fish feed by PLSR as a guideline to avoid false-positive and negative results. However, it is recommended that the TAG and TP analyses undergo method validation and verification (MVV) to determine the limit of detection (LOD) and quantification (LOQ) of the method before the dataset analysis. Also, this manuscript explained the PCA, DA and PLSR, which were the frequently utilized MDA for authentication, while other MDA such as multiple linear regression (MLR) and principal components regression (PCR) were not discussed. Since limited research focuses on fish feed testing, this manuscript may guide the researchers and testing laboratories to extend their scope of analysis and suggest applying HPLC-RID, DSC and MDA as an option for fish feed testing. The certification or regulatory bodies at the governmental level and testing laboratories could adapt this guideline to develop a standard of TAG and TP analyses for authentication of fish feed sources.

Supplementary Materials: Table S1: Normality test of Shapiro-Wilk after dataset transformations, Table S2: Normality test of Anderson-Darling after dataset transformations, Table S3: Normality test of Lilliefors after dataset transformation.

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Supplementary Materials

Table Supplementary 1. Normality test of Shapiro-Wilk after dataset transformations.

Variable[1,2]	*p*-value of Shapiro-Wilk[3,4,5]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Triacylglycerol (TAG)[1]									
LLLn	***0.0057***	**0.0185**	**0.0185**	**0.0185**	**0.0185**	**0.0185**	**0.0185**	**0.0185**	**0.0185**
LLL	0.1575	0.5358	0.5358	0.5358	0.5358	0.5358	0.5358	0.5358	0.5358
OLL	0.5867	*0.4699*	0.4699	0.4699	0.4699	0.4699	0.4699	0.4699	0.4699
PLL	0.2661	*0.1054*	0.1054	0.1054	0.1054	0.1054	0.1054	0.1054	0.1054
MPL	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
OOL	0.0501	***0.0491***	**0.0491**	**0.0491**	**0.0491**	**0.0491**	**0.0491**	**0.0491**	**0.0491**
POL	***0.0332***	0.0667	0.0667	0.0667	0.0667	0.0667	0.0667	0.0667	0.0667
PPL	***0.0196***	**0.0086**	**0.0086**	**0.0086**	**0.0086**	**0.0086**	**0.0086**	**0.0086**	**0.0086**
OOO	0.8236	*0.8747*	0.8747	0.8747	0.8747	0.8747	0.8747	0.8747	0.8747
POO	0.3895	*0.3916*	0.3916	0.3916	0.3916	0.3916	0.3916	0.3916	0.3916
PPO	***0.0210***	**0.0143**	**0.0143**	**0.0143**	**0.0143**	**0.0143**	**0.0143**	**0.0143**	**0.0143**
PPP	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOO	***< 0.0001***	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
SPO	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PPS	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOS	***< 0.0001***	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**
SSS	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
MMM	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PSO	***< 0.0001***	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Variable[1,2]	*p*-value of Shapiro-Wilk[3,4,5]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Thermal property (TP)[2]									
ICT	***0.0010***	**0.0234**	**0.0234**	**0.0234**	**0.0234**	**0.0234**	**0.0234**	**0.0234**	**0.0234**
CT1	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT2	0.1464	*0.1464*	0.1464	0.1464	0.1464	0.1464	0.1464	0.1464	0.1464
CT3	***0.0038***	**0.0030**	**0.0030**	**0.0030**	**0.0030**	**0.0030**	**0.0030**	**0.0030**	**0.0030**
CT4	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FCT	***0.0130***	**0.0352**	**0.0352**	**0.0352**	**0.0352**	**0.0352**	**0.0352**	**0.0352**	**0.0352**
IHT	***0.0002***	**0.0008**	**0.0008**	**0.0008**	**0.0008**	**0.0008**	**0.0008**	**0.0008**	**0.0008**
HT1	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT2	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT3	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT4	***0.0424***	**0.0436**	**0.0436**	**0.0436**	**0.0436**	**0.0436**	**0.0436**	**0.0436**	**0.0436**
HT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FHT	***0.0002***	**0.0050**	**0.0050**	**0.0050**	**0.0050**	**0.0050**	**0.0050**	**0.0050**	**0.0050**

Note:

[1]LLLn = dilinoleoyl-3-linolenileoyl glycerol, LLL = trilinoleoyl glycerol, MMM = trimyristoyl glycerol, dilinoleoyl-1-oleoyl glycerol (OLL),

PLL = dilinoleoyl-1-palmitoyl glycerol, MPL = myristoyl- palmitoyl-linoleoyl glycerol, OOL = dioleoyl-3-linoleoyl glycerol,

POL = palmitoyl-oleoyl-linoleoyl glycerol, PPL = dipalmitoyl-1-linoleoyl glycerol, OOO = trioleoyl glycerol, POO = dioleoyl-1-palmitoyl glycerol,

PPO = dipalmitoyl-3-oleoyl glycerol, PPP = tripalmitoyl glycerol, SOO = dioleoyl-1-stearoyl glycerol, PSO = palmitoyl-stearoyl-oleoyl glycerol,

PPS = dipalmitoyl-3-stearoyl glycerol, SSS = tristearoyl glycerol, SOS = 1,3-distearoyl-2-oleoyl and SPO = 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol.

[2]ICT = initial cooling temperature, CT = cooling temperature, FCT = final cooling temperature, IHT = initial heating temperature, HT = heating temperature and FHT = final heating temperature.

[3]Null hypothesis (H_0) = The triacylglycerols (TAGs) and thermal properties (TPs) of the dataset followed a normal distribution while alternative hypothesis (H_a) = The amino acids from the dataset did not follow a normal distribution at $p < 0.05$.

[4]Bold *p*-value indicated *p*-value < 0.05, thus this study accepted the H_a, indicated that the TAGs and TPs did not follow a normal distribution.

[5]Tansformation method with bold and italic p-value was selected as the best transformation method and the transformed observation was subjected to further dataset analysis.

Table Supplementary 2. Normality test of Anderson-Darling after dataset transformations.

Variable[1,2]	*p*-value of Anderson-Darling[3,4]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Triacylglycerol (TAG)[1]									
LLLn	***0.0075***	**0.0195**	**0.0195**	**0.0195**	**0.0195**	**0.0195**	**0.0195**	**0.0195**	**0.0195**
LLL	0.1273	*0.3316*	0.3316	0.3316	0.3316	0.3316	0.3316	0.3316	0.3316
OLL	0.8771	0.7271	0.7271	0.7271	0.7271	0.7271	0.7271	0.7271	0.7271
PLL	0.3244	0.1205	0.1205	0.1205	0.1205	0.1205	0.1205	0.1205	0.1205
MPL	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
OOL	0.0884	0.0898	0.0898	0.0898	0.0898	0.0898	0.0898	0.0898	0.0898
POL	0.0789	0.1097	0.1097	0.1097	0.1097	0.1097	0.1097	0.1097	0.1097
PPL	0.0586	***0.0225***	**0.0225**	**0.0225**	**0.0225**	**0.0225**	**0.0225**	**0.0225**	**0.0225**
OOO	0.6220	0.7029	0.7029	0.7029	0.7029	0.7029	0.7029	0.7029	0.7029
POO	0.5632	0.6529	0.6529	0.6529	0.6529	0.6529	0.6529	0.6529	0.6529
PPO	0.0612	***0.0376***	**0.0376**	**0.0376**	**0.0376**	**0.0376**	**0.0376**	**0.0376**	**0.0376**
PPP	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOO	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SPO	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PPS	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOS	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SSS	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
MMM	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PSO	**< *0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Variable[1,2]	*p*-value of Anderson-Darling[3,4]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Thermal property (TP)[2]									
ICT	***0.0008***	0.0608	0.0608	0.0608	0.0608	0.0608	0.0608	0.0608	0.0608
CT1	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT2	0.1715	0.1715	0.1715	0.1715	0.1715	0.1715	0.1715	0.1715	0.1715
CT3	***0.0020***	**0.0009**	**0.0009**	**0.0009**	**0.0009**	**0.0009**	**0.0009**	**0.0009**	**0.0009**
CT4	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FCT	***0.0217***	0.0536	0.0536	0.0536	0.0536	0.0536	0.0536	0.0536	0.0536
IHT	***< 0.0001***	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**	**0.0002**
HT1	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT2	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT3	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT4	0.0855	0.0924	0.0924	0.0924	0.0924	0.0924	0.0924	0.0924	0.0924
HT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FHT	***< 0.0001***	**0.0029**	**0.0029**	**0.0029**	**0.0029**	**0.0029**	**0.0029**	**0.0029**	**0.0029**

Note:

[1]LLLn = dilinoleoyl-3-linolenileoyl glycerol, LLL = trilinoleoyl glycerol, MMM = trimyristoyl glycerol, dilinoleoyl-1-oleoyl glycerol (OLL), PLL = dilinoleoyl-1-palmitoyl glycerol, MPL = myristoyl- palmitoyl-linoleoyl glycerol, OOL = dioleoyl-3-linoleoyl glycerol, POL = palmitoyl-oleoyl-linoleoyl glycerol, PPL = dipalmitoyl-1-linoleoyl glycerol, OOO = trioleoyl glycerol, POO = dioleoyl-1-palmitoylglycerol, PPO = dipalmitoyl-3-oleoyl glycerol, PPP = tripalmitoyl glycerol, SOO = dioleoyl-1-stearoyl glycerol, PSO = palmitoyl-stearoyl-oleoyl glycerol, PPS = dipalmitoyl-3-stearoyl glycerol, SSS = tristearoyl glycerol, SOS = 1,3-distearoyl-2-oleoyl and SPO = 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol.

[2]ICT = initial cooling temperature, CT = cooling temperature, FCT = final cooling temperature, IHT = initial heating temperature, HT = heating temperature and FHT = final heating temperature.

[3]Null hypothesis (H_0) = The triacylglycerols (TAGs) and thermal properties (TPs) of the dataset followed a normal distribution while alternative hypothesis (H_a) = The amino acids from the dataset did not follow a normal distribution at $p < 0.05$.

[4]Bold p-value indicated *p*-value < 0.05, thus this study accepted the H_a, indicated that the TAGs and TPs did not follow a normal distribution.

[5]Tansformation method with bold and italic p-value was selected as the best transformation method and the transformed observation was subjected to further dataset analysis.

Table Supplementary 3. Normality test of Lilliefors after dataset transformation.

Variable[1,2]	*p*-value of Lilliefors[3,4]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Triacylglycerol (TAG)[1]									
LLLn	***0.0218***	0.2267	0.2267	0.2267	0.2267	0.2267	0.2267	0.2267	0.2267
LLL	0.1147	*0.2023*	0.2023	0.2023	0.2023	0.2023	0.2023	0.2023	0.2023
OLL	0.8767	*0.4992*	0.4992	0.4992	0.4992	0.4992	0.4992	0.4992	0.4992
PLL	0.6168	*0.4011*	0.4011	0.4011	0.4011	0.4011	0.4011	0.4011	0.4011
MPL	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
OOL	0.1392	*0.1123*	0.1123	0.1123	0.1123	0.1123	0.1123	0.1123	0.1123
POL	0.2131	*0.2617*	0.2617	0.2617	0.2617	0.2617	0.2617	0.2617	0.2617
PPL	0.1835	*0.0553*	0.0553	0.0553	0.0553	0.0553	0.0553	0.0553	0.0553
OOO	0.5020	*0.5529*	0.5529	0.5529	0.5529	0.5529	0.5529	0.5529	0.5529
POO	0.6700	*0.7442*	0.7442	0.7442	0.7442	0.7442	0.7442	0.7442	0.7442
PPO	0.1776	*0.0808*	0.0808	0.0808	0.0808	0.0808	0.0808	0.0808	0.0808
PPP	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOO	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SPO	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PPS	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SOS	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
SSS	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
MMM	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
PSO	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Variable[1,2]	*p*-value of Lilliefors[3,4]								
	No transformation	**Standardize (n-1)**	**Standardize (n)**	**Centre**	**Standard deviation^{-1} (n-1)**	**Standard deviation^{-1} (n)**	**Rescale from 0 to 1**	**Rescale from 0 to 100**	**Pareto**
Thermal property (TP)[2]									
ICT	***0.0302***	0.1126	0.1126	0.1126	0.1126	0.1126	0.1126	0.1126	0.1126
CT1	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT2	0.1401	*0.1401*	0.1401	0.1401	0.1401	0.1401	0.1401	0.1401	0.1401
CT3	***0.0001***	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**
CT4	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
CT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FCT	***0.0094***	**0.0327**	**0.0327**	**0.0327**	**0.0327**	**0.0327**	**0.0327**	**0.0327**	**0.0327**
IHT	***0.0001***	**0.0004**	**0.0004**	**0.0004**	**0.0004**	**0.0004**	**0.0004**	**0.0004**	**0.0004**
HT1	***0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT2	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT3	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT4	0.0968	0.0741	0.0741	0.0741	0.0741	0.0741	0.0741	0.0741	0.0741
HT5	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
HT6	***< 0.0001***	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FHT	***0.0006***	**0.0010**	**0.0010**	**0.0010**	**0.0010**	**0.0010**	**0.0010**	**0.0010**	**0.0010**

Note:

[1]LLLn = dilinoleoyl-3-linolenileoyl glycerol, LLL = trilinoleoyl glycerol, MMM = trimyristoyl glycerol, dilinoleoyl-1-oleoyl glycerol (OLL), PLL = dilinoleoyl-1-palmitoyl glycerol, MPL = myristoyl- palmitoyl-linoleoyl glycerol, OOL = dioleoyl-3-linoleoyl glycerol, POL = palmitoyl-oleoyl-linoleoyl glycerol, PPL = dipalmitoyl-1-linoleoyl glycerol, OOO = trioleoyl glycerol, POO = dioleoyl-1-palmitoyl glycerol, PPO = dipalmitoyl-3-oleoyl glycerol, PPP = tripalmitoyl glycerol, SOO = dioleoyl-1-stearoyl glycerol, PSO = palmitoyl-stearoyl-oleoyl glycerol, PPS = dipalmitoyl-3-stearoyl glycerol, SSS = tristearoyl glycerol, SOS = 1,3-distearoyl-2-oleoyl and SPO = 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol.

[2]ICT = initial cooling temperature, CT = cooling temperature, FCT = final cooling temperature, IHT = initial heating temperature, HT = heating temperature FHT = final heating temperature.

[3]Null hypothesis (H_0) = The triacylglycerols (TAGs) and thermal properties (TPs) of the dataset followed a normal distribution while alternative hypothesis (H_a) = The amino acids from the dataset did not follow a normal distribution at $p < 0.05$.

[4]Bold p-value indicated *p*-value < 0.05, thus this study accepted the H_a, indicated that the TAGs and TPs did not follow a normal distribution.

[5]Tansformation method with bold and italic p-value was selected as the best transformation method and the transformed observation was subjected to further dataset analysis.