



UNIVERSITI PUTRA MALAYSIA

***DETECTION OF LARD IN FISH FEED AND OPTIMIZING THE  
PROCESSING CONDITIONS FOR PELLETED FISH FEED PRODUCTION  
USING CHEMOMETRIC AND RESPONSE SURFACE METHODOLOGY***

MOHAMED HANIFF HANAFY BIN IDRIS

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By

**MOHAMED HANIFF HANAFY BIN IDRIS**

**Thesis Submitted to the School Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirement of the Degree of Master of Science**

**February 2022**

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## **DEDICATIONS**

Bismillahirrahmanirrahim

Especially for:

Emak and Ayah,

My siblings,

My lectures,

My friends,

Thank you so much for the unwavering support, advice, and encouragement throughout this journey. May Allah bless you and reward you a thousand-fold.

Ameen.

Alhamdulillah.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of  
the requirement for the degree of Master of Science

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**February 2022**

**Chairman : Shuhaimi bin Mustafa, PhD**  
**Institute : Halal Products Research**

In order to save the cost of fish production, some small-scale farmers prefer to produce fish feed using inexpensive pelleting methods and ingredients. However, due to a lack of knowledge and expertise, the processing conditions of fish feed production are often neglected, and the basic ingredient such as oil is deliberately replaced with cheaper material such as lard. From a religious perspective, lard in any product is strictly prohibited, especially for the Muslim community. This research thus intends to optimize the processing conditions of the pelleting method using response surface methodology (RSM) and also to detect lard as an adulterant in fish feed using fatty acid (FA) and triacylglycerol (TAG)-based analyses separately, which are then integrated with chemometrics. The optimum processing conditions were determined based on the desired properties of bulk density, floatability, water absorption and water solubility indices of the fish feed produced. For lard detection, *sn*-2 FA composition was coupled with total FA composition to enhance the FA-based analysis by gas chromatography-mass spectrometry (GC-MS). Meanwhile, to improve the TAG-based analysis, thermal analysis by differential scanning calorimetry (DSC) was combined with TAG composition analysis by high-performance liquid chromatography (HPLC). These two types of analysis (FA and TAG-based) were then incorporated with chemometric techniques, namely principal component analysis (PCA), orthogonal partial least square-discriminant analysis (OPLS-DA), and orthogonal partial least square-regression (OPLS-R). Results of RSM revealed that the combination of water temperature at 70 °C, mixing speed at 75 rpm, and mixing time of 5 min were the optimal processing conditions in producing pelleted fish feed with the best quality. For lard detection, the enhanced-PCA model exhibited a preferable distribution pattern of fish feed by differentiating them into separate clusters without overlap. The OPLS-based prediction models constructed from the most discriminating variables pre-defined by the enhanced-PCA also showed good performances. The OPLS-DA model provided significant class discrimination between lard adulterated and non-adulterated fish feed; meanwhile, the OPLS-R model revealed a stronger relationship between the observed and predicted value of lard percentage with the increasing value of the coefficient of determination ( $R^2$ ). The

optimized processing conditions determined by RSM showed it was possible to produce relatively good quality pelleted fish feed comparable to extruded fish feed. In addition, the involvement of complementary analysis with chemometrics indicated that lard in fish feed could be detected and quantified more effectively, and its relevant biomarkers could also be discovered.

Keywords : Chemometrics, Fatty Acid, Fish feed, Lard, Triacylglycerol.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai  
memenuhi keperluan untuk ijazah sarjana Master Sains

**PENGESANAN LEMAK BABI DALAM MAKANAN IKAN DAN  
PENGOPTIMUMAN KEADAAN PEMPROSESAN BAGI PENGHASILAN  
MAKANAN IKAN PEMPELETAN MENGGUNAKAN KEMOMETRIK DAN  
METODOLOGI PERMUKAAN RANGSANGAN**

Oleh

**MOHAMED HANIFF HANAFY BIN IDRIS**

Februari 2022

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**Institut : Penyelidikan Produk Halal**

Bagi menjimatkan kos pengeluaran makanan ikan, beberapa penternak berskala kecil cenderung menghasilkan makanan ikan menggunakan kaedah pempeletan dan ramuan yang murah. Namun, disebabkan kekurangan ilmu dan kapakaran, keadaan pemprosesan penghasilan makanan ikan sering diabaikan, dan ramuan asas seperti minyak sengaja digantikan dengan bahan yang lebih murah seperti lemak babi. Dari sudut keagamaan, kehadiran lemak babi dalam sesuatu produk adalah dilarang terutama bagi umat Islam. Oleh itu, kajian ini bertujuan mengoptimumkan keadaan pemprosesan kaedah pelet menggunakan metodologi permukaan rangsangan (RSM) dan juga untuk mengesan lemak babi sebagai pencemar dalam makanan ikan menerusi analisis berasaskan asid lemak (FA) dan triasilgliserol (TAG) secara berasingan, yang kemudiannya digabungkan bersama kemometrik. Keadaan pemprosesan optimum ditentukan berdasarkan sifat-sifat seperti kepadatan pukal, kebolehapungan, indeks penyerapan air dan kelarutan air makanan ikan yang dihasilkan. Bagi pengesan lemak babi, komposisi asid lemak *sn*-2 digabungkan bersama komposisi keseluruhan asid lemak untuk menambahbaik analisis berasaskan asid lemak oleh kromatografi gas-spektrometri jisim (GC-MS). Manakala, untuk menambahbaik analisis berasaskan triasilgliserol, analisis termal oleh kalorimeter pengimbasan pembezaan (DSC) digabungkan bersama analisis komposisi triasilgliserol oleh kromatografi cecair berprestasi tinggi (HPLC). Kedua-dua analisis ini kemudiannya digabungkan bersama teknik-teknik kemometrik seperti analisis komponen utama (PCA), analisis diskriminan-kuasa dua terkecil separa ortogonal (OPLS-DA), dan regresi-kuasa dua terkecil separa ortogonal (OPLS-R). Keputusan RSM menunjukkan bahawa gabungan suhu air: 70 °C, kelajuan pencampuran: 75 rpm dan masa pencampuran: 5 minit merupakan keadaan pemprosesan optimum bagi menghasilkan makanan ikan pempeletan dengan kualiti terbaik. Bagi pengesan lemak babi, model PCA-yang-ditambahbaik menunjukkan corak taburan makanan ikan yang lebih dikehendaki dengan membezakan mereka kepada kluster tersendiri tanpa pertindihan. Model ramalan OPLS yang dibina daripada pembolehubah yang ditetapkan oleh PCA-yang-ditambahbaik juga menunjukkan prestasi yang baik. Model OPLS-DA menghasilkan diskriminasi kelas

yang signifikan antara makanan ikan yang dicemari lemak babi dan yang tidak tercemar, manakala OPLS-R memberikan hubungan baik antara peratusan lemak babi diperhatikan dan diramalkan dengan peningkatan nilai pekali penentuan ( $R^2$ ). Keadaan pemprosesan optimum yang ditentukan oleh RSM membuktikan adalah mungkin untuk menghasilkan makanan ikan pempeletan yang berkualiti setara dengan makanan ikan ekstrusi. Selain itu, penyertaan analisis pelengkap bersama kemometrik membuktikan bahawa lemak babi dalam makanan ikan boleh dikesan dan diukur dengan lebih berkesan, dan penanda-bio yang relevan juga dapat ditemukan.

Kata kunci: Kemometrik, Asid lemak, Makanan ikan, Lemak babi, Triasilgliserol,

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## **Declaration by Members of Supervisory Committee**

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>APPROVAL</b>	vi
<b>DECLARATION</b>	viii
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xvii
 <b>CHAPTER</b>	
<b>1      INTRODUCTION</b>	<b>1</b>
1.1     Research background	1
1.2     Hypothesis	3
1.3     Objectives	3
<b>2      LITERATURE REVIEW</b>	<b>4</b>
2.1     Aquaculture in Malaysia	4
2.2     Catfish farming	5
2.3     Nutrient requirements	6
2.4     Production process of fish feed	8
2.5     Response surface methodology	9
2.6     Aquafeed issue in Malaysia	10
2.7     Legislation related to animal feeds	11
2.8     Alternative dietary lipid sources	11
2.9     Analytical tools for authentication of lipids	14
2.9.1    Gas chromatography-mass spectrometry	14
2.9.2    High-performance liquid chromatography	15
2.9.3    Differential scanning calorimetry	15
2.10    Chemometric analysis	16
2.11    The use of chemometrics on analytical methods to detect lard adulteration in animal feed	18
<b>3      OPTIMIZATION OF PROCESSING CONDITIONS FOR PELLETED FISH FEED PRODUCTION USING RESPONSE SURFACE METHODOLOGY</b>	<b>19</b>
3.1     Introduction	19
3.2     Materials and method	20
3.2.1    Materials	20
3.2.2    Proximate analysis	20
3.2.3    Diet formulations	20
3.2.4    Experimental design	21
3.2.5    Fish feed preparation	23
3.2.6    Determination of physical properties of fish feed	23
3.2.6.1   Bulk density	23
3.2.6.2   Floatability	24

3.2.6.3	Water absorption index and water solubility index	24
3.2.6.4	Statistical analysis	24
3.3	Results and discussion	25
3.3.1	Composition of formulated fish feed	25
3.3.2	Data overview and statistical evaluation	26
3.3.3	The effect of processing conditions of physical properties of fish feed	30
3.3.4	Optimization of the processing condition for fish feed	36
3.4	Conclusion	37
<b>4</b>	<b>DETECTION OF LARD IN FISH FEED BY INCORPORATING <i>sn</i>-2 FATTY ACIDS INTO TOTAL FATTY ACID ANALYSIS COUPLED WITH CHEMOMETRICS</b>	
4.1	Introduction	38
4.2	Materials and methods	39
4.2.1	Materials	39
4.2.2	Diet formulations	40
4.2.3	Fish feed preparation	41
4.2.4	Oil extraction	41
4.2.5	2-monoacylglycerol spot isolation	41
4.2.6	Fatty acid methylation	41
4.2.7	Gas chromatography-mass spectrometer parameters	42
4.2.8	Statistical and chemometric analyses	42
4.3	Results and discussion	44
4.3.1	Overall fatty acid composition in fish feed	44
4.3.2	<i>sn</i> -2 fatty acid composition in fish feed	48
4.3.3	Data pre-processing	51
4.3.4	Principal component analysis	51
4.3.5	Orthogonal partial least square-discriminant analysis	54
4.3.6	Orthogonal partial least square-regression	57
4.3.7	Support vector machine, random forest, and multilayer perceptron-artificial neural network for comparative evaluation of OPLS-DA and OPLS-R models	59
4.3.8	Model validations of OPLS-DA and OPLS-R	60
4.4	Conclusion	61
<b>5</b>	<b>DETECTION OF LARD IN FISH FEED USING DIFFERENTIAL SCANNING CALORIMETRY AS A COMPLEMENTARY METHOD TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COMBINED WITH CHEMOMETRICS</b>	
5.1	Introduction	62
5.2	Materials and methods	63
5.2.1	Materials	63

5.2.2	Formulations and preparation of fish feed	63
5.2.3	Oil extraction	64
5.2.4	High-performance liquid chromatography of triacylglycerol	65
5.2.5	Differential scanning calorimetry	65
5.2.6	Statistical and chemometric analyses	65
5.3	Results and discussion	66
5.3.1	High-performance liquid chromatography of triacylglycerol	66
5.3.2	Differential scanning calorimetry analysis	72
5.3.3	Data pre-processing	84
5.3.4	Principal component analysis	84
5.3.5	Orthogonal partial least square-discriminant analysis	86
5.3.6	Orthogonal partial least square-regression	90
5.3.7	Support vector machine, random forest, and multilayer perceptron-artificial neural network for comparative evaluation of OPLS-DA and OPLS-R models	91
5.3.8	Model validations of OPLS-DA and OPLS-R	92
5.4	Conclusion	93
<b>6</b>	<b>SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	<b>95</b>
6.1	Summary and general conclusions	95
6.2	Recommendations for future research	95
<b>REFERENCES</b>		<b>97</b>
<b>APPENDICES</b>		<b>123</b>
<b>BIODATA OF STUDENT</b>		<b>141</b>
<b>LIST OF PUBLICATIONS</b>		<b>142</b>

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
3.1	Design matrix and the results of full factorial design of processing conditions for fish feed production	22
3.2	Proximate composition of each ingredient and formulation pre-defined by Winfeed 2.8 software	25
3.3	Analysis of variance (ANOVA) derived from dependent variable models	26
3.4	The model equation in terms of coded variables for physical properties of fish feed	27
3.5a-b	Estimated coefficient values for the model term and their interaction effects	28
3.6	Solutions for optimal processing conditions	37
4.1	Overall fatty acid composition (%) of different ratios of lard : palm oil in fish feed formulation	46
4.2	<i>sn</i> -2 fatty acid composition (%) of different ratios of lard : palm oil in fish feed formulation	49
4.3	Correlation scaled of loading vector and score vector for the discriminating variables along PC1 and PC2.	53
4.4	Performance of OPLS-DA Model 2, SVM-C, RF-C, MLP-ANN-C, OPLS-R Model 2, SVM-R, RF-R, MLP-ANN-R.	59
5.1a	Triacylglycerol composition (UUU and SUU) (%) of fish feed at different percentages of LD+PO and different ratios of LD to PO	68
5.1b	Triacylglycerol composition (SSU and SSS) (%) of fish feed at different percentages of LD+PO and different ratios of LD to PO	70
5.2a	Cooling thermogram parameters	74
5.2b	Heating thermogram parameters	79
5.3	Performance of OPLS-DA Model 2, SVM-C, RF-C, MLP-ANN-C, OPLS-R Model 2, SVM-R, RF-R, MLP-ANN-R.	92

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
3.1	Flow chart for chapter 3	21
3.2a	Response surface plot of BD for the effect of water temperature and mixing speed at a constant mixing time	30
3.2b	Response surface plot of BD for the effect of water temperature and mixing time at a constant mixing speed	31
3.2c	Response surface plot of BD for the effect of mixing speed and mixing time at a constant water temperature	31
3.3a	Response surface plot of FT for the effect of water temperature and mixing speed at constant mixing time	32
3.3b	Response surface plot of FT for the effect of water temperature and mixing time at a constant mixing speed	32
3.3c	Response surface plot of FT for the effect of mixing speed and mixing time at a constant water temperature	32
3.4a	Response surface plot of WAI for the effect of water temperature and mixing speed at a constant mixing time	33
3.4b	Response surface plot of WAI for the effect of water temperature and mixing time at a constant mixing speed	33
3.4c	Response surface plot of WAI for the effect of mixing speed and mixing time at a constant water temperature	34
3.5a	Response surface plot of WSI for the effect of water temperature and mixing speed at a constant mixing time	34
3.5b	Response surface plot of WSI for the effect of water temperature and mixing time at a constant mixing speed	35
3.5c	Response surface plot of WSI for the effect of mixing speed and mixing time at a constant water temperature	35
4.1	Flow chart for chapter 4	40
4.2a	PCA score scatter plot of model 1 (overall fatty acid composition)	52
4.2b	PCA biplot of model 1 (overall fatty acid composition)	52

4.3a	PCA score scatter plot of model 2 (combined overall fatty acid and <i>sn</i> -2 fatty acid compositions)	53
4.3b	PCA biplot of model 2 (combined overall fatty acid and <i>sn</i> -2 fatty acid compositions)	54
4.4a	OPLS-DA score scatter plot of model 1	55
4.4b	OPLS-DA score scatter plot of model 2	55
4.5a	OPLS-DA loading scatter plot of model 1	56
4.5b	OPLS-DA loading scatter plot of model 2	56
4.6a	VIP plot of the most discriminating fatty acids that relevant to OPLS-DA model 1	57
4.6b	VIP plot of the most discriminating fatty acids that relevant to OPLS-DA model 2	57
4.7a	OPLS actual values vs predicted values plot of model 1	58
4.7b	OPLS actual values vs predicted values plot of model 2	58
4.8a	Y-scrambling plot for OPLS-DA model 2	60
4.8b	Y-scrambling plot for OPLS-R model 2	61
5.1	Flow chart for chapter 5	64
5.2a	DSC cooling thermograms of TAG derived from fish feeds with 25% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	76
5.2b	DSC cooling thermograms of TAG derived from fish feeds with 50% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	77
5.2c	DSC cooling thermograms of TAG derived from fish feeds with 75% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	78
5.3a	DSC heating thermograms of TAG derived from fish feeds with 25% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	81
5.3b	DSC heating thermograms of TAG derived from fish feeds with 50% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	82

5.3c	DSC heating thermograms of TAG derived from fish feeds with 75% of LD+PO bi-mixture at different ratios of LD to PO (0:25, 25:75, 50:50, 75:25, 100:0)	83
5.4a	PCA score scatter plot of model 1 (triacylglycerol composition)	84
5.4b	PCA biplot of model 1 (triacylglycerol composition)	85
5.5a	PCA score scatter plot of model 2 (triacylglycerol composition combined with thermal properties)	85
5.5b	PCA biplot of model 2 (triacylglycerol composition combined with thermal properties)	86
5.6a	OPLS-DA score scatter plot of model 1	87
5.6b	OPLS-DA score scatter plot of model 2	88
5.7a	OPLS-DA loading scatter plot of model 1	88
5.7b	OPLS-DA loading scatter plot of model 2	89
5.8a	VIP plot of the most discriminating TAGs that are relevant to OPLS-DA model 1	89
5.8b	VIP plot of the most discriminating TAGs and thermogram parameters that relevant to OPLS-DA model 2	90
5.9a	OPLS actual values vs predicted values plot of model 1	90
5.9b	OPLS actual values vs predicted values plot of model 2	91
5.10a	Y-scrambling plot for OPLS-DA model 2	93
5.10b	Y-scrambling plot for OPLS-R model 2	93

## LIST OF ABBREVIATIONS

2-MAG	2-monoacylglycerol
BD	Bulk density
BTS	Bartlett's Test of Sphericity
CV-ANOVA	Analysis of variance testing of cross-validated predictive residuals
DSC	Differential scanning calorimetry
FA	Fatty acid
FAME	Fatty acid methyl ester
FT	Floatability
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
KMO	Kaiser-Mayer-Olkin
LD	Lard
LFF	Lard-containing fish feed
MLP-ANN	Multilayer perceptron-artificial neural network
OPLS-DA	Orthogonal partial least square-discriminant analysis
OPLS-R	Orthogonal partial least square-regression
PCA	Principal component Analysis
PO	Palm oil
POFF	Palm oil-containing fish feed
RF	Random forest
RMSECV	Root mean square error of cross-validation
RMSEE	Root mean square error of estimation
RMSEP	Root mean square error of prediction
RSM	Response surface methodology

SFA	Saturated fatty acids
<i>sn</i> -2	second stereospecific position
SSS	Trisaturated
SSU	Disaturated-monounsaturated
SUU	Monosaturated-diunsaturated
SVM	Support vector machine
TAG	Triacylglycerol
USFA	Unsaturated fatty acids
UUU	Triunsaturated
VIP	Variable importance of projection
WAI	Water absorption index
WSI	Water solubility index

# CHAPTER 1

## GENERAL INTRODUCTION

### 1.1 Research background

Catfish (*Clarias spp.*) is one of the freshwater fish species that contributes significantly to aquaculture production in Malaysia (Dauda et al., 2018). This species is well known for its high resistance to disease and ease to breed (Shourbela & Tohamy, 2020). Year after year, the evolutionary progress in the catfish industry has led to the introduction of hybrid catfish through cross-breeding between male *Clarias gariepinus* and female *Clarias macrocephalus* (Koolboon et al., 2014).

The improved features, particularly in terms of delicacy, have caused the market demand for this hybrid catfish species to increase rapidly. (Amang, 2020). However, there are several problems that farmers often encounter to meet market demand and maintain the sustainability of catfish farming. The main challenge is the cost of feed supply which is greatly influenced by two critical factors: processing techniques and ingredients used to produce fish feed. For savings purposes, these factors should be carefully considered as they represent more than 50% of the total cost of fish production (Rana et al., 2009).

In fish feed production, there are two main processing techniques commonly practiced by manufacturers, either by pelleting or extrusion process (Zhenhua, 2011). The pelleting process entails the compression phase of small particles to produce larger particles in the presence of appropriate water content, temperature, and pressure. Meanwhile, the extrusion process involves not only compression but also cooking and expansion phase of the feed, which requires a higher water content, temperature, and pressure than the pelleting process (Anonymous, 2019c).

In general, extruded feed is preferred by most farmers because its buoyancy and water stability are better than pelleted feed (Welker et al., 2018). The good physical properties of extruded feed make it easy for farmers to observe and manage fish feeding activities, which can indirectly prevent wastage and water contamination (Verdegem, 2013). However, the high operating costs and market prices of extruded feed have forced small-scale farmers to produce their fish feed using cheaper pelleting methods to save costs (Agrimag, 2019).

Even so, due to lack of expertise and experience, the fish feed produced is sometimes easily immersed and disintegrated when exposed to water. To address this issue, in addition to using good binding and floating agents, the processing conditions of the pelleting method need to be studied in more detail. Therefore, the optimum level of each processing condition could be well determined to produce high-quality pelleted fish feed comparable to the extruded feed. One of the suggested ways is to apply a well-used optimization approach namely Response Surface Methodology (RSM) (Irungu et al.,

2019). It is suitable for use in situations where two or more influence factors need to be optimized to obtain the desired responses (H. Wang et al., 2021; M. Kumari & Gupta, 2019)

Apart from the physical properties, the nutrient content is also one of the important factors in the production of fish feed related to the selection of the ingredients. The ingredients used are based on the nutrient requirements of the species and the age of the fish raised (Robinson et al., 2001). Fish feeds are composed of various ingredients such as fishmeal, soybean meal, cornmeal, starch, fish oil, vitamin, mineral, and so on. They can be divided into six major compound categories: moisture, protein, fat, ash, crude fibre, and nitrogen-free extract (Anonymous, 2019b). Protein and fats are the most important compounds in producing fish feed (Petricorena, 2014). The main function of protein is to enhance growth and health performance, while fat is important to supply energy to the fish (Craig & Helfrich, 2009).

Traditionally, fish oil is commonly used as a source of fat in fish feed production (Harlıoğlu, 2012). However, due to its high price, sometimes this oil is deliberately replaced by suppliers with cheaper fat sources to reduce production costs. Among the fats often used as a substitute are vegetable oil, chicken fat, and lard. However, as is well known, the presence of lard in the human food chain is a serious religious issue among the Muslim and Jewish communities (Dahimi et al., 2013). In addition, the consumption of swine and its derivatives is perspicuously haram and strictly prohibited (Department of Standard Malaysia, 2009). It is clearly stated in the Al-Quran (chapter 2, verse 173) where Allah has forbidden Muslims to eat only carrion, blood, and swine flesh and slaughtered animals, not for Allah's sake.

Many analytical methods have been used for the analysis of lard adulteration, such as gas chromatography-mass spectrometry (GC-MS) (Guntarti et al., 2020), differential scanning calorimetry (DSC) (Dahimi et al., 2014), high-performance liquid chromatography (HPLC) (Marikkar et al., 2003), fourier transform infrared spectroscopy (FTIR) (Man et al., 2014), nuclear magnetic resonance (NMR) (Fadzillah et al., 2015), and isotope-ratio mass spectrometry (IRMS) (Naquiah et al., 2015). However, the detection of lard was reported to be more difficult when the chemical composition of lard is almost similar to other fats such as chicken and palm oil (Yanty et al., 2018). Furthermore, the existence of lard as a minor component in a complex mixture makes lard detection more complicated (Al-Kahtani et al., 2014).

In such a situation, one of the possible ways to improve the capabilities of lard detection is by applying chemometric techniques to the data obtained from analytical methods. These chemometric techniques use a combination of knowledge related to mathematics, statistics, and logic-based methods to manage, explore and interpret data better and more effectively (Haswell, 1992). Worley & Powers (2016) have proved that the use of principal component analysis (PCA) with orthogonal partial least square-discriminant analysis (OPLS-DA) are powerful chemometric techniques for qualitative analysis. Meanwhile, Nitta et al. (2017) have shown that the application of principal component analysis with orthogonal partial least square-regression (OPLS-R) are excellent chemometric techniques for quantitative analysis.

At present, there is no specific method that has been published for detecting lard as an adulterant in fish feed. Hence, in addition to proposing optimal processing conditions for the pelleting method, this study also suggests the use of two fundamentally different analytical methods (fatty acid and triacylglycerol-based) along with their respective complementary method and combined with chemometric techniques in developing more reliable detection methods for lard in fish feed.

## **1.2 Hypothesis**

In this study, it is hypothesized that:

1. The use of RSM will provide a good description of the effects of each processing condition of pelleted fish feed production as well as their interactive and quadratic effects on fish feed quality, thereby facilitating the determination of optimal processing conditions.
2. Based on fatty acid analysis, the incorporation of *sn*-2 fatty acid composition with overall fatty acid composition coupled with chemometric techniques will increase the effectiveness of lard detection and quantification processes in fish feed.
3. Based on triacylglycerol analysis, the integration of triacylglycerol thermal properties with triacylglycerol composition combined with chemometric techniques will improve the capabilities of lard detection and quantification methods in fish feed.

## **1.3 Objectives**

Thus, the present study embarks on the following objectives:

1. To optimize the processing conditions of pelleted fish feed production using RSM.
2. To enhance the effectiveness of lard detection and quantification methods in fish feed by incorporating *sn*-2 fatty acid composition with overall fatty acid composition using GC-MS coupled with chemometric techniques.
3. To improve the detection and quantification processes of lard in fish feed by integrating DSC with HPLC data combined with chemometric techniques.

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