

UNDERSTANDING LIPID QUALITY VARIABILITY IN ASIA PACIFIC THROUGH COMPREHENSIVE LIPID EVALUATION TESTS

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➤ Supporting Information

ABSTRACT: The objective of this study was to investigate the variability in the oxidative quality and nutritional values of different lipid samples collected across Asia-Pacific region. The oxidative quality was evaluated through the peroxide value (PV) and malondialdehyde (MDA) content, while the free fatty acid (FFA) content and degree of fatty acid saturation (U/S ratio) were two essential parameters used to understand the nutritional values or metabolizable energy (ME) values of lipid samples. A total of 1221 lipid samples were collected and analyzed over a period of 10 years. The study showed high variability in oxidative quality between the lipid samples. Due to higher unsaturated fatty acid composition, the oxidative quality for most of the fish oil and soybean oil was at a less favorable range compared to rice bran oil, crude palm oil, and refined palm oil. The standard deviation of free fatty acids (FFA) content of soybean oil and refined palm oil was smaller compared to tallow, rice bran oil, crude palm oil, and fish oil. Fish oil and soybean oil had a higher standard deviation in U/S ratio. Variations in the FFA content and U/S ratio contributed to fluctuation in ME values.

Keywords: Fatty acid composition, Hydrolysis, Lipid quality, Metabolizable energy, Oxidation.

INTRODUCTION

Lipids are organic compounds comprising triglycerides which are soluble in organic solvents such as chloroform and hexane (Brian et al., 2015). The term "lipid" is often used interchangeably with fat and oil. Fat is solid at room temperature and of animal origin while oil is liquid at room temperature and of vegetable origin (Brian et al., 2015).

Lipids are expensive commodities and yet they are highly variable in composition and quality which contribute to variability in their energy density and energy content of the animal diets. It is important to quantify lipid quality variability for precise diet formulation (Wealleans et al., 2021). Inclusion of animal and vegetable-derived lipids in animal diets increases energy density because the energy in lipids is at least twice as high as other food nutrients such as carbohydrates and proteins (Ravindran et al., 2016). Apart from energy enhancement, the addition of lipids can also i) increase the diet palatability; ii) reduce the dust during feed milling; iii) improve the absorption of fat-soluble vitamins; iv) supply metabolically essential fatty acids such as linoleic acids, and v) allow for better absorption of nutrients from the diet by reducing the rate of feed passage through the gastrointestinal tract (Baião and Lara, 2005; Ravindran et al., 2016). Despite the numerous advantages of lipid supplementation, it is important to recognize that lipids are extremely susceptible to oxidative and hydrolytic rancidification. This, combined with improper storage conditions and fatty acid composition, results in quality variation (Robards et al., 1988).

Oxidation occurs in the presence of initiators such as oxygen, light, heat, and metal ions, which can cause lipids to be oxidized to hydroperoxides and carbonyls through a cascade of chemical reactions. The chemical composition of the lipids (i.e., degree of unsaturation in the fatty acid moiety of lipids) also plays a part in their susceptibility to the oxidation process (Robards et al., 1988). The extent of oxidative rancidity is commonly evaluated using the peroxide value (PV) and malondialdehyde (MDA) content to determine the degree of primary and secondary oxidation respectively (Cong et al., 2020). Lipid oxidation gives rise to unique, unpalatable odors and flavors (Robards et al., 1988). Oxidation also impacts energy digestibility and the stability of other nutrients in animal diets such as vitamins and carotenoids (Bonnie and Choo, 1999; Choe and Min, 2009; Shurson et al., 2015). This adversely affects the animal's feed intake and growth performance leading to poor return of investment (ROI) (Shurson et al., 2015; Esmail, 2018). Therefore, antioxidants are often added to fats and oils in feed to delay the onset of oxidation.

The presence of heat, moisture, and endogenous lipase lead to hydrolysis of lipids to free fatty acids (FFA) and glycerol. FFA content is often evaluated to determine the extent of hydrolytic rancidity (Robards et al., 1988). Apart from being a hydrolytic quality indicator, FFA content is also closely related to the nutritional values (i.e., metabolizable energy (ME) values) of the lipids (Wiseman and Salvador, 1991). Higher dietary FFA content was linked to a lower fat absorption rate in chickens (Rodriguez-Sanchez et al., 2019). According to Attech and Leeson (1985), lower fat absorption was likely

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due to the formation of insoluble soaps from the reaction between the carboxylic functional group of FFA and divalent minerals such as calcium and magnesium in the intestinal tract, which led to a lower fat retention rate in their intestine and with resultant lower ME values. Besides the negative contribution of high FFA content to the ME values (Wiseman and Blanch, 1994), degree of fatty acid saturation (U/S ratio) is also strongly related to fat digestion, i.e., unsaturated fatty acids have higher digestibility than saturated fatty acids (Thng et al., 2020). Wiseman et al. (1998) expressed the relationship of FFA content and U/S ratio to ME values in a mathematical equation (“Wiseman Equation”) which can be used to predict the ME values of lipids for broiler chickens of different ages.

Lipid samples were collected across the Asia-Pacific region between year 2010 and 2020, from different suppliers, to evaluate their oxidative quality and ME values. The oxidative quality of the lipids was determined using PV and MDA analyses while FFA content and U/S ratio of the lipids were analyzed to predict their ME values using the Wiseman Equation (Wiseman et al., 1998). The analyses showed that both oxidative and nutritional values of various lipid types were inconsistent, and variabilities were observed between the lipid types. This study highlights the importance of assessing lipid quality to help ensure animal performance and ROI in view of the variability of lipid quality in feed.

MATERIALS AND METHODS

Sample collection and preparation

A total of 1221 lipid samples of animal and vegetable origins (Figures 1 and 2) were collected from different suppliers across the Asia-Pacific region (Figures 3 and 4) from year 2010 to 2020. No written record with respect to the handling, storage condition, or treatment on the lipid samples were available prior to the sample receipt. All lipid samples were stored in plastic containers and kept in the chiller at 2 to 6 °C upon receipt. Prior to analysis, the samples were either thawed at room temperature or melted in the oven at 60 °C. Prolonged heating of samples in the oven was avoided to minimize the impact on lipid quality. All lipid samples were analyzed within one week from the receiving date.

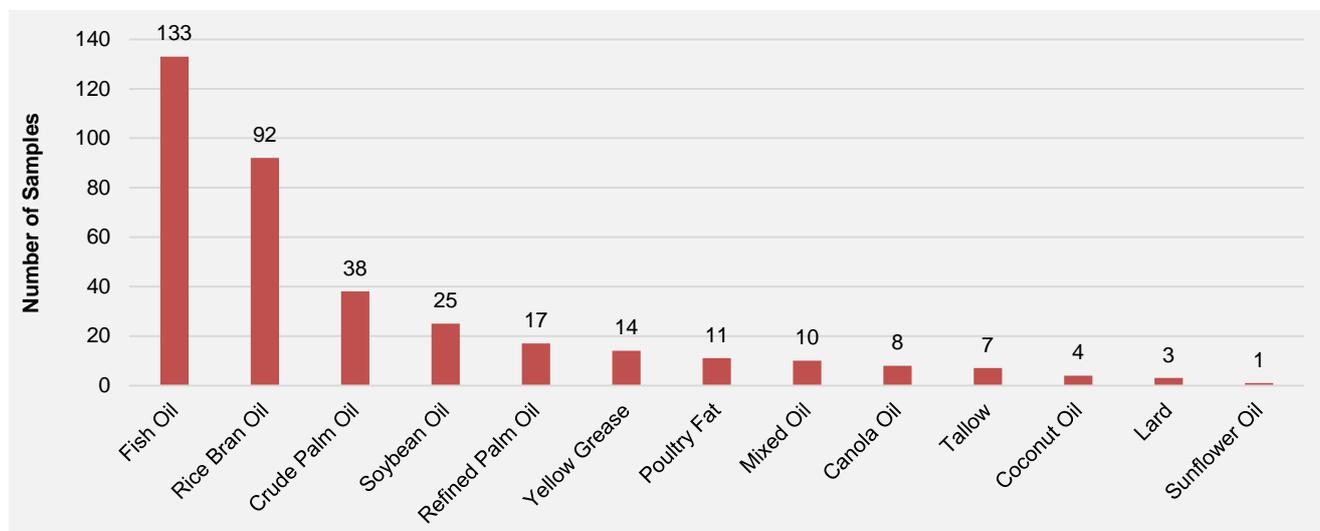


Figure 1 - Collection of different lipid types for oxidative quality study.

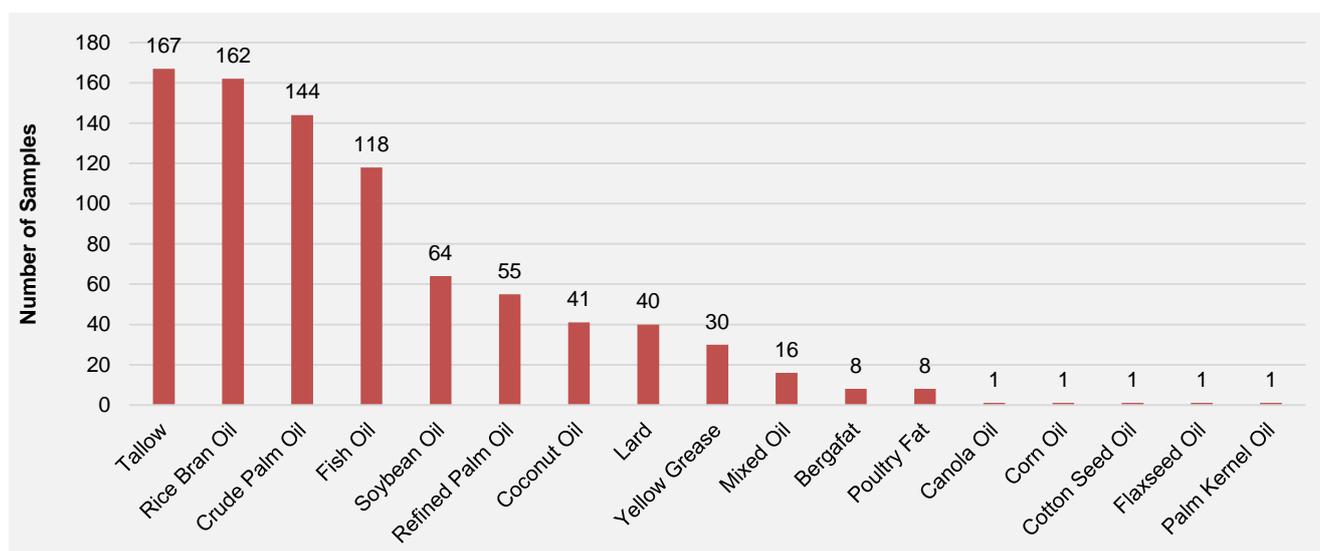


Figure 2 - Collection of different lipid types for FFA content, U/S ratio, and ME values study.

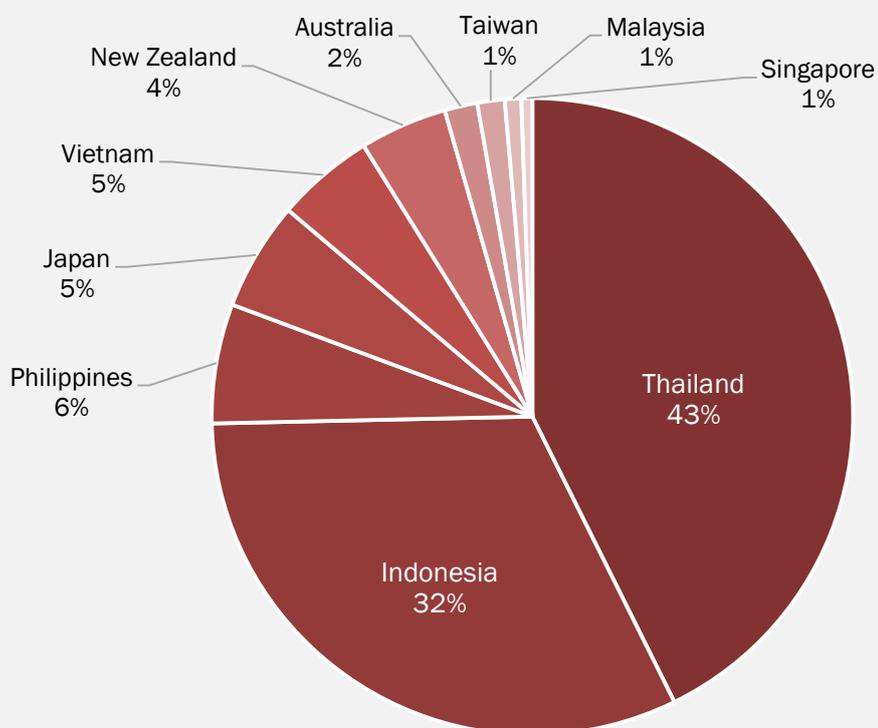


Figure 3 - Origin of lipid samples collected for oxidative quality study.

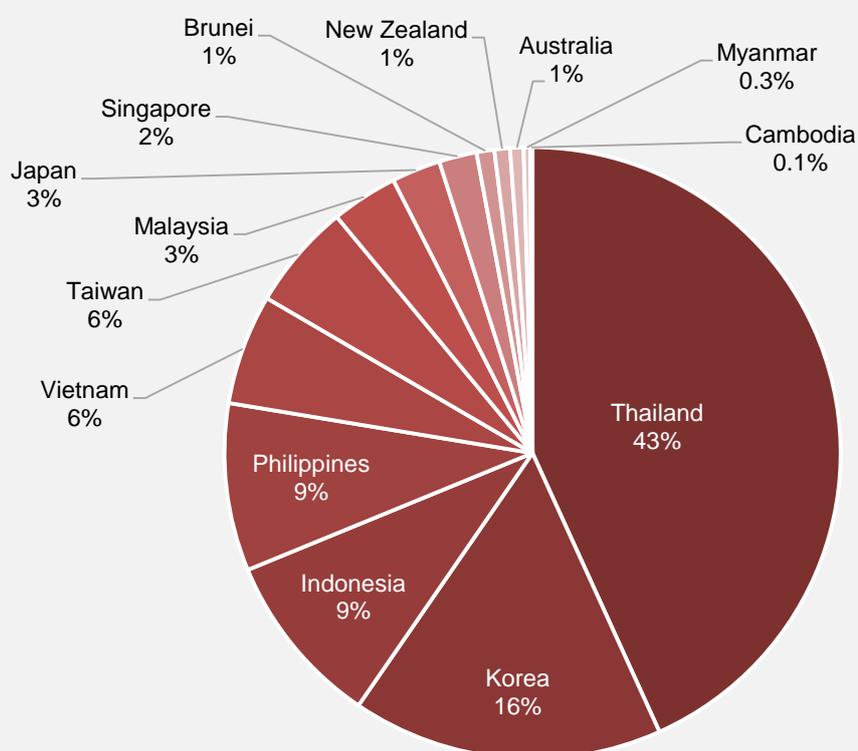


Figure 4 - Origin of lipid samples collected for FFA content, U/S ratio, and ME values study.

Instrumentation

A DL 50 GRAPHIX auto-titrator (Mettler-Toledo, United States) with DM140-SC and DG113-SG glass electrodes (Mettler-Toledo, United States) were used to determine peroxide value and free fatty acid content respectively. A 1100 Series high pressure liquid chromatography system with diode array detector (Agilent Technologies, United States) with ZORBAX StableBond C18 column (5 μm , 250 \times 4.6 mm) (Agilent Technologies, United States) was used to determine the malondialdehyde content. A 7890B gas chromatography system with flame ionization detector (Agilent Technologies, United States) with Supelco SPTM-2560 column (100 m \times 0.25 mm \times 0.20 μm) (Sigma-Aldrich, United States) was used for chromatographic separation of the fatty acid methyl esters.

Peroxide value (PV) determination

The PV of lipid samples was determined using an in-house method, modified from the American Oil Chemists' Society (AOCS) Official Method Cd 8-53 (AOCS, 2017). Thirty mL of acetic acid-chloroform mixture (Avantor, United States; Tedia, United States) was added to one gram of lipid sample in a titration cup and 0.5 mL of saturated potassium iodide solution (Merck, Germany) was subsequently added. The mixture was swirled for one minute and the cup was stored in the dark for another one minute. Thirty mL of distilled water was then added. The cup was attached to the auto-titrator, and the mixture was stirred at 50 % speed for five minutes before titrating it against standardized 0.01 N sodium thiosulphate solution (titrant) (Fisher Scientific, United States) to its potentiometric endpoint. The titrant was standardized against potassium dichromate (Merck, Germany) in potassium iodide solution acidified with 1 N hydrochloric acid (Merck, Germany) prior to analysis. The result was calculated based on the volume consumption of titrant and its concentration.

Malondialdehyde (MDA) content determination

The MDA content of lipid samples was determined using an in-house method, modified from the work published by Mendes et al. (2009). Dilution of 1,1,1,3,3-Tetraethoxypropane (TEP) (Sigma-Aldrich, United States) with 5 % trichloroacetic acid (TCA) solution (Sigma-Aldrich, United States) was used to prepare a series of standard solutions (0.2, 0.4, 0.8, 1.0, 2.0, and 5.0 µg/g). One hundred mL of 5 % TCA solution was added to 10 gram of lipid sample in a 150 mL glass beaker. The mixture was stirred for 15 minutes, and the aqueous layer of the mixture was filtered through a 0.45 µm PES syringe filter (Agilent Technologies, United States) to obtain a clear solution. Five mL of sample supernatant and standard solution were transferred to 15 mL centrifuge tubes filled with five mL of 0.2 % thiobarbituric acid (TBA) (Sigma-Aldrich, United States). The mixture was vortex agitated and the tubes were placed in a water bath with boiling water for 30 minutes. The tubes were then cooled, and the content was vortex agitated again before injection into high pressure liquid chromatography (HPLC) for analysis. The MDA-TBA adduct was separated isocratically with a HPLC mobile phase pumped at 0.7 mL/min and consisting of 0.03 M potassium dihydrogen phosphate solution (Merck, Germany), adjusted to pH 7 using 1 N sodium hydroxide solution (Merck, Germany) and methanol (Fisher Scientific, United States) in the ratio 55:45 (v/v), column thermostat at 30 °C, and detection wavelength of 532 nm. The total analysis run time was 10 minutes with post run of five minutes. The result was calculated based on the established calibration curve and 0.2 µg of TEP corresponded to 0.0065 µg of MDA.

Free fatty acid (FFA) content determination

The FFA content of lipid samples was determined using an in-house method, modified from the Association of Official Analytical Chemists (AOAC) Official Method 940.28 (AOAC, 2012). Fifty mL of 95 % ethanol (Aik Moh Paints and Chemicals, Singapore) was added to one gram of lipid sample in a titration cup. The cup was attached to the auto-titrator, and the mixture was stirred at 50 % speed for one minute before titration against 0.1 N sodium hydroxide solution (titrant) (Merck, United States) to its potentiometric endpoint. The result was calculated based on the volume consumption of titrant and its concentration. The FFA content was expressed either as % oleic acid, % palmitic acid, or % lauric acid depending on the lipid type.

Fatty acid profile analysis

The fatty acid composition of lipid samples was determined using an in-house method, modified from the Association of Official Analytical Chemists (AOAC) Official Method 969.33 (AOAC, 2012). In short, the fatty acids in the lipid samples were trans esterified into fatty acid methyl esters (FAME) to ease the chromatographic separation. Four mL of 0.5 M methanolic sodium hydroxide solution (Fisher Scientific, United States; Merck, Germany) was added to 40 mg of lipid sample in a 50 mL round bottom flask and refluxed until there were no visible lipid globules. Five mL of 14 % boron trifluoride in methanol (Sigma-Aldrich, United States) was then added and refluxed for another two minutes. Subsequently, 10 mL of heptane (Sigma-Aldrich, United States) was added and refluxed for another one minute. The content was then cooled to room temperature. Next, 15 mL of saturated sodium chloride solution (Merck, Germany) was added, and the flask was swirled vigorously. The top organic layer was filtered through an 0.2 µm RC syringe filter (Sartorius, Germany) attached to a syringe filled with sodium sulphate (Merck, Germany) and injected into a gas chromatography (GC) system for analysis. FAME were analyzed with helium as the carrier gas at a flow rate of 0.85 mL/min, split ratio of 40:1, injection volume of 0.4 µL, and inlet temperature of 260 °C. The GC oven temperature was programmed at 140 °C for five minutes and increased to 235 °C at 5 °C/min for 15 minutes, followed by 15 °C /min to 250 °C for five minutes. The total run time was 45 minutes. The percentage composition of each FAME in lipid samples was calculated with reference to Supelco 37 Component FAME Mix (Sigma-Aldrich, United States). The degree of fatty acid saturation (U/S ratio) of the lipid samples was then determined based on the percentage composition results.

Metabolizable energy (ME) prediction

The ME values of lipid samples for broilers of different ages were predicted using Wiseman Equation (Equation 1) with FFA content, U/S ratio, and empirical constants A, B, C, and D (Table 1).

$$\text{ME (kcal/kg)} = (A+B \times \text{FFA}+C \times e^{(D/U/S)})/0.004184 \quad (1)$$

Table 1 - The values of empirical constants A – D used in Wiseman Equation for the prediction of ME values of lipid samples for broilers of different ages (Wiseman et al., 1998).

Empirical constant	Young broilers (aged < 21 Days)	Old broilers (aged > 21 Days)
A (MJ/kg)	38.112 ± 1.418	39.025 ± 0.557
B (MJ/kg)	-0.009 ± 0.002	-0.006 ± 0.001
C (MJ/kg)	-15.337 ± 2.636	-8.505 ± 0.746
D	-0.506 ± 1.186	-0.403 ± 0.088

Statistical analysis

Descriptive statistics were calculated using Microsoft Excel 365.

RESULTS AND DISCUSSION**Oxidative quality (PV and MDA content)**

The peroxide value (PV) and malondialdehyde (MDA) content are two key parameters that quantify primary and secondary products generated during lipid oxidation. The results for PV and MDA content and variability are shown in Table 2 for the five major lipid types, namely fish oil, rice bran oil, crude palm oil, soybean oil, and refined palm oil. The standard deviation for PV values for these lipids ranged from 1.38 meq/kg for refined palm oil to 3.82 meq/kg for crude palm oil while that of MDA content ranged from as low as 0.12 µg/g for rice bran oil to 5.87 µg/g for fish oil. These results indicated that there were different degrees of oxidation across these five lipid types, and more importantly, that oxidative variability was present between samples belonging to the same lipid type. This could be attributed to numerous factors such as the possible presence of initiators such as heat and metal ions, as well as the chemical composition of the lipids. High temperature storage conditions and the existence of metal ions in the lipids can catalyze oxidation, leading to increased PV and MDA content. Additionally, lipids rich in unsaturated fatty acids are more prone to oxidation as compared to the more saturated lipids (Robards et al., 1988).

To describe the extent of oxidative rancidification of the lipids more effectively, Verleyen (2010) published an interpretation guideline comprising of four different oxidation stages based on the PV and MDA content of the lipids (Table 3). The oxidative stages of the samples from the five major lipid types tested in the current study were partitioned according to this guideline to examine oxidative variability (Figure 5). Accordingly, less than 40 % of the fish oil samples had PV and MDA contents in the range of acceptable oxidative quality equating to more than 60 % of the fish oil samples exhibiting signs of oxidation during the time of the study. Severe lipid oxidation accounted for 8.9 % of the fish oil samples analysed, which was the highest amongst the five major lipid types analysed. This can be attributed to the high susceptibility of oxidation in fish oil due to the presence of long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). LCPUFA are highly reactive towards oxidation due to (i.) a high number of double bonds and (ii.) position of double bonds in the backbone structure of the fatty acid as bisallylic carbons in LCPUFA have lower activation energy for hydrogen donation and free radical formation which favours lipid oxidation (Albert et al., 2013).

Table 2 - Descriptive statistical analysis for the oxidative quality of five major lipid types.

Lipid type	Sample size	PV (meq/kg) ^a				MDA Content (µg/g) ^{a, b}			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Fish oil	133	0.28	16.64	4.56	3.07	BDL	38.8	4.06	5.87
Rice bran oil	92	0.54	14.17	4.09	2.89	BDL	0.43	0.35	0.12
Crude palm oil	38	0.50	21.97	4.39	3.82	BDL	2.79	1.38	0.94
Soybean oil	25	0.36	15.68	6.21	3.75	BDL	0.23	NA	NA
Refined palm oil	17	0.72	5.45	2.39	1.38	BDL	0.31	NA	NA

^a Min = minimum; Max = maximum; SD = standard deviation; ^b BDL = below detection limit; NA = not applicable; The detection limit of MDA content was 0.2 µg/g; The mean and standard deviation of MDA content for soybean oil and refined palm oil were not tabulated as there was only one sample for each lipid type with MDA content found to be greater than the detection limit.

Table 3 - Guideline on interpreting the oxidative quality of different lipid types.

Oxidative quality	PV (meq/kg)	MDA Content (µg/g)	
		Fish oil	All other oils
Acceptable	<5	<2	<1
Onset of oxidation	5-10	2-5	1-2
Progressed oxidation	10-20	5-10	2-4
Severe oxidation	>20	>10	>4

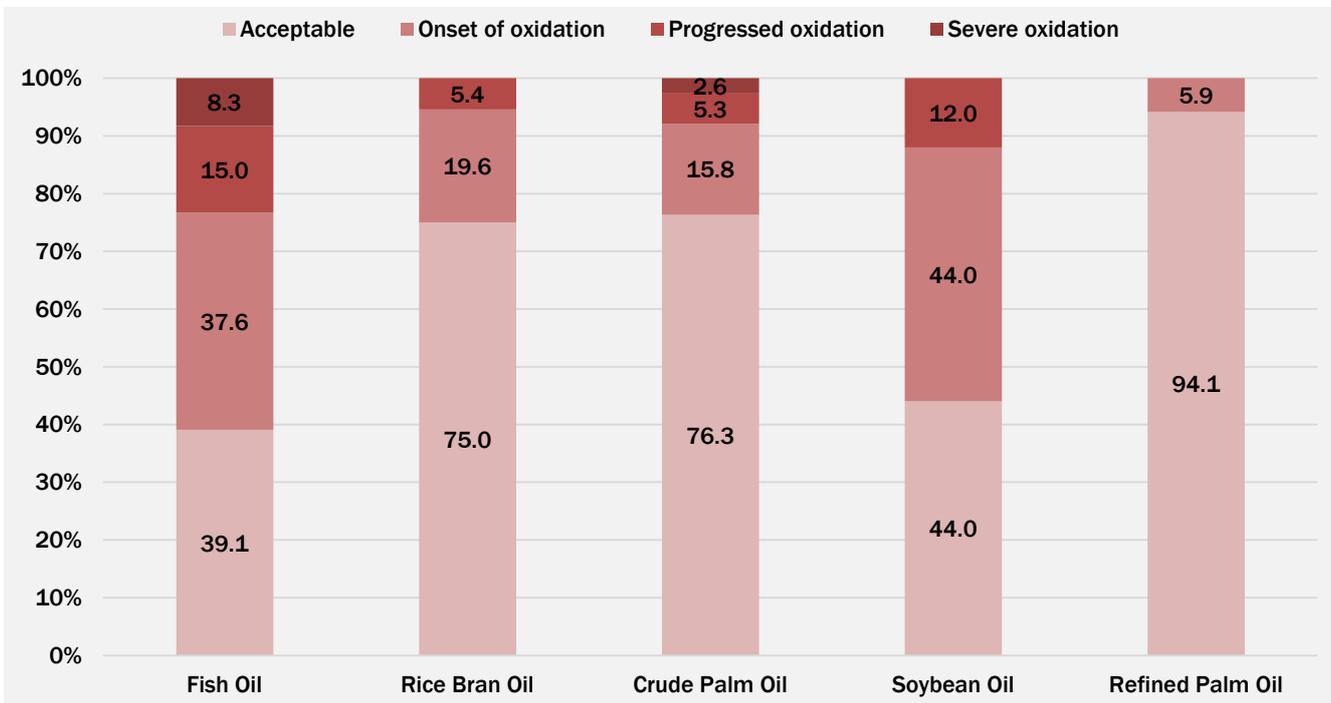


Figure 5 - Oxidative status partition percentages of 5 major lipid types.

Interestingly, the proportion of rice bran oil, crude palm oil, and refined palm oil samples with acceptable oxidative quality were higher compared to the soybean oil samples. Rice bran oil samples involved in the study were of crude oil origin, likely to contain high amounts of endogenous antioxidants such as tocopherols and oryzanols. These antioxidants help to retard oxidation rate (Yoon and Kim, 1994). The presence of natural antioxidants and the absence of highly unsaturated fatty acids in the crude palm oil and refined palm oil samples also made them comparatively stable (Berger, 2003). Severe lipid oxidative deterioration was observed in one of the crude palm oil samples, but this was likely due to the prolonged storage of the lipid under unfavorable conditions prior to receipt. Due to the presence of high levels of mono- and polyunsaturated fatty acids in soybean oil (Kozłowska and Gruczyńska, 2018), it is more susceptible to oxidation as compared to rice bran oil, crude palm oil, and refined palm oil. Indeed, it was observed that 56% of the soybean oil samples were oxidized during the time of study. Apart from the partition percentages of the oxidative status of the lipids, alternative illustrations to visualize the variation in the oxidative quality of the lipids are shown in Figures 6 and 7.

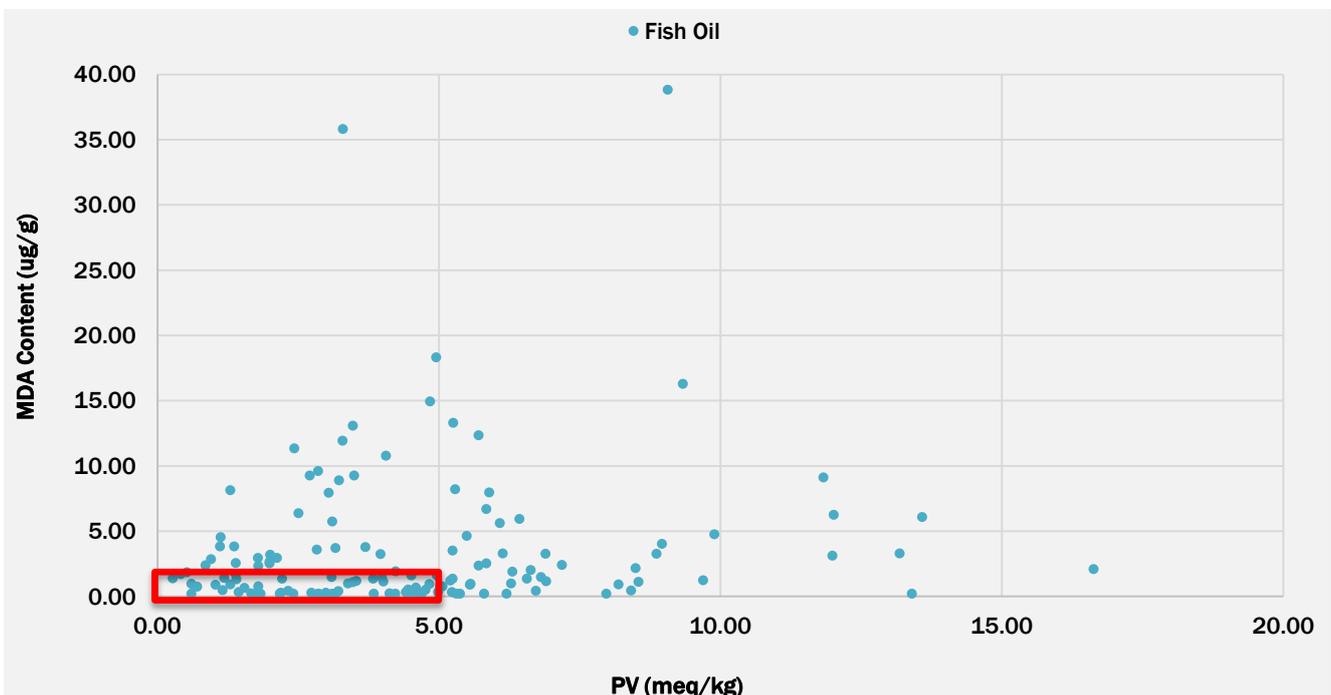


Figure 6 - Oxidative quality distribution chart of fish oil (Footnote: The marked region indicates where the oxidative quality of the fish oil samples was within the acceptable range).

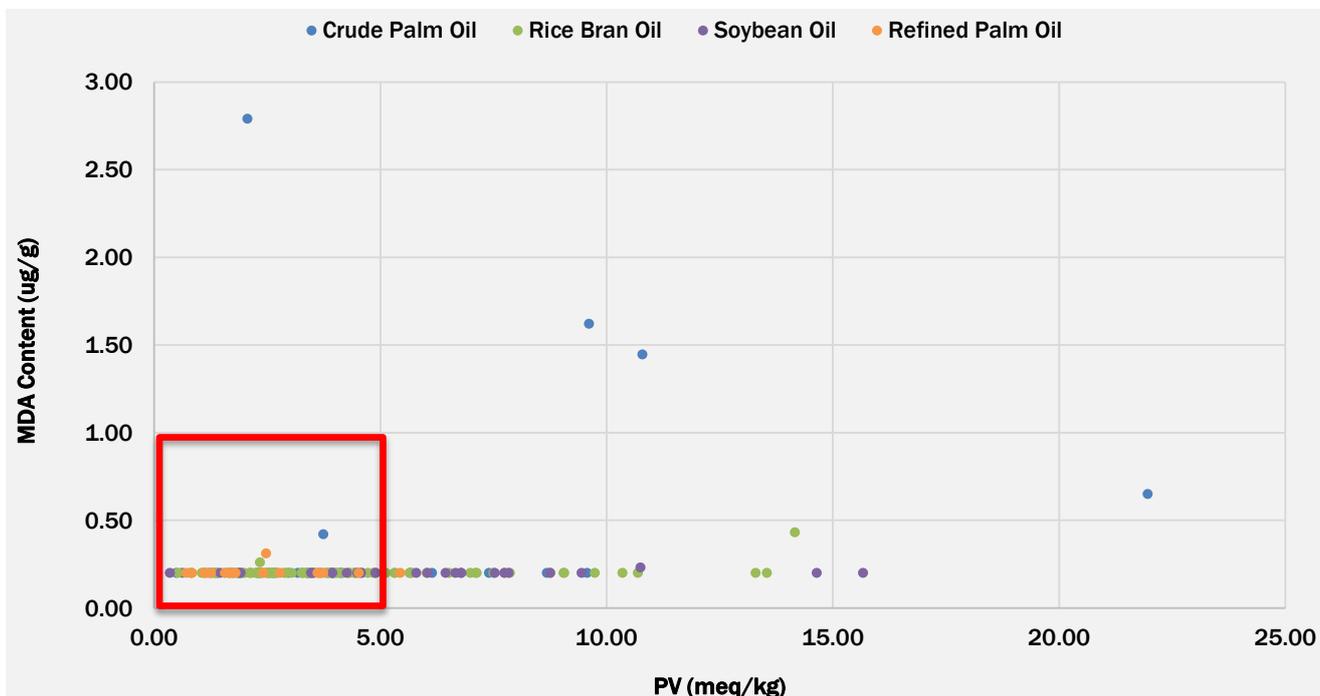


Figure 7 - Oxidative quality distribution chart of crude palm oil, rice bran oil, soybean oil, and refined palm oil samples. (Footnote: The marked region indicates where the oxidative quality of the lipid samples was within an acceptable range; The MDA content of the lipid samples which were below the detection limit were assigned with the value of 0.2 $\mu\text{g/g}$ to make sure the data points were valid to be plotted.)

FFA content and U/S ratio

The free fatty acid (FFA) content and degree of fatty acid saturation (U/S ratio) are two variables incorporated into the Wiseman Equation to (ME) values of the lipids for broilers of different ages (Wiseman et al., 1998). The results were presented in Table 4 for the six major lipid types, namely, tallow, rice bran oil, crude palm oil, fish oil, soybean oil, and refined palm oil.

The standard deviation of FFA content for the soybean oil and refined palm oil samples were relatively small (i.e., 0.8 % oleic acid and 0.7 % palmitic acid respectively). Similarly, their average FFA content (i.e., 1.0 % oleic acid and 0.5 % palmitic acid respectively) were lower compared to the other four lipid types. The small variability and low FFA content may be attributed to the post-processing steps in the manufacturing processes used to derive these lipids. Physical and chemical refining can contribute to the removal of FFA in crude lipids, and this helps to keep the FFA content of soybean oil and refined palm oil at a more consistent level (Robert, 2021). Conversely, the FFA content of the tallow, rice bran oil, crude palm oil, and fish oil samples were more widely spread, with their average FFA contents higher than soybean oil and refined palm oil samples. For tallow and fish oil samples, higher FFA content observed may be attributed to extraction processes (i.e., rendering and wet pressing method respectively) (National Renderers Association, 2003; Bonilla-Méndez JR and Hoyos-Concha, 2018) involving high temperature conditions which subsequently increase the rate of hydrolysis in the lipids (Robards et al., 1988). The raw material for rice bran oil and crude palm oil samples (i.e., rice bran and oil palm fruit mesocarp respectively) contain high lipase activity (Cadena et al., 2012; Brunschwiler et al., 2013) which can potentially cause enzymatic degradation of the lipids, resulting in increased FFA levels (Robards et al., 1988). The extraction processes and the composition of the raw materials play a part in the variability and the level of FFA content. Figure 8 illustrates the variation in the FFA content of the 6 major lipid types in the form of box plot diagram.

From Table 4, it was observed that the fish oil and soybean oil samples had relatively high average U/S ratios (i.e., 2.21 and 4.56 respectively). This is because soybean oil is rich in mono- and polyunsaturated fatty acids (Kozłowska and Gruczyńska, 2018) and fish oil generally contains higher proportions of LCPUFA (Albert et al., 2013). The standard deviations of the U/S ratios in the fish oil and soybean oil samples were relatively large (i.e., 1.38 and 1.34 respectively) compared to other four lipid types (Table 4). This can be closely related to their highly unsaturated lipid profile which is more susceptible to oxidation. Oxidation involves the breakdown of the double bonds in the unsaturated fatty acids with oxidation being inevitable. The standard deviation of the U/S ratio in the fish oil and soybean oil samples was greater than the other lipid types. Additionally, the variation in the fatty acid composition of fish oil samples due to different fish species (such as salmon and tuna, FAO/WHO, 2017), would also contribute to the U/S ratio variability.

The average U/S ratio for tallow, crude palm oil and refined palm oil samples was close to 1.00 making these lipid types more resistant towards the oxidative rancidification process. Although rice bran oil is made up of a higher proportion of unsaturated fatty acids as shown by the relatively high average U/S ratio of 2.71 (Table 4), the inherent presence of natural antioxidants helps to retard the oxidation process and keep their U/S ratio at a more consistent level (Yoon and Kim, 1994). The standard deviation of the U/S ratio for the rice bran oil samples was only 0.50. The variation in

measured U/S ratios may reflect the extent to which the lipid samples were exposed to the oxidation initiators. Figure 9 illustrates the variation in the U/S ratios of the six major lipid types in the form of box plot diagram.

Table 4 - Descriptive statistical analysis for the FFA content and U/S ratio of six major lipid types.

Lipid type	Sample size	FFA content (% oleic / % palmitic acid) ^{a, b}				U/S ratio ^a			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Tallow	167	0.3	51.8	2.6	4.9	0.34	3.22	1.29	0.40
Rice bran oil	162	3.2	68.2	12.6	7.9	1.31	4.55	2.71	0.50
Crude palm oil	144	0.1	49.6	7.2	7.8	0.81	3.62	1.14	0.41
Fish oil	118	0.4	31.1	4.6	5.8	0.56	9.52	2.21	1.38
Soybean oil	64	0.01	4.3	1.0	0.8	0.61	5.74	4.56	1.34
Refined palm oil	55	0.02	4.3	0.5	0.7	0.43	2.53	1.18	0.28

^a Min = minimum; Max = maximum; SD = standard deviation; ^b FFA content of crude palm oil and refined palm oil was expressed in % palmitic acid while FFA content of other lipid types was expressed in % oleic acid. The results expressed in % oleic acid can be converted into % palmitic acid by dividing the former results by 282 g/mol and followed by multiplying with 256 g/mol.

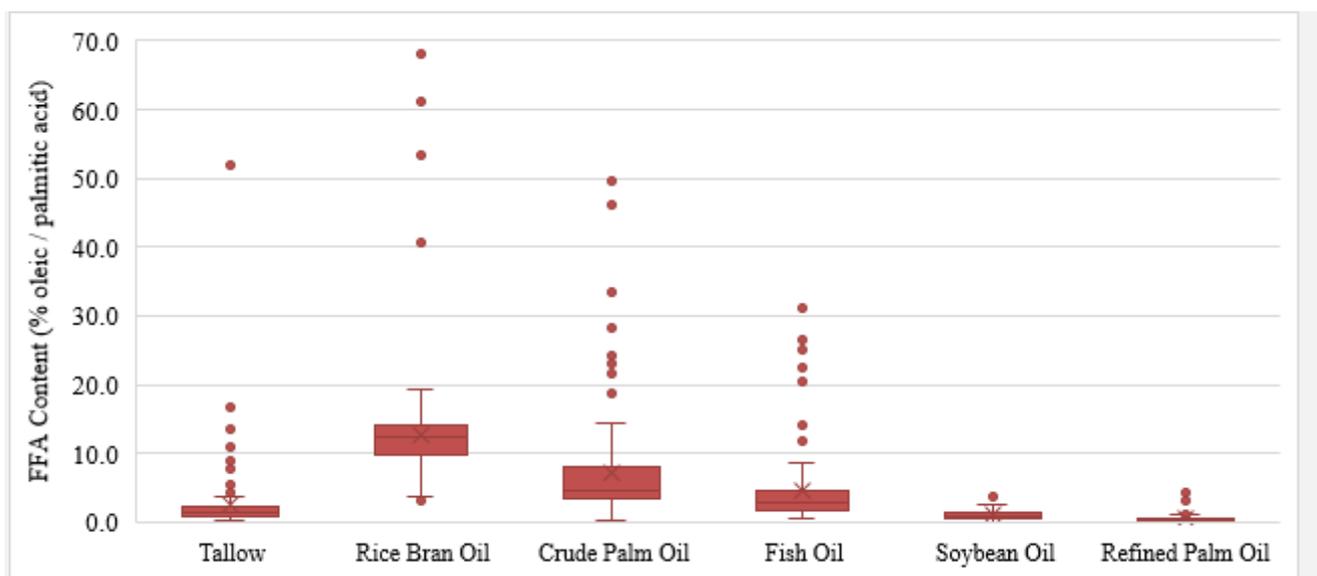


Figure 8 - FFA content variability of 6 major lipid types.

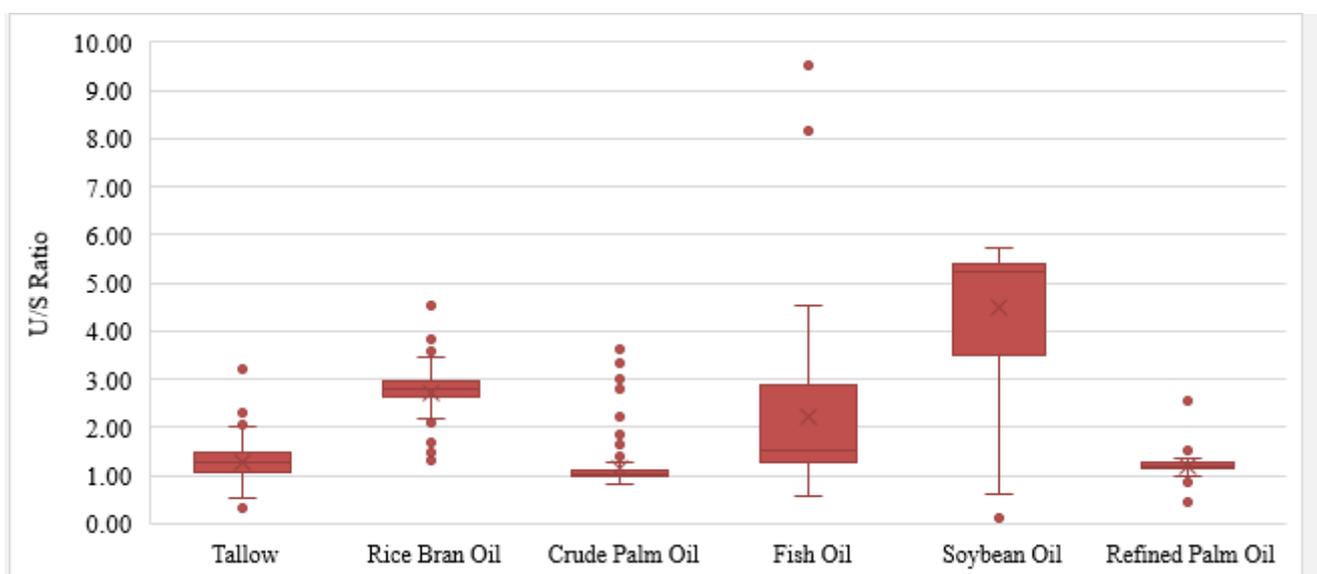


Figure 9 - U/S ratio variability of 6 major lipid types.

Nutritional values (ME values)

Different amplitudes of fluctuation in the FFA content and U/S ratio gave rise to diverse variability in the predicted ME values between and within the lipid types (Figures 10 and 11). The standard deviation of predicted ME values for the fish oil and soybean oil samples (i.e., 639 and 517 kcal/kg respectively for young broilers; 363 and 290 kcal/kg respectively for old broilers) was relatively high compared to other lipid types (Table 5). This was due to the relatively high variation in the U/S ratio for these lipid types (Table 4). In comparison to the U/S ratio, the effect of FFA variation of predicted ME values was less notable as FFA was only linearly correlated to the predicted ME values, as demonstrated in the Wiseman Equation. This explains the observation of having a higher predicted ME value variability for the soybean oil samples even though the standard deviation of their FFA content was small (i.e., 0.8% oleic acid, Table 4). As already referred to, the presence of natural antioxidants in rice bran oil facilitated the retardation of oxidative rancidification and kept the U/S ratio at a more consistent level (Table 4). Hence the standard deviation of predicted ME values for young and old broilers for the rice bran oil samples (i.e., 264 and 152 kcal/kg respectively) was relatively small (Table 5).

The average predicted ME values for the rice bran oil, fish oil, and soybean oil samples (i.e., 7637, 7370, and 8356 kcal/kg respectively for young broilers; 8195, 8079, and 8658 kcal/kg respectively for old broilers) were higher than other lipid types (Table 5) as they contained higher mean U/S ratios than the other lipid types with unsaturated fatty acids being more digestible than saturated fatty acids (Tanchaoenrat et al., 2014). The average predicted ME values of the lipids for old broilers (>21 days old) were higher than young broilers (<21 days old, Table 5). This could be attributed to the fact that young broilers produce less bile acids and pancreatic lipase than the older birds. This in turns reduces the chicks' ability to digest and absorb dietary lipids (Arshad et al., 2020). It is also noteworthy that the variability of the predicted ME values for young broilers was consistently more pronounced than for the older broilers.

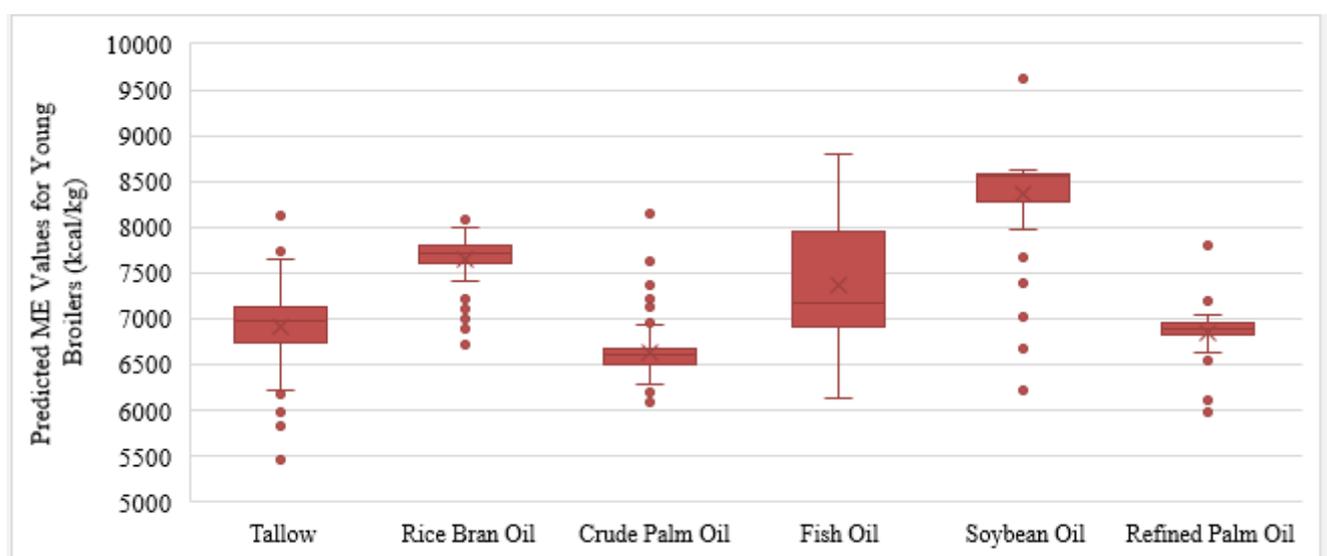


Figure 10 - Variation in the predicted ME values of six major lipid types for young broilers (aged <21 Days).

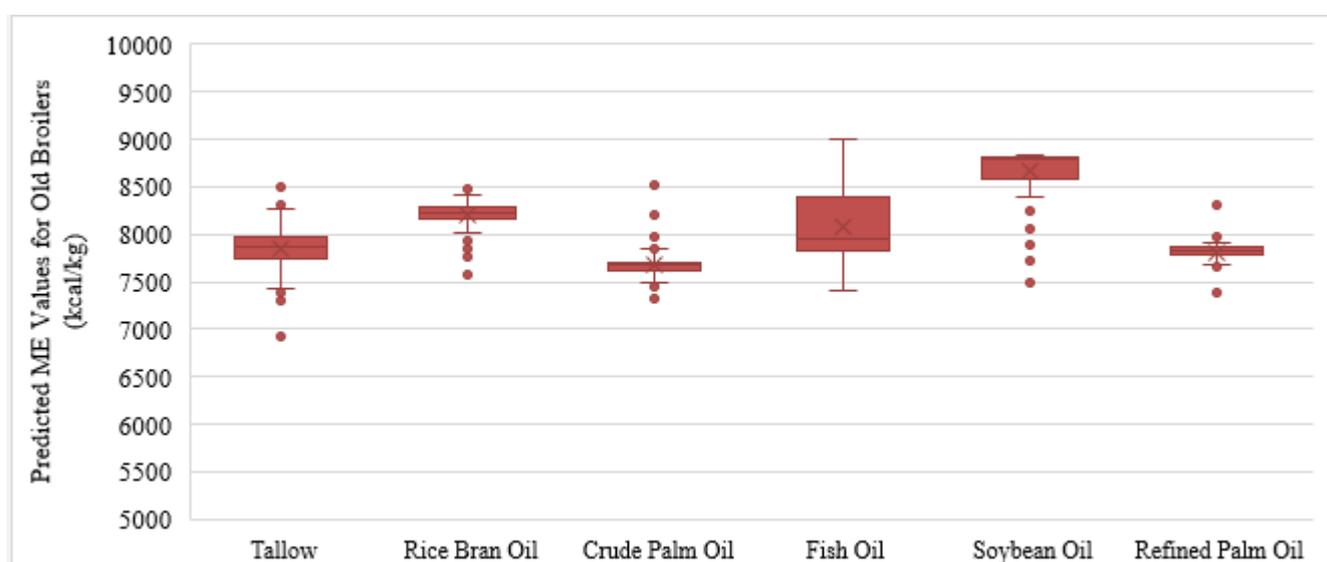


Figure 11 - Variation in the predicted ME values of six major lipid types for old broilers (aged >21 Days).

CONCLUSION

The data from this study showed that the variability of oxidative and nutritional values was observed between the different lipid types as well between the same lipid type. The subsequent impact of these variabilities on the predicted broiler ME values, as calculated using the Wiseman Equation, were presented. To minimize the unfavorable consequences of lipid quality variability on the animal performance and return of investment, laboratory analysts and nutritionists are thus recommended to conduct thorough laboratory analyses regularly to examine the oxidative and nutritional values of lipids. Based on the lipid evaluation information, nutritionists will be able to make informed formulation decisions and apply antioxidants into the lipids when necessary to help protect the quality of their feed formulation.

DECLARATIONS

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Authors' contribution

J.X. TING proposed the design of study, prepared the manuscript, and performed the laboratory analysis. A. THNG, H.R. TAY, G.H. SOO, and H.C. ONG assisted with the laboratory analyses. All the authors read and approved the final manuscript.

Conflict of interests

The authors declared that there is no conflict of interest.

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REFERENCES

- Albert BB, Cameron-Smith D, Hofman PL, and Cutfield WS (2013). Oxidation of Marine Omega-3 Supplements and Human Health, *BioMed Research International*, 2013: 464921. DOI: <https://doi.org/10.1155/2013/464921>
- AOAC (2012) Association of Official Analytical Chemists. Official Methods of analysis, 19th Edition. Washington, D.C. pp.12. http://www.aocofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=2902
- AOAC (2012) Association of Official Analytical Chemists. Official Methods of analysis, 19th Edition. Washington, D.C. pp.19-20. http://www.aocofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=1733
- AOCS (2017) American Oil Chemists' Society. Official Methods and Recommended Practices of the AOCS, 7th Edition. Cd 8-53. DOI: <https://myaccount.aocs.org/PersonifyEbusiness/Store/Product-Details?productId=217949329>
- Arshad M, Bhatti S, Hassan I, Rahman M, and Rehman M (2020). Effects of Bile Acids and Lipase Supplementation in Low-Energy Diets on Growth Performance, Fat Digestibility and Meat Quality in Broiler Chickens. *Brazilian Journal of Poultry Science*, 22(2):1-8. DOI: <https://doi.org/10.1590/1806-9061-2020-1258>
- Attech JO, and Leeson S (1985). Influence of age, dietary cholic acid, and calcium levels on performance, utilization of free fatty acids, and bone mineralization in broilers. *Poultry Science*, 64(10):1959-1971. DOI: <https://doi.org/10.3382/ps.0641959>
- Baião NC, and Lara LJC (2005). Oil and Fat in Broiler Nutrition. *Brazilian Journal of Poultry Science*, 7(3):129-141. DOI: <https://doi.org/10.1590/S1516-635X2005000300001>
- Berger KG (2003). Palm Oil, in *Encyclopedia of Food Sciences and Nutrition*, ed. by Caballero B. Academic Press, London, 4325-4331. DOI: <https://doi.org/10.1016/B0-12-227055-X/01348-1>
- Bonilla-Méndez JR, and Hoyos-Concha JL (2018). Methods of Extraction, Refining and Concentration of Fish Oil as A Source of Omega-3 Fatty Acids. *Corpoica Ciencia y Tecnología Agropecuaria*, 19(3):645-668. DOI: https://doi.org/10.21930/rcta.vol19_num2_art:684
- Bonnie TYP, and Choo YM (1999). Oxidation and Thermal Degradation of Carotenoids. *Journal of Oil Palm Research*, 2(1):62-78. DOI: <http://jopr.mpob.gov.my/wp-content/uploads/2013/07/jopr11n1-p71.pdf>
- Brian JK, Trey AK, and Gerald CS (2015). Characteristics of lipids and their feeding value in swine diets. *Journal of Animal Science and Biotechnology*, 6(1):30. DOI: <https://doi.org/10.1186/s40104-015-0028-x>
- Brunschwiler C, Heine D, Kappeler S, Conde-Petit B, and Nyström L (2013). Direct Measurement of Rice Bran Lipase Activity for Inactivation Kinetics and Storage Stability Prediction. *Journal of Cereal Science*, 58(2):272-277. DOI: <https://doi.org/10.1016/j.jcs.2013.06.007>
- Cadena T, Prada F, Perea A and Romero H (2012). Lipase Activity, Mesocarp Oil Content, And Iodine Value In Oil Palm Fruits *Ofelaeis Guineensis*, *Elaeis Oleifera*, And The Interspecific Hybrid *O×G* (*E. Oleifera*×*E. Guineensis*). *Journal of the Science of Food and Agriculture*, 93(3):674-680. DOI: <https://doi.org/10.1002/jsfa.5940>
- Choe E, and Min DB (2009). Mechanisms of Antioxidants in the Oxidation of Foods. *Comprehensive Reviews in Food Science and Food Safety*, 8(4):345-358. DOI: <https://doi.org/10.1111/j.1541-4337.2009.00085.x>
- Cong S, Dong W, Zhao J, Hu R, Long Y and Chi X (2020). Characterization of the Lipid Oxidation Process of Robusta Green Coffee Beans and Shelf Life Prediction during Accelerated Storage. *Molecules*, 25(5):1157. DOI: <https://doi.org/10.3390/molecules25051157>
- Esmail SH (2018). Feed Flavours for Cattle: The Benefits. DOI: <https://www.allaboutfeed.net/animal-feed/feed-additives/feed-flavours-for-cattle-the-benefits/>

- Food and Agricultural Organization of the United Nations World Health Organization (FAO/WHO) (2017). Codex Alimentarius Commission. Standard for fish oils codex stan 329. Joint FAO/WHO, Rome, Italy. DOI: https://www.iffco.com/system/files/downloads/Codex%20Standard%20for%20Fish%20oils%20CXS_329e_Nov%202017.pdf
- Kozłowska M and Gruczyńska E (2018). Comparison of the oxidative stability of soybean and sunflower oils enriched with herbal plant extracts. *Chemical Papers*, 72(10):2607-2615. DOI: <https://doi.org/10.1007/s11696-018-0516-5>
- Mendes R, Cardoso C and Pestana C (2009). Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. *Food Chemistry*, 112(4):1039-1045. DOI: <https://doi.org/10.1016/j.foodchem.2008.06.052>
- National Renderers Association (2003). Pocket Information Manual – A Buyer's Guide To Rendered Products. DOI: http://assets.nationalrenderers.org/pocket_information_manual.pdf
- Ravindran V, Tanchaenrat P, Zaefarian F and Ravindran G (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. *Animal Feed Science and Technology*, 213:1–21. DOI: <https://doi.org/10.1016/j.anifeeds.2016.01.012>
- Robards K, Kerr AF and Patsalides E (1988). Rancidity and its Measurement in Edible Oils and Snack Foods. A Review. *The Analyst*, 113(2):213-224. DOI: <https://doi.org/10.1039/an9881300213>
- Robert SZ (2021). Alkali Refining. DOI: <https://lipidlibrary.aocs.org/edible-oil-processing/alkali-refining>
- Rodriguez-Sanchez R, Tres A, Sala R, Garcés-Narro C, Guardiola F, Gasa J and Barroeta AC (2019). Effects of dietary free fatty-acid content and saturation degree on lipid-class composition and fatty-acid digestibility along the gastrointestinal tract in broiler starter chickens. *Poultry Science*, 98(10):4929-4941. DOI: <https://doi.org/10.3382/ps/pez253>
- Shurson GC, Kerr BJ and Hanson AR (2015). Evaluating the Quality of Feed Fats and Oils and Their Effects on Pig Growth Performance. *Journal of Animal Science and Biotechnology*, 6(10):1-11. DOI: <https://doi.org/10.1186/s40104-015-0005-4>
- Tanchaenrat P, Ravindran V, Zaefarian F and Ravindran G (2014). Digestion of Fat and Fatty Acids Along the Gastrointestinal Tract of Broiler Chickens. *Poultry Science*, 93(2):371-379. DOI: <https://doi.org/10.3382/ps.2013-03344>
- Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). The Use of Predicted Apparent Metabolizable Energy Values to Understand the Oil and Fat Variability in Broilers. *Online Journal of Animal and Feed Research*, 10(4):150-157. DOI: <https://doi.org/10.51227/ojaf.2020.21>
- Verleyen, T (2010). Oxidation Key Issue in Use of Oils and Fats for Feed. Available from All About Feed. DOI: <https://www.allaboutfeed.net/Nutrition/Raw-Materials/2010/2/Oxidation-key-issue-in-use-of-oils-and-fats-for-feed-AAF011469W/>
- Wealleans AL, Bierinckx K, Witters E, Benedetto MD and Wiseman J (2021). Assessment of the quality, oxidative status and dietary energy value of lipids used in non-ruminant animal nutrition. *Journal of The Science of Food and Agriculture*, 101(10):4266-4277. DOI: <https://doi.org/10.1002/jsfa.11066>
- Wiseman J and Blanch A (1994). The Effect of Free Fatty Acid Content on the Apparent Metabolizable Energy of Coconut / Palm Kernel Oil for Broiler Chickens Aged 12 and 52 Days. *Animal Feed Science and Technology*, 47(3-4):225-236. DOI: [https://doi.org/10.1016/0377-8401\(94\)90126-0](https://doi.org/10.1016/0377-8401(94)90126-0)
- Wiseman J, Powles J and Salvador F (1998). Comparison between Pigs and Poultry in the Prediction of the Dietary Energy Value of Fats. *Animal Feed Science and Technology*, 71(1-2):1-9. DOI: [https://doi.org/10.1016/S0377-8401\(97\)00142-9](https://doi.org/10.1016/S0377-8401(97)00142-9)
- Wiseman J and Salvador F (1991). The Influence of Free Fatty Acid Content and Degree of Saturation on the Apparent Metabolizable Energy Value of Fats Fed to Broilers. *Poultry Science*, 70(3):573-582. DOI: <https://doi.org/10.3382/ps.0700573>
- Yoon SH and Kim SK (1994). Oxidative stability of high-fatty acid rice bran oil at different stages of refining. *Journal of the American Oil Chemists' Society*. 71:227-229. DOI: <https://doi.org/10.1007/BF02541562>