UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF OMEGA-3 CONTENT IN EGG OF CHICKEN FED WITH COMMON FEED AND EXTRUDED FLAXSEED

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DETERMINATION OF OMEGA-3 CONTENT IN EGG OF CHICKEN FED WITH COMMON FEED AND EXTRUDED FLAXSEED

BY

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A project submitted as partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

APPROVAL

This project entitled "Determination of omega-3 content in eggs of chicken fed with common feed and extruded flaxseed" was prepared by Nur Hanis binti Zulkfili and submitted to the Faculty of Medicine and Health Sciences as a partial fulfilment of the requirement of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

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LIST OF ABBREVIATIONS

ALA; C18:3	α-linolenic acid
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CH_2Cl_2/MeOH	Dichloromethane-methanol
CHD	Coronary Heart Disease
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FAME	Fatty acid methyl ester
FFQ	Food Frequency Questionnaire
GC	Gas Chromatography
H_2O	Water
IBM SPSS	IBM Statistical Package for the Social Sciences
K_2CO_3	Potassium Carbonate
KCl	Potassium Chloride
LNA; C18:2	linoleic acid
MANS	Malaysian Adult Nutrition Survey
MeHg	Methylmercury
Omega – 3 FA	Omega – 3 Fatty Acids
Omega – 3 PUFAs	Omega – 3 Polyunsaturated Fatty Acids (PUFAs)

LIST OF ABBREVIATIONS

PPB	Part Per Billion
RCF	Relative Centrifugal Force
RNI	Reference Nutrient Intake
SRM	Standard Reference Material
TEI	Total Energy Intake
V/V	Volume concentration of a solution
W/V	Percent Concentration weight / volume

ABSTRACT

DETERMINATION OF OMEGA-3 CONTENT IN EGG OF CHICKEN FED WITH COMMON FEED AND EXTRUDED FLAXSEED

Nur Hanis Zulkfili

As today, eggs make an important remark to the human diet. Egg can also be a good source to be fortified with omega-3 polyunsaturated fatty acid (EPA and DHA) which differ based on the hen's breed and their feeding. Several studies revealed that omega-3 PUFAs can lower blood pressure and serve as anti-inflammation which aid in cardioprotection. It is proven that Malaysian have deficient omega-3 fatty acid consumption, and this nutrient is mainly obtained from fish-based supplement. However, the major concern about this is the exposure of methylmercury found in fishes. Hence, this study was conducted to determine and compare omega-3 content in egg of chicken fed with common feed and extruded flaxseed. The eggs were provided by the company and were randomly collected according to their days. Next, the egg yolks were separated from albumen by using egg yolk separator before they were homogenized for total lipid and omega 3 content analysis. Omega 3 analysis consist of cold lipid extraction, methylation of fatty acid and gas chromatography. Samples were prepared in duplicate form and analyzed by using One-way ANOVA to determine mean and standard deviation. The result showed that there was no significant difference in total lipid content with ($p > 0.05$) between control and treatment group. Whereas for EPA content, Treatment Day 14^{th} (1.06%) was significantly different with ($p < 0.05$) when compared with other group and selected days. As for DHA content, there was no significant difference when compared between the group and selected days. Nonetheless, the treatment group still have a higher amount of DHA compared to the control group. Overall, feeding hens with flaxseed can increase omega-3 content in egg and Day 14^{th} is the optimum feeding days to enrich omega-3.

ABSTRAK

PENENTUAN KANDUNGAN OMEGA-3 DALAM TELUR AYAM YANG DIBERI MAKANAN UMUM DENGAN YANG DIPERTINGKATKAN DENGAN BIJI RAMI YANG TELAH DIEKSTRUSI

Nur Hanis Zulkfili

Seperti hari ini, telur memainkan peranan yang penting kepada diet manusia. Telur juga boleh menjadi sumber yang baik untuk diperkaya dengan asid lemak tidak tepu omega-3 (EPA dan DHA) yang berbeza berdasarkan spesies ayam dan makanannya. Beberapa kajian telah menunjukkan bahawa omega-3 dapat menurunkan tekanan darah dan berfungsi sebagai anti radang yang membantu dalam perlindungan kardio. Namun begitu, sudah terbukti bahawa rakyat Malaysia kekurangan pengambilan asid lemak tidak tepu omega-3, dan nutrien ini juga boleh diperolehi terutamanya dari makanan berasaskan ikan. Walau bagaimanapun, kebimbangan utama mengenai ini adalah pendedahan metilmercuri yang terdapat dalam ikan. Oleh itu, kajian ini dilakukan untuk mengetahui dan membandingkan kandungan omega-3 dalam telur ayam yang diberi makanan umum dengan yang dipertingkatkan dengan biji rami yang telah diekstrusi. Telur ini disediakan oleh sebuah syarikat dan dikumpulkan secara rawak mengikut hari yang telah ditetapkan. Seterusnya, kuning telur dipisahkan dari putih telur dengan menggunakan pemisah kuning telur sebelum diselaraskan untuk analisis kandungan lemak dan omega 3. Analisis omega 3 ini terdiri daripada ekstrak lemak sejuk, metilasi asid lemak dan kromatografi gas. Sampel disediakan dalam bentuk pendua dan dianalisis dengan menggunakan ANOVA sehala untuk menentukan purata dan sisihan piawai. Hasil kajian menunjukkan bahawa tiada perbezaan yang signifikan dalam jumlah kandungan lemak dengan ($p > 0.05$) antara kumpulan kawalan dan kumpulan rawatan. Manakala untuk kandungan EPA, hari rawatan ke-14 (1.06%) jauh berbeza dengan ($p < 0.05$) jika dibandingkan antara kumpulan dan hari. Bagi kandungan DHA pula, tiada perbezaan yang signifikan jika dibandingkan antara kumpulan dan hari. Walaupun begitu, kumpulan rawatan masih mempunyai jumlah DHA yang lebih tinggi berbanding kumpulan kawalan. Secara keseluruhan, ayam yang diberi makan dengan biji rami dapat meningkatkan kandungan omega-3 dalam telur dan hari ke-14 adalah hari makan yang optimum untuk diperkaya omega-3.

CHAPTER 1
INTRODUCTION

1.1 Background

Modifications in agricultural practice over the past 50 years have expanded the ability of the nation to provide food to its population by increased productivity, increased food abundance and lower seasonal dependency (Kearney, 2010). People these days have more access to food either it is from animal or plant sources, hence they will have more options to choose on types of food that they want to consume. Nevertheless, this will establish a food pattern among themselves which is influenced by several factors namely economic status and food availability. A national study conducted by local researchers found that white rice and hen eggs contributed significantly to the most popular weekly foods consumed with 2.48 cups and 1.58 pieces per serving respectively (MANS, 2014).

For the past thousand years, chickens and men have coexisted. Chicken specifically known as 'poultry' or 'fowl' is a term to define captive-raised domesticated birds in which their meat, eggs, and feathers were utilized by humans. Domestic fowl which commonly found in India, Myanmar, Malaysia, and Thailand were originally from the Red Jungle Fowl (*Gallus gallus*) and wild jungle fowl (*Gallus domesticus*) (Crawford, 1990; Elson, Gleadthorpe, Vale, & Uk, 2011)   However, it was the Roman who initiated fowl to be used as laying hens by developing their breeds for agricultural industries. As of today, eggs make an important remark to the human diet. Both of their distinct part either the egg yolk and albumen, deliver important nutrients for human development and growth (Hegde, Panse, Atakare, & Kadam, 2016). Other than that, Fraeye et al., (2012) stated that egg is a good source to be fortified with omega-3 polyunsaturated fatty acid which differ based on the hen's breed and their feedings.

According to Endo and Arita (2016), they stated that omega-3 (n-3) polyunsaturated fatty acids (PUFA) are considered as cardio-protective. Several studies revealed that omega-3 PUFAs can lower blood pressure and serve as anti-inflammation which aid in cardioprotection (Lorente-Cebrián et al., 2013; Nestel et al., 2015). However, there are inconsistent findings to determine the efficacy of omega-3 in attending cardiovascular diseases. Despite these circumstances, extensive scientific findings support the advantages of consuming long-chain omega-3 polyunsaturated fatty acids (PUFA) in terms of cardiovascular morbidity and mortality risk (Bowen, Harris, & Kris-Etherton, 2016; Hammad, Pu, & Jones, 2016; Roy & Le Guennec, 2017). Omega-3 fatty acids that are essential in the diet and human physiology are α-linolenic acid (ALA; C18:3), eicosapentaenoic acid (EPA; C20:5), and docosahexaenoic acid (DHA; C22:6).

Animals, including humans, do not have delta-15 desaturase enzymes thus, they cannot synthesize a-linolenic acid on their own (Calder, 2013). Besides, EPA and DHA can be synthesized from α-linolenic acid (ALA; C18:3), which also explain the reason it is an essential fatty acid. This fatty acid can be obtained from a major source of plants such as flaxseed, canola, soy, and walnut. Nonetheless, the conversion pathway of EPA and DHA from α-linolenic acid is ineffective as it is competitively hindered by dietary linoleic acid (LNA; C18:2) (Elkin, Kukorowski, Ying, & Harvatine, 2018). The consumption of marine-derived EPA and DHA is therefore necessary for beneficial health effects. Examples of marine-derived EPA and DHA are fatty fish marine such as salmon, mackerel, herring, lake trout, sardines, and marine fish oil.

However, due to several factors, such as the growing demand for fish causes a disparity between demand and supply for fish and fishery products. (Tocher, Betancor, Sprague, Olsen, & Napier, 2019). Other than causes the need to ameliorate in aquaculture production, it is simply insufficient to satisfy human necessities. Therefore, another alternative or substitute for non-fish sources of omega-3 PUFA is required. This is where microalgae and fortified foods such as dairy products, eggs, and meats come in.

As diverse functional foods fortified with omega-3 are evolved, one of the most accepted foods is omega-3-enriched egg (Hegde, Panse, Atakare, & Kadam, 2016) Fraeye et al., (2012) stated that egg is well received as human food and food ingredients. Egg which is rich in protein sources contains all nine essential amino acids such as histidine, leucine, and lysine (Hedge et al., 2016). Other than rich in protein, they also stated that eggs offer a significant amount of vitamins and minerals except Vitamin C. Hence, an egg is the optimal choice to fulfil the requirement of omega-3 PUFA for human nutrition.

To incorporate omega-3 PUFA in egg, altering the hen's diet is important. Flaxseed or flaxseed oil can be introduced in hen's feeding due to their high level of α-linolenic acid (Hedge et al., 2016). Flaxseed or also known as Linseed is a colourless to yellowish oil extracted from dried, matured of the flax plant (*Linum Usitatissimum*). Ganorkar and Jain (2013) explained that this crop is native to the Mediterranean and West Asia. A growing portion of the flaxseed crop is commercially used, whether immediately or after storage (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014). They also stated that due to its nutrition composition, flaxseed is used in various food products. Thus, flaxseed or specifically extruded flaxseed were introduced in the hen’s feeding to improve fatty acid composition in the eggs. Hence, this study was conducted to study omega-3 content in layer eggs between chicken fed with common feed and feed with enriched extruded flaxseed on selected days.

1.2 Problem statement

Throughout the Malaysian background, intakes of omega-6 PUFA (LA) are recommended at 3-7% of total energy intake (TEI) based on 2000 kcal. Whereas omega-3 PUFA is recommended at 0.3-1.2% daily (equivalent to 0.67g-2.67g) TEI (RNI, 2017). This range omega-3 FA intake is proposed based on the current omega-3 quality of Malaysian daily diets and the ability to increase consumption without substantial changes in dietary habits. However, based on reports reviewed by Kock, Sivalingam, Azlinda, Siew, and Sim (2012), they stated that Malaysians have deficient omega-3 fatty acid consumption mainly among pregnant and lactating women.

This statement was also supported by a local study conducted by Lee, Shahar, Yusoff, and, Chin (2013) among Malays and Chinese elderly individuals aged 60 years and above in *Klang* Valley. Based on interviewer-administered semi-quantitative Food Frequency Questionnaire (FFQ) and 3 days' food record, they found that these elderly consumed about 47 to 1209 mg/day and 93 to 1194 mg/day respectively which was below the RNI recommendation (0.67g-2.67g/day). However, there are not many recent studies that assess omega 3 intake in Malaysia. Nevertheless, it can still be concluded that omega-3 intake among the Malaysian community was still low and affect the ratio of 1:2 for omega-6 and omega-3 fatty acid (Kock et al., 2012; Lee et al., 2013).

Omega-3 is essential for the human diet due to its beneficial health effects (Lorente-Cebrián et al., 2013; Nestel et al., 2015; Albert, Cameron-Smith, Hofman, &

Cutfield, 2013). However, since omega-3 PUFA is mainly found in marine fish, the major concern is the exposure of methylmercury (MeHg) due to human activities or even present naturally (Elkin et al., 2018; Ahmad et al., 2016). According to Anual et al. (2018), they found median mercury concentration was high among fishes such as oxeye scad (*lolong kuning*), mangrove red snapper (*ikan merah*) and also torpedo scad (*cencaru*). Approximately 95 % of MeHg in consumed fish are transported into the human blood system and maximum blood levels of MeHg are achieved within 4 –14 hours of consumption (Kershaw, Dhahir, & Clarkson, 1980). Ahmad et al. (2016) also revealed that marine fishes such as yellowtail and yellow-stripe scads, torpedo scad and red snapper are the types of fish that are frequently consumed among Malaysian adult in Peninsular Malaysia. This will then pose a health risk, especially in neurological problems if overconsumption.

Besides, according to a study conducted by Hu, Laird, and Chan (2017), they found the harmful effects of mercury in the marine fish reduced the useful effects of EPA and DHA. This review was based on a study among 2072 participants aged 18–79 to determine the association between Canadian Inuit traditional diet (rich in marine fish) and myocardial infarction risk. Furthermore, many factors affect the fatty acid composition of fish. For example, their species, types of feeding, or even geographical location can alter the fatty acid composition in fish (Tasbozan & Gocke, 2017). For types of feedings, wild marine fish contains more omega-3 as they feed on phytoplankton and zooplankton that are abundant in omega-3 PUFA. While for farm fish, some have high omega-6 due to the consumption of cereal and vegetable oil as their feeding.

Other than that, food choices might be influenced especially among health-conscious people as well as people who simply prefer to not eat fish. Welch, Shakya-Shrestha, Lentjes, Wareham, and Khaw (2010) stated that although fish has a high amount of EPA and DHA, a large proportion of the population consumes little or no fish. According to the Malaysian Adult Nutrition Survey (MANS, 2014), they found Malaysian adults (18-59 years old) consume about 1.6 servings per day of medium marine fish with the prevalence around 29.4 %. Hence, substitutes for the omega-3 source of diet are much needed.

There are many omega-3 enriched foods in the market to replace marine fish and seafood. These include fortified cereals, dairy products, eggs, salad dressings, and spreads. As fortified food became increasingly popular, extensive research needed to be done to support the benefit from the consumption of it. Smith, Beamer, Matak, and Jaczynski (2018) explained that eggs have become popular functional foods because of their low cost, functional versatility, and high nutritional value. As the egg is mostly consumed worldwide, the fatty acid composition of egg yolk, where the lipids are accumulated, is closely related to the type of lipids ingested by the laying hens (Fraeye et al., 2012). Thus, the hen's diet plays a significant role in determining the concentration of omega-3 PUFA in eggs.

In pursuit to increase omega-3 content in poultry products, implementation of fish oil and flaxseed has been a common practice. Bowen et al. (2016), also explained that fish oils such as menhaden, salmon, and algal oils were enumerated to foods, or animals are given feed containing EPA and/or DHA to enhance their tissues. However, the sensory quality of omega-3 PUFA enriched eggs might be impacted due to the presence of off-flavours and also depletion in lipid stability. The presence of fishy odour and fishy off-flavour is an unpleasant side effect of substituting standard diet with omega-3 PUFA enriched fed diet to the hens. Likewise, possible negative effects represent a drawback to the industry. Thus, extensive research is required to examine the possible effect of omega-3 PUFA enriched diet and the best alternative to fortified omega-3 in food products.

Flaxseed is a rich source of omega-3 fatty acid namely α-linolenic acid (ALA), soluble and insoluble fibres, lignans which responsible for antioxidants; Ganorkar & Jain, 2013; Ivanov, Rashevskaya, & Makhonina, 2011; Singh, Mridula, Rehal, & Barnwal, 2011). However, as stated by Flax Council of Canada (2016), these important flaxseed growing countries are Canada, China, United States, India, and Ethiopia. As for Malaysia, this source of omega-3 was imported from oversea which increased the production cost. Hence, this study was conducted to determine optimum feeding days of flaxseed that should be incorporated to maximize the production of fatty acid composition in the egg.

1.3 Significance of the study

This study was conducted to investigate omega-3 content in layer eggs between chicken fed with common feed and feed with enriched extruded flaxseed on selected days. Omega-3 content in layer eggs can be used as a substitute for other omega-3 rich sources including marine fish which have several issues among health-conscious people and lower consumption among Malaysian adults. Besides that, this study can help to fill the gap in understanding and knowledge about optimum feeding days to minimize the cost production.

Other than that, the findings of this study can also provide baseline data for further reference and research. This is because an egg has many benefits in terms of nutritional value and widely accepted among Malaysian. Other than that, the egg is also used as other ingredients in producing baked and cooking products. Hence, by enriching omega-3 content in eggs, it can increase the nutritional value of the product itself.

An egg is high in protein and offers a significant amount of vitamins and minerals except vitamin C (Hedge et al., 2016). As eggs contain many nutrients, this study is beneficial for many agencies and the food industry as they can use this finding to improve their products. All in all, the food industry can meet consumer's demands and needs for healthy and nutritious products.

Last but not least, nutritionists could play an important role in promoting good dietary behaviour and a healthy lifestyle among consumers. Hence, they can improve their overall nutritional and health status to increase their daily performance.

1.4 Objectives

1.4.1 General objective

To study Omega-3 content in layer eggs between chicken with common feed and feed with enriched extruded flaxseed on selected days.

1.4.2 Specific objective

1. To measure the total fat amount in layer eggs between chicken fed with common feed and enriched extruded flaxseed on day 7th, 14th, 21st, and 28th.

2. To determine and compare EPA content in layer eggs between chicken fed with common feed and enriched extruded flaxseed on day 7th, 14th, 21st, and 28th.

3. To determine and compare DHA content in layer eggs between chicken fed with common feed and enriched extruded flaxseed on day 7th, 14th, 21st, and 28th.

1.5 Hypothesis

There is a significant difference for total lipid content in layer eggs between chicken with common feed and feed with enriched extruded flaxseed on day 7th, 14th, 21st, and 28th.

There is a significant difference for Omega-3 content in layer eggs between chicken with common feed and feed with enriched extruded flaxseed on day 7th, 14th, 21st, and 28th.

CHAPTER 2

LITERATURE REVIEW

2.1 Omega-3

Dietary fat is essential for the human body to maintain homeostasis (Lorente-Cebrián et al., 2013). A fatty acid is one of dietary fat that is subdivided into saturated and unsaturated fatty acids which is further categorized according to the number of double bonds present within the chain. Polyunsaturated fatty acid mainly omega-3 PUFA is a synthetic fatty acid that can be derived from the diet or naturally formed (Mustapha, 2013). This essential fatty acid namely docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5) have drawn significant interest due to their health benefits such as lowering TAG and blood pressure (Bowen, Harris, & Kris-Etherton, 2016; Hammad, Pu, & Jones, 2016; Roy & Le Guennec, 2017).

2.1.1 Role of omega 3

Omega-3 has been reported to be advantageous for human health. Pro-inflammatory activities of omega 3 are generally accepted to have led to cardioprotective function (Endo & Arita, 2016; Bowen et al., 2016; Venturini, Simão, Urbano, & Dichi, 2015; Albert et al., 2013). A randomized controlled trial in Japan by Yokoyama et al., (2007) showed that, in addition to a statin, therapy with a highly diluted prescription regimen of EPA effectively reduced cardiovascular disease in patients with previous myocardial infarction via cholesterol-independent process. Other than that, reviews by Mozaffarian and Wu (2011) also supported the desirable impact of omega-3 PUFA in cardiovascular symptoms such as anti-inflammation, plasma TAG, and blood pressure-lowering properties in most of the animal studies. However, further clinical findings are required to prove omega-3 PUFA competency as cardio-protective due to inconsistent findings on these health effects.

2.1.2 Benefit of omega-3 on health

Based on RNI (2017), they reported that important fatty acid namely linoleic acid (C18:2) and α-linolenic acid (C18:3), that could not be produced naturally, are particularly critical in infant diets since birth to 2 years. Omega-3 needs to be raised above normal to promote fetal development, particularly for the eyes and brain (Mustapha, 2013; Swanson, Block, & Mousa, 2012). As reported by Nesheim and Yaktine (2007), based on animal studies, the withdrawal of omega-3 during pregnancy affects the visual of the fetus. Besides, as human brain development started approximately in the third trimester of pregnancy, DHA is essential for the mother to deliver them across the placenta during pregnancy and in breast milk after birth (Calder, 2013). DHA which makes about more than half of fatty acid present in the segment of the retina is essential for optimum sensory, neurological, and child behavioural development.

Next, omega-3 fatty acid is also recognized as a nutrient with the ability to inhibit the ageing process in unhealthy conditions (Rosney, Haron, Salleh, & Shahar, 2018). Based on their study conducted among 90 elderlies from 3 Peninsular States (Selangor, Kelantan, and Perak), they found that the composition of PUFA, such as omega-3 and omega-6 was higher among successful seniors compared to normal ageing for both men and women. This successful ageing was characterized based on Hamid, Momtaz, and Ibrahim (2012), such as they are free of serious diseases, have a decent quality of life, and feeling good about their health. Hence, it can be concluded that these fatty acids were able to deter pathological condition which is associated with the ageing process.

Furthermore, review and meta-analysis by Mazereeuw, Lanctôt, Chau, Swardfager, and Herrmann (2012) and Fotuhi, Mohassel, and Yaffe (2009) have found that high omega-3 PUFA intake can reduce Alzheimer risk and cognitive decline among elderly. However, these epidemiological studies have several biases and the association can also be influenced by other factors namely a healthy way of living and a healthy diet. Hence, further researches are needed to study the association of omega-3 PUFA and the reduction of diseases among the elderly.

2.1.3 Sources of omega 3

As omega 3 can provide many health benefits, it can also be obtained from various sources. Ryckebosch, Bruneel, Muylaert, and Foubert (2012) stated that the primary source of omega 3 PUFA is cold-water fish. However, due to several factors such as types of feedings, the geographical location which affect the fatty acid composition and possible contamination of methylmercury, other sources for this fatty acids are recommended (Elkin et al., 2018; Tasbozan & Gocke, 2017; Ahmad et al., 2016)

Ganesan, Brothersen, Mcmahon, Ganesan, and Brothersen (2014) explained that α-Linolenic acid (ALA) is typically found in plant and animal dietary oils, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are obtained through aquatic sources such as fish, algae, and fish oils.

Apart from that, omega-3 PUFA can also be fortified in infant formula, dairy products, meat, eggs, and also bakery products. Agostoni (2008) proposed that in an attempt to enrich human milk, dietary changes to PUFA-rich oils are indicated for lactating mothers, followed by improvements for the caloric intake of lipids. However, for commercial dairy products, meat, and eggs, they are currently fortified with omega-3 PUFA from flaxseed, fish oil, and microalgae through animal feedings (Ganesan et al., 2014). At the same time, certain aspects need to be considered regarding this inclusion criterion. This is due to the possible deprivation of fatty acid during the digestive process of ruminant (Maia, Chaudhary, Figueres, & Wallace, 2007; Lock & Bauman, 2004).

Whereas for bakery products, different types of fat are introduced based on the variety of bread and therefore wheat can be easily enhanced by incorporating omega 3 PUFA-rich fats during bread processing (Ganesan et al., 2014).

2.2 Egg

Stadelman and Cotterill (1995) stated that egg is among the few products consumed globally and therefore the egg industry is a significant part of the world food sector. The egg sector is subdivided into areas of specialization such as feed formulation, replacement of pullet rearing, laying flock management, and egg marketing. This management is important to maintain the quality of eggs that will be distributed to the consumer. As the egg is usually marketed in a form of hard shell, they have been commercialized widely especially from White Leghorn hen species in the United States due to their high rate of lay and adaptability to diverse climates (Stadelman & Cotterill, 1995).

As for the egg industry in Malaysia, Federation of Livestock Farmers Association of Malaysia reported that in 2018, there were about 11 942 652 627 hen eggs that were produced weekly. All the eggs are advertised as fresh in-shell eggs by wholesalers. Currently, three small egg processing plants are in operation to manufacture various types of liquid eggs to meet particular bakery and confectionery requirements. There have been two major companies of egg marketing in Malaysia which are LTKM Bhd and Lay Hong Berhad (Nutriplus). Besides, there is also a growth in the production of "designer eggs" to meet the demand for "healthier" eggs namely low cholesterol and high omega 3. This is where altering the hen's diet by the inclusion of omega-3 PUFA plays a significant role to meet the demands of the community.

2.2.1 Egg nutrient content

Miranda et al. (2015) mentioned eggs are an inexpensive yet highly nutritious product, offering essential nutrients which give an impact on human wellbeing. According to a study conducted by Kuang, Yang, Zhang, Wang, and Chen (2018), they found that eggs contain abundant important proteins, fats, vitamins, minerals, and bioactive compounds. Roughly a medium-sized boiled egg contains nearly 80 kcal energy with 0.56 g carbohydrate, 6.29 g protein, and 5.3 g total fat. Out of 5.3 g total fat, 1.6 g is saturated, 2.0 g is monounsaturated, 0.7g is polyunsaturated, and another 186 mg is cholesterol (Kuang et al., 2018). As for micronutrients, the egg contains a variety of minerals such as magnesium, calcium, zinc, phosphorus and iron and most vitamins namely niacin, thiamin, riboflavin, folate, vitamin A, vitamin B6 except got vitamin C. However, pressure, age, hen nutrition, and environmental factors may affect their compositions and the net amount.

2.3 Enrichment of Omega-3

2.3.1 Flaxseed

Many studies have reported the advantages of consuming flaxseed. According to Flax Council of Canada, flaxseed is considered a highly nutritious food as they contain about 55-57% of the essential omega-3 fatty acid, ALA, soluble and insoluble fibre which aid in lowering cholesterol level and lignan as an antioxidant. Several studies have been performed to determine flaxseed effectiveness as a hen diet to increase omega-3 fatty acids in the eggs. Based on a study by Cherian and Quezada (2016), they have observed that eggs from hens fed on camelina and flaxseed diet have a significant difference in the amount of α-linolenic acid, DHA and immunoglobulin IgY concentration when compared with control egg in which the hens were fed on corn-soybean basal diet. Dietary flaxseed was also reported to be enriched in total omega-3 content in egg yolk compared to soy-based feed (Elkin et al., 2018). Apart from that, the cost of flaxseed is cheaper than chia seeds and fish oil which have similar effects on influencing omega-3 content in egg yolk (Coorey, Novinda, Williams, & Jayasena, 2015). However, a study reported that the inclusion rate of flaxseed should be below 10% as higher than that amount can affect the sensory taste of the egg and reduce the growth performance of the hens itself (Anjum, Haider, Khan, Sohaib, & Arshad, 2013).

2.3.2 Fish oil

Fish oil is one of the popular ingredients to be enriched in the hen diet due to the high amount of EPA and DHA. However, Fraeye et al. (2012) reported that long-chain omega-3 PUFA (EPA and DHA) accommodate more double bonds than ALA which are more susceptible to oxidative breakdown. This process will then destroy biological tissues and has been associated with numerous diseases such as cancer and atherosclerosis. Apart from that, the quality of omega-3 PUFA fortified eggs might be impacted due to the presence of off-flavours and also depletion in lipid stability (Bowen et al., 2016). The sensory panels describe the off-flavours in the egg that were enriched with fish oil as “fishy taste” which will affect the sensory evaluation and the marketing of the eggs (Fraeye et al., 2012). Marine sources of omega-3 FA offer the benefit of direct incorporation

2.3.3 Chia seeds

Chia seeds are the edible seeds of Salvia Hispanic, a flowering plant indigenous to Central America in the mint family *(Lamiaceae*), or the similar *Salvia columbariae* of the southwestern America and Mexico. It is a novel and grey with black and white spots with hydrophilic properties. These seeds provide a good amount of omega-3 FA, fibre, antioxidants, iron, and calcium. Research to determine the omega-3 fatty acid profile of eggs from laying hens fed diets supplemented with chia, fish oil, and flaxseed was carried out by Coorey et al. (2015). Based on their findings, they found the fatty acid composition of egg yolk was significantly affected by the dietary treatments. The inclusion of chia seeds produced the highest concentration of omega-3 content without significant change in sensory properties.

2.4 Fatty acid analysis

2.4.1 Cold lipid extraction

Cold lipid extraction was used for this study as this method can prevent oxidation of polyunsaturated fatty acids. This process was modified by using a solvent with low toxicity such as hexane and ethanol (Biondo et al., 2015). This extraction was done by using reagents and chemicals such as dichloromethane-methanol 2:1 (v/v) (CH2Cl2/MeOH) which helps to extract the lipid while 50mg/L butylated hydroxytoluene (BHT) acts as an antioxidant.

From the lipid extraction method, fatty acid methyl ester (FAME) was produced by alkali catalyzed reaction between fat and methanol in the presence of a base. This methyl ester is relatively more stable and able to provide a quantitative sample for gas chromatography (GC) analysis. Another reason to use FAME is that this fatty acid has low polarity compared to free fatty acid thus, they are more amenable for GC analysis.

Besides that, the cold lipid extraction method is considered as a high-quality method. This is because egg yolk has high moisture, more than 80% which is suitable when we freeze the samples first at -20 °C. Other than that, this method can achieve optimal extraction as it allows the extraction for both polar and non-polar based on the water-methanol ratio.

2.4.2 Soxhlet method

The extraction of soxhlet is considered practical in which the analyte is concentrated as a whole from the matrix or extracted from specific interfering substances. It becomes one of the most important techniques in the field of environmental extraction. This method was done by placing the sample in a thimble-holder and gradually filled with condensed fresh solvent from a distillation flask (García-Ayuso, Velasco, Dobarganes, & Luque de Castro, 1999). As the liquid reaches a point of excess, a siphon aspires the entire contents of the thimble-holder and loads it back into the distillation flask, bringing the analytes collected in the bulk liquid. The operation is repeated until it achieves full extraction.

Giergielewicz-Mozajska, Dabrowski, & Namieśnik (2001) stated that although many people preferred to use soxhlet as their method of choice, it is time-consuming and uses huge amounts of organic solvent which increase waste and risk the health due to the hazardous effects from the vapour produced. Ramola et al. (2019) also mentioned based on their findings between Bligh and Dyer and soxhlet extraction methods, they found that soxhlet only managed to extract saturated fatty acids and monounsaturated fatty acids. While Bligh and Dyer extraction, various amounts of different saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids were able to be obtained.

2.4.3 Gas chromatography

Gas chromatography (GC) is the leading separation and analysis of volatile compounds (McNair & Miller, 2009). It was used for the gas, liquid, and solid analysis with these generally dissolved in volatile solvents. During GC, the sample is vaporized and transported through the column in mobile gas phase. For most analyses, sample partitions (equilibrate) into and out of the stationary liquid phase, depending on their solubility at a specific temperature in the stationary phase. The component of the sample which is called solutes will then separate from one another based on their relative vapour pressure and affinities for the stationary phase.

Within GC, they are divided according to the state of the stationary phase (McNair & Miller, 2009). For example, if the stationary phase is solid, the technique is called gas-solid chromatography whereas if it is in a liquid state, the technique is called gas-liquid chromatography.

They reported that the capillary column was reliable in providing resolution such as they can distinguish 450 components in the aroma of coffee. Sensitive detectors such as flame ionization detectors can quantity 50 ppb of organic compounds with a relative standard deviation of approximately 5% (Dobrokhotov & Larin, 2019). Gas chromatography automated systems are also able to handle many samples per day with minimal period.

CHAPTER 3

METHODOLOGY

3.1 Samples

Purposive sampling method was employed in selecting the eggs (sample) for this study where the eggs were purposefully provided by a company from Kuala Lumpur. The selected eggs were from Bovan Brown poultry breed, aged 30 weeks that were reared in a closed house system managed by the company with the use of standard commercial managements of layer birds.

These hens were fed on two types of feedings which are normal manufacture feedings (control group) and common feed with 1% extruded flaxseed (treatment group). The samples were collected randomly according to weeks from the farm and were analyzed for their total lipid and omega-3 content.

3.2 Reagents and chemicals

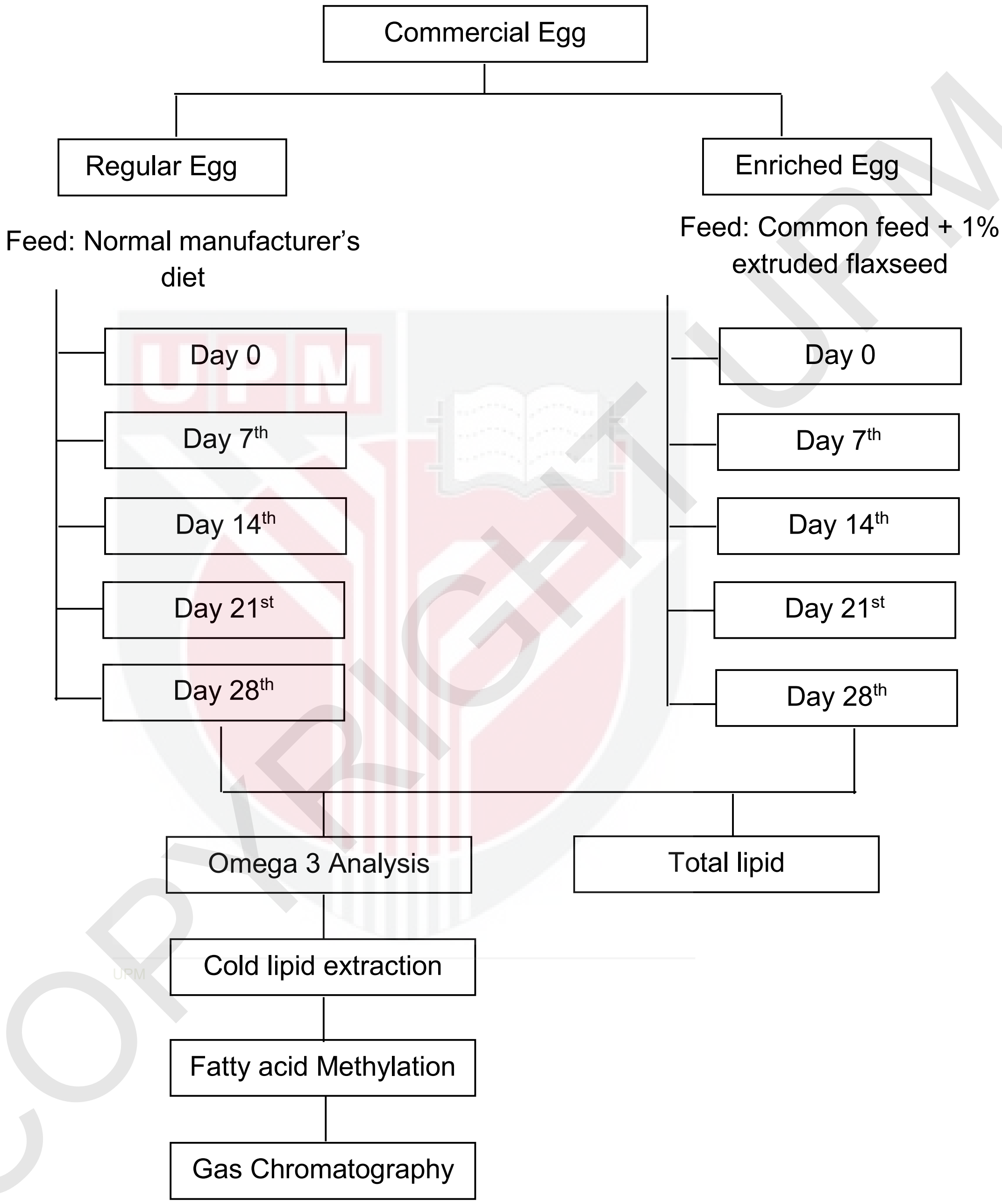
Dichloromethane-methanol 2:1 (v/v) (CH2Cl2/MeOH) (combined 2.6 L dichloromethane and 1.3 L methanol in a 4 L amber bottle), 50mg/L BHT for initial homogenization of sample (added 50 mg butylated hydroxytoluene to a final volume of 1 L of reagent 1 (CH2Cl2/MeOH) in a volumetric flask), 0.88% KCl in H2O (w/v) (dissolved 17.6 g KCl into 2 L deionised water), Water-methanol (1:1 v/v) (H2O/MeOH) (combined 1 L each of water and methanol in a beaker), 0.44% KCl in H2OMethanol (3:1) (combined them in a 4 L amber bottle)

Acetyl chloride/methanol: C2H3ClO/MeOH (1:10) (cooled down 50 ml of methanol and 5 ml of Acetyl chloride in an ice bath for a while before adding drop by drop of Acetyl chloride in Methanol), C23:0 (approx. 3.75 mg/ml in toluene), Potassium Carbonate: K2CO3 (1M) (weighed 13.82 g of K2CO3 before dissolve and diluted them to 100 ml of deionized water), Hexane

3.3 Sampling preparation

Based on Wang, Sunwoo, Cherian, and Sim (2000), egg yolks (sample) were separated from albumen by using egg yolk separator. The samples then homogenized according to their groups and selected days and freeze them at – 20 ºC for the analysis of total lipid and omega 3. Moreover, each sample has similar amount of eggs with (N = 4) according to their groups and selected days except for Treatment Day 21st, the (N=1). This is due to most of the sample for that day had spoiled and could not be used. Hence, it would affect the result later on.

3.4 Experimental design



3.5 Procedure Omega-3 Analysis

The analysis of omega-3 fatty acid was divided into 2 major stages; cold lipid extraction and methylation of fatty acid. After this process was done, the sample underwent gas chromatography analysis. However, only egg yolk was analyzed as it contains almost all lipid in the whole egg. This method was based on Folch, Lees, and Sloane Stanley (1956) with modification by Ways and Hanahan (1964).

The first procedure for omega-3 analysis was cold lipid extraction. This cold lipid process was done to prevent the oxidation of polyunsaturated fatty acids. It was modified by using low toxicity solvents which were hexane and ethanol (Biondo et al., 2015). This method started by homogenizing 2 g of egg yolk for 1 min with an aliquot of CH2Cl2/MeOH (2:1)+BHT followed by the filtration of that mixture onto a No 1. filter paper. The solid residue then suspended and homogenized again for another 1 min with the previous aliquot. The step was repeated before combined filtrates (60 ml circa) was transferred to a separation funnel. Next, 30 ml of 0.44% KCl in H2O/MeOH (3:1) was added. After that, the mixture was shaken thoroughly and allowed to settle (min 4 hours or overnight).

The bottom layer containing the purified lipid was recovered into a round bottom flask. Almost all the solvent was evaporated within the rota evaporator. The lipid then recovered with an appropriate aliquot of CH2Cl2/MeOH (2:1) and transferred to a 10 ml volumetric flask, and makeup to 10ml. Out of that 10 ml, 6 ml of the solvent with the extracted lipid was transferred to a dried, pre-weighed 7ml glass vial and used for the quantification of total lipid. The vials were dried for total lipids in the oven overnight at 50°C. Afterwards, the temperature was increased up to 100°C for another ½ hour. Lastly, it was cooled in a desiccator and weighed. Another remaining of 3 ml solvent with the extracted lipid was then used for the analysis of the fatty acid

This process started by transferring the portion of lipid into a Pyrex vial. Then, the lipid was evaporated to dryness by flushing them with nitrogen. A 200µl of C23:0 (3.75mg/ml in toluene) was added with the exact amount of the internal standard recorded. After that, 2 ml of acetyl chloride/methanol (C2H3ClO /MeOH (1:10) (freshly prepared) was added and shaken. The sealed Pyrex vial was then placed in the oven at 100 °C for 1 hour (or 50 °C overnight). Afterwards, it was cooled in a fume cupboard before adding another 2 ml of K2CO3 (1M). Later, 3 ml of n-Hexane was added and shaken well before it was vortex for about 10 seconds. The sample was centrifuged for 3 minutes at 1000 rcf and was filled in a GC vial (~1.5 mL). Lastly, fatty acid methyl ester was isolated and identified by using Agilent Technologies hp88 GC System with an injection volume of 1 µl, injector and the detector temperature are 250°C. Sample identification was done by comparing peak and retention time between standard graphs and sample graphs by using y = mx + c formula.

3.6 Calculation of Total lipid

Total lipid (g) was calculated based on Shahidi (2005) by using the formula:

$$Weight\ of\ dried\ vial\ with\ extracted\ lipid\ - Pre\ weight\ of\ dried\ vial$$

Whereas for the percentage of total lipid was calculated by using the formula:

$$\frac{Weight\ of\ lipid\ extracted\ (g)}{Weight\ of\ sample\ (g)}\ x\ 100\%$$

3.7 Comparison between Standard reference material (SRM 1546a)

Standard reference material (SRM) is mainly intended to verify methods for evaluating the fatty acids, cholesterol, calories, nutrients, vitamins and amino acids in canned meat products and related materials. SRM may also be used for quality control when assigning values to in-house reference. This homogenate is a mixture of pork and chicken products blended in a commercial process which contains about 85 g each can. Hence, this homogenate was prepared according to Folch, Lees, and Sloane Stanley (1956) with modification by Ways and Hanahan (1964) method and comparison was done with SRM certified value. Based on the comparison, only EPA reached the certified value with (0.1594 ± 0.000).

3.8 Data Analysis

These samples were prepared in duplicate form. The data for total lipid and omega-3 content in the sample eggs were analyzed by using IBM SPSS version 24. Descriptive analysis was used to determine mean and standard deviation. Whereas for analytical analysis, One-way ANOVA and Tukey's post hoc test were used to determine and compare significant differences of total lipid and omega3 content in control and treatment samples.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Total lipid content

Total lipid content was determined by measuring the weight of vial and extracted lipid with pre-weight of vial (Shahidi, 2005). This total content of lipid was conveyed in g /100 g and converted to a percentage before analyzed using One-way ANOVA. As for SRM 1546a, the value of total lipid content was 12.25 g/100 g in which it does not achieve the certified value of 18.96 g/100 g.

Table 1: Total lipid content between control and treatment group by selected days

	Mean % ± SD	
Days	**Control**	**Treatment**
0	14.25 ± 0.35^{a}	14.25 ± 0.35^{a}
7th	11.75 ± 0.35^{bc}	11.50 ± 0.00^{b}
14th	11.00 ± 0.71^{b}	13.00 ± 0.71^{ab}
21st	13.25 ± 0.35^{ac*}	8.75 ± 0.35^{c*}
28th	11.00 ± 0.71^{b*}	14.00 ± 0.71^{a*}

($^{ab\ cd}$) – comparison between days in a column
Means with different superscript within each group are significantly different at $p < 0.05$
(*) – indicates a significant difference in the same row between control and treatment groups ($p<0.05$)

As shown in Table 1, there was no significant difference in total lipid content between Day 7th, 14th and 28th in the control group. There was also no significant difference in total lipid content between Day 7th and 14th in the treatment group. In general, it can be concluded that there was no major significant difference in total lipid content between groups and selected days.

Antruejo et al. (2012) findings showed a similar result with this study when they compared total lipid content between flaxseeds, chia seeds and maize feedings in a similar period with ($p < 0.05$). They reported for feeding parts which contain α-linolenic acid (ALA) regardless of its sources have no significant difference with non-α-linolenic sources. This finding was also similar to Neijat, Ojekudo, and House (2016) in which they found that dietary supplementation either flaxseed or algal DHA does not have a significant effect on total lipid content in egg yolk.

One of the possible reason is that enriching egg with omega-3 does not directly increase the total lipid content. However, this nutrient altered the lipid composition in the egg yolk. Based on Antruejo et al. (2012), they reported that palmitic fatty acid content was significantly higher in the yolk from hens fed on traditional maize diet (23.25%) compared to hens fed on α-linolenic sources such as flaxseed (19.75%) and chia seed (19.75%).

According to Sim and Qi (1995), they explained that high dietary consumption of PUFA may be related as this has been shown to decrease liver production of saturated fatty acids. This palmitic acid was also shown to increase the risk of coronary heart disease (CHD) (Sim & Qi, 1995). As a result, it is highly recommended to reduce the intake of saturated fatty acid in our diet.

4.2 Eicosapentaenoic acid (EPA) content

EPA concentration was determined by using a standard curve obtained from 37 FAME mix standard analysis with the equation of $y = 0.3855x - 0.485$ ($R^2 = 0.998$) at a retention time of 25.5 min. Then, EPA concentration was converted into gram before divided with total lipid and multiplied with a hundred per cent. Afterwards, one-way ANOVA analysis was conducted. As for SRM 1546a, eicosanoic acid certified value was used since EPA value was not available. Based on the result, EPA reached the range with 0.1594 g/100 g compared to the certified value of 0.0329 g/100 g.

Table 2: EPA content between control and treatment group by selected days

	Mean % ± SD	
Days	**Control**	**Treatment**
0	0.38 ± 0.00^{a}	0.38 ± 0.00^{a}
7th	0.38 ± 0.00^{a}	0.43 ± 0.01^{a}
14th	$0.41 \pm 0.01^{a*}$	$1.06 \pm 0.04^{b*}$
21st	$0.27 \pm 0.00^{b*}$	$0.67 \pm 0.03^{c*}$
28th	$0.42 \pm 0.03^{a*}$	$0.82 \pm 0.03^{d*}$

($^{ab\ cd}$) – comparison between days in a column
Means with different superscript within each group are significantly different at $p < 0.05$
(*) – indicates a significant difference in the same row between control and treatment groups ($p<0.05$)

Based on Table 2, EPA content was higher in the treatment group and was significantly different on Day 14th ($p < 0.05$) when compared with other selected days. Whereas for the control group, there was no significant difference except for Day 21st. Neijat et al. (2016) further explained that omega-3 formation in the egg was highly affected with ($p < 0.0001$) by the source of omega-3 in the diet with flaxseed oil-fed gather on average of 10-fold ALA when compared with a control group (cereal-based diet). This was one of the possible reason why treatment group have a higher amount of EPA compared to control. As ALA can be converted to EPA, it can also affect the amount of omega-3 content in the egg.

Moreover, feeding hens with flaxseed longer than 14 days did not significantly increase EPA content. Neijat et al. (2016) asserted similar result when algal DHA was used in which it deposited highest levels of DHA in Day 14th (Week 2) and stabilized until Day 28th (Week 4). Hence, it can be concluded that egg fortification significantly increased during the first 14 days and reached a plateau on the same day. Later, EPA content remained constant although there was a further supplementation. This confirms that maximum enrichment day of omega 3 content in egg yolk was on Day 14th (Week 2).

Lemahieu et al. (2015) observed that there was a declination in omega-3 content between Day 14th until Day 25th after hitting maximum amount in 2 weeks, in which they proposed the possible reason was a reduction in egg yolk weight. Similar to the changes in egg yolk weights, the total lipid content in egg yolk also escalate within hens age but remains unchanged in mature hens (Nielsen, 1998). The authors confirmed that these findings were apparent when they compared eggs that were fed on ALA sources between week 4 and 6 have similar content of total lipid compared to week 2.

Furthermore, Neijat et al. (2016) stated that the production of an egg is low in the first week. Hence, since it takes about 7 days for egg yolk material disposition, eggs in week 2 (Day 14th) will have a greater accumulation of yolk material which result in a better amount of omega- 3 PUFA.

4.3 Docosahexaenoic acid (DHA) content

Similar to EPA, DHA concentration was also determined based on a standard curve from gas chromatography analysis of 37 FAME mix standard with y = 0.3742 x – 0.6538 (R^2 = 0.9976) at a retention time of 29.4 min. Subsequently, DHA content was then converted to a percentage before analysis of One-way ANOVA was carried out. Whereas for the comparison of SRM 1546a, ALA certified value was used as DHA value was inaccessible. The value of DHA material obtained was 0.012 g/100 g where it does not meet the approved value of 0.133 g/100 g.

Table 3: DHA content between control and treatment group by selected days

	Mean % ± SD	
Days	**Control**	**Treatment**
0	0.30 ± 0.00^{a}	0.30 ± 0.00^{acd}
7th	0.26 ± 0.00^{a*}	0.36 ± 0.00^{b*}
14th	0.34 ± 0.02^{a}	0.34 ± 0.00^{bc}
21st	0.27 ± 0.00^{a}	0.31 ± 0.00^{cd}
28th	0.29 ± 0.02^{a*}	0.34 ± 0.00^{bc}

($^{ab\ cd}$) – comparison between days in a column
Means with different superscript within each group are significantly different at $p < 0.05$
(*) – indicates a significant difference in the same row between control and treatment groups ($p<0.05$)

As shown in table 3, the control group showed no significant difference between those 5 selected days. Likewise, for the treatment group, there was also no major significant difference between those 5 days. Nonetheless, DHA content was higher in the treatment group compared to the control group.

Lemahieu et al., 2015 reported similar findings in which feeding supplementation of ALA yield a higher amount of ALA and DHA in the egg yolk compared to control group. Yannakopoulos, Tserveni-Gousi, and Christaki (2005) also explained that eggs that were enriched with ALA contain a higher level of omega 3 PUFA particularly DHA type, compared to a regular egg with (120mg versus 0mg). This is because flaxseed lack of DHA (Ayerza & Coates, 2001) which also explained why the treatment group have a higher amount of DHA compared to the control group.

Besides, it also confirmed that there was a conversion of DHA from the precursor of ALA under enzymatic influence of desaturase and elongase (Cherian & Sim, 1991). However, the process is not efficient as there was a low activity of the desaturase enzyme and also competition with other fatty acids for the enzyme. Lemahieu et al. (2015) also stated that the main source DHA (Algal DHA) have a higher amount of DHA compared to the main source of ALA (flaxseed) as it was more readily available. Hence, it can be concluded that ALA enrichment was rather inefficient in egg yolk especially if the main reason is to increase DHA specifically as only overdoing the amount of flaxseed can lead a significant difference of DHA content.

CHAPTER 5
CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Conclusion

It can be concluded that there was no significant difference in total lipid content between groups and selected days. Nevertheless, many studies had reported that eggs that were fortified with ALA have a lower amount in saturated fatty acids such as palmitic, and stearic acid with $p < 0.05$ (Yannakopoulos et al., 2005). This will then assist in lowering the risk of cardiovascular disease and improve omega 6 and omega 3 ratio (1:2) due to the increase of omega 3 content in egg yolk. Eggs that were enriched with omega-3 also have a higher amount of EPA and DHA compared to regular egg. Apart from that, it can also be said that Day 14th is the optimum feeding days to enrich omega 3 in an egg as after 28th day (week 4), omega 3 have reached saturation. Overall, ALA source specifically flaxseed is useful to be used as enrichment of omega 3 content in an egg.

5.2 Limitations and recommendations

One of the limitation in this study is that there is no comparison between different rates of flaxseed. Only 1% of extruded flaxseed was used as the treatment group. Hence if there are different rates of extruded flaxseed, an optimum amount of extruded flaxseed can be determined. However, the inclusion rate should be below than 5%. This is because according to study, they reported that providing flaxseed more than 5% would affect the growth performance of hens and affect the sensory quality of the egg (Anjum et al., 2013). Thus, future study should include different rate of extruded flaxseed namely 1%, 3% and 5%. Moreover, this study also did not make a comparison between different kinds of eggs such as quail egg and duck egg. If such study includes different kinds of egg, it will be more advantageous as different people have different preference of food and food accessibility. Last but not least, it was evident that only two types of fatty acid were analyzed in this study which are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since there are possibilities of fortification of omega-3 might impact other fatty acid composition, other fatty acids including saturated fatty acid should also be analyzed to determine their associations.

References

Agostoni, C. (2008). Role of long-chain polyunsaturated fatty acids in the first year of life. *Journal of Pediatric Gastroenterology and Nutrition*, *47 Suppl 2*, 15–18. https://doi.org/10.1097/01.mpg.0000338811.52062.b2

Ahmad, N. I., Mahiyuddin, W. R. W., Mohamad, T. R. T., Ling, C. Y., Daud, S. F., Hussein, N. C., … Sulaiman, L. H. (2016). Fish consumption pattern among adults of different ethnics in Peninsular Malaysia. *Food and Nutrition Research*, *60*. https://doi.org/10.3402/fnr.v60.32697

Albert, B. B., Cameron-Smith, D., Hofman, P. L., & Cutfield, W. S. (2013). Oxidation of marine omega-3 supplements and human health. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/464921

Anjum, F. M., Haider, M. F., Khan, M. I., Sohaib, M., & Arshad, M. S. (2013). Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. *Lipids in Health and Disease*, *12*(1), 1–12. https://doi.org/10.1186/1476-511X-12-13

Antruejo, A., Azcona, J. O., Garcia, P. T., Gallinger, C., Rosmini, M., Ayerza, R., … Ayerza, R. (2012). Omega-3 enriched egg production : the effect of α -linolenic ω -3 fatty acid sources on laying hen performance and yolk lipid content and fatty acid composition. *British Poultry Science*, *1668*(52:6, 750–760). https://doi.org/10.1080/00071668.2011.638621

Anual, Z. F., Maher, W., Krikowa, F., Hakim, L., Ahmad, N. I., & Foster, S. (2018). Mercury and risk assessment from consumption of crustaceans, cephalopods and fish from West Peninsular Malaysia. *Microchemical Journal*, *140*(April), 214–221. https://doi.org/10.1016/j.microc.2018.04.024

Ayerza, R., & Coates, W. (2001). Omega-3 enriched eggs: The influence of dietary α-linolenic fatty acid source on egg production and composition. *Canadian Journal of Animal Science*, *81*(3), 355–362. https://doi.org/10.4141/A00-094

Biondo, P. B. F., Dos Santos, V. J., Montanher, P. F., Junior, O. D. O. S., Matsushita, M., Almeida, V. C., & Visentainer, J. V. (2015). A new method for lipid extraction using low-toxicity solvents developed for canola (Brassica napus L.) and soybean (Glycine max L. Merrill) seeds. *Analytical Methods*, *7*(23), 9773–9778. https://doi.org/10.1039/c5ay02243f

Bowen, K. J., Harris, W. S., & Kris-Etherton, P. M. (2016). Omega-3 Fatty Acids and Cardiovascular Disease: Are There Benefits? *Current Treatment Options in*

Cardiovascular Medicine, *18*(11). https://doi.org/10.1007/s11936-016-0487-1

Calder, P. C. (2013). Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology? *British Journal of Clinical Pharmacology*, *75*(3), 645–662. https://doi.org/10.1111/j.1365-2125.2012.04374.x

Cherian, G., & Quezada, N. (2016). Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed. *Journal of Animal Science and Biotechnology*, *7*(1), 1–8. https://doi.org/10.1186/s40104-016-0075-y

Coorey, R., Novinda, A., Williams, H., & Jayasena, V. (2015). Omega-3 Fatty Acid Profile of Eggs from Laying Hens Fed Diets Supplemented with Chia, Fish Oil, and Flaxseed. *Journal of Food Science*, *80*(1), S180–S187. https://doi.org/10.1111/1750-3841.12735

Dobrokhotov, V., & Larin, A. (2019). Multisensory Gas Chromatography for Field Analysis of Complex Gaseous Mixtures. *ChemEngineering*, *3*(1), 13. https://doi.org/10.3390/chemengineering3010013

Elkin, R. G., Kukorowski, A. N., Ying, Y., & Harvatine, K. J. (2018). Dietary High-Oleic Acid Soybean Oil Dose Dependently Attenuates Egg Yolk Content of n-3 Polyunsaturated Fatty Acids in Laying Hens Fed Supplemental Flaxseed Oil. *Lipids*, *53*(2), 235–249. https://doi.org/10.1002/lipd.12016

Elson, H. A., Gleadthorpe, A., Vale, M., & Uk, M. (2011). *Housing and Husbandry of Laying Hens : past , present and future*. *46*(2), 16–24.

Endo, J., & Arita, M. (2016). Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. *Journal of Cardiology*, *67*(1), 22–27. https://doi.org/10.1016/j.jjcc.2015.08.002

Federation of Livestock Farmers Association of Malaysia.(2018).*Table egg production.*Retrieved from http://www.flfam.org.my/index.php/industry-info/the-poultry-industry/table-egg production

Flax Council of Canada, F. (2016). *Growing flax*. 61. Retrieved from http://flaxcouncil.ca/growing-flax/

Folch, J., Lees, M., & Sloane Stanley, G. . (1956). *A simple method for the isolation and purification of total lipides from animal tissues*. https://doi.org/10.1016/j.athoracsur.2011.06.016

Fotuhi, M., Mohassel, P., & Yaffe, K. (2009). Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: A complex association.

Nature Clinical Practice Neurology, *5*(3), 140–152. https://doi.org/10.1038/ncpneuro1044

Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K., & Foubert, I. (2012). Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Research International*, *48*(2), 961–969. https://doi.org/10.1016/j.foodres.2012.03.014

Ganesan, B., Brothersen, C., Mcmahon, D. J., Ganesan, B., & Brothersen, C. (2014). *Fortification of Foods with Omega-3 Polyunsaturated Fatty Acids Fortification of Foods with Omega-3*. *8398*. https://doi.org/10.1080/10408398.2011.578221

Ganorkar, P. M., & Jain, R. K. (2013). Flaxseed - A nutritional punch. *International Food Research Journal*, *20*(2), 519–525.

García-Ayuso, L. E., Velasco, J., Dobarganes, M. C., & Luque De Castro, M. D. (1999). Accelerated extraction of the fat content in cheese using a focused microwave-assisted Soxhlet device. *Journal of Agricultural and Food Chemistry*, *47*(6), 2308–2315. https://doi.org/10.1021/jf981095f

Giergielewicz-Mozajska, H., Dabrowski, L., & Namieśnik, J. (2001). Accelerated solvent extraction (ASE) in the analysis of environmental solid samples - Some aspects of theory and practice. *Critical Reviews in Analytical Chemistry*, *31*(3), 149–165. https://doi.org/10.1080/20014091076712

Goyal, A., Sharma, V., Upadhyay, N., Gill, S., & Sihag, M. (2014). Flax and flaxseed oil: an ancient medicine & modern functional food. *Journal of Food Science and Technology*, *51*(9), 1633–1653. https://doi.org/10.1007/s13197-013-1247-9

Hamid, T. A., Momtaz, Y. A., & Ibrahim, R. (2012). Predictors and prevalence of successful aging among older Malaysians. *Gerontology*, *58*(4), 366–370. https://doi.org/10.1159/000334671

Hammad, S., Pu, S., & Jones, P. J. (2016). Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. *Lipids*, *51*(5), 507–517. https://doi.org/10.1007/s11745-015-4113-x

Hegde, M. V., Panse, M. L., Atakare, S. P., & Kadam, S. S. (2016). Omega-3 fatty acids: Keys to nutritional health. *Omega-3 Fatty Acids: Keys to Nutritional Health*, 1–610. https://doi.org/10.1007/978-3-319-40458-5

Hu, X. F., Laird, B. D., & Chan, H. M. (2017). Mercury diminishes the cardiovascular protective effect of omega-3 polyunsaturated fatty acids in the modern diet of Inuit in Canada. *Environmental Research*, *152*, 470–477. https://doi.org/10.1016/j.envres.2016.06.001

Ivanov, S., Rashevskaya, T., & Makhonina, M. (2011). Flaxseed additive application in dairy products production. *Procedia Food Science*, *1*(Icef 11), 275–280. https://doi.org/10.1016/j.profoo.2011.09.043

Kearney, J. (2010). Food consumption trends and drivers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2793–2807. https://doi.org/10.1098/rstb.2010.0149

Kershaw, T. G., Dhahir, P. H., & Clarkson, T. W. (1980). The relationship between blood levels and dose of methylmercury in man. *Archives of Environmental Health*, *35*(1), 28–36. https://doi.org/10.1080/00039896.1980.10667458

Kock, W. N. T., Sivalingam, N., Azlinda, H., Siew, R. W., & Sim, L. C. (2012). Omega-6 and Omega-3 fatty acid nutrition amongst Malaysians are far from desirable. *International E-Journal of Science, Medicine and Education*, *6*(2), 4–9.

Kuang, H., Yang, F., Zhang, Y., Wang, T., & Chen, G. (2018). The Impact of Egg Nutrient Composition and Its Consumption on Cholesterol Homeostasis. *Cholesterol*, *2018*. https://doi.org/10.1155/2018/6303810

Lee, L. K., Shahar, S., Yusoff, N. A. M., & Chin, A. V. (2013). Validation of a food frequency questionnaire in assessing the omega-3 polyunsaturated fatty acids intake for malays and Chinese elderly in Malaysia. *Sains Malaysiana*, *42*(11), 1625–1632.

Lemahieu, C., Bruneel, C., Ryckebosch, E., Muylaert, K., Buyse, J., & Foubert, I. (2015). Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. *Journal of Functional Foods*, *19*, 821–827. https://doi.org/10.1016/j.jff.2015.04.021

Lock, A. L., & Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids*, *39*(12), 1197–1206. https://doi.org/10.1007/s11745-004-1348-6

Lorente-Cebrián, S., Costa, A. G. V., Navas-Carretero, S., Zabala, M., Martínez, J. A., & Moreno-Aliaga, M. J. (2013). Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. *Journal of Physiology and Biochemistry*, *69*(3), 633–651. https://doi.org/10.1007/s13105-013-0265-4

Maia, M. R. G., Chaudhary, L. C., Figueres, L., & Wallace, R. J. (2007). Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie* *van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *91*(4), 303–314. https://doi.org/10.1007/s10482-006-9118-2

MANS. (2014). Malaysian Adult Nutrition Survey 2014. In *2014*. https://doi.org/10.1007/s13398-014-0173-7.2

Mazereeuw, G., Lanctôt, K. L., Chau, S. A., Swardfager, W., & Herrmann, N. (2012). Effects of omega-3 fatty acids on cognitive performance: A meta-analysis. *Neurobiology of Aging*, *33*(7), 1482.e17-1482.e29. https://doi.org/10.1016/j.neurobiolaging.2011.12.014

McNair, H. M., & Miller, J. M. (2009). Basic Gas Chromatography 2nd Edition. In *Journal of Chemical Education* (Vol. 75). https://doi.org/10.1021/ed075p1094

Miranda, J. M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J. A., Lamas, A., … Cepeda, A. (2015). Egg and egg-derived foods: Effects on human health and use as functional foods. *Nutrients*, *7*(1), 706–729. https://doi.org/10.3390/nu7010706

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, *58*(20), 2047–2067. https://doi.org/10.1016/j.jacc.2011.06.063

Mustapha, M. S. (2013). *Role of Omega 3 fatty acids during pregnancy and lactation* (p. Vol 4 (3) The Malaysian Journal of Nursing). p. Vol 4 (3) The Malaysian Journal of Nursing.

Neijat, M., Ojekudo, O., & House, J. D. (2016). Effect of flaxseed oil and microalgae DHA on the production performance, fatty acids and total lipids of egg yolk and plasma in laying hens. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *115*(October), 77–88. https://doi.org/10.1016/j.plefa.2016.10.010

Nesheim, M. C., & Yaktine, A. L. (2007). Seafood choices: Balancing benefits and risks. In *Seafood Choices: Balancing Benefits and Risks*. https://doi.org/10.17226/11762

Nestel, P., Clifton, P., Colquhoun, D., Noakes, M., Mori, T. A., Sullivan, D., & Thomas, B. (2015). Indications for Omega-3 Long Chain Polyunsaturated Fatty Acid in the Prevention and Treatment of Cardiovascular Disease. *Heart Lung and Circulation*, *24*(8), 769–779. https://doi.org/10.1016/j.hlc.2015.03.020

Ramola, B., Kumar, V., Nanda, M., Mishra, Y., Tyagi, T., Gupta, A., & Sharma, N. (2019). Evaluation, comparison of different solvent extraction, cell disruption methods and hydrothermal liquefaction of Oedogonium macroalgae for biofuel production.

Biotechnology Reports, *22*, e00340. https://doi.org/10.1016/j.btre.2019.e00340

RNI. (2017). *Recommended Nutrient Intakes for Malaysia 2017*.

Rosney, N., Haron, H., Salleh, M., & Shahar, S. (2018). Comparison of fatty acid profiles between successful and usual aging elderly. *Sains Malaysiana*, *47*(8), 1819–1826. https://doi.org/10.17576/jsm-2018-4708-21

Roy, J., & Le Guennec, J. Y. (2017). Cardioprotective effects of omega 3 fatty acids: origin of the variability. *Journal of Muscle Research and Cell Motility*, *38*(1), 25–30. https://doi.org/10.1007/s10974-016-9459-z

Ryckebosch, E., Bruneel, C., Muylaert, K., & Foubert, I. (2012). Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. *Lipid Technology*, *24*(6), 128–130. https://doi.org/10.1002/lite.201200197

Shahidi, F. (2005). Extraction And Measurement Of Total Lipids. *Handbook of Food Analytical Chemistry*, *1–2*, 425–435. https://doi.org/10.1002/0471709085.ch12

Singh, K. K., Mridula, D., Rehal, J., & Barnwal, P. (2011). Flaxseed: A potential source of food, feed and fiber. *Critical Reviews in Food Science and Nutrition*, *51*(3), 210–222. https://doi.org/10.1080/10408390903537241

Smith, E., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2018). Storage stability of egg sticks fortified with omega-3 fatty acids. *Journal of the Science of Food and Agriculture*, *98*(9), 3452–3461. https://doi.org/10.1002/jsfa.8858

Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 Fatty Acids EPA and DHA : Health. *Journal of Advanced Nutrition*, *3*, 1–7. https://doi.org/10.3945/an.111.000893.Omega-3

Tasbozan, O., & Gocke, M. A. (2017). Fatty acids in fish scales. *Marine Biology*, *157*(7), 1567–1576. https://doi.org/10.1007/s00227-010-1430-8

Tocher, D. R., Betancor, M. B., Sprague, M., Olsen, R. E., & Napier, J. A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. *Nutrients*, *11*(1), 1–20. https://doi.org/10.3390/nu11010089

Venturini, D., Simão, A. N. C., Urbano, M. R., & Dichi, I. (2015). Effects of extra virgin olive oil and fish oil on lipid profile and oxidative stress in patients with metabolic syndrome. *Nutrition*, *31*(6), 834–840. https://doi.org/10.1016/j.nut.2014.12.016

Wang, Y., Sunwoo, H., Cherian, G., & Sim, J. S. (2000). *Fatty Acid Determination in Chicken Egg Yolk : A Comparison of Different Methods*. 1168–1171.

Ways, P. and Hanahan, D. J. (1964) *Characterization and quantification of red cell lipids in normal man*. Journal of Lipid Research. 1964; 5: 318-328

Welch, A. A., Shakya-Shrestha, S., Lentjes, M. A. H., Wareham, N. J., & Khaw, K. T. (2010). Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the precursor-product ratio of α-linolenic acid to long-chain n-3 polyunsaturated fatty acids: Results. *American Journal of Clinical Nutrition*, *92*(5), 1040–1051. https://doi.org/10.3945/ajcn.2010.29457

Yannakopoulos, A., Tserveni-Gousi, A., & Christaki, E. (2005). Enhanced egg production in practice: The case of bio-omega-3 egg. *International Journal of Poultry Science*, *4*(8), 531–535. https://doi.org/10.3923/ijps.2005.531.535

Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y., Ishikawa, Y., … Shirato, K. (2007). Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet*, *369*(9567), 1090–1098. https://doi.org/10.1016/S0140-6736(07)60527-3

Appendices

Figure 1: Standard curve of 37 FAME mix standard for EPA

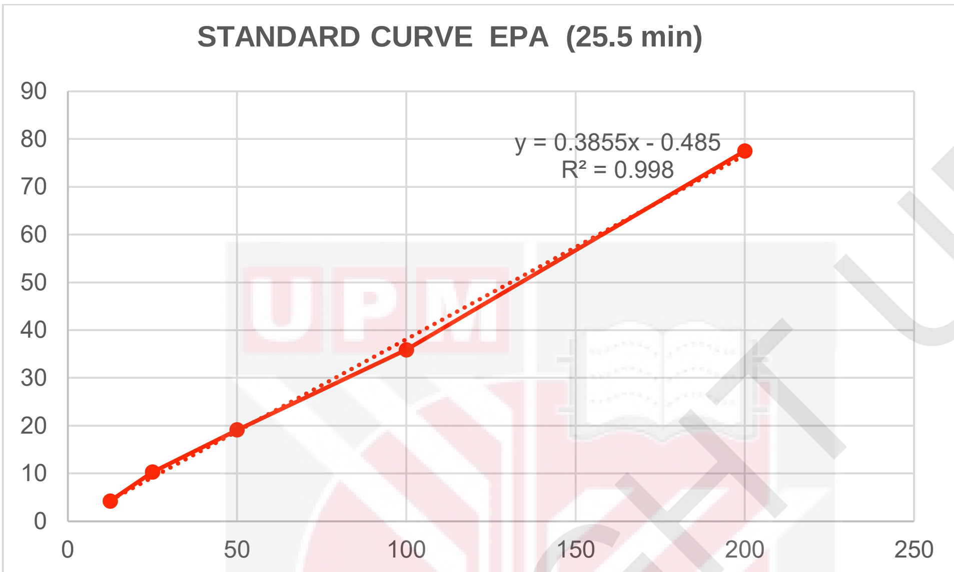


Figure 2: Standard curve of 37 FAME mix standard for DHA

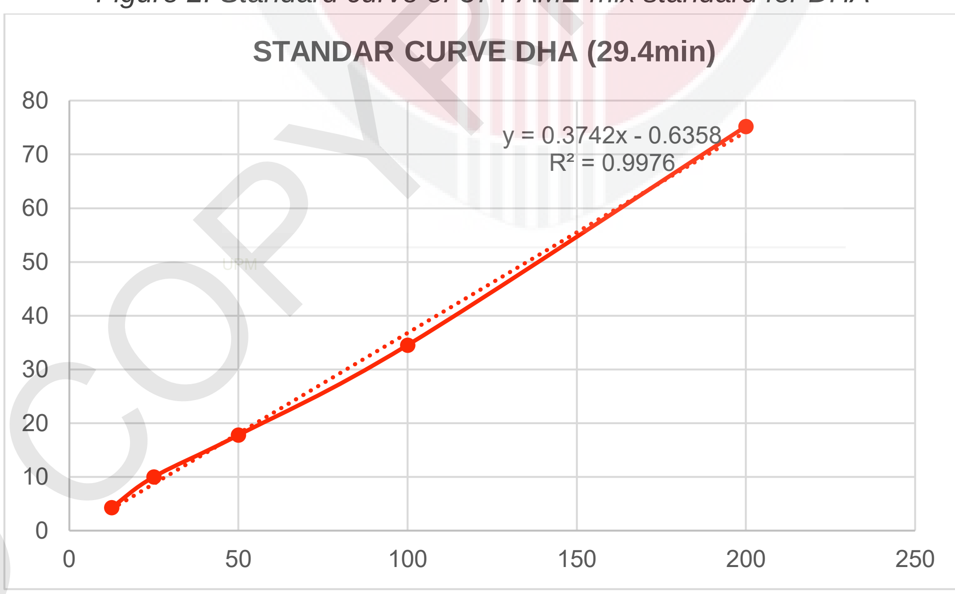


Table 4: Total lipid of Standard reference material comparison (SRM 1456a)

Reference Values for Proximate and Calories	Current analysis value
18.96 ± 0.40	12.25 ± 0.00

Table 5: EPA and DHA of Standard reference material comparison (SRM 1456a)

	Fat (g/100 g)	
Types of fatty acid	**Certified Mass Fraction Value for fatty acids as free fatty acids**	**Current analysis value**
Eicosanoic Acid (C20:0)	0.0329 ± 0.0009	0.1594 ± 0.0000
α-Linolenic Acid (C18:3 n-3)	0.133 ± 0.020	0.012 ± 0.000