Seasonal Variation and Regression Prediction of Fatty Acid Compositions in Tuna Oil from Three Tuna Species (*Katsuwonus pelamis, Thunnus tonggol* and *Euthynnus affinis*)

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Abstract

Tuna heads, by-products from canned tuna industry, are used as raw materials for processing fish oils. Lipid composition of tuna head is a good source of beneficial fatty acids, especially Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). They are the important keys of fish oil quality. The propose of this research work was aimed to determine the fatty acid composition in the heads of three different tuna species (*Katsuwonus pelamis*, *Thunnus tonggol*, and *Euthynnus affinis*) collected from canned tuna industry in Samut Sakhon Province, Thailand in different seasons. The results showed that high quality tuna oil processed from *E. affinis* sample caught between February and May had the highest contents of PUFA with 46.25% and 28.86% of DHA contents (p<0.05). The percentage of major fatty acids composition of tuna heads predicted by regression correlation from the fatty acid profiles over three different periods between 2014 to 2015 from February to May, from June to September, and from October to January were used to study seasonal variation. The spawning season of tunas (October to January) gave the lowest DHA, Omega-3 and PUFA compositions compared to the other two periods of time.

Keywords: Seasonal variation, Regression prediction, Fatty acids, Tuna head

1. Introduction

Tuna fish is rich in Omega-3 Polyunsaturated fatty acids (Omega-3 PUFAs) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which it is unique and not synthesized by terrestrial animals (Ali *et al*., 2013). Normally, tuna fish oil can be processed by using by-products from canned tuna industry. Those wastes or by-products including heads, bones, intestines, and liver that are approximately 30–35% of total weight of tuna (Das *et al*., 2011).

Omega-3 PUFAs are important nutritional elements for human health. They are important for growth development during the last trimester of the fetus. In addition, they contain hormones known as eicosanoids, which are involved in several metabolism processes of the human body. Eicosanoid hormones are mainly related to risk reduction of cardiovascular disease (Smuts *et al*., 2003 and Kaur *et al*., 2014).

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However, the composition of fatty acid in fish fluctuates widely according to several factors mainly species and seasonal factors (Saito *et al*., 1995). The amounts of DHA in lipids greatly change within species as well as during climatic seasons (Greenberg *et al*., 2008). This notion of seasons having an effect on lipid and fatty acid composition was recently corroborated in a study by Mourente and Tocher (2009). Mourente and Tocher (2009) reported that the seasonal accumulation of sufficient reserves may be a necessary for maturity in some fishes and particularly in migrating tuna. The lipid storage allows tuna to swim across the ocean for long time even during that time the food is limited (Mourente *et al*., 2002).

In 2016, Food and Agriculture Organization of the United Nations (FAO) reported that the top six exporters of canned tuna for the first half of the year of 2016 in order were: Thailand, Ecuador, Spain, China, Indonesia and Mauritius, consecutively. There are tuna factories in Europe, however most of the processing plants are established in Thailand (Fair Trade Center, 2008). Tuna industry in Thailand mostly uses *K. pelamis*, *E. affinis,* and *T. tonggol* as the major raw material for canned tuna processing (Fair Trade Center, 2008 and Nootmorn, 2015). Exporting markets of tuna products in Thailand are increasing dramatically to 501,391 tons in 2006. In 2016, Thailand is the biggest tuna products exporter with 559.612 tones. However, with a dramatically increasing production of tuna products that bring about a considerable quantity of wastes, which are needed to be utilized. Tuna heads are the rich source of Omega-3 polyunsaturated lipids (Omega-3 PUFAs) that are mostly used for processing tuna fish oil. However, different tuna species and seasonal capturing can affect to amount of Omega-3 PUFAs composition of the oil in tuna head.

The aim of research study was to evaluate the seasonal variation of fatty acid using fatty acid profile in tuna heads from three different tuna species captured in different seasons throughout the year to predict a main group of fatty acid composition in tuna species, mostly used as the raw material for canned tuna processing.

2. Materials and Methods

2.1 Materials

Tuna samples were received from the tuna factory in Samut Sakhon, Thailand. The samples were three different tuna species (*Katsuwonas pelamis, Thunnus tonggol* and *Euthynnus affinis*) that captured in the periods of seventeen months between May 2014 and September 2015. All samples were grouped into three fishing seasons (February to May, June to September, and October to January) from the Western Pacific Ocean. The frozen whole tunas were transported to the laboratory of Department of Fishery Products at Kasetsart University, Thailand within three hours and were stored at -20°C until laboratory analysis.

2.2 Separation of fish oil from tuna heads

Frozen tuna samples from the same season from different years were mixed and grouped into three different seasons. Sample group 1 represented tuna samples which caught between February to May. Group 2 and 3 represented the samples which caught between June to September and between October to January, respectively. Before oil separation processing, whole body of frozen tunas were thawed overnight at 4°C. Then, tuna head were separated from the other parts. According to the method of Chantachum *et al*. (2000) with a slight modification, tuna oil was extracted from tuna heads using the wet-reduction process. Head samples were heated by steam cooking at 85°C for 15 minutes. A hydraulic press was applied to fish oil extracted from heated samples. Tuna head oil was recovered by centrifugation at 12,000 rpm for 10 minutes at 25°C.

2.3 Fatty acid analysis

Oil samples (25 mg) were transferred into 16x150 screw cap test tubes containing 0.1 mg of the internal standard (C23:0). Fatty acid methyl esters were then identified by an Agilent 7890A gas chromatograph (GC) (Agilent Technologies, USA) equipped with a flame ionization detector (FID) and integrator using derivatization techniques following the AOAC method (2000). DB-WAX capillary column 30 m × 0.25 mm (J&W Scientific, USA) was used with helium as the gas carrier at a flow rate of 30 ml/min. The initial temperature of the column was set at 170°C and then increased to 225°C at a rate of 1°C per minute. After that, they were held at 220°C for an additional 20 minutes. The temperature of the detector was set at 270°C, while the temperature of the injection port was maintained at approximately 250°C. Retention times of fatty acid methyl ester (FAME) were used to identify chromatographic peaks by comparison with PUFA No.3 from Menhaden Oil standards (Supelco, USA). The peak area was quantified as the percentage of total saponifiable lipid (AOAC, 2000).

2.4 Statistical analysis

Seasonal variation of the fatty acids was determined in triplicate samples using a 3x3 factorial that provided in completely randomized design (CRD). The analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences between sample groups with the significant at $p<0.05$

In addition, the Principal Component Analysis (PCA) were performed on the eight groups of fatty acid samples (EPA, DHA, PUFAs, MUFAs, SFAs, Omega-3, Omega-6, and Omega-9). The linear regression analysis was used to predict the correlation of each fatty acid group as an independent variable, and Omega-3 as a dependent variable.

3. Results and Discussion

3.1 Fatty Acids Profile and Total Fat Contents of Tuna Head Oil During Three Different Seasons

PUFAs were the largest components in tuna head oil, following by SFAs and MUFAs in all tuna species. The highest means value of PUFAs and SFA found in *E. affinis* during February to May were 46.25 and 36.35% respectively. Moreover, PUFAs, MUFAs, Omega-3, Omega-9, and DHA showed the significant seasonal variations (p<0.05) in each tuna species. The nutrition value of tuna head oil showed the DHA and EPA contents with the highest mean of DHA was 28.86% from *E. affinis* caught during February and May compared to *T. tonggol*, with highest EPA caught during October and January was 9.04% (Table 1).

Both season and species had highly significant effects on DHA contents (p<0.05), which was not found in the SFAs composition from *K. pelamis* and *T. tonggol*. Nordgarden (2003) reported similar seasonal changes in fatty acid compositions in fish due to metabolic changes in their β-oxidation capacity. However, Central Institute of Fisheries Technologies. (1993) reported that fatty acid composition revealed profound variation among fish species. Saito *et al.* (1995) stated that accumulation of DHA in the muscle tissues of tuna at more than 20% of total fatty acids, which was higher than *Solea solea,* a non-migratory fish species.

Table 1 Fatty acids groups (the percentage of total saponifiable lipid) in tuna head oil during the different fishing period

Fatty acid	Tuna	February-May	June-September	October-January
PUFAs	*K. pelamis*	42.56±0.45^{Ba}	39.02±1.55^{Bb}	35.41±2.29^{Bc}
	T. tonggol	44.15±0.65^{Aa}	42.79±0.06^{Ab}	40.91±0.40^{Ac}
	E. affinis	46.25±0.00^{Aa}	43.43±0.16^{Ab}	40.88±0.12^{Ac}
MUFAs	*K. pelamis*	17.47±0.01^{Ab}	15.47±0.62^{Ac}	21.28±0.25^{Aa}
	T. tonggol	16.29±0.93^{Bb}	15.03±0.01^{Bc}	21.46±0.05^{Ba}
	E. affinis	16.05±0.00^{Ab}	16.55±0.06^{Ac}	21.51±0.04^{Aa}
SFAs	*K. pelamis*	34.02±0.16^{Aa}	34.70±0.18^{Aa}	32.99±1.92^{Ab}
	T. tonggol	31.78±3.93^{Ba}	33.13±0.06^{Ba}	30.63±0.09^{Bb}
	E. affinis	36.35±0.00^{Aa}	33.03±0.25^{Aa}	30.72±0.06^{Ab}
Omega-3	*K. pelamis*	36.18±0.17^{Ba}	32.66±1.32^{Bb}	29.29±2.10^{Bc}
	T. tonggol	37.48±0.41^{Aa}	35.92±0.06^{Ab}	33.92±0.07^{Ac}
	E. affinis	38.88±0.00^{Aa}	36.57±0.10^{Ab}	33.86±0.14^{Ac}

Table 1 Fatty acids groups (the percentage of total saponifiable lipid) in tuna head oil during the different fishing period (continue)

Fatty acid	Tuna	February-May	June-September	October-January
Omega-6	*K. pelamis*	3.12±0.01Bb	2.79±0.43Bb	3.78±0.80Ba
	T. tonggol	3.74±0.08Ab	3.76±0.01Ab	4.41±0.01Aa
	E. affinis	4.03±0.00Ab	3.60±0.01Ab	4.43±0.10Aa
Omega-9	*K. pelamis*	10.18±0.01Ab	7.70±0.54Ac	12.88±2.46Aa
	T. tonggol	8.11±0.41Bb	7.24±0.10Bc	10.60±0.03Ba
	E. affinis	8.58±0.21Bb	8.07±0.08Bc	10.64±0.02Ba
EPA	*K. pelamis*	4.68±0.02Bb	4.59±0.57Bb	4.96±0.31Ba
	T. tonggol	5.52±0.11Bb	5.02±0.12Bb	9.04±0.13Ba
	E. affinis	5.36±0.03Ab	5.61±0.06Ab	8.82±0.01Aa
DHA	*K. pelamis*	27.76±0.15Ba	24.67±0.35Bb	21.98±0.59Bc
	T. tonggol	27.97±0.08Ba	26.95±0.02Bb	19.99±0.03Bc
	E. affinis	28.86±0.04Aa	27.11±0.02Ab	20.11±0.11Ac
total fat content	*K. pelamis*	11.68±0.04Aa	9.55±0.11Ab	6.58±0.30Ac
(%dry weight)	*T. tonggol*	11.13±0.33Aa	9.57±0.21Ab	6.77±0.55Ac
	E. affinis	11.40±0.88Aa	9.64±0.12Ab	7.61±0.09Ac

Note: *K. pelamis; Katsuwonas pelamis, T. tongol; Thunnus tongol, E. affinis; Euthynus affinis,*
PUFA; polyunsaturated fatty acid, MUFA; monounsaturated fatty acid, SFA; saturated fatty acid
Values are expressed as mean±SD (n=3); Different sets of letters on each value of the same row (a, b, c) and column (A, B, C) show a significant difference ($p<0.05$).

Chen *et al*. (2007) reported that PUFAs were not automatically synthesized within the human body; therefore, EPA and DHA were required for human consumption in the diet. Nevertheless, EPA was considered as the main precursor of prostaglandin, thromboxane, and leukotrienes synthesis, while DHA was considered as the main element in membrane phospholipids of brain and retina cell, as well as a human health dietary supplements (Spiller, 1995). Tuna is perceived as a fatty pelagic fish with a high amount of DHA in its muscle compared to other lean pelagic fish such as cod, haddock and pollack with fatty acids in their livers and other internal organs (Aubourg *et al*., 2002). Alkio *et al*. (2000) determined DHA as the main element in tuna fish oil production at 18.3%; higher than other fish like Baltic herring and menhaden with DHA content at around 6.4% and 8.8%, respectively. However, lipid content of fish varies with species, diet, geographic origin, season, and reproduction.

Figure 1 shows the fatty acid profiles. Concentrations of eighteen fatty acids of three tuna species (*K. pelamis*, *T. tonggol,* and *E. affinis*) were determined for three different seasons. Results showed the remarkable changes in fatty acid composition over the three periods (February to May, June to September, and October to January). For all tuna samples, fatty acids with high concentrations over the year were DHA (C22:6n3) with mean values 27.76±0.15%, 27.97±0.08%, and 28.87±0.05% for *K. pelamis*, *T. tonggol* and *E. affinis* in summer (Feb to May), respectively. While, EPA (C20:5n3) from *K. pelamis*, *T. tonggol* and *E. affinis* were shown a mean values as 4.68±0.02%, 5.52±0.11%, and 5.35±0.04% in summer (February to May), respectively; palmitic acid (C16:0) with mean values 23.14±0.10%, 22.11±0.77%, and 23.03±0.00% in summer (February to May), respectively; and oleic (C18:1) with mean values 12.01±0.69%, 10.22±0.03%, and 10.25±0.02% for winter (October to January), respectively.

The most abundant Omega-3 PUFAs was DHA (C22:6n3) followed by EPA (C20:5n3). February to May showed the highest DHA contents in all tuna species, while *E. affinis* had the best nutrition value with 27.76% and 8.82% DHA and EPA contents, respectively. Popovic *et al*. (2011) studied in Southern Bluefin tuna which represented the highest level of DHA and Omega-3 fatty acid, known for its benefit human heart disease, and highly favored in Japan.

Seasons affect main fatty acids content such as EPA and DHA which are the target of fish oil extraction. The highest DHA content was found between February and May, with a slight decrease between June and September followed by October and January, respectively. In contrast, EPA content was highest between October and January, followed by June to September and February to May, respectively. Castell *et al*. (1972) reported that lipid storage varied during reproductive and nutrition periods, especially during reproduction when lipids were transported from the liver and the muscles to the gonads to enhance their development. Therefore, fatty acid content decreased during the reproduction period. In addition, when fish have enough food they can control their reproduction cycles which are directly associated with food abundance. If there is food scarcity in their environment the variation is low, but if it is abundant, the variation is higher during the year (Kluytmans and Zandee, 1974; Ackman *et al*., 1975; Kinsella *et al*., 1977; Mute *et al*., 1989; Kendemir and Polat, 2007).

Johnson (2009) determined that fatty acid composition in skipjack tuna was related to the reproductive demand by demonstrating fluctuations over the maturation period. Seasonal variations in EPA and DHA were recorded by Kacar *et al*. (2016) who reported that the percentage of EPA decreased after the reproduction season (September). DHA was the main PUFA in *Silurus triostegus* with maximum percentage in November (17.10%), while in March (before the reproduction period), the concentration of EPA decreased to its lowest value (6.59%). (Kacar *et al*., 2016)

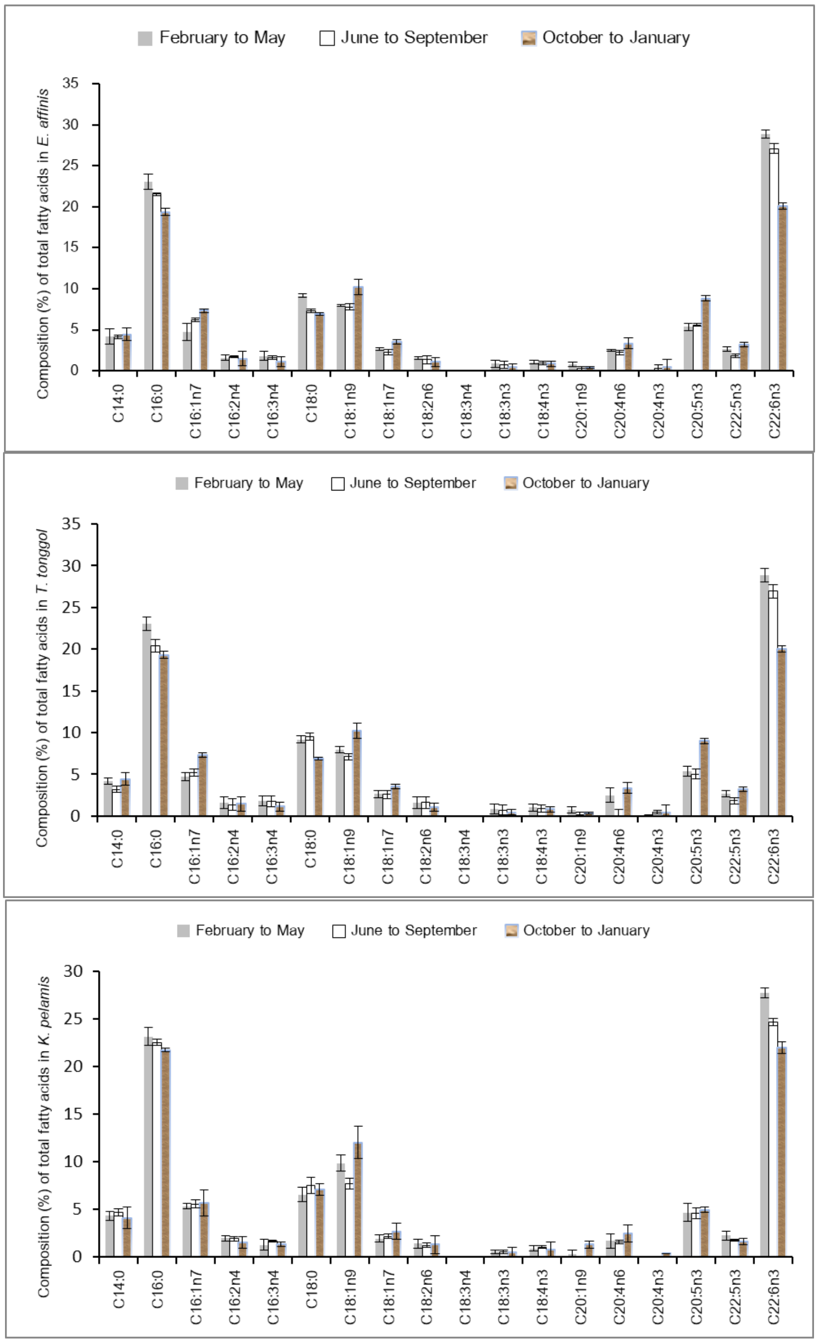

Figure 1 Fatty acid profile of tuna head oil extracted from *K. pelamis, T. tonggol,* and *E. affinis*

C14:0; Myristic acid, C16:0; Palmitic acid, C16:1n7; Palmitoleic acid, C16:2n4; C16:3n4; C18:0; Stearic acid, C18:1n9; Oleic acid, C18:1n7; Vaccenic acid, C18:2n6; Linoleic acid, C18:3n4; octadecatrienoic acid, C18:3n3; α-Linolenic, C18:4n3; Stearidonic acid, C20:1n9; Eicosenoic, C20:4n6; Arachidonic acid, C20:4n3; Eicosatetraenoic acid, C20:5n3; Eicosapentaenoic acid, C22:5n3; Docosapentaenoic acid, C22:6n3; Docosahexaenoic acid

Tropical tuna such as *K. pelamis, T. tonggol,* and *E. affinis,* are all migratory species. During migration, they usually swim toward coastal waters to feed and spawn. The migration begins in September (Chiou and Lee, 2004). Interestingly, relationships between the spawning season of tuna and the lowest amounts of fatty acid composition were observed. During October to January in the spawning period, fatty acids were lower than during the other periods. Concentrations of eight fatty acids (C16:0, C16:2n4, C16:3n4, C18:0, C18:2n6, C18:3n3, C18:4n3, and C22:6n3) from all eighteen fatty acids decreased from the period before (February to May and June to September), respectively.

3.2 Principal Component and Regression Analysis of Fatty Acid Composition in Tuna Heads

Figure 2 represented Principal Component Analysis (PCA), which was classified fatty acid group. The figures also described the relationship between fatty acids composition in tuna oil samples. The components were linear combinations of the original variables and were determined so that the first component explained the largest part of the total variance. This means that correlated variables were explained by the same component and less correlated variables by different components. In the present analysis, the two components explained 55.57% and 24.89%, respectively, of the total variance in the eight variables. Samples with similar values for the variables explained by the component appeared close together. Therefore, fatty acid grouping could be used in the following evaluations. After PCA gave the components of fatty acid that effected to the three species of tuna sample. After that, the linear regression was evaluated as the following model:

$$Omega3 = 0.597(DHA) + 0.854(EPA) + 0.472(PUFA) - 0.379(Omega6) - 3.329$$

This model showed R square with 99.7%, *p*-value <0.001. Durabin-Watson Statistic showed no auto-correlation (2.053). Rui *et al*. (2013) reported that the regression and correlation analysis were frequently used to measure the strength of association between two variables X and Y, using the regression recorded positive and negative correlations between experimental factors. Similar to Jahan *et al*. (2005) who found the neutral lipid was a factor related to flavor in cooked chicken breast by PCA.

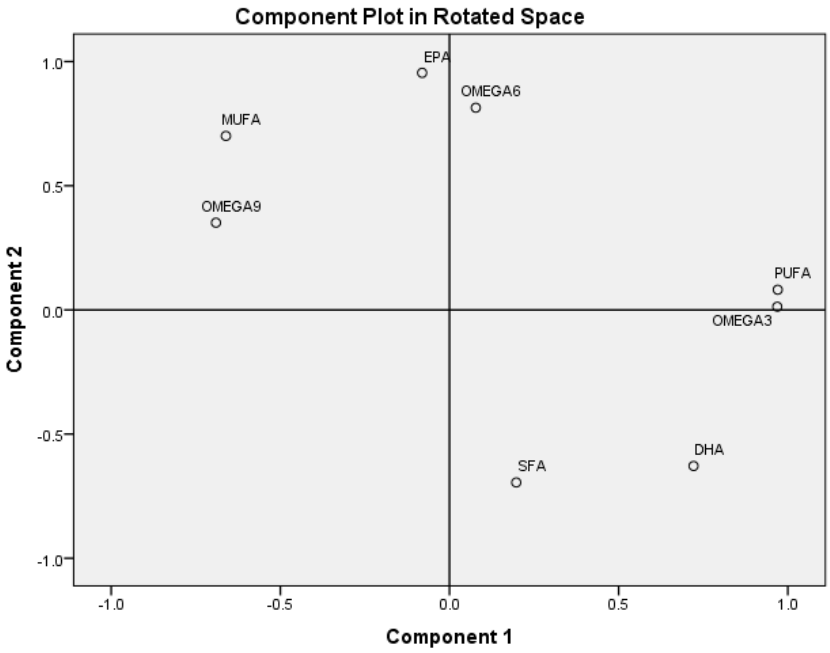

Figure 2 Score plot of principal components 1 and 2 for tuna head samples; EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; OMEGA3: Omega-3 fatty acid; OMEGA6: Omega-6 fatty acid and Omega-9: Omega-9 fatty acid

4. Conclusions

Seasonal variation of beneficial fatty acids such as EPA, DHA, Omega-3 and Omega-6 compositions in tuna heads were predictable by regression correlation analysis of the fatty acid profiles in three tuna species (*K. pelamis, T. tonggol* and *E. affinis*) over the year. Our results also demonstrated that the spawning season of tuna (October to January) provided the lowest nutritive value of tuna compared to other seasons. Nonetheless, a larger data set is necessary for a deeper understanding of the relationships between the seasons and fatty acid signatures with regarding to the tuna fish oil industry and the marine ecosystem.

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