C20:2n-6	0.33 & 0.45	0.23 & 0.32	0.30 & 0.44	-	-	1.07 ± 0.01	0.27 ± 0.03	0.15 ± 0.05
C20:3n-6 (DGLA)	2.70 & 2.74	2.82 & 2.66	2.71 & 2.72	2.93	-	0.11 ± 0.00	0.13 ± 0.01	0.38 ± 0.04
C20:3n-3	0.49 & 0.66	0.17 & 0.18	0.18 & 0.19	1.25	-	0.10 ± 0.00	-	-
C20:4n-6	4.44 & 4.39	4.62 & 4.35	4.50 & 4.47	1.55	-	0.20 ± 0.01	1.76 ± 0.04	0.28 ± 0.02
C20:5n-3 (EPA)	2.73 & 3.14	2.49 & 2.69	2.32 & 2.41	6.12	0.1	5.52 ± 0.02	3.80 ± 0.24	5.44 ± 0.08
C21:0	1.27 & 1.60	0.97 & 1.53	1.17 & 1.34	-	-	0.33 ± 0.03	0.51 ± 0.05	-
C22:0	0.17 & 0.28	0.14 & 0.16	0.14 & 0.13	1.85	5.1	-	0.28 ± 0.03	0.22 ± 0.04
C22:1n-9	0.18 & 0.31	0.00 & 0.00	0.00 & 0.00	-	0.1	-	0.37 ± 0.01	0.16 ± 0.01
C22:2n-6	0.24 & 0.36	0.07 & 0.25	0.00 & 0.07	-	-	0.37 ± 0.01	-	-
C22:6n-3 (DHA)	23.29 & 22.28	24.14 & 22.72	23.70 & 23.41	8.65	18.8	32.06 ± 0.30	22.15 ± 0.04	8.54 ± 0.04
C23:0	0.42 & 0.65	0.23 & 0.39	0.26 & 0.26	-	-	1.91 ± 0.01	-	-
C24:0	0.82 & 1.14	0.49 & 0.79	0.66 & 0.65	1.05	0.0	0.40 ± 0.06	0.39 ± 0.05	-
C24:1	2.44 & 2.84	2.43 & 2.50	2.29 & 2.25	-	-	0.68 ± 0.01	1.04 ± 0.04	-
∑ SFA*	33.71 & 33.50	34.49 & 34.86	27.99 & 35.94	27.96	-	38.08 ± 0.16	47.41 ± 0.11	-
∑ MUFA	25.39 & 24.73	25.95 & 24.77	35.12 & 25.22	27.71	-	20.94 ± 0.07	23.27 ± 0.24	-
∑ PUFA	36.87 & 33.87	36.95 & 35.74	34.40 & 24.28	44.34	-	40.98 ± 0.23	29.31 ± 0.19	-
∑ ω-3	26.99 & 26.50	27.10 & 26.06	26.50 & 26.27	-	-	-	-	-
ω-3:ω-6	2.73 & 2.62	2.75 & 2.69	3.35 & 2.72	-	-	-	-	-

LA= Linoleic acid; GLA= Gamma linolenic acid; ALA= Alpha linolenic acid; DGLA= dihomo gamma linolenic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ∑SFA= sum of Saturated Fatty Acid; ∑MUFA= sum of Monounsaturated Fatty Acid; ∑PUFA= sum of Polyunsaturated Fatty Acid

* The sums of SFA, MUFA and PUFA in each experiment were calculated separately

Fatty acid composition

The fish oil extracted from yellowfin tuna heads using three different autoclaving durations was subjected to fatty acid analysis to determine the amount of saturated, monounsaturated and polyunsaturated fatty acids present in the oils. Thirty different fatty acids were identified in the present study and the results are presented in Table 3. The results indicated that the fish oil from yellowfin tuna heads contains saturated fatty acids (27.99 - 35.94 %), monounsaturated fatty acids (24.73 - 35.12 %) and polyunsaturated fatty acids (24.28 – 36.95 %). The most prominent saturated and monounsaturated fatty acids present in the extracted oils were palmitic acid (17.17 – 20.21 %) and oleic acid (15.31 – 27.05 %), respectively. The major polyunsaturated fatty acid present in the extracted oil from yellowfin tuna heads was docosahexaenoic acid (DHA) (22.28 – 24.14 %). The total omega-3 fatty acids present in the extracted oils were in the range of 26.06 – 27.10 %. The ratios of omega 3/omega 6 fatty acids present in the extracted oils were in the range of 2.62 – 3.35.

Oil colour

The colour values of the present study, given in Table 2, indicate that all yellowfin tuna oils had moderate L* values, signifying dark colour appearances of the product. All oils had very low positive a* values, indicating a slightly reddish colour, and a positive low value of b* colour, indicating yellowish colour. Thus, all oils extracted by autoclaving have shown an intense brown colour. In a comparison of three heat durations of autoclaving, there were no significant differences found among treatments for all values ($P > 0.05$). In the present study, the colour values (Table 2) indicated that all yellowfin tuna oils had moderate L* values, signifying dark colour appearances of the product. All oils had very low positive a* values, indicating a slightly reddish colour, and a positive low value of b* colour, indicating yellowish colour. Thus, all oils extracted by autoclaving have shown an intense brown colour. In a comparison of three heat durations of autoclaving. No significant differences were found among treatments for all values ($P > 0.05$).

DISCUSSION

This study provides some insight into the use of autoclaving as an effective and potential wet reduction process for extracting fish oil especially by using tuna heads, which is a frequent discard from processing plants. The proximate composition of tuna heads reported by Nguyen et al. (2011) was slightly higher than the ash and lipid contents reported in this study but almost similar moisture content, as 11.8 ± 1.1%, 13.5 ± 0.1% and 59.0 ± 1.1%, respectively for ash, lipid and moisture contents of yellowfin tuna head. The dissimilarities in the proximate composition of the same species from the different parts of the world could be due to the physiological factors (migrations or spawning), and natural factors (food), as those affect the chemical composition especially a high deviation of the lipid fraction of fish (Borlongan & Benitez 1992; Pradhan et al. 2015).

Autoclaving, one of the pre-treatments which could use for the wet reduction process, has shown increasing yield and recovery percentages of oil at the initial autoclaving durations but prolonged autoclaving harms both the yielding and recovery percentages. The wet reduction process, which consists of three basic steps: cooking, pressing and centrifugation (Fang et al. 2019), is considered as one of the common methods used in the industrial-scale for the production of fish oil. The cooking step in this process causes to coagulation of the fish protein and facilitates the separation of liquids and solids by mechanical force. Moreover, fat cells are also disrupted in higher temperatures, to release the oil fraction of fish into the liquid phase (Bimbo 1990). Generally, autoclaving can be considered as a better cooking method as it produces high volumes of fish oil without using any chemicals during extraction. The results obtained by Pudtikajorn & Benjakul (2020) for skipjack tuna eyeballs have also confirmed that increasing autoclaving duration has affected the extraction yield to decrease after a certain autoclaving duration showing that there was some residual oil remaining in the sample. Proteins generally endure irreversible denaturation at higher temperatures for an extended period even at neutral pH (Weijers et al. 2003). Thus, it can be assumed that denaturation of the proteins has occurred when applying heat for a prolonged duration and a firm structure could be

formed, which acts as a barrier to release oil any further.

Comparing the properties of oil extracted from yellowfin tuna heads such as oil acidity, peroxide value, *p*-anisidine value, TOTOX value, moisture, colour, fatty acid composition and ATR-FTIR measurements have provided some insight into the quality of the oil for different autoclaving durations. One of the important quality parameters of oil is acidity which is related to the presence of acidic compounds including Free Fatty Acids (FFA) and other non-lipid acids. The formation of FFA is mainly caused by hydrolysis of triacylglycerides, whereas non-lipid acids like acetic acid compounds are known as acids generated during raw material spoilage. Thus, acidity depends on some characteristics of oil-related to the extraction procedure, oil composition and the freshness of raw material (Rubio-Rodríguez et al. 2012). The mean acid values of oils, reported in this study, for three autoclaving durations were higher than the WHO standard limit (2017) set for fish oil as 3.0 mg KOH g^{-1}. But, considering the FFA % of extracted fish oils resulting from all three autoclaving durations were found to be within the acceptable limit of 1% - 7% according to the standard value stated by Bako et al. (2017). However, irregular high values of free fatty acid content may be due to the heat variation during the extraction process and also due to the presence of moisture. Chantachum et al. (2000) also stated that the presence of both moisture and heat can induce hydrolysis of oil. In the case of autoclaving, heat may induce the hydrolysis of triglyceride molecules of the oil and caused the release of free fatty acids in high amounts from the glycerol backbone. Moreover, the increase of FFA value at 45 min of autoclaving may be due to the extended contact time with water that induces the oil hydrolysis. This has also been explained by García-Moreno et al. (2014), where FFA content of sardine oil increased with increased pressing stages which allow for a larger contact time of oil with stick water. In the wet reduction process, a high amount of water tends to increase the hydrolysis reaction rate of fat and oil (Pudtikajorn & Benjakul 2020). Further, a high level of oil acidity is an indication of hydrolytic rancidity which indicates the need for further refining before it can be used for a specific purpose.

Peroxide value (PV) is a useful indicator of the extent of primary oxidation the oil has undergone. If PV is low, higher in the quality of oil about oxidation status. Fish oil consists of a high amount of PUFA, which are highly reactive with oxygen and many factors including light, heat, hemeproteins and ions of heavy metals can speed up lipid oxidation (EFSA Panel 2010). As described in previous studies, the high temperature of the wet reduction process can increase lipolysis, and as a result of that, the formation of free fatty acids which are highly reacted with oxygen than the esterified form may occur (Suseno et al. 2015). However, there was a decreasing trend of peroxide value after a certain stage of autoclaving duration that might probably be due to less oxygen dissolved in the material as explained by Suseno et al. (2015) where the temperature of water increased, the amount of dissolved oxygen will reduce. Nevertheless, all peroxide values obtained in this study were below 5 mEq/kg oil which is the standard value for fish oils specified by WHO (2017).

The *p*-AnV is used for measuring the non-volatile secondary products of oxidation. At the end of the oxidation process, hydroperoxides are degraded and secondary products of oxidation are generated, such as aldehydes, acids, epoxide monomers, alcohols, ketones, dienals, lactones, hydroxy components, polymer compounds, etc (Pudtikajorn & Benjakul, 2020). In this study, a high temperature of 121 °C was used in the autoclaving process and some volatile oxidation products could have been evaporated. However, non-volatile oxidation products can be simultaneously decomposed, as indicated by the lower *p*-AnV. Generally, a longer autoclaving duration can enhance the decomposition of hydroperoxides, leading to the formation of numerous secondary oxidation products. In the present study, *p*-AnV has increased with the progression of autoclaving duration. However, all *p*-AnV of extracted fish oils were below 20 which is the standard value for fish oils specified by WHO (2017).

The total oxidation (TOTOX) value is a quality parameter related to the presence of different compounds such as aldehydes, ketones, hydroperoxides etc. which are mainly generated by degradation of PUFA in the presence of pro-oxidants such as oxygen, high temperatures, light and metal compounds (Rubio-Rodríguez et al. 2012). The allowable limit of TOTOX value stated by CODEX standards for human consumption is ≤

26 WHO (2017). The resulted TOTOX values in this study were well below the maximum allowable limit, thus indicating the low rancidity level of the extracted oils by autoclaving. Deepika et al (2014) extracted oil from salmon by-products and reported TOTOX values for gut, heads and frames as 6.11, 10.73 and 1.00, respectively. The TOTOX value can be increased for some fish oils due to harsh extraction conditions and extended storage time.

The moisture contents of oil should be very low for extending its oxidative stability. As fish oil has highly unsaturated fatty acids, a pro-oxidant like moisture can accelerate both oxidative and hydrolytic rancidity. In the present study, the moisture content is around zero indicates good stability in the storage.

Significant information on the oxidative status of oils can be acquired by studying the absorbance and frequency values of several bands of infrared spectra. The ATR-FTIR spectra of tuna oil obtained is almost similar to those of other fish oils described in the literature. As described by Karunathilaka et al. (2018), the differences appeared in absorbance of bands in higher wavenumber region from 3,100 – 2,700 cm^{-1} can be attributed to omega-3 PUFA contents. Furthermore, the band near 3,013 cm^{-1} is especially representing =C–H stretching vibrational mode of unsaturated fatty acids. The decrease of peak intensities around 2,923 cm^{-1} and 2,853 cm^{-1} regions could be due to the reduction of double bonds inside fatty acids by oxidation during a longer autoclaving duration. According to Plans et al. (2015) shift in the carbonyl group (1,744 – 1,741 cm^{-1}) of the oils depending on the type of FAs esterification. Moreover, during a longer autoclaving duration, the ester bond of triacylglycerols can be cleaved by hot water (Pudtikajorn & Benjakul 2020). The increase of peak intensity near 1,149 cm^{-1} was observed in the present study and this has been proved to be related to the proportion in the sample of saturated acyl groups and a similar trend at band 1,163 cm^{-1} was observed by Liang et al. (2013) in walnut oils during heating. Thus, that variation indicates the increase of saturated fatty acids with prolonged autoclaving duration. Moreover, the increase of peak intensity around 966 cm^{-1} with increasing holding time is caused by the formation of trans fatty acids. The possible reason for this could be that oil extraction processes are generally undergone in different thermal treatments which accelerate lipid oxidation and formation of *trans* fat (Tsuzuki et al. 2010). The selected peak intensities (absorbance) of oils extracted from autoclaving for a longer autoclaving duration markedly changed in comparison with oils extracted from less autoclaving duration indicating a clear effect of autoclaving duration on the oxidative state of oil.

Similar to the results of Nazir et al (2017), a slight difference was found in the fatty acids profile of oil from yellowfin tuna heads analysed in this study. Anyhow, according to both the studies PUFA content was the highest compared to SFA and MUFA contents. The fatty acid compositions have slight variations in total SFA, MUFA and PUFA contents with respect to autoclaving durations. When increasing the autoclaving duration from 15 min to 30 min, an increase of total SFA can be noticeable as confirmed by the ATR-FTIR spectra at the wavenumber region near 1,149 cm^{-1}. There was a slight variation of that pattern in the first replicate of 45 min autoclaved sample as total SFA has decreased as mentioned in Table 3. However, the total SFA of the second replicate of 45 min autoclaved sample has increased further, and it is similar to the trend shown in the ATR-FTIR spectra. When comparing all heat treatments, variation in total MUFA content was not significant except the first replication in 45 min autoclaved sample which has recorded a high value of total MUFA. In the present study, the most prominent MUFA is oleic acid and it is an important finding as diets rich in oleic acid are associated with a reduced risk for developing type 2-diabetes (de Oliveira et al., 2017). Similarly, Nazir et al. (2017) also reported the fatty acid profile of oil from yellowfin tuna heads with a predominance of oleic acid (20.45 %). The most important component in fish oil is PUFA, especially long-chain PUFA such as EPA and DHA. In all extracted oils, DHA was the most prominent fatty acid and the relative abundance of DHA has a minimal difference with increasing autoclaving duration. Generally, PUFA in fish oil undergoes both oxidative and hydrolytic rancidity with ease. Therefore, the process conditions of the extraction method are a significant factor in extracting fish oil. Nazir et al. (2017) recommended the wet reduction process works best in extracting DHA and EPA compared to the other two extraction methods (acid silage method, solvent extraction) that they evaluated. The effect of autoclaving duration on fish oil is also reflected in the total

PUFA content as it has slightly decreased with progressing autoclaving duration. In ATR-FTIR spectra, the peak intensity at 3,013 cm^{-1} which represents =C–H stretching vibrational mode of unsaturated fatty acids has decreased slightly from 15 min to 30 min and decreased further when progressing autoclaving duration up to 45 min. This behaviour is also confirmed by the fatty acid composition of the extracted oils in the present study. Thus, it seems that the level of fatty acid decomposition slightly increases with the longer autoclaving duration of oil extraction from yellowfin tuna heads. When comparing the autoclaving duration of 15 min and 30 min, only a slight change in total PUFA content as influenced by autoclaving time was noted. The total omega-3 PUFA content in the extracted oils has not drastically changed as increasing the autoclaving duration. Similar to that omega-3 to omega-6 ratio is also indicating that the fatty acid composition of yellowfin tuna oil is rich in omega-3 PUFA than the omega-6 PUFA which is a beneficial feature for upgrading the product for human consumption.

Oil colour is an important physical property for consumer perception related to the presence or absence of impurities because it requires high-cost processing to obtain an acceptable light coloured oil (Okada & Morrissey 2007). The intense brown colour of extracted oils could be due to the proteins and carbohydrates in the tuna head which degraded during heat treatment in the extraction process and those degraded products might contribute to a darker colour (Aidos et al. 2003). Moreover, lipid oxidation can also lead to a darker colour of oil (Choe and Min 2006). It also indicates the possibility of co-extraction of pigments along with oil in the wet reduction process. Although the colour values of each oil determined in the present study were not statistically significant, Bako et al. (2017), has reported that heat treatment for a prolonged period has improved the dark colour of mockery oil compared to the oil extracted without heat treatment.

CONCLUSIONS

Due to the high percentage yield (5.37±0.22 %) and greater oil characteristics within the acceptable limits for edible oils, the most suitable autoclaving duration for extracting oil from yellowfin tuna heads is 30 min at 121 °C. Moreover, the wet reduction process using autoclaving as the pre-treatment can be considered as an environmentally friendly approach, which produces no chemical wastage and produces oil from tuna heads with an acceptable recovery percentage of 70.87 ± 2.97 % and high-quality oxidative stability. It is also recommended that oil from yellowfin tuna head is a good source of PUFA, especially DHA. Due to its high acid values that exceeded the standard limit, the oil refinery process would be required before any further applications targeting human consumption.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

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