Effect of autoclaving as a pre-treatment in the wet reduction process for extracting fish oil from yellowfin tuna heads

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Abstract Various studies have focused on extracting fish oil using a variety of methods, yet, the wet reduction process is the common industrial method as it is environmentally friendly. However, the use of autoclaving in extracting oils from fish or fish by-products are comparatively less. Therefore, this study was conducted to extract crude oils from the yellowfin tuna (*Thunnus albacares*) head, the lipids richest body part compared to processing offcuts like skin, viscera etc., by a simple wet reduction process using an autoclave at 121 °C as the pre-treatment with different holding times (15, 30, and 45 min). The maximum extraction yield of 5.37±0.22 % was recorded at 30 min holding time of autoclave. No significant changes in peroxide value, moisture content and colour values were observed for the different autoclaving durations ($P > 0.05$). Conversely, acid value, *p*-anisidine value and total oxidation value (TOTOX) were increased significantly with a longer autoclaving duration of 45 min ($P < 0.05$). However, all peroxide values, *p*-anisidine value, TOTOX value were below the standard values stated by the World Health Organization (WHO) except the acid values which shows the need for refinery before any applications. Based on the spectra of Attenuated total reflectance - Fourier transform infrared (ATR-FTIR), autoclaving for a longer time resulted in lower intensity at wavenumbers around 3,012 - 3,013 and 1,741 - 1,744 cm^{-1} and shifting of bands to lower wavenumbers showing its effect on the structural changes of molecules in the oil. All extracted oils resulted in a high PUFA content than MUFA and SFA contents based on the fatty acid composition. The DHA (C22:6, n3) was the most prominent fatty acid in all the oil samples. However, PUFA content was reduced slightly by increasing the autoclaving duration at 45 min. Therefore, this study concludes autoclaving at 121°C for 30 minutes as the optimum pre-treatment conditions to extract fish oil from yellowfin tuna heads using the wet reduction process.

Keywords: Autoclaving, Fish oil, Oil extraction, *Thunnus albacares,* Wet reduction

INTRODUCTION

"Tuna" or "Thunnini" are generally used names for the fish belonging to the Family *Scombridae*, Genus *Thunnus*, and is a key marine product with greater economic value (Sunoko & Huang 2014). Yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*), bigeye tuna (*Thunnus obesus*), and albacore tuna (*Thunnus alalunga*) are four species of tuna that have been identified as major tuna species caught in the Indian ocean (Lecomte et al. 2017). As one of the major fishing

countries in South Asia, Sri Lanka exports processed tuna which significantly contributes to the gross economic development of the country (Yamao and De Silva 2006). Hence, more than 20 tuna processing plants are operating in the country with a total annual capacity of 14,252 Mt for the year 2019 (MFARD 2020). Processing of tuna at an industrial scale produces a large amount of offcuts such as skins, heads, viscera, bones, and fins. Generally, these fish leftovers, which comprise 20 - 80% of the original fish, have a huge contribution to global waste accumulation (Alfio et al. 2021). Hence, all of these discarded materials have been in the research spotlight in recent decades, to develop ways to maximize biochemical heterogeneity and process them into useful products derived from proteins, amino acids, bioactive peptides, enzymes, gelatin, collagen, minerals and oil (Ghaly et al. 2013).

For decades, it has been known that fish oils consisted of substances that have valuable dietary and medicinal properties as well as physical properties that are useful for human well-being. Long-chain omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are rich in fish oil have health benefits for the indication of anti-inflammatory effects, prevention of cardiovascular diseases, and development of the brain (Kris-Etherton et al. 2002; Lands 2005). Among several other sources of omega-3 fatty acids, tuna residues present elevated nutritional quality and a high potential for human consumption (de Oliveira et al. 2017). Moreover, it has been shown that the average value of EPA and DHA in tuna oil can be approximately 6.5% and 22.9%, respectively (Shimada et al. 1997). Many studies have shown that tuna heads can be recognized as the richest part of lipids than the other tuna offcuts (Ferdosh et al. 2015; Nguyen et al. 2011).

Many research studies have been focused on extracting fish oil using several methods, including enzymatic extraction (Gbogouri et al. 2006; Linder et al. 2005), solvent extraction, even though it is not practicable on an industrial scale (Adeniyi and Bawa, 2006), and supercritical fluid extraction, which results in high yields but the high capital cost limits its use on an industrial scale (Rubio-Rodríguez et al. 2012). However, the wet reduction process which comprises cooking, pressing and centrifuging to recover fish oil is the common industrial method and is considered to be environmentally friendly (Chantachum et al. 2000). As reported by Nazir et al. (2017), the wet reduction method was found to be the most effective and potential method in extracting oils from yellowfin tuna heads with yields of 12.80%. However, several studies have been conducted to assess the wet reduction process in extracting fish oil using different heating mechanisms in the cooking step such as steaming, direct cooking, and double boiling to denature the proteins associated with oil and liberate oil to a greater extent. Apart from that, the autoclaving technique is another heating mechanism that is generally used for sterilisation purposes; but it is also recognized as a cell disruption method in extracting lipids from biomass (Gupta et al. 2019). This technique uses high temperature and pressure to disrupt cells to facilitate the release of oil (Rakesh et al. 2015). According to Gupta et al. (2019), the main advantage of autoclaving is its easy operation. Many research has been conducted in assessing the effectiveness of autoclaving as a pre-treatment in extracting oils from microalgae by Lee et al. (2010) and Surendhiran & Vijay (2014). However, the use of autoclaving in extracting oils from fish or fish offcuts are comparatively less. Therefore, this study aimed to extract fish oil from yellowfin tuna heads using autoclaving as the heating method and to characterise the extracted oil in assessing the effectiveness of the autoclaving method.

METHODOLOGY

Raw material preparation

Heads of yellowfin tuna (*Thunnus albacares*) were obtained from a fish processing plant in Ja-Ela, Sri Lanka. Samples were transported in ice, in which the temperature of heads was around 0 - 2 °C. After manual cutting of heads into small pieces, they were minced using a domestic mixer grinder to make a homogenous mixture. Samples were kept in polyethylene bags and stored at - 20 °C until further experiments.

Oil extraction

A physical method called the wet reduction process was used for oil extraction, according to the method of Crexi et al. (2009) with some modifications.

After being thawed overnight at 4 °C, the sample was further thawed with running cold water until the core becomes free of ice. The tuna head sample (400 g) was transferred into a 600 mL Pyrex glass beaker covered with Aluminium foil and placed in the autoclave (JEIO Tech, ST-50G, Korea). Autoclaving at 121 °C was applied for three different time durations (15, 30 and 45 min) with three replicates for each. Subsequently, all samples were allowed to be cooled down at room temperature and samples were pressed manually using a pressing gun covered with a cheesecloth. The pressed liquids were subjected to centrifugation (Sigma 6-16KHS, Germany) at 13,000 × g at 48 °C for 10 min. The top phase containing oil was gently separated into an amber glass bottle with the aid of a 1 mL pipette and stored at -20 °C until used for analysis.

Analytical methods

Raw material characterization

The following parameters were determined in triplicate for the raw material (raw tuna head): total lipid according to the method described by Bligh and Dyer (1959). The moisture and ash contents were determined gravimetrically using approximately 5.0 g of a sample portion according to the methods of the Association of Official Analytical Chemists (AOAC 1990).

Extraction yield and recovery

The yield was reported as the percentage of fish oil extracted from yellowfin tuna head as follows:

$$\text{Yield } \% = \frac{\text{weight of fish oil extracted by wet reduction process}}{\text{Initial weight of tuna head sample}} \times 100$$

Recovery of extracted fish oil was calculated as follows:

$$\text{Recovery } \% = \frac{\text{weight of fish oil extracted by wet reduction process}}{\text{weight of oil extracted from Bligh \& Dyer method}} \times 100$$

Free fatty acid percentage (FFA%)

The FFA% was determined using the titration method according to the method described in AOCS Official Method Cd 3d-63 (AOCS 2009). Fish oil sample (2.5 g) was mixed with 50 mL of 95% neutral ethyl alcohol and swirled. The solutions were titrated, using phenolphthalein as an indicator, with 0.1 N KOH until the pinkish colour was observed at the endpoint. The FFA concentration in fish oil was calculated as a percentage of oleic acid. The expression is given as:

$$\%\ \text{FFA as oleic acid} = \frac{28.2\ \times \text{Volume of KOH (mL)} \times \text{Normality of KOH solution (molL}^{-1})}{\text{Weight of sample (g)}}$$

where 28.2 = Molecular weight of Oleic acid divided by ten (g mol^{-1})

$$\text{Acid value (mg KOHg}^{-1}) = \frac{56.1\ \times V_{KOH}\ \times C_{KOH}}{\text{Weight of oil (g)}}$$

where 56.1 = Molecular weight of KOH (g mol^{-1})

Peroxide value (PV)

The PV was analysed using a titration method according to AOCS Official Method Cd 8b-90 (AOCS 2009). Fish oil (5.00 g) was dissolved in 30 mL of acetic acid/chloroform mixture (3: 2). Thereafter, 1 mL of saturated KI was added, and the mixture was kept in dark for 5 min. DW (50 mL) was added and the mixture was shaken. Then, 0.5 mL of 1% starch solution, an indicator, was mixed and shaken well. The mixture was then neutralized with 0.1 N $Na_2S_2O_3$ until the dark blue colour had faded. PV was calculated as follows:

$$\text{Peroxide value (mEqkg}^{-1}) = \frac{\text{Volume of Na}_2\text{S}_2\text{O}_3\ (mL) \times \text{ Concentration of Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{Weight of oil (g)}}$$

para-Anisidine value (*p*-AnV)

p-AnV was analyzed using the AOCS official method Cd 18-90 (AOCS, 1998). About 2.0 g of sample was weighed into a 25 mL volumetric flask and topped up with isooctane (S_1). Subsequently, 1 mL of 0.5% *p*-anisidine in acetic acid (w/v) was added to 5 mL of sample solution (S_2). The mixed solution was kept in the dark for 10 min. Additionally, pure isooctane without and with added *p*-anisidine were used as B_1 and B_2, respectively. The absorbance of S_1, S_2, B_1 and B_2 was read at 350 nm. *p*-AnV was calculated as follows:

$$p - AnV = \frac{25\ mL\ \times (1.2\ \times (AS_2 - AB_2) - (AS_1 - AB_1))}{Weight\ of\ fish\ oil\ (g)}$$

where; AS1 = Absorbance at 350 nm of S_1

AS2 = Absorbance at 350 nm of S_2

AB1 = Absorbance at 350 nm of B_1

AB2 = Absorbance at 350 nm of B_2

TOTOX value

The TOTOX of fish oil can reflect the content of oxidation products including primary and secondary oxidation products of fish oil. It is a synthetically oxidation index, calculated by PV and *p*-AnV through the following equation;

TOTOX value = 2PV + *p*-AnV

Moisture content

The moisture content of extracted oils was determined using the standard method of ISO 662:2016 (ISO, 2016). A portion of oil (around 5 g) was weighed and kept for 1 h in the drying oven set at 103 °C. After that, it was allowed to cool at room temperature in the desiccator and then weighed to the nearest 0.001 g. The operations of heating, cooling and weighing were repeated until a constant weight is obtained.

Attenuated total reflectance - Fourier transform infrared (ATR-FTIR) Measurements

The method described by Daoud et al. (2019) was used with slight modifications for ATR-FTIR measurement. FTIR spectrometer (Spectrum 2; PerkinElmer, Waltham, MA, USA) coupled to an ATR accessory (Zinc selenide crystal) was used to obtain ATR-FTIR spectra. Each sample (20 µL of fish oil) was spread directly on the crystal surface and the absorbance spectrum was collected. A dry and empty ATR cell was used as a reference. The ATR spectra were averaged over 4 scans in the range of 4,000 – 550 cm^{-1} and recorded with a resolution of 4 cm^{-1}. The cleanliness of the ATR crystal was assured by acetone. All analyses were carried out at 22 ºC.

Fatty acids composition

Fatty acid methylester (FAME) was prepared according to the method described by Lei et al. (2016) with slight modifications. Lipid samples (about 70 mg) were weighed into clean and dried sample vials, then 2 mL of 0.5 M NaOH methanol solution was added to each sample vial. The vial was tightly capped and incubated at 100 °C for 5 min. After cooling, 2 mL of 14% boron trifluoride (BF_3)/methanol solution were added and the sample was vortexed for 1 min. Incubation at 100 °C for 5 min allowed the methylation. After the reaction, the FAME was extracted by adding 2 mL hexane and 2 mL saturated NaCl solution, followed by vortex for 2 min and centrifugation for 3 min at 2000 rpm. The top layer was separated and treated with anhydrous sodium sulphate.

The prepared FAME was then taken for analysis by using Shimadzu GC-2030 gas chromatogram equipped with a fused silica capillary column 30 m x 0.32 mm id (Restek Corp., USA) and a flame ionization detector (FID). The temperatures of the injector and the FID were set at 250 °C. The column temperature was programmed as follows; Initial at 140 °C (holding for 10 min), then increasing up to 185 °C at a rate of 3 °C/min, (holding for 5 min at 185 °C), then ramped up to 235 °C at a rate of 5 °C/min (holding for 12 min at 235 °C). The injection volume was 1.0 µL. Each treatment was analyzed by duplicating two independent experiments.

Oil colour

The colour values of crude fish oil were measured using a Chroma Meter (CR-400, Konica Minolta, INC, Japan). Each fish oil sample (5 mL) was transferred into a glass test tube and subsequently, the bottom of the test tube was placed directly on the Chroma meter. The colour values were expressed as lightness (L*), redness/greenness (a*) and yellowness/blueness (b*). If the L* value is low, it indicates darkness (Haq *et al*., 2018), and certain colour of L* can be considered as correspondent to a value of a greyscale, between black (L* = 0) and white (L* = 100) (Chakraborty & Joseph 2015). Negative values of a* attributed for greenish colours and positive values for the reddish ones, whereas negative values of b* attributed for bluish colours and positive values for the yellowish ones (García-Moreno et al. 2013).

Statistical analysis

For all experiments, triplication was used with three different sets of samples. For comparison of means, one-way analysis of variance (ANOVA) and Tukey's post-hoc test were used at $P < 0.05$ statistical significance. Statistical Package for the Social Sciences (SPSS) software (New York, NY, USA) was used for statistical analysis.

RESULTS

The proximate composition analysis of the raw material of Yellowfin tuna head resulted 60.99 ± 0.61% of moisture, 7.58 ± 0.51% of lipid and 5.20 ± 0.12% of ash content. Different oil yields and recovery percentages were resulted based on autoclave durations (Table 1). When autoclaving at 121 ºC, the extracted % oil yield increases with increasing autoclaving duration i.e 4.81 ± 0.45 and 5.37 ± 0.22 % yields resulted at 15 and 30 minutes, respectively, but a longer autoclaving period (45 min) resulted in a low % of yield, 3.49 ± 0.30 % ($P < 0.05$). Subsequently, the highest recovery percentage was recorded by autoclaving at 30 minutes (70.87 ± 2.97 %) followed by 15 minutes (63.52 ± 5.95 %) ($P > 0.05$).

Oil quality parameters

Oil acidity

The FFA contents and acid values of all the extracted oils obtained from Yellowfin tuna heads showed differences with different autoclaving durations (Table 2). A higher acid value (AV) represents a larger amount of free fatty acids, thus increasing AV. When autoclaving duration increased as 15 min, 30 min, and 45 min, acid value resulted in a slight increase as 4.91 ± 0.25, 4.90 ± 0.59, and 7.93 ± 0.65 mg KOH g^{-1}, respectively ($P < 0.05$) (Table 2). Further, as shown in Fig. 1, FFA content was low in oils extracted for 15 min and 30 min ($P > 0.05$) and the oil with the highest FFA content was obtained with a longer autoclaving duration of 45 min ($P < 0.05$).

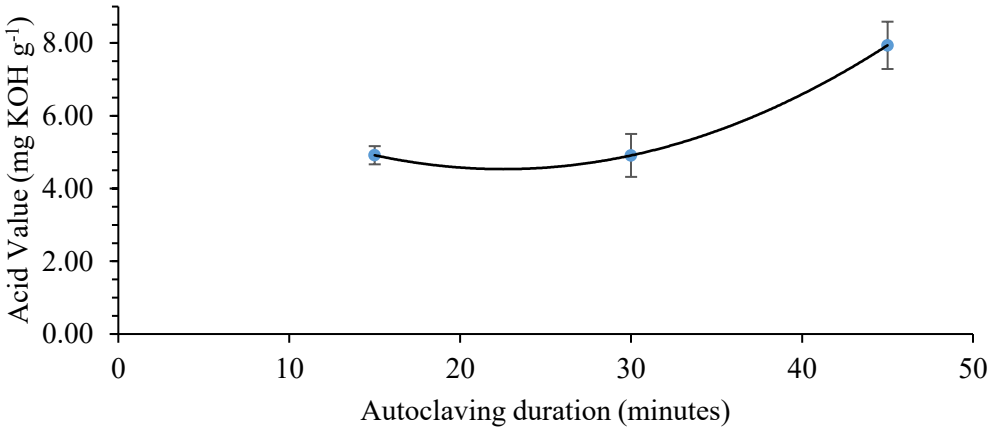

Fig 1 Variation of acid value (AV) with autoclaving duration (The vertical bars in the data points represent the standard deviation of the mean)

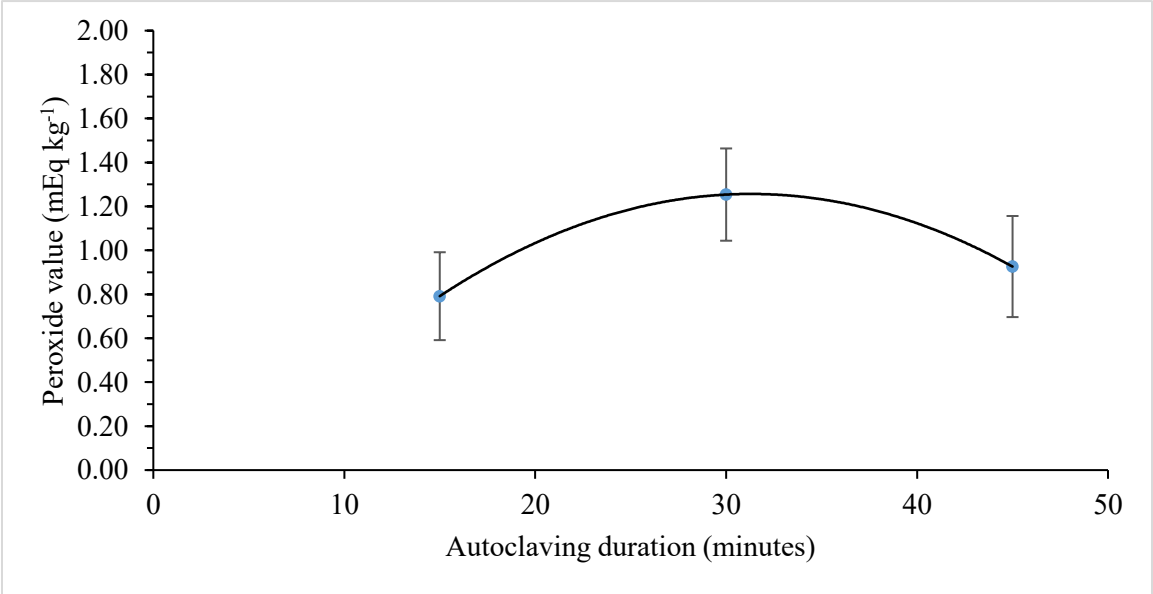

Fig 2 Variation of peroxide value (PV) with autoclaving duration (The vertical bars in the data points represent the standard deviation of the mean)

Table 1 Comparison of percentage yield (%) and percentage recovery (%) of oil extracted from yellowfin tuna heads for different autoclaving durations at 121 ºC and results of previous studies for different extraction methods and body parts of different species.

	Autoclaving duration (min)			Steaming 30 min Yellowfin tuna heads (Nazir et al. 2017)	Autoclaving 20 min Skipjack tuna eyeballs (Pudtikajorn and Benjakul 2020)	Direct cooking 20 min Skipjack tuna liver (Fang et al. 2019)	Double boiling 2 h Common Kilka edible muscles (Sayyad and Ghomi 2017)
	15	30	45				
Yield %	4.81 ± 0.45[a*]	5.37 ± 0.22[a]	3.49 ± 0.30[b]	12.80	16.49	-	-
Recovery %	63.52 ± 5.95[a]	70.87 ± 2.97[a]	46.06 ± 3.98[b]	-	64.06	56.76±1.57	54.28 ± 2.35

*Different lowercase letters in the same raw denote significant differences ($P < 0.05$)

Table 2 Percentage free fatty acid (FFA%), acid value (AV), peroxide values (PV), *p*-Anisidine value (*p*-AnV), total oxidation value (TOTOX value), percentage moisture of oil from yellowfin tuna heads extracted for different autoclaving durations at 121 ºC

Autoclaving duration	**FFA%**	**AV (mg KOH g^{-1})**	**PV (mEq kg^{-1})**	***p*-AnV**	**TOTOX value**	**Moisture %**	**Colour values**		
							L*	a*	b*
15 min	2.47 ± 0.13[b*]	4.91 ± 0.25[b]	0.79 ± 0.20	10.49 ± 0.21[c]	12.08 ± 0.58[b]	0.25 ± 0.07	28.19 ± 1.56	0.59 ± 0.20	2.59 ± 1.20
30 min	2.46 ± 0.29[b]	4.90 ± 0.59[b]	1.25 ± 0.21	16.63 ± 0.32[b]	19.14 ± 0.58[a]	0.18 ± 0.08	27.87 ± 0.48	0.63 ± 0.10	2.42 ± 0.31
45 min	3.98 ± 0.32[a]	7.93 ± 0.65[a]	0.92 ± 0.23	17.56 ± 0.33[a]	19.41 ± 0.73[a]	0.21 ± 0.04	26.92 ± 0.58	0.69 ± 0.01	2.26 ± 0.37

*Different lowercase letters in the same column denote significant differences ($P < 0.05$)

PV

In this study, the mean peroxide values (PV) of extracted fish oil were not significantly different among the groups ($P > 0.05$). However, the parabolic line in Fig. 2 shows that there is an increasing trend of PV with increasing autoclaving duration up to 30 min but decreasing of PV when autoclaving duration is extended further up to 45 min.

p-AnV

A drastic increase of *p*-AnV (from 10.49 to 17.56) were noticeable as autoclaving duration progressed from 15 to 45 min (Table 2). The mean *p*-AnV of extracted fish oils were significantly different among the groups ($P < 0.05$).

TOTOX value

The total oxidation value (TOTOX) of all oil samples extracted from autoclaving for different durations were between 12.08 and 19.41 (Table 2). There was a drastic increase of TOTOX value when progressing the autoclaving duration from 15 min to 30 min, but only a slight increment of TOTOX value was observed when autoclaving duration increased from 30 min to 45 min ($P < 0.05$).

Moisture content

The moisture content of oil was ranged from 0.18 to 0.25 % in all the extracted oils and the mean moisture contents of extracted fish oils were not significantly different between the groups ($P > 0.05$) (Table 2).

ATR-FTIR spectra

In this study, spectral changes were similar among all heat treatments and the spectra of tuna oil extracted with three different autoclaving durations in the 4,000 - 500 cm^{-1} spectral range are shown in Fig. 3. The assignment of functional groups responsible for IR absorption peaks of oil extracted from yellowfin tuna head using an autoclave for 15 min (Fig. 3) is as follows: 3,013 cm^{-1} (C– H stretching vibration of the cis-double bond); 2,923 cm^{-1} (symmetric stretching vibration shoulder of CH_3); 2,853 cm^{-1} (asymmetric stretching vibration of CH_2); 1,744 cm^{-1} (C=O stretching vibrations); 1,464 cm^{-1} (bending vibrations of the CH_2 and CH_3); 1,378 cm^{-1} (bending vibrations of CH_2 groups); 1,149 cm^{-1} (C–O stretching vibration); 966 cm^{-1} (associated with trans fatty acids); 721 cm^{-1} (rocking vibrations of CH_2 groups) (Cebi et al. 2017; Vongsvivut et al. 2012). In a comparison of the other two FTIR spectra shown in Fig. 3 related to the autoclaving duration of 30 min and 45 min, an increase or decrease of certain bands positions in some of the wavenumber regions was observed related to the autoclaving duration of 15 min. In this study, only the regions which were prominent for the FTIR spectrum of oil were evaluated.

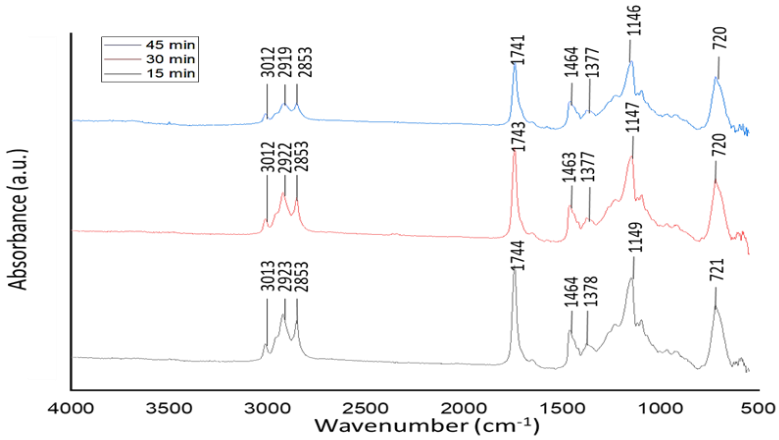

Fig 3 FTIR spectra of oil from yellowfin tuna heads extracted at different autoclaving durations at wavenumber from 4,000 to 500 cm^{-1}

According to Fig. 4A, a decrease in the peak intensity (absorbance) near 3,013 cm^{-1} and shifting of the band into a lower wavenumber (3,012 cm^{-1}) were observed when a longer autoclaving duration was used. Around 2,923 cm^{-1} and 2,853 cm^{-1} regions, the peak intensities were also decreased with increasing autoclaving duration. The intensity of the band at 1,744 cm^{-1} has tended to decrease while shifting the band in the wavenumber from 1,744 cm^{-1} to 1,741 cm^{-1} with a longer autoclaving duration (Fig. 4B). Another band was present in all samples of oil near 1,149 cm^{-1}, and its intensity increased with a longer autoclaving duration (Fig. 4C). The fingerprint region is another important region that lies between 900 and 1,200 cm^{-1}, which reflects the properties or spectral features specific to the molecules; for instance, the band at 966 cm^{-1} is associated with C-H out of plane deformation vibration of trans double bonds (Birkel & Rodriguez-Saona 2011). As shown in Fig. 4D, the peak intensity has increased with increasing autoclaving duration indicating the formation of trans fatty acids when using a longer autoclaving duration.

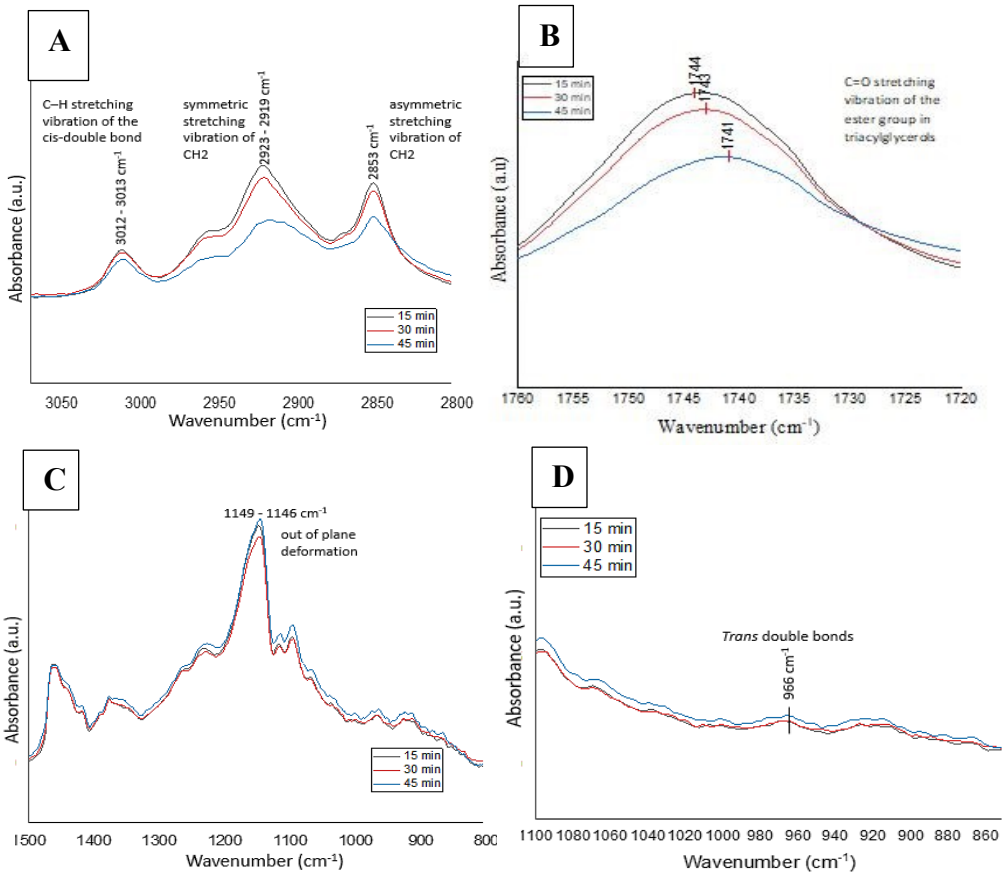

Fig 4 FTIR spectra of oil from yellowfin tuna heads extracted at different autoclaving durations; (A) wavenumber of 3,050 – 2,800 cm-1; (B) wavenumber of 1,760 – 1,720 cm-1; (C) wavenumber 1,500 - 800cm-1; (D) wavenumber of 1,100 – 860 cm-1

Table 3 Fatty acid composition (FID response area relative percentages) of oils from Yellowfin tuna heads extracted by wet reduction process for different autoclaving durations at 121 ºC

Fatty acids	Autoclaving durations (min)			Steaming 30 min	Steaming 30 min	Autoclaving 20 min	Direct cooking 20 min	Double boiling 2 h
	15	30	45	Yellowfin tuna heads (Nazir et al. 2017)	Skipjack tuna heads (non-precooked) (Chantachum et al. 2000)	Skipjack tuna eyeballs (Pudtikajorn and Benjakul 2020)	Skipjack tuna liver (Fang et al. 2019)	Common Kilka edible muscles (Sayyad and Ghomi 2017)
	Relative abundance in percentage (Values of replicate 1 & 2 are given)							
C12:0	0.00 & 0.00	0.00 & 0.00	0.02 & 0.03	-	-	-	-	-
C13:0	0.00 & 0.00	0.00 & 0.00	0.03 & 0.03	-	-	-	-	-
C14:0	2.43 & 2.34	2.59 & 2.58	2.70 & 2.59	2.18	5.8	4.05 ± 0.02	1.49 ± 0.04	6.94 ± 0.13
C14:1n-5	0.17 & 0.00	0.08 & 0.14	0.03 & 0.03	0.08	-	-	-	-
C15:0	0.65 & 0.74	0.82 & 0.79	0.87 & 0.86	2.12	1.9	1.58 ± 0.01	0.88 ± 0.03	-
C15:1	0.00 & 0.00	0.08 & 0.00	0.00 & 0.06	-	-	-	-	-
C16:0	18.49 & 17.72	20.21 & 18.61	20.10 & 20.11	10.80	30.3	24.08 ± 0.12	31.67 ± 0.21	22.13 ± 0.19
C16:1n-7	4.97 & 4.78	5.27 & 4.99	4.90 & 5.14	5.79	6.4	6.78 ± 0.02	2.86 ± 0.03	10.89 ± 0.08
C17:0	1.24 & 1.12	1.09 & 1.21	1.01 & 1.08	-	-	-	2.29 ± 0.05	1.25 ± 0.10
C17:1n-7	0.87 & 0.79	0.76 & 0.82	0.48 & 0.77	-	-	-	0.49 ± 0.02	0.95 ± 0.02
C18:0	6.89 & 6.69	6.71 & 7.49	0.00 & 7.62	7.03	7.9	5.19 ± 0.12	9.67 ± 0.22	5.05 ± 0.13
C18:1n-9	16.09 & 15.31	16.97 & 15.68	27.05 & 16.47	20.45	16.5	13.04 ± 0.07	18.17 ± 0.23	29.09 ± 0.21
C18:2n-6 (LA)	1.74 & 1.67	1.78 & 1.68	0.07 & 1.70	5.16	2.0	1.30 ± 0.02	0.80 ± 0.03	2.11 ± 0.02
C18:3n-6 (GLA)	0.48 & 0.43	0.30 & 0.46	0.29 & 0.27	3.16	0.6	0.08 ± 0.01	0.40 ± 0.01	1.13 ± 0.09
C18:3n-3 (ALA)	0.42 & 0.49	0.33 & 0.42	0.32 & 0.27	3.19	-	0.16 ± 0.00	-	-
C20:0	1.32 & 1.23	1.23 & 1.32	1.04 & 1.24	1.24	0.6	0.55 ± 0.00	0.22 ± 0.03	1.39 ± 0.07
C20:1n-9	0.67 & 0.70	0.36 & 0.62	0.36 & 0.50	0.14	2.0	0.43 ± 0.04	0.33 ± 0.01	1.85 ± 0.01

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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