International Food Research Journal 30(3): 668 - 682 (June 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Optimisation of ultrasound-assisted lipid extraction in the pretreatment of purple-spotted bigeye fish skin

*Chongkhong, S.

Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand

Article history

Received:
13 March 2022
Received in revised form:
11 July 2022
Accepted:
3 December 2022

Keywords

fish lipid, purple-spotted bigeye, ultrasound-assisted extraction, lipid oxidation, defatting

Abstract

The present work investigated the defatting of purple-spotted bigeye fish skin to obtain lipid prior to hydrolysis for gelatine production. Lipid was extracted using ultrasound-assisted extraction (UAE). The drying temperature of the skin on lipid recovery and preliminary kinetic of UAE were studied before a two-stage extraction was optimised by employing response surface methodology. Qualities of lipids and of the remaining solids after extraction were then analysed. The optimal drying temperature was found to be 80°C. The kinetic study that followed indicated that the highest extraction rate was achieved at 30°C, and from UAE using ethanol (UAEE) and sequential UAE using hexane (UAEH), the maximum percentage of total lipid recovery was 91.326 at 30°C, 37 kHz, and 60 min. The qualities determined by peroxide, conjugated diene, thiobarbituric acid reactive substances, and acid values showed that the extracted lipid could be suitable for industry, while the remaining solid could be used as material for gelatine production.

DOI
https://doi.org/10.47836/ifrj.30.3.11

Introduction

Thailand is the largest producer of surimi in Southeast Asia, with a growth rate of 5 - 10% per annum, and 130,000 metric tons produced in 2018 (Pascal, 2019). Over 20 surimi factories in the country use demersal fish species as raw materials since they are abundant low-value fishes in the Gulf of Thailand and the Andaman Sea. Species including threadfin bream (*Nemipterus* spp.), lizardfish (*Saurida* spp.), and bigeye (*Priacanthus* spp.) have appropriate characteristics to be processed into export-quality surimi (Siriraksophon *et al.*, 2009). During surimi production, the processing generates large quantities of liquid and solid wastes. The composition of the solid wastes, constituting 50 - 70% of the original raw fish, comprise head, viscera, skin, and bone (Mekpiroon *et al.*, 2016).

Although fish wastes or by-products constitute a potential alternative source of proteins and oils, these have been mainly used for animal feed. A more complete exploitation of fish raw within the concept of "zero waste" is, therefore, a necessary and urgent issue. Moreover, rather than investment in solid waste management, costs could be reduced *via* their transformation into marketable value-added products such as collagen/gelatine and oil/lipid, thereby resulting in better income for all parties.

Fish gelatine and lipid have been applied in the food and pharmaceutical industries. Fish skin, a by-product of the fish industry, is a prime material for producing gelatine (Alipal *et al.*, 2021) and polyunsaturated fatty acid (PUFA)/fish oil (Rubio-Rodríguez *et al.*, 2012). It generates somewhat higher yields than other by-products (Kittiphattanabawon *et al.*, 2016).

Typical conversion of the by-products into gelatine involves four major steps: pretreatment, hydrolysis, extraction, and recovery/drying. Nevertheless, applications of fish gelatine as supplements or food ingredients are limited, mainly due to unpleasant odour and turbidity (Sae-Leaw and Benjakul, 2015). Fish skin contains high lipid content, which is oxidised during hydrolysis or extraction at elevated temperatures. This leads to the generation of fish odour in the products (Sae-Leaw *et al.*, 2016a), while turbidity is caused by unfilterable particulate matter formed by the fat/lipid released

*Corresponding author.
Email: sininart.c@psu.ac.th

from the raw fish skin (Cole, 2012). The defatting of fish skin prior to extraction of gelatine is thus an important step (Sae-Leaw *et al.*, 2016b).

Defatting or lipid extraction from fish by-products can be accomplished *via* various means. Conventional methods include Soxhlet, pressing, and solvent extraction, whereas innovative methods involve extraction processes such as supercritical fluid extraction (Rubio-Rodríguez *et al.*, 2012), microwave-assisted extraction (Hu *et al.*, 2017), and ultrasound-assisted extraction (Stevanato and da Silva, 2019). With conventional methods, the extraction temperatures that provide the highest yields of lipids are in the range of 60 - 90°C, or near the boiling point of the solvent used. However, lipid extraction of dried fish skin containing protein as the main content should be performed at a lower temperature. Ultrasound-assisted extraction (UAE) is an alternative utilised to lower temperature, reduce extraction time, and decrease solvent consumption over conventional methods. UAE can therefore be employed to diminish the degradation of protein and the binding of lipid and protein at high temperatures. It is also relatively convenient to operate and scale up to the industrial process level (Rajha *et al.*, 2019).

Lipids can be non-polar or polar. Non-polar or mildly polar lipids such as triacylglycerols, sterols, and tocopherols are highly soluble in hexane, benzene, and other stronger polar solvents such as alcohols. Polar lipids such as glycolipids and phospholipids require polar solvents like ethanol and chloroform. Lipid solubility in a solvent involves appropriate proportion of polar and non-polar lipid groups in a complex cellular matrix, and some studies have indeed used solvent mixtures having both polar and non-polar forms (Sinthusamran *et al.*, 2018). However, the use of a single solvent would facilitate easier recovery of the solvent, thereby reducing waste and the overall cost.

Purple-spotted bigeye (*Priacanthus* spp.), a demersal fish species, is commonly used in surimi industries due to its high gel-forming ability. The skin of purple-spotted bigeye is a sticky thick sheet firmly attached with fine small scales containing complex cellular matrices associated with different bonds. Therefore, skin preparation, type of solvent, and optimal extraction methods are prerequisites. In addition, the freshness of raw fish and preservation of freshness are prime factors affecting the quality of extracted lipids, and the rate of lipid oxidation that causes fish odour (Yarnpakdee *et al*., 2012).

Up until 2021, the defatting of fish skin to obtain fish lipid 'prior to hydrolysis' in gelatine production had not yet been practiced. The removed fat is generally washed to render skin with less fat. Defatting the skin of purple-spotted bigeye by UAE to obtain first the lipid was investigated in the present work.

Fish skin preparation for lipid extraction is usually performed by size dissection and drying. Drying is one of the most necessary steps prior to lipid extraction since the yield of extracted lipid is dependent upon components of the dried material, especially its moisture content, drying temperature, and duration (Ratti *et al*., 2008). The drying temperature of the fresh skin of the purple-spotted bigeye was thus investigated first.

The extraction rate, the proportion of lipid and non-lipid components, and the quality of extracted lipid are all influenced by temperature (Jabar *et al*., 2015). As a result, before optimising extraction, the optimal range of extraction temperature had to be determined. Since no prior literature is available on the kinetic of UAE of the purple-spotted bigeye skin, a preliminary study was conducted to evaluate the effect of temperature prior to determination of the optimal range of extraction factors, followed by a two-stage extraction: UAE using ethanol (UAEE) and sequential UAE using hexane (UAEH) to enhance the lipid yields. Corresponding extraction factors were optimised employing response surface methodology (RSM). The quality of the optimal lipid was subsequently determined.

An anticipated added benefit from the present work would be that the solid fractions (skins with lowered lipid) remaining after removal of the liquid fractions (lipid-solvent fraction), which retain a high protein/collagen content not soluble in organic solvents, could be used as material for the production of gelatine or protein hydrolysates in future work, with a production process to lower fish odour and to reduce turbidity of the gelatine for better food applications. For this, volatile compounds from the remaining solid fraction were also analysed and assessed in the present work.

Materials and methods

Chemicals

Ethanol and hexane were of analytical grade and purchased from Boss Supply and Service Co., Ltd. Hat Yai, Songkhla, Thailand.

Selection and preliminary processing of purple-spotted bigeye skin

Fresh purple-spotted bigeye fish skin was obtained from Pae Khai-Lium, a minced fish factory that is a supplier to Pacific Fish Processing Co., Ltd., Songkhla, Thailand. The fish skin was brought from the factory twice, once for the entire study, and once for repeated trials. Every experiment was duplicated twice to estimate the variance in results. The selected skin was required to have characteristics like tightly arranged scales, glossy, retaining its natural colour and fresh smell. The remaining flesh attached to the skin was manually removed, and the cleaned skin was rinsed in a dilute acetic acid-water solution. The washed skin was set aside, well drained for an hour in a refrigerator, placed in sealed plastic bags, and stored at -20°C, not exceeding one month before usage.

Chemical composition analyses of the thawed fresh skin were conducted following methods stipulated by the Association of Official Analytical Chemist (AOAC, 2000). The chemical composition included moisture (drying at 95 - 100°C), protein (Kjeldahl method), fat (Soxhlet method), ash (incineration at 550 - 600°C), and carbohydrate (subtraction method).

Preparation of skin

Preliminary UAE of skins at various drying temperatures was conducted to determine the optimal drying temperature-time for skin preparation.

Prior to lipid extraction, the skin was dissected into approximately 0.5 cm^2 pieces. The cut skin was then dried in a hot chamber until it retained about 8% moisture in order to not adversely affect the extraction, especially with polar solvents (Balasubramanian *et al.*, 2013), and so the temperature-time of drying would be optimised. According to Bako *et al.* (2017), proteins could coagulate, and cell membranes could be disrupted at drying temperatures of 60 - 90°C to facilitate leakage out of the bound water and oil. The dried skins were then kept in a desiccator until they were at an ambient temperature. After that, the skins were extracted to investigate their lipid yields under fixed predetermined conditions, *e.g.*, a 1:10 (w/v) ratio of skin to ethanol, 30°C, 37 kHz ultrasonic frequency, and 60 min. The skin sample dried under the optimal drying temperature-time that provided the highest yield in the preliminary UAE was stored in a sealed plastic bag, and placed in a desiccator until utilised in further experiments.

Effect of extraction temperature (preliminary kinetic data)

Sample at a sample-to-ethanol ratio of 1:10 (w/v) (Sae-Leaw *et al.*, 2016a; 2016b) was put in screw-capped bottles. Lipid extraction was processed employing an ultrasound water bath (Elma Schmidbaure GmbH, Germany, 950 Watt) with a frequency of 37 kHz, at 30 - 60°C for 10 - 60 min. After extraction, each mixture was filtered through a Whatman No. 4 filter paper. The liquid (lipid-solvent) fraction was evaporated using a rotary evaporator (Heidolph, Heating bath Hei-NAP, EU) at 45°C and 175 mbar, placed in an oven at 105°C for 2 h to remove residual solvent and purify the lipid product, kept in a desiccator for 1 h until it was at an ambient temperature, and then weighed to estimate the lipid yield.

Optimisation of lipid extraction

Lipid extraction using UAE

Kinetic data provided optimal temperature for extraction, and helped to assign the range of factors for optimisation to find the highest yield. Samples were extracted in two stages with different solvent to enhance efficacy of the extraction. The first UAE employed ethanol (UAEE) and the second used hexane (UAEH).

For UAEE, the sample was mixed with ethanol at a sample-to-solvent ratio of 1:10 (w/v). Extraction conditions were varied by RSM. Each extraction was then processed to purify the lipid product as earlier described, and the yield was determined.

After finding the optimal condition that provided the highest yield for UAEE, its solid fraction obtained after filtration was used in UAEH. Similar experimental conditions as for the UAEE were applied, except that the solvent was now hexane instead of ethanol.

Determination of optimal conditions

RSM, a statistical method, was employed to optimise the extraction conditions. Full factorial design with three factors provided nine experimental cases to investigate responses in lipid yields from UAEE and UAEH. Each case was repeated twice. The responses were processed statistically by analysis

of variance (ANOVA). Yield (*Y*) and recovery (*R*) of the extracted lipids were calculated using Eq. 1 and Eq. 2, respectively.

Y (%) = [weight of lipid extracted by UAE (g) / weight of dried skin sample (g)] × 100 (Eq. 1)

R (%) = [weight of lipid extracted by UAE (g) / weight of total lipid extracted by Soxhlet method (g)] × 100 (Eq. 2)

Quality of extracted lipids

Determination of lipid oxidations

The liquid fractions obtained under optimal extraction conditions that provided highest yields from UAEE and UAEH were evaporated using a rotary evaporator at 45°C and 175 mbar. Residual solvents were removed by nitrogen flushing to purify the lipid. Each lipid sample was poured into a brown glass bottle, and then tightly sealed with nitrogen at an ambient temperature. These were stored at -40°C prior to analyses of lipid oxidations and chemical compositions.

Oxidations of the lipid samples were considered from peroxide value (PV) determined by titration (Low and Ng, 1978), conjugated diene value (CD) determined by the International Union of Pure and Applied Chemistry (IUPAC, 1992) method, thiobarbituric acid reactive substances value (TBARS) determined by the method detailed in Buege and Aust (1978), anisidine value (AnV) determined by the American Oil Chemists' Society (method Cd 18-90; AOCS, 1992b), and acid value (AV) determined by titration (method Cd 3a 63(89); AOCS, 1992a).

Determination of lipid chemical compositions

Determination of fatty acid profiles followed the fine-tuned method by Muhammed *et al.* (2015). The lipid sample was first methylated to prepare fatty acid methyl ester (FAME). The FAME was then analysed using gas chromatography with an auto-injector and flame ionisation detector (GC-FID Agilent 7890B, Santa Clara, CA, USA). The oven temperature was maintained at 80°C, the injector at 250°C, and the detector at 270°C, with an inlet pressure of 31.624 psi. Septum purge and column flows were set at 3.0 and 1.0 mL/min, respectively.

Determination of volatile compounds in solid fractions

Volatile compounds in the raw / fresh purple-spotted bigeye skin were analysed. Residual volatile compounds in the solid fraction obtained from UAEE (named SFE) and the solid fraction obtained from UAEH (named SFH) at optimal conditions were also determined to consider the use of skins with lowered lipid (the SFE and the SFH) as materials for producing gelatine with less fish odour in the future. A solid-phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) was employed to determine the volatile compounds following the method of Iglesias and Medina (2008) with minor modifications. These compounds were analysed by the Office of Scientific Instrument and Testing, Prince of Songkla University, Hat Yai campus, Thailand.

Results and discussion

Properties of fresh purple-spotted bigeye skin

The compositions of fresh skin (62.23% moisture based on wet basis) in percentage order were 21.19% protein, 12.13% ash, 3.62% fat, and 0.83% carbohydrate. The moisture was within the general range for fresh conditions (Harianti, 2020). For demersal fishes, the protein content was found to be slightly lower than that of gilthead sea bream (24.78%) (Pateiro *et al.*, 2020), while ash and fat contents were much higher as compared to that of lizardfish (1.16% ash and 1.76% fat) (Jaziri *et al.*, 2021). These were reportedly caused by differences in fish environmental conditions (Khitouni *et al.*, 2014). High ash content was related to the thickness of skin affected by mineral availability surrounding their habitat (Muralidharan *et al.*, 2013). For carbohydrate, the percentage was similar to those reported by Jaziri *et al.* (2021) and Njinkoue *et al.* (2016).

The flesh of purple-spotted bigeye fish is high in protein and its skin is also relatively high in protein. Eighty percent of the skin protein, a fibrous protein, is collagen, and could be developed into valued products including protein hydrolysates, collagen, and gelatine. Nevertheless, pretreatment processes must be rigorously considered to reduce the fat content to improve product quality. To assess the full potential of the skin, it should be pretreated with solvent extraction to first obtain the lipid, leading to

gains in both the lipid (liquid fraction) and skin with less lipid content (solid fraction) that could be used as material to produce valuable products.

Drying of fresh skin

Using 200 g of cut skin sample as the beginning wet weight, the time durations required to reach 8% moisture at various drying temperatures are shown in Table 1. Clearly, drying time required to reach 8% moisture decreased with increasing drying temperature.

Fresh fish are sensitive to deterioration by enzymes and oxidation of lipids. Although drying is a form of preservation, it leads to changes in sensory characteristics and nutrition values. These changes depend on the drying conditions and specific characteristics of that particular material. Drying influences properties of raw material, be it water, protein, carbohydrate, or lipid. For material with high protein, drying at temperatures that are too high or for too long usually causes progressed protein/amino acid degradation. For materials high in lipid, drying at a high temperature under an atmosphere with free oxygen will cause oxidation and rancidity problems. Regarding the effect of drying on carbohydrate, high temperatures could cause the burning of sugar, known as caramelisation. Therefore, temperatures that are too high or heating for long durations is not recommended as they increase the binding of lipids and proteins, thus more difficult to extract with organic solvents (Moreau, 2005).

In general, raw materials are dried prior to oil extraction at 40 - 60°C for soft materials such as fish fillets (Mujaffar and Sankat, 2011), and at 80 - 100°C for hard materials such as seeds (Joven *et al.*, 2020). Soft materials, having more porous structures and more free water, hence allowing water molecules to more easily move and traverse, resulting in a faster drying rate than hard materials.

The bigeye skin, though flexible, is hard and tough so it is not easily dried. Moreover, drying at too high a temperature can render the skin cell walls to shrink too rapidly and cause the skin surface to become even harder, thus making it more difficult for moisture to be removed (Di Domenico, 2014). A moderate temperature of 80°C was considered suitable for drying of fresh fish skin, of which its major component is water, to retain most properties of the fresh material (Kosuke *et al.*, 2006).

Fortunately, fresh skin contains low amount of fat and little carbohydrate. Table 1 shows that the optimal drying temperature was 80°C, as evidenced by its highest recovery (55.7%) derived from preliminary UAE of the dried skin.

Table 1. Drying time for fresh skin to reach 8% moisture and lipid recovery from the preliminary UAE *vs*. drying temperature.

Drying temperature (°C)	Time to reach 8% moisture (min)	Lipid recovery (%)
60	420 ± 2	52.679 ± 0.006
70	370 ± 2	54.917 ± 0.002
80	340 ± 2	55.692 ± 0.002
90	320 ± 2	53.784 ± 0.004

Values are mean ± standard deviation.

Effect of extraction temperature and time on lipid recovery

Lipid recovery from UAEE at 37 kHz *vs*. temperature and time is presented in Table 2. The highest recovery was found at 30°C and 60 min.

The reaction rate of the lipid extraction from the skin for the UAEE can be derived from kinetic using Eq. 3.

$$dR_E/dt_e = kR_E^n \quad \text{(Eq. 3)}$$

where, R_E = percentage of lipid recovery from UAEE; k = extraction constant; t_e = extraction time (min); and n = reaction order.

The term dR_E / dt_e is positive because recovery increased with time. Based on Eq. 3, plots of ln (dR_E / dt_e) *vs*. ln R_E using differential method on values in Table 2 were linear. Values n obtained from straight-line slopes with average R^2 of 0.989 provided the first order kinetics, while extraction rate constants were calculated from the slopes. It was observed, the rate constant decreased with increasing temperature.

For the fixed ultrasonic power of 950 W, and ultrasonic times not over 30 min, the lipid recovery increased with increasing temperature; however, the recovery trend was reversed with ultrasonic times longer than 30 min (Table 2). More cavitation bubbles formed with higher ultrasonic power which accelerated the penetration of solvent into the cells, and improved the mass transfer rate of lipid from the cells into the solvent (Gholivand *et al.*, 2014). Nevertheless, there were no improvements in recovery when temperatures were increased for over 40 min. The decrease in recovery with increasing temperature could be due to the formation of more bound lipids.

However, for any fixed temperature, lipid recovery increased with increasing time, though its rate slowed at 50°C and higher. This implied that sufficiently good recoveries of lipid from the skin could be obtained using UAEE at temperatures not greater than 50°C, together with an ultrasonic time of not less than 40 min.

Table 2. Lipid recovery from UAEE (R_E) at 37 kHz *vs*. temperature and time.

Time	R_E (%) at temperature (°C)			
(min)	30	40	50	60
10	22.010 ± 0.007	24.770 ± 0.006	27.530 ± 0.007	30.290 ± 0.009
20	28.760 ± 0.006	30.550 ± 0.007	32.340 ± 0.009	34.140 ± 0.012
30	35.500 ± 0.006	36.330 ± 0.007	37.160 ± 0.010	37.980 ± 0.011
40	42.250 ± 0.005	42.110 ± 0.008	41.970 ± 0.010	41.830 ± 0.013
50	49.000 ± 0.004	47.890 ± 0.008	46.790 ± 0.011	45.680 ± 0.012
60	55.690 ± 0.002	53.670 ± 0.009	51.600 ± 0.012	49.530 ± 0.015

Values are mean ± standard deviation.

Optimisation

From the results on preliminary kinetic, a combination of temperatures ranging from 30 to 45°C; 37 to 130 kHz ultrasonic frequency; and 30 to 60 min ultrasonic time was adopted for RSM optimisation.

Effect of UAEE on dried skin sample

The experimental conditions and their results are tabulated in Table 3. These were used in regression models on lipid recovery obtained from UAEE (R_E) on the sample (Eq. 4), and that obtained from UAEH (R_H) on SFE (Eq. 5):

$$R_E(\%) = 14.031 + 0.225 * T - 0.284 * f + 0.907 * t + 0.004 * T * f - 0.010 * T * t + 0.002 * f * t \quad \text{(Eq. 4)}$$

$$R_H(\%) = 13.420 - 0.379 * T - 0.213 * f + 1.300 * t + 0.010 * T * f - 0.023 * T * t - 0.003 * f * t \quad \text{(Eq. 5)}$$

where T, f, and t = temperature (°C), ultrasonic frequency (kHz), and time (min), respectively.

The analysis of variance, as illustrated in Table 4, for $p < 0.05$ indicated a significant effect on the result. Determination coefficients R^2 of 0.996 from UAEE, and 0.995 from UAEH satisfactorily indicated good accuracy of the regression models.

Figures 1a, 1b, and 1c present RSM plots from the model results based on Eq. 4 for R_E. It can be seen that temperature exerted only minor effects on lipid recovery, whereas effects from ultrasonic frequency and time were more prominent. From Figures 1a and 1b, there appeared to be no noticeable improvements in recovery due to temperature. Under the conventional method, an increase in temperature decreased the viscosity of the solvent and the strength of its intermolecular forces, thus allowing for better penetration into the cellular matrix, and increasing the rate of extraction (Efthymiopoulos *et al.*, 2018). However, UAEE at 30 - 40°C could also provide high extraction rates and sufficiently good recoveries. This mild temperature condition had an added benefit in preserving the protein/collagen contained in the solid fraction that would be used as material for further study.

In Figures 1a and 1c, the extraction was efficient at frequencies below 45 kHz with a power of 950 W. A lower frequency produced larger cavitation

bubbles that had more time to grow before they exploded, while more cavitation bubbles were produced with an increase in power. This was in agreement with Krishnan *et al.* (2015) who reported that oil yield increased when amplitude level or bubble size increased. Meanwhile, Ivanovs and Blumberga (2017) showed that the efficiency of sample preparation could be improved using a low frequency of 16 - 100 kHz coupled with a high power of 100 - 1000 W.

In Figures 1b and 1c, for any temperature and frequency, the amount of recovery increased when time increased. Result extrapolation interestingly indicated that a longer time (> 60 min) should provide a higher recovery (> 55%), thus should be further investigated, or a second extraction carried out. For the same extraction time of 60 min and the same 1:10 ratio of sample-to-ethanol, 55.691% lipid was recovered employing UAE at 30°C as compared to 9.945% in our solvent-extraction study conducted at 78°C. When compared with a Soxhlet extraction using a ratio of 1:50, also at 60 min and 78°C, the recovery was 19.337%. It was thus concluded that UAE could shorten the time and reduce the solvent over conventional methods.

Table 3. Experimental conditions and results on lipid extractions.

Experiment #	Condition			%Recovery		
	***T* (°C)**	***f* (kHz)**	***T* (min)**	R_E	R_H	R_{tot}
1	30	37	60	55.691 ± 0.002	35.635 ± 0.003	91.326 ± 0.005
2	45	130	30	32.983 ± 0.009	27.155 ± 0.007	60.138 ± 0.016
3	45	130	60	53.122 ± 0.008	22.873 ± 0.006	75.995 ± 0.014
4	30	37	30	35.884 ± 0.005	21.409 ± 0.004	57.293 ± 0.009
5	45	37	30	36.851 ± 0.006	9.779 ± 0.005	46.630 ± 0.011
6	30	130	60	50.497 ± 0.008	29.945 ± 0.004	80.442 ± 0.012
7	40	80	45	41.381 ± 0.007	21.464 ± 0.003	62.845 ± 0.010
8	30	130	30	25.138 ± 0.007	21.713 ± 0.003	46.851 ± 0.010
9	45	37	60	53.177 ± 0.006	15.829 ± 0.006	69.006 ± 0.012

Values are mean ± standard deviation.

Table 4. Analysis of variance (ANOVA) of the models, R_E (Eq. 4) and R_H (Eq. 5), for lipid extractions.

Term	R_E		R_H	
	Std. error	***p*-value**	**Std. Error**	***p*-value**
Constant	9.481	0.277	7.329	0.209
T	0.238	0.445	0.184	0.176
f	0.062	0.044	0.048	0.047
t	0.178	0.036	0.138	0.011
T*f	0.001	0.093	0.001	0.010
T*t	0.004	0.160	0.003	0.021
f*t	< 0.001	0.142	< 0.001	0.034
R^2	0.996		0.995	
R^2 adjusted	0.983		0.979	
Std. error	1.403		1.084	

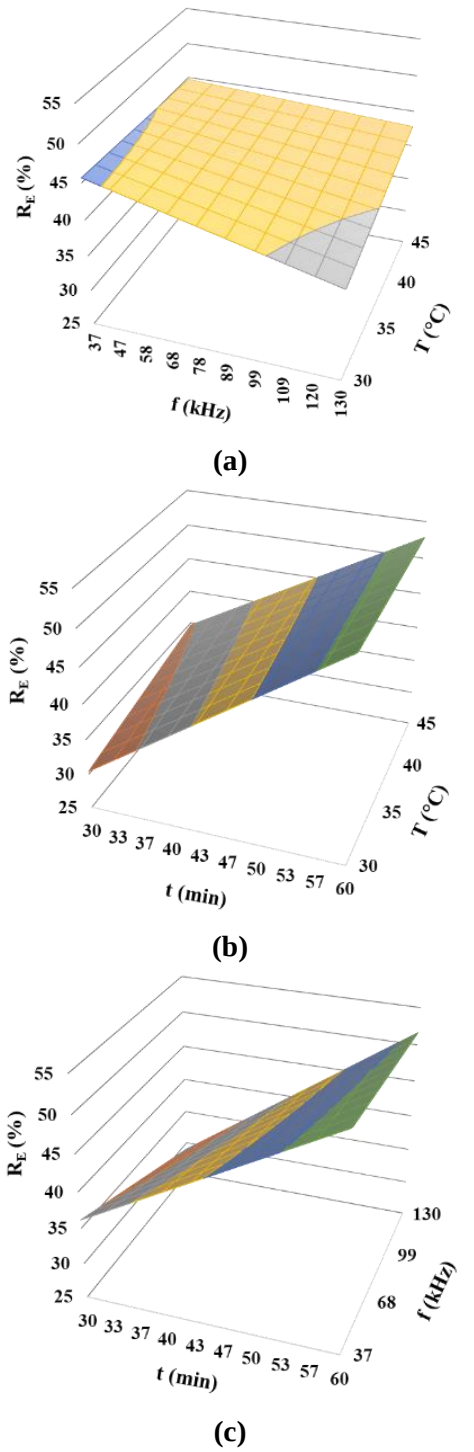

Figure 1. RSM plots of recovery *vs*. temperature, frequency, and time from UAEE.

Effect of UAEH on SFE

The solid fraction obtained after UAEE (SFE) at the optimal condition was used for UAEH. The regression model for recovery was represented by Eq. 5, and RSM plots of the effects of temperature, frequency, and time are shown in Figures 2a, 2b, and 2c. The results of these three factors on extractions with ethanol (UAEE) and sequential hexane (UAEH) showed similar effects that both the frequency and the time were significant ($p < 0.05$). For UAEH, though the individual effect of temperature was not significant, all its interactive effects were clearly significant. UAEH required a lower operating temperature than UAEE since the lower viscosity, lower surface tension, and boiling point of hexane affected cavitation, hence better penetration, upon which lipid recovery is dependent.

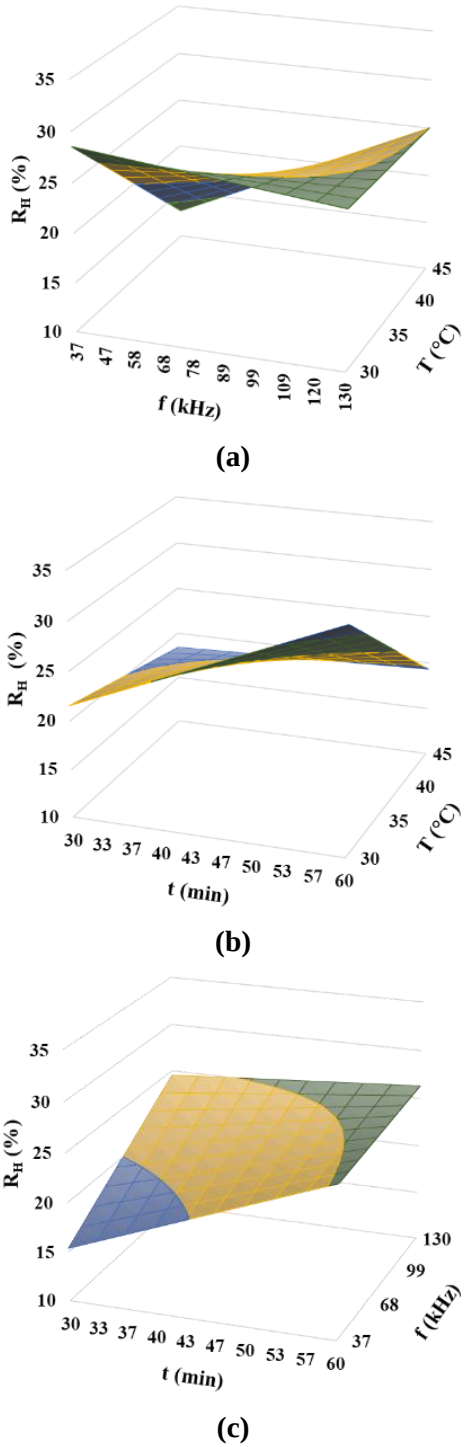

Figure 2. RSM plots of recovery *vs*. temperature, frequency, and time from UAEH.

Overall effects of both extractions

Under the same conditions, 30°C, 37 kHz, and 60 min, the maximal recovery of lipid obtained was 55.691% from UAEE, and 35.635% from UAEH. From RSM optimisation Eqs. 4 and 5, and coincidentally from Experiment#1 in Table 3, the highest percentages of recovery from the complex cellular skin were obtained under comparatively mild conditions on temperature and frequency. The total recovery under optimal conditions of UAEE and UAEH of 91.326% indicated that the lipid content of the skin had been reduced from 3.620 to 0.314%. Therefore, the goal of pretreatment for gelatine production, of which the fat content should not exceed 1% prior to the extraction step, was achieved.

The single-solvent two-stage extraction allowed easy recovery of each individual solvent, making it cost saving and beneficial for the environment. Note that the nine cases for UAEE and UAEH shown in Table 3 had been dually replicated to estimate the average variance in yield measures. The water contents of the reused solvents, analysed by the Karl Fischer method, were respectively < 1.5% for ethanol and < 0.2% for hexane. Solvents with these water contents were reused for duplication, and the presence of trace water had shown no adverse effects on the extractions.

Lipid quality

Lipid oxidation

Oxidations of lipids obtained from UAEE and UAEH are shown in Table 5.

PV and CD of lipid represent primary oxidation products. Both are affected by various factors including light, heat, extraction method, oxygen saturation, and fatty acid composition (Gulzar and Benjakul, 2018). PV is a measure of peroxide and hydro-peroxide concentrations formed in the initial stage of lipid oxidation, while CD is formed when fatty acid radicals derived from removal of hydrogen at the polyunsaturated fatty acid (PUFA) double-bond position react with oxygen and become fatty acid peroxides. Peroxides are subsequently bonded to other PUFA double bonds to form a CD structure (Shima and Shakashita, 2016). Lipid from fish skin has high amounts of PUFA that are highly reactive to oxygen.

From Table 5, the PV of lipid obtained from UAEH was very much higher than that from UAEE. The very high amount of PV in our UAEH lipid was most probably due to air exposure in the mixture filtration step after UAEE that had allowed oxygen to be incorporated into the sample, and accelerated lipid oxidation in the UAEH. Although most lipids extracted using UAE showed high PV by cavitation effect (Gulzar and Benjakul, 2020), the PV from our UAEE was not high, *i.e.* < 20 mEq peroxide/Kg, which is acceptable for food products according to Bimbo (1990). This could be because UAEE was performed at low temperature (30°C) with limited oxygen in the screw-capped bottle.

For CD, the value obtained from UAEH was two-thirds that of UAEE. However, their absolute magnitudes were not significantly different like that of PV.

TBARS indicates the presence of volatile polar secondary lipid oxidation products such as aldehydes and ketones, while AnV indicates the presence of non-volatile secondary oxidation products including acids, epoxide monomers, hydroxy components, and polymer compounds. As compared to TBARS and AnV values of lipid obtained from optimal UAEE, their decrease in optimal UAEH showed that smaller amounts of peroxides had been further oxidised into

Table 5. Colour, PV, CD, TBARS, AnV, and AV of extracted lipids from UAEE and UAEH.

Analysis	Quality of extracted lipid	
	UAEE	UAEH
Colour (Gardner liquid standard)	Red-gold (12)	Gold (7)
Peroxide value (PV, mEq peroxide/Kg lipid)	18.18	300.00
Conjugated diene (CD)	18.05	11.53
TBARS (mg MDA/Kg lipid)	1.25	0.47
Anisidine value (AnV)	39.94	31.31
Acid value (AV, mg KOH/g)	2.55	1.44

secondary oxidation products. Cavitation generating heat and pressure could have inactivated enzymes including lipase, phospholipase, and lipoxygenase, while low operating temperature could have activated bio-antioxidant action or enzyme inhibitor, thus leading to less oxidation of the lipid from the UAEH. From these results, the TBARS values from both lipid samples were at an acceptable level, *i.e.* < 2 mg MDA/kg according to Connel (1995), while AnV values were still high, *i.e.* > 20 according to Standard for Fish Oil (2017), thus indicating a high proportion of non-volatile oxidation products.

For lipid quality consideration, although PV indicated the oxidative deterioration of lipid, it should be considered in tandem with TBARS which monitors the progress of oxidation. A high PV may reflect either an increase in hydro-peroxide formation or a decrease in decomposition while the TBARS value would ascertain whether the PV value found earlier is indeed detrimental to taste stability. The flavour stability of lipid could be improved by antioxidants (Steele, 2004).

Lastly, AVs of all lipid samples fell within the standard limit for edible oil, *i.e.*, < 3.0 mg KOH/g according to Standard for Fish Oil (FAO, 2017). The further decrease of AV in UAEH from UAEE showed that the released free fatty acids (FFAs) were less prone to oxidation, as evidenced earlier by the decreased values in CD, TBARS, and AnV. These combined results indicated that the obtained lipids were within specification limits, and could be applied in numerous related industries including the food, pharmaceutical, and cosmetics industries.

Lipid compositions

Table 6 shows the fatty acid profiles of lipids obtained from UAEE and UAEH at optimal conditions. Palmitic acid was the most plentiful fatty acid in both samples, followed by DHA (docosahexaenoic acid) and stearic acid.

Among the fatty acid groups in both samples, saturated fatty acid (SFA), possessing a stable structure, was most prevalent. After UAEE, UAEH yielded a similar percentage in monounsaturated fatty acid (MUFA) and a slight increase in PUFA. The 11.8% increase in polyene index (ratio of DHA + EPA to C16:0) from UAEE reconfirmed the decrease in AnV and TBARS. Hence, lipids extracted by UAE employing low temperature and limited oxygen demonstrated abilities to retard lipid oxidation or rancidity.

Volatile compounds in raw skin and solid fractions

Table 7 details the abundance of volatile compounds in aldehydes, alcohols, and ketones detected in the raw bigeye skin, and in skins with lowered lipid (SFE and SFH).

Freshness, cleanliness, and preservation of raw fish are important factors affecting the rate of lipid oxidation. Volatile compounds can form, and their contents increase with longer storage times (Yarnpakdee *et al.*, 2012). The most noticeable volatile compound used as an index of lipid oxidation in many kinds of food is the aldehydes (Ross and Smith, 2006). Among the aldehyde compounds analysed in the present work, the most prevalent which formed in the raw skin was benzaldehyde, followed by hexadecanal and hexanal. However, the compounds found were low in content and no heptanal, a prime indicator of flavour deterioration in fish products (Maqsood and Benjakul, 2011), was detected in the raw skin. One reason could be that the raw in the present work was carefully selected for freshness, and properly cleaned before storage, meaning that most of the volatile compounds had not yet formed.

Alcohols and ketones are lipid oxidation products derived from decomposition of hydro-peroxides (Iglesias and Medina, 2008). Although the raw fish skin contained hexanol and octanol contributing to fish odour (Yarnpakdee *et al.*, 2012), they had not been detected in SFE and SFH. Formed from oxidation of arachidonic acid (a crucial contributor to off flavour), 1-octen-3-ol was the main volatile compound in the raw skin. Fortunately, after UAEE had eradicated most of it, UAEH was able to completely remove the compound from SFH.

The lower amounts of compounds in SFE (the material for UAEH) were generally in accordance with the lower TBARS and AnV values of the lipid obtained from UAEH as detailed in Table 5. The results seen in Tables 5 and 7 is that oxidation of lipids and decomposition of hydro-peroxides to secondary oxidation products were less pronounced in SFH when compared respectively with SFE and the raw skin. More compounds in the solid fractions had been removed with progressing extraction stages using a different single solvent in each stage at a low temperature.

Table 6. Fatty acid profile of lipids obtained from UAEE and UAEH.

Fatty acid composition	Fatty acid name	Lipid obtained from UAEE (%)	Lipid obtained from UAEH (%)
Saturated			
C12:0	Lauric	-	0.09
C14:0	Myristic	4.82	4.48
C15:0	Pentadecanoic	1.45	1.34
C16:0	Palmitic	28.38	26.09
C17:0	Heptadecanoic	2.01	0.08
C18:0	Stearic	12.00	12.25
C20:0	Arachidic	0.69	0.73
C21:0	Heneicosanoic	0.12	0.18
C22:0	Docosanoic	0.43	0.47
C23:0	Tricosanoic	3.34	3.37
C24:0	Lignoceric	0.32	0.35
ΣSFA		53.56	49.43
Monounsaturated			
C14:1	Myristoleic	0.17	0.16
C16:1	Palmitoleic	0.18	0.15
C17:1	*cis*-10-Heptadecanoic	0.48	0.43
C18:1 *trans* 9	Elaidic	0.24	0.23
C18:1 *cis* 9	Oleic	3.08	2.88
C20:1	*cis*-11-Eicosenoic	0.40	0.40
C22:1 *cis* 13	Erucanoic	-	0.18
C24:1 *cis* 15	Nervonic	0.62	0.69
ΣMUFA		5.17	5.12
Polyunsaturated			
C18:2 *cis* 9, 12	Linoleic	1.23	1.24
C18:3 *cis* 6, 9, 12	Gamma-Linolenic	0.16	0.11
C18:3 *cis* 6, 9, 15	Alpha-Linolenic	0.24	0.29
C20:2	*cis*-11, 14-Eicosadienoic	0.59	0.64
C20:3	*cis*-8, 11, 14-Eicosatrienoic	-	0.10
C20:4	*cis*-5, 8, 11, 14-Eicosatraenoic	-	0.12
C22:2	*cis* 13, 16-Docosadienoic	0.15	0.23
C20:5 (EPA)	*cis*-5, 8, 11, 14, 17-Eicosatrienoic	3.20	3.43
C22:6 (DHA)	*cis*-4, 7, 10, 13, 16, 19-Docosahexaenoic	13.40	14.91
ΣPUFA		18.97	21.07
Other		22.30	24.38

Table 7. Volatile compound abundance in raw skin and solid fractions.

Compound	Raw skin	SFE	SFH
Aldehyde			
Hexanal	0.42	ND	ND
Octanal	0.09	ND	ND
Nonanal	0.33	0.59	ND
5-Ethylcyclopent-1-enecarboxaldehyde	0.07	ND	ND
Benzaldehyde	1.48	0.48	0.28
(2E,6Z)-2,6-Nonadienal	0.11	ND	ND
Tetradecanal	0.36	0.27	ND
(E)-Hexadec-2-enal	0.15	ND	ND
Pentadecanal	0.10	0.10	ND
Hexadecanal	0.90	0.62	0.54
17-Octadecenal	0.01	ND	ND
Octadecanal	0.03	ND	ND
Alcohol			
1-Hexanol	0.11	ND	ND
1-Octen-3-ol	1.91	0.14	ND
1-Heptanol	0.60	ND	ND
1,5-Octadien-3-ol	0.26	ND	ND
Cyclooctyl alcohol	0.64	ND	ND
Ketone			
2-Octanone	0.02	ND	ND
1-phenyl-ethanone	0.50	ND	ND
3-Undecen-2-one	0.28	ND	ND

Values are expressed as abundance ($\times 10^8$). ND: not detectable.

Fish odour is commonly generated from compounds with unsaturated fatty acids (UFAs, both MUFA and PUFA), particularly phospholipid in the polar lipids group. To lessen fish odour in gelatine production, fish skin with low amounts of polar lipids should be used. Bound lipids require polar solvents for extraction, and ethanol (a polar solvent) is suitable to be selectively used first (UAEE) to remove some compounds from the raw skin. The results in Table 6 confirm that most compounds could be removed in this first step. To further enhance the yields on the extraction of most other lipids in the fish skin which are non-polar, hexane (a non-polar solvent) was subsequently used (UAEH).

This combination offered a cost-effective alternative to fish lipid production where waste was nearly eradicated in the process, and provided viable products for both the fish lipid and the protein material with lowered lipid (< 1%) used for gelatine production. Furthermore, this pretreatment of fish skin by the proposed two-stage lipid extraction could efficiently decrease the formation of secondary oxidation products in both the lipid and lessened-lipid skin. The freshness of fish skin and the low-temperature two-stage process yielded low formations of secondary oxidation products, which meant that the SFH raw material for gelatine production was nearly devoid of odour-inducing volatiles. The feasibility for its usage for gelatine production with fewer problems of odour and reduced turbidity from less skin fat was thus confirmed.

Conclusion

Ultrasound-assisted extraction was an effective method for the extraction of lipids from fish skin. As compared to conventional methods, it could provide a higher yield in less time and with reduced solvent consumption. Fresh purple-spotted bigeye fish skin should be dried at 80°C before UAE. Kinetic data indicated that UAE at a low temperature of 30°C provided the highest extraction rate. A two-stage lipid extraction (pretreatment) to obtain fish lipid prior to hydrolysis in gelatine production resulted in two useful product gains namely the lipids (liquid fractions) characterised within the standard limit that could be applied for related industries, and the solid fractions remaining after removal of the liquid fractions that retained high protein with low lipid, which can consequently be used as material for the production of gelatine or protein hydrolysates.

Acknowledgement

The author would like to express appreciation to Mr. Wiwat Sutiwipakorn, a former engineering lecturer, Prince of Songkla University, for his assistance with the English in this paper.

References

Alipal, J., Mohd Pu'ad, N. A. S., Lee, T. C., Nayan, N. H. M., Sahari, N., Basri, H., ... and Abdullah, H. Z. 2021. A review of gelatin: Properties, sources, process, applications, and commercialization. Materials Today - Proceedings 42(1): 240-250.

American Oil Chemists' Society (AOCS). 1992a. Method Cd 3a 63(89) - Acid value. United States: AOCS Press.

AOCS 1992b. Method Cd 18-90 - *p*-anisidine value. United States: AOCS Press.

Association of Official Analytical Chemist (AOAC). 2000. Official methods of analysis of AOAC International. 17th ed. United States: AOAC.

Bako, T., Umogbai, V. I. and Awulu, J. O. 2017. Criteria for the extraction of fish oil. Agricultural Engineering International - CIGR Journal 19(3): 120-132.

Balasubramanian, R. K., Doan, T. T. Y. and Obbard, J. P. 2013. Factors affecting cellular lipid extraction from marine microalgae. Chemical Engineering Journal 215-216: 929-936.

Bimbo, A. 1990. Production of fish oils. New York: Van Nostrand Reinhold.

Buege, J. A. and Aust, S. D. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52: 302-310.

Cole, C. G. B. 2012. Gelatine clarity. Retrieved on June 09, 2021 from website: https://www.gelatin.co.za/Gelatine%20Clarity.pdf

Connel, J. J. 1995. Control of fish quality. 4th ed. England: Fishing News Books Ltd.

Di Domenico, C. N. B. 2014. Fish drying: A review. Revista Brasileira de Engenharia de Pesca 7(1): 1-18.

Efthymiopoulos, I., Helliera, P., Ladommatosa, N., Russo-Profilia, A., Eveleigha, A., Alievb, A., ... and Mills-Lampteyc, B. 2018. Influence of solvent selection and extraction temperature on yield and composition of lipids extracted from spent coffee grounds. Industrial Crops and Products 119: 49-56.

Food and Agriculture Organization (FAO). 2017. CXS 329-2017 - Standard for fish oil. Italy: FAO.

Gholivand, M. B., Yamini, Y. and Dayeni, M. 2014. Optimization and comparison of ultrasound-assisted extraction of estragole from tarragon leaves with hydro-distillation method. Analytical and Bioanalytical Chemistry Research 1(2): 99-107.

Gulzar, S. and Benjakul, S. 2018. Ultrasound waves increase the yield and carotenoid content of lipid extracted from cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*). European Journal of Lipid Science and Technology 120: 1-11.

Gulzar, S. and Benjakul, S. 2020. Impact of pretreatment and atmosphere on quality of lipids extracted from cephalothorax of Pacific white shrimp by ultrasonic assisted process. Food Chemistry 309: 125732.

Harianti. 2020. Characterization chemical composition of skin and head bones barracuda (*Sphyraena jello*) as collagen raw material. IOP Conference Series - Earth and Environmental Science 564: 012046.

Hu, B., Li, C., Zhang, Z., Zhao, Q., Zhu, Y., Su, Z. and Chen, Y. 2017. Microwave-assisted extraction of silkworm pupal oil and evaluation of its fatty acid composition, physicochemical

properties and antioxidant activities. Food Chemistry 231: 348-355.

Iglesias, J. and Medina, I. 2008. Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. Journal of Chromatography A 1192: 9-16.

International Union of Pure and Applied Chemistry (IUPAC). 1992. Standard methods for the analysis of oils, fats, and derivatives. United Kingdom: IUPAC.

Ivanovs, K. and Blumberga, D. 2017. Extraction of fish oil using green extraction methods: A short review. Energy Procedia 128: 477-483.

Jabar, J. M., Lajide, L., Adetuyi, A. O., Owolabi, B. J., Bakare, I. O., Abayomi, T. G. and Ogunneye, A. L. 2015. Yield, quality, kinetics and thermodynamics studies on extraction of *Thevetia peruviana* oil from its oil bearing seeds. Journal of Cereals and Oilseeds 6(5): 24-30.

Jaziri, A. A., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. and Huda, N. 2021. Chemical composition of lizardfish surimi by-product: Focus on macro and micro-minerals contents. Current Research in Nutrition and Food Science 9(1): 52-61.

Joven, J. M. O., Gadian, J. T., Perez, M. A., Caingles, J. G., Mansalaynon, A. P., Ido, A. L. and Arazo, R. O. 2020. Optimized ultrasonic-assisted oil extraction and biodiesel production from the seeds of *Maesopsis eminii*. Industrial Crops and Products 155: 112772.

Khitouni, I. K., Mihoubi, N. B., Bouain, A. and Rebah, F. B. 2014. Seasonal variation of the chemical composition, fatty acid profiles and mineral elements of *Diplodus Annularis* (Linnaeus, 1758) caught in the Tunisian coastal water. Journal of Food and Nutrition Research 2(6): 306-311.

Kittiphattanabawon, P., Benjakul, S., Sinthusamran, S. and Kishimura, H. 2016. Gelatin from clown featherback skin: Extraction conditions. LWT - Food Science and Technology 66: 186-192.

Kosuke, N., Jin, Li. Y., Fukumuroa, Z., Andoa, M. and Akaishi, Y. A. 2006. Low-temperature desiccant-based food drying system with airflow and temperature control. Journal of Food Engineering 75: 71-77.

Krishnan, V. C. A., Kuriakose, S. and Rawson, A. 2015. Ultrasound assisted extraction of oil from rice bran: A response surface methodology approach. Journal of Food Processing and Technology 6: 454.

Low, L. and Ng, C. 1978. Laboratory manual on analytical methods and procedures for fish and fish products. In Hasegawa, H. (ed). Singapore: Southeast Asian Fisheries Development Center.

Maqsood, S. and Benjakul, S. 2011. Oxidation of lipid and protein in horse mackerel (*Trachurus*) mince and washed minces during processing and storage. Food Chemistry 124: 875-883.

Mekpiroon, A., Lerdrattranataywee, W. and Jutidamrongphan, W. 2016. Perspective of waste utilization in seafood industry. International Journal of Management and Applied Science 2(9): 2394-7926.

Moreau, R. A. 2005. Extraction and analysis of food lipids. In Otles, S. (ed). Methods of Analysis of Food Components and Additives, p. 97-110. United States: CRC Press.

Muhammed, M. A., Domendra, D., Muthukumar, S. P., Sakhare, P. Z. and Bhaskar, N. 2015. Effects of fermentatively recovered fish waste lipids on the growth and composition of broiler meat. British Poultry Science 56(1): 79-87.

Mujaffar, S. and Sankat, C. K. 2011. The effect of temperature on the drying characteristics of salted shark fillets. The West Indian Journal of Engineering 33: 19-25.

Muralidharan, N., Shakila, R. J., Sukumar, D. and Jeyasekaran, G. 2013. Skin, bone and muscle collagen extraction from the trash fish, leather jacket (*Odonus niger*) and their characterization. Journal of Food Science and Technology 50: 1106-1113.

Njinkoue, J. M., Gouado, I., Tchoumbougnang, F., Ngueguim, J. H. Y., Ndintech, D. T., Fomogne-Fodjo, C. Y. and Schweigert, F. J. 2016. Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian coast: *Pseudotolithus typus* (Bleeker, 1863) and *Pseudotolithus elongatus* (Bowdich, 1825). Nutrition and Food Science Journal 4: 27-31.

Pascal, G. 2019. Future seafood: Surimi market update. In the 12th Surimi School Asia. Bangkok, Thailand.

Pateiro, M., Munekata, P. E. S., Domínguez, R., Wang, M., Barba, F. J., Bermúdez, R. and Lorenzo, J. M. 2020. Nutritional profiling and

the value of processing by-products from gilthead sea bream (*Sparus aurata*). Marine Drugs 18: 101.

Rajha, H., Abi-Khattar, A., EI Kantar, S., Boussetta, N., Lebovka, N., Maroun, R. and Vorobiev, E. 2019. Comparison of aqueous extraction efficiency and biological activities of polyphenols from pomegranate peels assisted by infrared, ultrasound, pulsed electric fields and high-voltage electrical discharges. Innovative Food Science and Emerging Technologies 58: 102212.

Ratti, C., Belkacemi, K. and Gutie, L. 2008. Food chemistry effect of drying method on the extraction yields and quality of oils from Quebec Sea buckthorn (*Hippophae rhamnoides L.*) seeds and pulp. Food Chemistry 106(3): 896-904.

Ross, C. F. and Smith, D. M. 2006. Use of volatiles as indicators of lipid oxidation in muscle foods. Comprehensive Reviews in Food Science and Food Safety 5: 10-25.

Rubio-Rodríguez, N., de Diego, S. M., Beltrán, S., Jaime, I., Sanz, M. T. and Rovira, J. 2012. Supercritical fluid extraction of fish oil from fish by-products: A comparison with other extraction methods. Journal of Food Engineering 109: 238-248.

Sae-Leaw, T. and Benjakul, S. 2015. Physico-chemical properties and fishy odour of gelatin from seabass (*Lates calcarifer*) skin stored in ice. Food Bioscience 10: 59-68.

Sae-Leaw, T., Benjakul, S. and O´Brien, N. M. 2016a. Effect of pretreatments and drying methods on the properties and fishy odor/flavor of gelatin from seabass (*Lates calcarifer*) skin. Drying Technology 34: 53-65.

Sae-Leaw, T., Benjakul, S. and O´Brien, N. M. 2016b. Effect of pretreatments and defatting of seabass skins on properties and fishy odor of gelatin. Journal of Food Biochemistry 40: 741-753.

Shima, M. and Sakashita, H. 2016. Analysis of the concentration of conjugated diene structures in glyceryl trilinoleate during oxidation. Food Science and Technology Research 22(6): 733-738.

Sinthusamran, S., Benjakul, S., Kijroongrojana, K., Prodpron, T. and Agustini, T. W. 2018. Yield and chemical composition of lipids extracted from solid residues of protein hydrolysis of pacific white shrimp cephalothorax using ultrasound-assisted extraction. Food Bioscience 26: 169-176.

Siriraksophon, S., Pangsorn, S. and Laong-manee, P. 2009. The surimi industry in Southeast Asia: Trend and demand for raw materials. Southeast Asian Fisheries Development Center 7(2): 1-7.

Steele, R. 2004. Understanding and measuring the shelf-life of food. 1st ed. United States: CRC Press.

Stevanato, N. and da Silva, C. 2019. Ultrasound-assisted extraction using ethanol as solvent and assessment of its potential for ester production. Industrial Crops and Products 132: 283-291.

Yarnpakdee, S., Benjakul, S., Nalinanonb, S. and Kristinssonc, H. G. 2012. Lipid oxidation and fishy odour development in protein hydrolysate from Nile tilapia (*Oreochromis niloticus*) muscle as affected by freshness and antioxidants. Food Chemistry 132: 1781-1788.