Cholesterol and alpha-tocopherol contents of fish and other seafood from the Straits of Malacca

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Abstract

This study was conducted to determine the cholesterol and alpha-tocopherol contents of 20 marine fish and four other seafood from the Straits of Malacca. Cholesterol and alpha-tocopherol contents of the fish and other seafood were determined using high-performance liquid chromatography. The results showed that most of the fish contained low amounts of cholesterol, except sixbar grouper *(Epinephelus fasciatus)*, long-tailed butterfly ray (*Gymnura* sp.), yellowstripe scad *(Selaroides leptolepis)*, cuttlefish *(Sepia officinalis)*, large-scale tongue sole *(Cynoglossus arel)*, and longtail shad *(Hilsa macrura)* that contained high amounts of cholesterol (119.39-353.97 mg/100 g wet samples). Indian mackerel *(Rastrelliger kanagurta)*, giant seaperch *(Lates calcarifer)*, prawn *(Metapenaeus affinis)*, and moonfish *(Trachinotus blochii)* had high alpha-tocopherol contents (462-989 μg/100 g wet sample). Regular consumption of fish and other seafood is highly recommended partly due to the high alpha-tocopherol content. Due to the high cholesterol in certain types of fish, consumption of the fish fillets of sixbar grouper, long-tailed butterfly ray, yellowstripe scad, cuttlefish, and large scale tongue sole should be <100 g per day and <50 g per day for longtail shad. Validation of the analytical method also showed a high accuracy and reproducibility of the HPLC method.

Introduction

Cholesterol and alpha-tocopherol are the main components of the unsaponifiable lipid fraction of fish and seafood. These nutritionally significant types of lipids are also part of the essential data in food composition database. Cholesterol, the principal steroid in animal-based food, is a well-known precursor of bile acids, steroid hormones, and vitamin D (Grosvenor and Smolin, 2002). High dietary intake of cholesterol has been implicated in atherosclerosis (McNamara, 2000; Tziakas *et al.*, 2013). There is a growing interest of the public to assess their intake of dietary cholesterol, as well as to know the types of high cholesterol foods. Alpha-tocopherol, the fat-soluble vitamin, is an important antioxidant to combat free radical-induced diseases, particularly protection of membrane and lipoprotein particles (Kalogeropoulos *et al.*, 2007). It has been gaining recognition as an important nutrient in many types of foods.

Studies on the unsaponifiable lipid components (cholesterol and alpha-tocopherol) in fish and seafood have been conducted in Italy (Orban *et al.*, 2011) and Portugal (Afonso *et al.*, 2013). Ten species of marine fish obtained from Malaysian market have cholesterol content of 37.1-49.1 mg/100 g (Osman *et al.*, 2001), but alpha-tocopherol content was not determined. For most of the data, the lipid component was determined independently following extraction, saponification, and separation steps of the unsaponifiable fraction. In recent years, study on unsaponifiable lipid has become part of the routine analysis. A few lipid components have been simultaneously determined (Lopez-Cervantes *et al.*, 2006). In this simultaneous quantification, sample was directly saponified, followed by extraction,

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Table 1. List of samples with narrow range of weight and length

Common name	Local name	Scientific name	Habitat	Range of weight (g)	Range of length (cm)	Fat content (g/100 g muscle)[1]
Hardtail scad	Cencaru	*Megalapsis cordyla*	Pelagic	100-250	21-28	1.53 ± 0.15[a]
Golden snapper	Jenahak	*Lutjanus johnii*	Demersal	490-510	30-35	1.29 ± 0.41[a]
Indian mackerel	Kembung	*Rastrelliger kanagurta*	Pelagic	50-100	14-20	1.80 ± 0.62[a]
Indian threadfin	Kurau	*Polynemus indicus*	Demersal	350-1450	36-59	0.85 ± 0.21[a]
Malabar red snapper	Merah	*Lutjanus argentimeculatus*	Demersal	580-760	28-37	1.37 ± 1.10[a]
Dorabwolf herring	Parang	*Chirocentrus dorab*	Pelagic	200-900	40-71	1.22 ± 0.22[a]
Long-tailed butterfly ray	Pari	*Gymnura* sp.	Demersal	1300-1700	32-36	0.93 ± 0.12[a]
Large-scale tongue sole	Sebelah/Lidah	*Cynoglossus arel*	Demersal	50-100	24-32	0.70 ± 0.10[a]
Spanish mackerel	Tenggiri papan	*Scromberomorus guttatus*	Pelagic	200-450	30-42	1.05 ± 0.06[a]
Black pomfret	Bawal hitam	*Parastromateus niger*	Pelagic	780-1040	33-42	2.33 ± 0.11[a]
Silver pomfret	Bawal putih	*Pampus argentus*	Pelagic	100-200	15-25	2.09 ± 0.93[a]
Sixbar grouper	Kerapu	*Epinephelus fasciatus*	Demersal	480-750	33-36	3.46 ± 3.46[ab]
Japanese threadfin bream	Kerisi	*Nemipterus japonicus*	Demersal	100-230	18-25	2.70 ± 0.37[ab]
Yellowstripe scad	Selar kuning	*Selaroides leptolepis*	Pelagic	50-100	16-20	2.12 ± 0.50[a]
Gray eel-catfish	Sembilang	*Plotosus* sp.	Demersal	350-600	40-50	3.04 ± 0.59[ab]
Fourfinger threadfin	Senangin	*Eleutheronema tetradactylum*	Pelagic	150-300	27-32	2.10 ± 0.25[a]
Giant seaperch	Siakap	*Latescalcarifer*	Demersal	700-1000	38-42	2.68 ± 0.79[ab]
Fringescale sardinella	Tamban	*Clupea fimbriata*	Pelagic	20-40	13-17	3.00 ± 2.40[ab]
Moonfish	Nyior-nyior	*Trachinotus blochii*	Demersal	400-1400	31-47	6.89 ± 2.76[b]
Longtail shad	Terubuk	*Hilsa macrura*	Pelagic	928	40-45	23.15 ± 0.00[c]
Cuttlefish	Sotong	*Sepia officinalis*	-	20-45	12-18	1.35 ± 0.28[a]
Prawn	Udangputih	*Metapenaeus affinis*	-	10-20	12-17	1.06 ± 0.10[a]
Cockles	Kerang	*Anadara granosa*	-	10-20	2-5	1.93 ± 1.28[a]
Oyster	Tiram	*Ostrea* sp.	-	100-300	14-48	1.24 ± 0.00[a]

[1]Fat content of samples as reported by previous study (Nurnadia *et al*., 2011).
[2]Different superscript lowercase letters (a-h) denote significant difference at $P < 0.05$ (Tukey's post-hoc test).

therefore, reduce the occurrence of oxidation during extraction. The method also offers high recovery (98-100%) of the constituents, thus providing a more accurate and reliable composition data.

Due to the lack of information on cholesterol and alpha-tocopherol of certain types of fish and seafood in Malaysian Food Composition Database (Tee *et al*., 1997), there is a need to determine the cholesterol and alpha-tocopherol contents in fish and seafood obtained from the Straits of Malacca. Realizing the importance of cholesterol and alpha-tocopherol in fish fillet that affect human health, choosing the low cholesterol and high alpha-tocopherol fish fillet is a good choice. According to the reported data on cholesterol in fish and seafood, only a limited type of these foods has been tabulated in the ASEAN Food Composition Table (Puwastien *et al*., 1999). Therefore, the aim of this study was to determine cholesterol and alpha-tocopherol contents of fish and seafood obtained from the Straits of Malacca using high-performance liquid chromatography (HPLC).

Materials and Methods

Chemicals and reagents

Methanol, chloroform, butylated hydroxytoluene, and potassium hydroxide were analytical grades, and methanol and acetonitrile used for HPLC analysis were HPLC grade. These chemicals and solvents were obtained from Merck (Darmstadt, Germany). Alpha-tocopherol and cholesterol were used as standards, where the chemicals were purchased from Sigma Chemical Co. (MO, USA).

Sample preparation

A stratified random sampling procedure was applied in the selection of fish and seafood samples (Greenfield and Southgate, 2003) between June to December, 2011. Ten fish landing areas along the Straits of Malacca were identified with the help of Malaysian Fisheries Development Authority. Each landing area represented the strata of the parent population and within each level, where the samples were collected randomly. The locations of each stratum were marked as L1 through L10 (Figure 1).
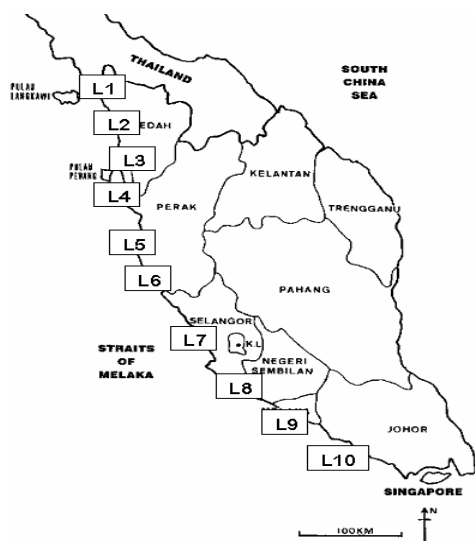
At each of the collection site, samples were

Figure 1. Location of samples collection sites

chosen randomly according to the available species. The samples were collected from the specified landing area, where the fish and shellfish had been caught within a period of 0-36 h. All of the fish and shellfish samples were immediately stored in ice boxes and transported to the laboratory. A total of 24 types of fish and other seafood were obtained from several visits to the fish landing area. The fish and other seafood were individually measured for total weight (g) and length (cm). The samples with a total weight within a narrow range were used as primary samples (Table 1). The samples were beheaded, gutted, washed, and filleted. These primary samples were placed in sealed plastic bags and frozen at -40°C. All these samples were analyzed within two weeks.

Due to the logistic reasons, pilot studies of the total fat contents were performed previously to compare the variability in geographical and physiological differences for the collected samples. The non-significant differences between the nutrient contents of the samples obtained from different locations suggested that primary samples can be composited based on different geographical locations (Greenfield and Southgate, 2003). Before the analysis, three composite samples from the same species were prepared based on the same weight of the fish fillet. Fish samples of L1, L2, L3, and L4 were homogenized and labelled as Composite 1, fish samples of L5, L6, and L7 were labelled as Composite 2, and fish samples from L8, L9, and L10 as Composite 3 (Figure 1). The composited samples were analyzed independently, and the data were presented as mean values for each species.

Fat extraction

Extraction of fat was performed based on the methods reported by Bligh and Dyer (1959) and Kinsella *et al.* (1977). Representative samples of the fish filets (30 g) were homogenized in a Waring blender for 2 min using a mixture of methanol (60 ml) and chloroform (30 ml). Then, 30 ml of chloroform and 30 ml of distilled water were added to the mixture. The homogenate was stirred with a glass rod and filtered through a Whatman No. 1 filter paper on a Buchner funnel with slight suction. The filtrate was transferred to a separation funnel. The clear phase (bottom phase) was poured into a 250 ml round-bottom flask and concentrated using a rotary evaporator at 40°C. The extractions were performed under minimal light exposure. The extracted lipids were kept in amber glass bottles and flushed with nitrogen. The bottles were immediately stored at -20°C before analysis.

Analysis of cholesterol and alpha-tocopherol

Cholesterol and alpha-tocopherol were extracted from the lipid fraction based on the method reported previously (Sanchez-Machado *et al.*, 2002; Lopez-Carventas *et al.*, 2006). A sub-sample of 0.40 g (± 0.001 g) was weighed in a screw-top assay tube. Two hundred microliters of pyrocatechol solution were used as antioxidant. Next, 5 ml of KOH solution (0.5 M in methanol) was added and immediately vortexed for 20 s. The tubes were placed in a water bath at 80°C for 15 min, removed every 5 min, and vortexed for 15 s. After chilled in ice water, 1 ml of distilled water and 5 ml of hexane were added to the tube. The mixture was rapidly vortexed for 1 min and centrifuged for 2 min at 425 ×g. Three millilitres of the upper phase were transferred to another test tube and dried under nitrogen. The semi-solid residue (extract) was redissolved with 3 ml of HPLC mobile phase solution [methanol: acetonitrile: water (68: 28: 4, v/v/v)] and membrane filtered (pore size 0.50 μm; Whatman, Clifton, New Jersey, USA). Finally, an aliquot of 20 μl of the extract was injected into an Agilent 1100 series HPLC system (Agilent, USA). Prior to HPLC analysis, the extracts were kept at -20°C. As a precaution, amber vials were used in order to minimize visible light degradation during analysis.

HPLC analysis

Chromatographic analysis was performed using an analytical scale (25 cm × 0.4 cm I.D.) C18 column with a particle size of 5 μm (Agilent, USA). The HPLC conditions were as follows: mobile phase [methanol: acetonitrile: water, (68:28:4, v/v/v)], flow rate of 1.4 ml/min, and column temperature of 36°C. Detection was performed using a diode-array detector,

at 208 nm for both alpha-tocopherol and cholesterol. Alpha-tocopherol and cholesterol in the samples were identified by comparing retention times of the samples with the retention time of standards of both compounds. Spiking test was performed to confirm the retention of alpha-tocopherol and cholesterol. Quantification was performed by calculating the concentrations of the compounds based on the standard calibration curves obtained.

Method validation

Method validation was performed to ensure the HPLC method used for the determination of alpha-tocopherol and cholesterol was highly reliable and able to produce reproducible data for future references. The standards were prepared based four different concentrations for cholesterol (500, 1000, 2500, and 5000 µg/ml) and alpha-tocopherol (5.0, 10.0, 25.0, and 50.0 µg/ml). The linearity parameters, which included linear regression (y = mx + c) and correlation coefficient (R^2), were obtained from the linear relationship between peak area and concentrations of cholesterol and alpha-tocopherol standards.

Precision tests for repeatability (within-day) and reproducibility (between-days) were performed. Cholesterol and alpha-tocopherol contents were determined for the five samples that randomly selected from the 24 samples. For repeatability (within-day precision), each sample was analyzed in a single day applying the same procedure that used to determine the cholesterol and alpha-tocopherol contents. The results were reported as relative standard deviation (RSD), minimum, and maximum values of five replicates for the cholesterol determination, and four replicates for the alpha-tocopherol determination. For reproducibility (between-days precision) test, samples were analyzed using the same procedure for four different days, representing four replicates for each. Alpha-tocopherol was analyzed for three different days, representing three replicates of each of the samples. The results were also presented as RSD of the replicates, coupled with minimum and maximum values for cholesterol and alpha-tocopherol.

The recoveries of the cholesterol and alpha-tocopherol were determined using three different concentrations of cholesterol standards (50.0, 100.0, and 150.0 µg/g extracted fat) and alpha-tocopherol standards (2500, 5000, and 7500 µg/g extracted fat). Four samples were randomly selected and used for the recovery analysis. Recoveries of each standard compound at different concentrations were determined by comparing the contents of cholesterol and alpha-tocopherol in individual sample with and without the addition of the standards. The results were presented as the percentages of recovery.

Statistical analysis

The data were analyzed using SPSS (Statistical Package of Social Science) version 17.0. Two different sets of statistics, namely descriptive statistics and inferential statistics, were applied. The results were presented as mean ± standard deviation (SD) and one-way analysis of variance, followed by Tukey's post-hoc comparisons to compare the differences between the mean values of cholesterol and alpha-tocopherol in different types of fish and other seafood.

Results and Discussion

Cholesterol and alpha-tocopherol contents

The cholesterol and alpha-tocopherol contents of the fish and other seafood samples are shown in Table 2. The cholesterol content of the samples was expressed as milligram per gram (mg/g) fat and milligram per 100 gram (mg/100 g) wet sample. The values were calculated based on the percentage of fat determined in 100 gram of fish fillet (Table 1) as reported previously (Nurnadia *et al.*, 2011).

The results showed that the fats extracted from the samples contained low levels of cholesterol (<50 mg/g). The cholesterol content determined in fish and other seafood samples ranged from 27.13 to 353.97 mg per 100 g wet sample. A total of six types of the fish samples (sixbar grouper, long-tailed butterfly ray, yellowstripe scad, cuttlefish, large-scale tongue sole, and longtail shad) contained high levels of cholesterol, where the values were 119.39, 143.25, 155.51, 157.16, 193.03, and 353.97 mg per 100 g wet samples, respectively). Although the cholesterol level of these fish fillet and seafood samples was high, a high level of omega-3 fatty acid was also found in these samples. The omega-3 to omega-6 ratios of these samples were the highest for cuttlefish (ω-3/ω-6=15.29, P/S=0.89), followed by long-tailed butterfly ray (ω-3/ω-6=13.29, P/S=1.30), large-scale tongue sole (ω-3/ω-6=7.80, P/S=1.10), yellowstripe scad (ω-3/ω-6=6.37, P/S=1.71), sixbar grouper (ω-3/ω-6=3.65, P/S=0.92), and longtail shad (ω-3/ω-6=0.76, P/S=0.36). A high ratio of omega-3 over omega-6 fatty acid is good for lowering blood cholesterol and has other health benefits (Nurnadia *et al.*, 2013). Besides, four fish samples had higher levels of cholesterol (Table 2) than the levels reported previously. Osman *et al.* (2001) reported that the cholesterol contents of long-tailed butterfly

Table 2. Cholesterol and alpha-tocopherol contents

Samples	Cholesterol (mean ± SD)		Alpha-tocopherol (mean ± SD)	
	mg/g oil	mg/100 g wet sample	mg/g oil	mg/100 g wet sample
Black pomfret	18.56 ± 0.66[cde]	43.25 ± 1.54[de]	nd	nd
Silver pomfret	21.11 ± 0.19[de]	44.10 ± 0.40[e]	nd	nd
Hardtail scad	35.40 ± 0.12[fgh]	54.15 ± 0.18[f]	nd	nd
Golden snapper	49.55 ± 0.12[i]	63.92 ± 0.14[g]	nd	nd
Indian mackarel	22.52 ± 0.18[e]	40.53 ± 0.32[cde]	257.03 ± 2.51[f]	462.65 ± 4.52[e]
Sixbar grouper	34.50 ± 0.05[fgh]	119.39 ± 0.17[k]	nd	nd
Japanese threadfin bream	33.12 ± 0.31[fg]	89.44 ± 0.82[i]	8.77 ± 2.76[ab]	23.68 ± 7.45[a]
Indian threadfin	48.12 ± 0.21[i]	40.90 ± 0.18[cde]	nd	nd
Malabar red snapper	30.16 ± 0.11[f]	41.32 ± 0.16[cde]	101.49 ± 1.25[d]	139.04 ± 1.71[c]
Moonfish	4.35 ± 0.09[a]	30.00 ± 0.62[ab]	143.54 ± 8.31[e]	989.00 ± 57.27[g]
Dorab wolfherring	38.60 ± 0.10[gh]	47.09 ± 0.11[ef]	nd	nd
Long-tailed butterfly ray	154.03 ± 0.08[m]	143.25 ± 0.07[l]	67.51 ± 17.17[cd]	62.78 ± 15.97[ab]
Large-scale tongue sole	275.76 ± 3.71[n]	193.03 ± 2.59[n]	29.57 ± 4.07[ab]	20.70 ± 2.85[a]
Yellowstripe scad	73.35 ± 0.43[j]	155.51 ± 0.90[m]	nd	nd
Gray eel-catfish	21.77 ± 0.28[de]	66.19 ± 0.84[g]	nd	nd
Fourfinger threadfin	21.38 ± 0.08[de]	44.91 ± 0.16[e]	43.58 ± 0.88[bc]	91.52 ± 1.85[bc]
Giant seaperch	13.22 ± 0.09[bc]	35.43 ± 0.25[bcd]	176.43 ± 7.68[e]	472.84 ± 20.59[e]
Fringe scale sardinella	10.39 ± 1.02[ab]	31.17 ± 3.05[ab]	nd	nd
Spanish Mackarel	94.60 ± 0.07[k]	99.34 ± 0.06[j]	nd	nd
Longtail shad	15.29 ± 0.02[bcd]	353.97 ± 0.33[o]	nd	nd
Cuttlefish	116.41 ± 0.29[l]	157.16 ± 0.40[m]	nd	nd
Prawn	32.54 ± 0.33[fg]	34.49 ± 0.35[abc]	779.56 ± 37.99[g]	826.34 ± 40.27[f]
Cockles	40.36 ± 1.07[h]	77.90 ± 2.04[h]	155.31 ± 3.13[e]	299.74 ± 6.05[d]
Oyster	21.88 ± 9.66[de]	27.13 ± 11.98[a]	nd	nd

[1]Different letters in same column denote significant difference at P < 0.05 (Tukey's post-hoc test); nd-not detected.

ray, yellowstripe scad, gray eel-catfish, and Spanish mackerel were 37.1, 47.3, 46.9, and 41.1 mg/100 g wet samples, respectively. Meanwhile, the other six fish samples (fourfinger threadfin, black pomfret, silver pomfret, fringe-scale sardinella, hardtail scad, and Indian mackerel) had cholesterol contents within the previously reported range (Osman *et al*., 2001).

The result for alpha-tocopherol contents in the fish fillet and other seafood samples were expressed as microgram per gram (μg/g) oil and microgram per 100 gram (μg/100 g) wet sample (Table 2). The values were calculated based on the percentage of fat determined in 100 g of the fillet. Among the samples analyzed, only ten samples contained alpha-tocopherol. These samples had a wide variation in alpha-tocopherol content. Large-scale tongue sole contained the lowest alpha-tocopherol content (20.70 μg/100 g wet sample), followed by Japanese threadfin bream (23.68 μg/100 g wet sample), and long-tailed butterfly ray (62.78 μg/100 g wet sample).

Although the fat extracted from prawn contained the highest level of alpha-tocopherol in oil (779.56 μg/g oil), the prawn muscle contained 1.06% of fat. Thus, 100 g of its wet muscle contained 826.34 μg alpha-tocopherol, which was significantly lower (P < 0.05) than the alpha-tocopherol in moonfish

(6.89% fat content). The alpha-tocopherol content of moonfish was 989.0 μg/100 g wet sample. Among the fish and seafood samples that contained alpha-tocopherol, the levels were ranged from 20.70 to 989.00 μg/100 g wet sample. The results suggest that some species of these marine fish and seafood are good sources of alpha-tocopherol.

In general, fish and shellfish obtained from the Straits of Malacca were highly nutritious. Thus, regular consumption of these fish and seafood are highly recommended for the functional effect (Nurnadia *et al*., 2011; Nurnadia *et al*., 2013). It is recommended that an increase in consumption of fish fillet to at least 100 g per day could be able to decrease the risk of coronary heart disease (Otsuka *et al*., 2000). Therefore, a 50% increase in consumption of fish and seafood will contribute to an increased intake of alpha-tocopherol.

Ministry of Health Malaysia recommended that daily cholesterol intake shall not exceed the limit of 200 mg (Ministry of Health, 2005). Therefore, the amount of fish and seafood intake should be monitored due to cholesterol content in the seafood. Increase consumption of high cholesterol seafood is hazardous to the individual with metabolic syndrome. Besides, intake of fish fillets of sixbar grouper,

Table 3. Precision of cholesterol analysis

Sample	Mean of cholesterol content (mg/g oil)	Repeatability (within-day precision) data of cholesterol (n=5)				Reproducibility (between-days precision) data of cholesterol (n=4)			
		Max	Min	SD	RSD (%)	Max	Min	SD	RSD (%)
Indian mackerel	22.84	23.03	22.64	0.16	0.70	23.10	22.67	0.14	0.61
Malabar red snapper	19.94	20.19	19.69	0.20	1.00	20.32	20.10	0.07	0.35
Moonfish	4.22	4.41	4.03	0.15	3.55	4.50	4.21	0.09	2.07
Giant seaperch	13.13	13.22	13.05	0.07	0.53	13.11	12.92	0.06	0.46
Prawn	32.11	33.36	30.85	1.01	3.15	33.06	32.01	0.33	1.01

Table 4. Precision of alpha-tocopherol analysis

Sample	Mean alpha-tocopherol content (mg/g oil)	Repeatability (within-day precision) data of alpha-tocopherol (n=4)				Reproducibility (between-days precision) data of alpha-tocopherol (n=3)			
		Max	Min	SD	RSD (%)	Max	Min	SD	RSD (%)
Indian mackerel	258.71	259.20	258.48	0.33	0.13	258.59	258.48	0.06	0.02
Malabar red snapper	101.36	101.75	101.17	0.27	0.27	101.35	101.17	0.10	0.10
Moonfish	143.54	155.50	137.43	8.31	5.79	142.83	137.43	2.88	2.06
Giant seaperch	176.43	183.92	167.40	7.68	4.35	183.92	172.84	5.84	3.25
Prawn	812.09	909.67	745.04	7.21	0.88	820.26	745.04	3.80	0.49

long-tailed butterfly ray, large scale tongue sole, yellowstripe scad, and cuttlefish should be limited to less than 100 g a day. Moreover, consumption of fillet of longtail shad should be limited to less than 50 g a day.

Method validation

Method validation was performed for the HPLC analysis of cholesterol and alpha-tocopherol based on several tests, such as linearity, precision (repeatability and reproducibility), and recovery tests. Linear relationships were observed for the cholesterol and alpha-tocopherol standards, where the correlation coefficient (R^2) ranged between 0.998 and 0.999. The precision test was performed for within-day (repeatability) and between-days (reproducibility) variabilities. The RSD values for the cholesterol content determined in the fish and seafood samples are shown in Table 3. The results showed that, for repeatability test, the RSD values for the cholesterol content ranged between 0.53-3.55%. The RSD values for reproducibility test were 0.35-2.07%. The results of repeatability and reproducibility for the alpha-tocopherol content determined in the samples are depicted in Table 4. The RSD values of the alpha-tocopherol content were

0.13-5.79% for the repeatability test and 0.02-3.25% for the reproducibility test. However, the RSD values of the repeatability and reproducibility tests were low. The results revealed that the analytical method was reliable, where it was able to produce accurate results for the multiple determinations of cholesterol and alpha-tocopherol contents in the fish and other seafood.

Conclusion

Cholesterol contents were low in most of the fish and seafood samples, except for the few types of the samples. The alpha-tocopherol contents were high in some of the samples. These findings provide important and useful data for Malaysian communities in choosing the best fish or seafood that contain low level of cholesterol and rich in alpha-tocopherol. Regular consumption of fish and seafood caught from the Straits of Malacca is highly recommended as these fish and seafood are highly nutritious and contribute to health beneficial effect. The intake of high cholesterol-containing fish or seafood should be monitored, especially in people with high blood cholesterol or heart-related diseases. The validation tests also proved that the analytical method used were

of high accuracy and able to produce reliable data for determination of cholesterol and alpha-tocopherol in local fish and seafood.

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