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Effect of boiling and steaming on the profile fatty acids and cholesterol in muscle tissue of molluscs

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Abstract

Molluscs are consumed by many communities and believed to use as a reliable drug. This research was aimed to study the changes of proxymate composition, cholesterol, and fatty acid composition due to processing methods and to determine the best method resulting in the best quality product. The experiment consisted of preparation and characterization of raw materials, determination of salt concentration in boiling water (1%, 1.5%, 2%, 2.5%, dan 3%), and determination of the processing method (fresh, boiling, steaming, and boiled with salt). The processing method significantly affected ($\alpha = 0.05$) proxymate composition (moisture, protein, ash and fat) and fatty acid content (oleic acid, EPA, DHA), but insignificantly affected cholesterol content. The smallest reduction for: fat content occured in *C. javanica* after steaming (38.07%), oleic acid content in *Cerithide obtusa* after steaming(12.69%), EPA content in *P. Canaliculata* after steaming (3.70%), and DHA content in *P. Canaliculata* after steaming (2.10%) repsctively. The highest ratio of omega 3: omega 6 was found in the boiled *C. javanica* (2.14). The highest cholesterol content was found in fresh P.canaliculata (0.101 mg/100 g). The best treatment method for muscle heating was steaming, because it gave the lowest effect on proxymate composition and essential fatty acids.

Introduction

Snails in Indonesia were used as a source of food and medicine. Several species of snails were believed to be used as medicine, such as sea snail *(Cerithide obtusa)*, Asiatic clam *(Corbicula javanica)*, channeled apple snail *(Pomacea canaliculata)*. People often used a mollusc to cure wounds, liver, typhoid, fever, itching, and to increase stamina. According to Purwaningsih (2012), sea snail *(C. obtusa)* had strong antioxidant activity with IC_{50} value at 58.19 ppm. Utilization of snails is usually after processing, thus the processing of material will affect content of nutrients. Purwaningsih *et al.* (2011a) stated that the cooking process decreased mineral content in the Asian green mussel *(Perna viridis)*. Other studies conducted in sea snail by Purwaningsih *et al*. (2011b) showed that the boiling and steaming treatment decreased mineral content (calcium, phosphorus, potassium, iron and zinc). Research on the protein content in Fasciolaria salmo done by Purwaningsih *et al.* (2013) showed that the boiling and steaming treatment decreased protein content.

Marine biota contain saturated and unsaturated fatty acid compounds, particularly polyunsaturated fatty acids (PUFAs). Babu *et al.* (2010) showed that the dominant fatty acid of *Bursa spinosa* (A Mesogastropod from Tamil Nadu, Southeast coast of India) was SFA (38.73%) and most of which were C16:0 (22.37%) and C14:0 (9.4%), PUFA of 36.12% and MUFA contributed 4.31% of the total fatty acids. According to Freije & Awadh (2010), omega-3 PUFA, eicosapentaenoic acid (20:5) (EPA) and Docosahexaenoic acid (20:6) (DHA) play important roles against cardiovascular disease, as well as improvement of study skills, and body's immune system. Information about fatty acids and cholesterol contents, as well as the influence of processing treatments such as boiling, boiling in salt water, and steaming on a variety of snails are still limited. The purpose of the study is to determine the best heating treatment for molluscs with respect to changes in fatty acid, proximate composition and cholesterol contents during the process as well as edible portion and taste acceptability.

Materials and Methods

Materials research

The samples used in this study were sea snail *(Cerithide obtusa)*, asiatic clam *(Corbicula javanica)*, and channeled apple snail *(Pomacea canaliculata)*. Sea snail *(Cerithide obtusa)* was harvested from Kalipasir, Palembang, South Sumatra. Asiatic

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clam *(Corbicula javanica)*, and channeled apple snail *(Pomacea canaliculata)* were collected from Situ Gede, Bogor, West Java. These molluscs were washed under running tab water. Then, they were separated from shell and sliced. The slices were subsequently randomly devided into 4 groups. Three groups were cooked by each cooking methodes, and than analyzed.

Endible portion

Endible portion of molluscs was estimated by Nurjanah *et al*.(1996). The endible portion is the percentage ratio between the weight of the material parts that can be used with a total weight of material. Rendemen value is used to determine the economic value of a product or material.

Heat-treatments

The further stage was to determine the optimum salt concentration in the boiling treatment with salt (1%, 1,5%, 2%, 2.5%, dan 3%). The taste acceptability was evaluated using hedonic test (SNI-01-2346-2006). Test hedonic for taste parameters is done by 30 semi-trained panelists. The boiling treatment was performed at 100^0C for 8 minutes, while steaming was done at 100^0C for 10 minutes.

Chemical analysis

Chemical analysis which comprised proximate analysis (AOAC 2005), fatty acids profile analysis (Mondello *et al.* 2006), and cholesterol analysis (Sutharshiny and Sivashanthini 2011; Cook 1958), ratio of omega 3: omega 6 (WHO 2008) on fresh meat, boiled, steamed and boiled with salt meat were performed.

Preparation of fatty acids methyl ester was carried out according to the method of Mondello *et al*. (2006). Crude oil extract (20 µL) from snails samples were trans-esterified in a pyrex tube by using 200 µL of borontrifluoride-methanol (20% BF3) reagent and heating at 100°C for 30 min. After cooling, 200 µL of n-hexane and 800 µL of distilled water were added to the mixture, which was then agitated manually for 1 min and centrifuged for 2 min. Approximately 100 µL of the upper n-hexane layer was transferred to a 150 µL glass insert for 2 ml vials after diluting the extracted hexane to obtain a suitable chromatographic response. Fatty acids were identified by comparing the retention times of FAME mixture with the standard myristic acid palmitic acid, stearic acid, oleic acid, linoleic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and etc. Three replicate GC analyses were performed and the results were expressed in GC area % as mean values ± standard deviation. The fatty acid composition of snails oil triacyglyserol was directly analyzed using Gas Chromatography (GC) after methylesterification. One µL of each fatty acid methyl ester (FAME) sample was injected (split ratio 15:1) into a GC 17 A-SHIMADZU Gas Chromatography (Shimadzu Scientific Inc., USA) with flame ionization detector (GC-FID). A BPX 70 (SGE, Australia) column consisting of a 30 m x 0.32 mm fused silica capillary coated with 70 % cyanopropyl olysilphenylene-siloxane of 0.25 µm film thickness was used, with Hydrogen as the carrier gas at constant linear velocity (28 cm/s). The injector temperature was 250°C and the detector temperature 280°C. The oven was programmed as follows: 80°C for 2 min, 5°C/min to 200°C for 10 min and 10°C/min to 230°C for a further 10 min. Total analysis time was 49 min and the last major fatty acid (24:1 n-9) was eluted at approximately 30 min. Chromatographic peaks were identified by comparing retention times with the PUFA standard.

Cholesterol content of molluscs was estimated by the Sutharshiny and Sivashanthini (2011) method. Extracted lipids were treated with ferric chloride, acetic acid mixture and sulphuric acid and the colour developed was observed. After 20 min absorbance was read at 560 nm in a spectrophometer (LABOMED, UVD-3000). The absorbance readings were plotted in a calibration curve and the relevant cholesterol concentrations were computed.

Statistical analysis

The experimental design for the determination of optimal salt concentration used Kruskal-Wallis, while for the determination of the best processing method applied randomized complete block design, with three replications. If the ANOVA F test showed significant effects, then followed by Duncan's test.

Results and Discussions

Edible portion

Molluscs that live in water with constant change of current will grow better than do in coral or in the flowing waters (Suwignyo *et al*. 2005). The size and weight of molluscs were observed in this study (Table 1), and differences of those were influenced by their growth. The growth is affected by internal and external factors. The external factors are habitat, season, water temperature, type of food, and environment; while internal factors are age, size, gender, eating habits, and other biological factors.

Molluscs are a filter feeder animals, and that fact probably makes molluscs to have small edible

portion. Food particles and other particles could settle in the digestive tract (Turgeon 1988). The three types of snails had various edible portion. The highest edible portion was found in *Pomacea canaliculata* (21.84%), and the lowest was measured in *Corbicula javanica* (17.65%).

Determination of salt concentration

Hedonic test of mollusc muscles boiled with salt showed that the taste scores were in the range of 4.57 (neutral) to 6.20 (rather like). The Kruskal-Wallis test revealed that the salt concentration affected the taste acceptability of mollusc muscle. Multiple comparisson test indicated that the taste scores of mollusc muscle boiled with 2.5% salt was significantly higher compared to those of muscle prepared using 1%, 1.5% and 2% salt; but insignificantly different from the scores of muscle boiled with 3% salt. Based on statistical analysis, boiled muscle showing the most acceptable taste was prepared using 2.5% salt.

Proximate composition of the mollusc muscle

Moisture, ash, protein, and fat contents in molluscs muscle were presented in Table 2. The moisture content of fresh mollusc muscle ranged from 72.10 to 85.38% and became from 67.23 to 81.05% after treatment. That was because the concentration of fluid in the snail meat is more concentrated than the concentration of boiling water, so the water moves into the flesh snail.

Analysis of variance at 95% confidence level indicated that the processing influenced the moisture content of mollusc muscle. Duncan's test showed that the moisture content of fresh mollusc muscle was significantly higher than that of boiled, boiled with salt, and steamed mollusc muscle. This was similar to the results from a study conducted by Weber *et al.* (2008) that treatment with hot water reduced moisture content of silver catfish fillets.

Analysis of variance at 95% confidence level indicated that the processing influenced the protein content of mollusc muscle. The best processing methods is steaming, because the decreasing of protein is lower. Duncan's test revealed that the protein content of fresh mollusc muscle was significantly higher than the protein content of boiled mollusc musce with salt and steamed muscle mollusc. The highest decrease of protein content was found in *P.canaliculata* muscle boiled with salt (58.85%), and the lowest decrease was occured in steamed *C. obtusa* (21.48%). Protein from mollusc was unstable and could be denatured by the increasing of temperature. This is because the content of myofibrils protein of molluscs is high, the content of myofibril protein of fish about 66-77%. Myofibril proteins more soluble in salt water. This fact was supported by Selcuk *et al.* (2010) showing the protein content might change depend on the type of species and the processing methods. Unlusayin *et al*. (2010) showed that the protein content of fresh shrimp *Penaeus semisulcatus* was 83.81% (db) and then decreased to 79.15% (db) after boiling using salt .

Analysis of variance at 95% confidence level indicated that the heating process affected the fat content of mollusc muscle. Duncan's test exhibited that the fat content of fresh mollusc muscle was significantly higher than the fat content of boiled, boiled with salt, and steamed muscle mollusc. The highest decrease of fat content was found in boiled muscle of *P.canaliculata* in salt water (71.69%), and the lowest decreasing of fat content was occured in steaming treatment of *C. Javanica* muscle (38.07%). Temperature and heating period can affect the fat content of food. Decreased levels of fat was different by the Weber (2008) and Bakar (2008) method, because differences in materials and processing period. The processing with heating method could break down fat components into volatile products such as aldehydes, ketones, alcohols, acids, and hydrocarbons which would be evaporated up by heating.

The above finding was supported by study of Weber *et al.* (2008) demonstrating that the fat content of silver catfish (Rhamdia quelen) decreased by 6% after boiling. In addition, Bakar *et al.* (2008) showed that the fat content of the Scomberomorus guttatus was decreased by 5% after steaming.

Analysis of variance at 95% confidence level showed that the heating process influenced ash content of mollusc muscle. Duncan's test informed that the ash content of fresh mollusc muscle was lower than boiled with salt. The heating treatment decreased ash content. The lowest decrease of ash content was found in steamed muscle of *Cerithide obtusa* (13.08%). Boiling with salt water increased ash content, the highest increase was found in muscle of *C. javanica* (49.07%). This research was supported Unlusayin *et al.* (2010) which stated that the ash content of fresh shrimp *(Penaeus semisulcatus)* increased from 7.68% (db) to 9:40% (db) after being boiled in salt water.

Fatty acid profile of the mollusc muscle

Fatty acid composition of three species of molluscs and fatty acids content in molluscs after treatment were presented in Table 3. The SFA content of fresh *C. obtusa* ranged from 0.08 to 2.11%, *C. javanica* was ranged from 0.13 to 9.96%, and SFA

Table 1. Physical characteristics for each of mollusc

Mollusca Species	Length (cm)	Width (cm)	Thick (cm)	Weight (g)	Edible Portion (%)
Sea snail (*C. obtusa*)	4.53 ± 1.01	3.75 ± 0.35	1.73 ± 0.18	1.80 ± 0.13	19.69 ± 1.23
Asiatic clam (*C. javanica*)	2.07 ± 0.36	1.67 ± 0.28	0.99 ± 0.29	2.70 ± 0.34	17.65 ± 1.45
C. apple snail (*P.canaliculata*)	2.56 ± 0.19	1.84 ± 0.19	1.45 ± 0.13	4.54 ± 0.80	21.84 ± 1.08

Description: n:3

Table 2. The chemical composition of mollusc muscle

Mollusca Species	Treatment	Water (%)	Ash (%)	Fat (%)	Protein (%)
Sea snail (*C. obtusa*)	Fresh	72.10 ± 1.02^{d}	7.80 ± 0.87^{bc}	4.71± 0.78^{a}	62.72± 0.87^{b}
	Boiled	72.69 ±2.30^{d}	6.78± 1.02^{c}	1.81±0.56^{c}	45.66± 1.23^{d}
	Steamed	68.18± 1.90^{e}	6.56± 1.20^{c}	2.26± 0.43^{bc}	49.25± 0.88^{c}
	Boiled with salt	68.98± 3.01^{e}	11.11± 1.35^{a}	1.76± 0.42^{c}	44.05± 1.88^{d}
Asiatic clam (*C. javanica*)	Fresh	85.38± 0.26^{a}	5.83± 1.02^{cd}	4.99± 0.87^{a}	67.34± 0.63^{a}
	Boiled	80.90± 2.20^{b}	4.16± 0.78^{d}	2.83± 0.62^{b}	39.51± 1.44^{e}
	Steamed	81.05± 1.15^{b}	4.14± 0.89^{d}	3.09± 0.76^{b}	42.27±1.02^{de}
	Boiled with salt	80.90± 2.21^{b}	8.69± 1.13^{b}	1.98± 0.37^{c}	31.31± 1.48^{f}
Channeled apple snail (*P.canaliculata*)	Fresh	77.40± 0.73^{c}	5.44± 1.53^{cd}	4.38± 0.88^{a}	62.12± 0.50^{b}
	Boiled	67.23± 1.10^{ef}	4.40± 1.02^{d}	1.24± 0.85^{d}	33.11± 1.21^{f}
	Steamed	68.36± 1.2^{e}	4.00± 1.06^{d}	2.70± 0.75^{b}	37.01± 0.88^{e}
	Boiled with salt	67.23± 2.32^{ef}	6.67± 1.02^{c}	1.24± 0.67^{d}	25.56± 1.32^{g}

Description: db: dry basis ; n:3

content in fresh *P. canaliculata* was ranged from 0.13 to 5.75% of the total fat content. The content of MUFA for fresh *C. obtusa* was 0.11 to 11:19%, *C. javanica* was 0.23 to 3.78%, and MUFA content of fresh *P. canaliculata* was 0.33 to 6.44% of the total fat content. PUFA content of fresh *C. Obtusa* was 0.43 to 3.52% of the total fat content, *C. javanica* was 1.12 to 3.01% of the total fat content, and *P. canaliculata* was 2.01 to 6.67% of the total fat content. Therefore, overall FA composition depended primarily on the snail species, rather than the way of cooking.

The highest fatty acids content in fresh muscle of *Cerithide obtusa* was monounsaturated fatty acids (11.75%). This was diferent with Babu *et al.* (2010) showed that the largest fatty acid in *Bursa spinosa* (A Mesogastropod from Tamil Nadu, Southeast coast of India) was saturated fatty acids/ SFA (38.73%), The highest fatty acids in *Corbicula javanica* and *Pomacea canaliculata* were saturated fatty acids, and its value were 18.0% and 14.78%. Fatty acid content from snail depent on this habitats. Coelho *et al*. (2011) showed that Mud snails (H. Ulvae) colonizing the mudflat significantly higher levels of 20:1n-9 (eicosenoic acid), SFA, MUFA, and 20:4n-6 (arachidonic acid, AA) than the seagrass meadow. Both groups of unsaturated fatty acids exhibited statistically significant differences between habitats. Marine primary producers have characteristic fatty acids and these are transferred into the storage lipids of higher trophic organism with unchanged or recognizable forms (Dalsgaard *et al.* 2003; Shin *et al*. 2008). The variation of fatty acid composition depends on species, food availability, age, geographical area, season and salinity (Ozogul and Ozogul 2005; Ozyurt *et al*. 2006). The most dominant MUFA of all mollusc was oleic acid (C18: 1). Oleic acid content were 11.19% for *Cerithide obtusa*, 3.22% for *C. javanica*, and 6.44% for *Pomacea canaliculata*. Go *et al.* (2002) showed oleic acid content of marine gastropods were 13.09% for *Monodonta turbinata*, 10.62% for *Gibula cineraria*, and 13.16% for *Littorina neritoides*.

Results of analysis of variance at 95% (α=0.05) confidence level indicated that the processing method influenced oleic acid content of mollusc muscle. The results of Duncan's test showed that the oleic acid content of steamed mollusc muscle was different with mollusc muscle which was boiled and boiled with salt. Oleic acid content of snail decreased after processing, the lowest decrease of oleic acid content was found in steamed muscle of *Cerithide obtusa* (12.69%), and the highest one was *C. Javanica* (92.24%) after being boiled with salt. It was consistent with a study carried out by Weber *et al*. (2008) that oleic acid content of Silver catfish *(Rhamdia quelen)* decreased after boiling was performed at approximately 98 °C (water temperature) for 12 min. Marichamy *et al*. (2009) stated that the composition of fatty acids in

Table 3. Fatty acid composition of mollusc muscle

Fatty acid (%)	Fresh	Boiled	Steamed	Boiled with salt
Sea snails (*C. obtusa*)				
(C12:0)	0.08 ±0.01^{b}	0.04 ±0.00^{c}	0.03 ±0.00^{c}	0.04 ±0.00^{c}
(C14:0)	0.47 ±0.00^{e}	0.25 ±0.00^{e}	0.36 ±0.00^{e}	0.25 ±0.00^{e}
(C16:0)	1.15 ±0.02^{d}	0.28 ±0.00^{e}	0.21 ±0.00^{e}	0.28 ±0.00^{e}
(C18:0)	2.11 ±0.04^{c}	1.48 ±0.01^{d}	1.57 ±0.02^{d}	1.48 ±0.02^{d}
(C20:0)	0.16 ±0.00^{b}	0.15 ±0.00^{b}	0.14 ±0.00^{c}	0.15 ±0.00^{b}
(C22:0)	-	-	-	-
(C24:0)	0.60 ±0.01^{a}	0.52 ±0.00^{a}	0.34 ±0.00^{b}	0.52 ±0.00^{a}
∑ SFA	4.03	2.72	2.65	2.72
(C14:1)	0.11 ±0.00^{e}	0.15 ±0.00^{e}	0.19 ±0.00^{e}	0.10 ±0.00^{e}
(C16:1)	0.25 ±0.00^{e}	0.34 ±0.00^{e}	0.40 ±0.00^{d}	0.21 ±0.00^{e}
(C18:1)	11.19 ±0.02^{a}	8.92 ±0.02^{b}	9.77 ±0.02^{ab}	5.27 ± 0.01^{c}
(C20:1)	0.08 ±0.00^{d}	0.01 ±0.00^{e}	0.13 ±0.00^{d}	0.08 ±0.00^{e}
∑ MUFA	11.75	9.41	10.49	5.66
(C18:2n6)	3.36 ±0.02^{b}	1.35 ±0.01^{c}	1.52 ±0.01^{c}	0.79 ±0.01^{e}
(C18:3n3)	0.43 ±0.00^{c}	0.33 ±0.00^{c}	0.37 ±0.00^{c}	0.18 ±0.00^{c}
(C20:4n6)	3.52 ±0.03^{c}	2.99 ±0.02^{c}	3.49 ±0.02^{c}	2.19 ±0.02^{d}
∑ PUFA	7.31	4.67	5.38	3.16
EPA (C20:5n3)	2.96 ±0.03^{c}	1.11 ±0.01^{d}	0.89 ±0.00^{de}	0.41 ±0.00^{e}
DHA (C22:6n3)	2.38 ±0.04^{b}	1.60 ±0.02^{c}	2.27 ±0.02^{b}	1.40 ±0.01^{d}
EPA+DHA	5.34	2.71	3.16	1.81
Asiatic clam (*C. javanica*)				
(C12:0)	0.13 ±0.00^{a}	0.04 ±0.00^{c}	0.04 ±0.00^{c}	0.03±0.00^{c}
(C14:0)	1.99 ±0.02^{c}	1.61 ±0.01^{c}	1.27 ±0.01^{d}	1.40±0.00^{d}
(C16:0)	9.96 ±0.04^{a}	5.51 ±0.04^{c}	8.37 ±0.05^{a}	5.65±0.03^{c}
(C18:0)	2.49 ±0.04^{c}	0.93 ±0.02^{e}	1.51 ±0.01^{d}	1.17±0.01^{d}
(C20:0)	0.21 ±0.00^{a}	0.12 ±0.00^{c}	0.18 ±0.00^{a}	0.13±0.00^{c}
(C22:0)	-	-	-	-
(C24:0)	-	-	-	-
∑ SFA	14.78	8.21	12.37	8.38
(C14:1)	0.23 ±0.01^{d}	0.18 ±0.01^{e}	0.24 ±0.01^{d}	0.59 ±0.00^{c}
(C16:1)	3.78 ±0.07^{a}	2.58 ±0.04^{b}	3.73 ±0.04^{a}	3.30 ±0.04^{a}
(C18:1)	3.22 ±0.09^{d}	1.63 ±0.06^{e}	2.64 ±0.09^{d}	0.25 ±0.00^{f}
(C20:1)	1.03 ±0.04^{a}	0.39 ±0.01^{c}	0.67 ±0.02^{b}	0.88 ±0.00^{a}
∑ MUFA	8.36	4.78	7.28	5.45
(C18:2n6)	1.12 +0.02d	0.61 +0.01e	0.98 +0.03d	0.58 +0.00e
(C18:3n3)	2.32 +0.01a	1.27 +0.04b	2.01 +0.07a	1.23 +0.01b
(C20:4n6)	3.01 +0.06c	1.11 +0.05e	1.90 +0.05d	1.27 +0.02e
∑ PUFA	6.45	2.99	4.89	3.08
EPA (C20:5n3)	2.10 +0.04c	1.39 +0.01d	1.47 +0.01d	1.04+0.01d
DHA (C22:6N3)	1.72 +0.02c	1.00 +0.03d	1.44 +0.03d	1.15+0.02d
EPA+DHA	3.82	2.39	2.91	2.19
Channeled apple snail (*P. canaliculata*)				
(C12:0)	0.13 ±0.00^{a}	0.09 ±0.00^{b}	0.09 ±0.00^{b}	0.08 ±0.00^{b}
(C14:0)	2.84 ±0.01^{a}	2.24 ±0.00^{b}	2.50 ±0.01^{a}	1.86 ±0.01^{c}
(C16:0)	6.90 ±0.03^{b}	5.78 ±0.01^{c}	6.12 ±0.01^{b}	4.80 ±0.01^{c}
(C18:0)	6.19 ±0.03^{a}	4.45 ±0.03^{b}	4.14 ±0.02^{b}	3.77 ±0.02^{b}
(C20:0)	0.21 ±0.00^{a}	0.19 ±0.00^{a}	0.19 ±0.00^{a}	0.19 ±0.00^{a}
(C22:0)	1.26 ±0.00^{a}	0.60 ±0.00^{b}	1.11 ±0.00^{a}	0.87 ±0.00^{b}
(C24:0)	0.52 ±0.00^{a}	0.38 ±0.00^{b}	0.51 ±0.00^{a}	0.25 ±0.01^{c}
∑ SFA	18.05	14.04	15.68	11.93
(C14:1)	1.00 ±0.01^{a}	0.75 ±0.00^{b}	0.75 ±0.00^{b}	0.87 ±0.00^{a}
(C16:1)	0.88 ±0.01^{c}	0.81 ±0.01^{c}	0.59 ±0.00^{d}	0.79 ±0.01^{c}
(C18:1)	6.44 ±0.09^{c}	3.27 ±0.04^{d}	5.30 ±0.04^{c}	2.37 ±0.01^{de}
(C20:1)	0.33 ±0.00^{c}	0.32 ±0.00^{c}	0.25 ±0.00^{cd}	0.30 ±0.00^{c}
∑ MUFA	8.65	5.15	6.88	4.33
(C18:2n6)	6.67 ±0.05a	6.06 ±0.06a	6.27 ±0.01a	5.45 ±0.03a
(C18:3n3)	2.01 ±0.01a	1.15 ±0.02b	1.60 ±0.01b	1.47 ±0.01b
(C20:4n6)	5.48 ±0.03a	4.15 ±0.02b	4.75 ±0.03ab	4.04 ±0.04b
∑ PUFA	14.16	11.36	12.62	10.96
EPA (C20:5n3)	5.14 ±0.02a	3.51 ±0.05b	4.95 ±0.03a	2.76 ±0.02c
DHA (C22:6N3)	2.86 ±0.01a	2.34 ±0.02b	2.80 ±0.03a	2.37 ±0.03b
∑ EPA+DHA	8.00	5.84	7.75	5.13

Description: n:3

fish muscle changed after cooking process, depend on temperature, wide contact surface, size of fish, and the initial fat content.

Analysis of variance at 95% (α=0.05) confidence level indicated that the processing method affected EPA content of mollusc muscle. Duncan's test showed that EPA content of boiled and steamed mollusc muscle was significantly higher with mollusc muscle boiled with salt. EPA content of mollusc decreased after processing, the lowest reduction of EPA content was found in steamed muscle of *P. Canaliculata* (3.70%) and the highest one was shown by *Cerithide obtusa* (86.15%) after boiling with salt.

Sioen *et al.* (2006) stated that the cooking methods can bring about fat migration from the fillets during cooking. FFA content of the raw food was significantly reduced by all the cooking conditions. The loss of volatile FFA occurred during heating, leading to a decreased FFA content.

Results of analysis of variance at 95% (α=0.05) confidence level indicated that the processing method affected DHA content of mollusc muscle. The results of Duncan's test showed that the DHA content of mollusc muscle boiled and boiled with salt was different with steamed mollusc muscle. DHA content of mollusc decreased after processing, the lowest

Table 4. Ratio n3/n6 of mollusc muscle

Type	Fresh	Boiled	Steamed	Boiled with salt
Sea snail (*C.obtusa*)	0.84	0.68	0.71	0.67
Asiatic clam (*C.javanica*)	1.48	2.14	1.71	1.85
Channeled apple snail (*P.canaliculata*)	0.82	0.68	0.85	0.70

Description: n:3

Table 5. Cholesterol content of mollusc muscle (mg/100 gr)

Species/Type	Fresh	Boiled	Steamed	Boiled with salt
Sea snail (*C.obtusa*)	0.044 ± 0.002^{c}	0.040 ± 0.001^{c}	0.041 ± 0.001^{c}	0.033 ± 0.001^{c}
Asiatic clam (*C.javanica*)	0.079 ± 0.003^{b}	0.072 ± 0.001^{b}	0.073 ± 0.003^{b}	0.074 ± 0.002^{b}
Channeled apple snail (*P.canaliculata*)	0.101 ± 0.002^{a}	0.091 ± 0.002^{a}	0.091 ± 0.002^{a}	0.082 ± 0.002^{a}

Description: n:3

decrease of DHA content was encountered in steamed mollusc muscle of *P. Canaliculata* (2.10%) and the highest decrease was found in boiled *C. Javanica* (41,86%). Kolakowska *et al*. (2001) studied the effect of heating on fish lipids in sprat, herring and bream. They found decreased in DHA by 20% after 1 h heating at 100 °C; a 45% decrease after 15 min heating at 160 °C and a 70% loss after 1 h at 160°C. EPA under the same conditions reported losses of less than 20%. PUFA, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA), are considered to be susceptible for oxidation during heating and other culinary treatments (Sant'Ana & Mancini-Filho 2000). PUFA autooxidation is catalysed by heat, light, trace metals or enzymes, and involves in free radical generation (Zuta *et al*. 2007).

The highest ratio of omega3: omega-6 was found in boiled muscle of *C. javanica* (2.14) and the lowest one was found in boiled muscle of *P. canaliculata* and *C. obtusa* (0,68). The ratio of omega-3:omega-6 is presented on Table 4. WHO (2008) in OSU (2012) recommended the minimal ratio of omega-3: omega-6 was 0.35. The group of omega-3 were linolenat (C18:3 n-3), eicosapentaenoic acid /EPA (C20:5 n-3), and docosahexaenoic acid/DHA (C22:6 n-3); and group of omega-6 were linoleat (C18:2 n-6), Cis-8,13,14 eikosatrienoat (C20:4 n-6). The higher ratio of omega 3: omega 6, the better food material was provided .

Gladyshev *et al.* (2006) stated that the ratio of omega-3 to omega-6 fatty acids, the levels of C18:2 n-6, DHA and EPA content showed the greatest influence. The ratios ranged from 1.48:1 in raw King Salmon to 0.56:1 in deep fried King Salmon. According to Shirai *et al*. (2002), the n-3/n-6 ratio (0.3) of silver catfish was low when it compared to Japanese catfish (1), but similar to Thai catfish (0.2).

Cholesterol content of the mollusc muscle

Results of analysis of variance at 95% (α=0.05) confidence level indicated that the type of mollusc influenced the cholesterol content of mollusc muscle. The results of Duncan's test showed that cholesterol content of sea snail *(C. obtusa)* was significantly lower with Asiatic clam *(C. javanica)*, and channeled apple snail *(P. canaliculata)*. The highest cholesterol content was shown by fresh *P. canaliculata* (0.101 mg/100 gr) and the lowest was revealed by muscle of *C. obtusa* (0.033 mg/100 gr) after boiling with salt (Table 5).

The differences of cholesterol content was caused by several factors, such as species, food availability, age, sex, water temperature, geographic location, and season (Sampaio *et al.* 2006). Cholesterol content of P. canaliculata was higher than mixed clam (0.034 mg/100 gr), blue mussel (0.023 mg/100 gr), Japanese oyster (0.076 mg/100 gr), scallop (0.050 mg/100 gr), crab (0.053 mg/100 gr), tuna (0.050 mg/100 gr), and beef (0.054 mg/100 gr); but more lower than shrimp (0.132 mg/100 gr) and egg yolk (1.030 mg/100 gr) (Okuzumi and Fujii 2000).

Conclusion

The fatty acid profile showed that the highest fatty acid of *C. obtusa* (sea snail) was monounsaturated fatty acid (MUFA), but *C. javanica* and *P. canaliculata* (freshwater snail) had the highest saturated fatty acid (SFA). The processing method gave significant effects (α = 0.05) on the content of chemical components (moisture, protein, ash and fat); fatty acid profile (oleic acid, EPA, DHA); but did not affect the content of cholesterol. The chemical components and fatty acid content of snail were decreased after processing. The best treatment method was steaming, because it gave the lowest effect in decreasing of chemical

components and fatty acids.

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