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Effect of Various Storage Temperatures on Stability of Refined Sardine (*Sardinella* sp.) Oil Capsule

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Abstract: Oxidation level in fish oil was highly affected by storage temperature. This study aimed to evaluate the stability of sardine oil capsule at room (27-32°C), chilling (7-8°C) and freezing (-3°C) temperatures in addition to observe the degumming effect on its stability. Parameters used to determine the stability including free fatty acid value, peroxide value, p-Anisidine value, total oxidation value and fatty acid profile of sardine oil. The result showed that oxidation occurred during storage which marked by the increase of parameters' value. The degumming treatment and freezing storage temperature gave the best result to keep the stability of sardine oil capsule. The highest concentration of PUFA, EPA and DHA obtained from capsule-without-degumming (ND) and stored at freezing temperature were 39.78%, 15.85% and 18.31%. The highest concentration of PUFA produced by capsule-with-degumming (D) was 40.13% at freezing temperature, whereas the highest concentration of EPA and DHA were 14.27% and 16.46% respectively obtained by room temperature treatment.

Key words: Capsule · Sardine Oil Stability · Refining · Sardine Oil · Temperature

INTRODUCTION

Fish meal industry is one of main industry in fisheries sector which obtained crude fish oil as its by-product form. Total production of fish meal in Indonesia was 139.459 ton in 2014 [1]. Each ton sardine that made fish meal could produce 50 kg crude oil as a by-product [2] where with this number, crude oil has a potential to be develop as a raw material in fish oil industries.

Unsaturated fatty acid holds many advantages for human health. It prevents coronary heart, cancer, cholesterol increase and degenerative diseases [3]. Sardine contains high amount of unsaturated fatty acid especially omega 3. Sardine fish oil contained omega 3 of 29.09% [4], 26.02% [5], 22.45% [6] and 20.41% [7]. The high amount of unsaturated fatty acid in the sardine oil makes this product easily oxidized. Oxidation in fish oil can be affected by storage temperature, oxygen, water, pro-oxidant, antioxidant [8] and metal [4].

Temperature was one of important factor which affects the quality of fish oil. The optimum storage temperature is able to maintain the quality of fish oil by reducing its oxidation rate [9]. Fish oil purification process affects the quality of fish oil produced. Degumming process in fish oil purification improves the quality of fish oil by decreasing the number of free fatty acid (FFA), peroxide value (PV), p-anisidine and total oxidation (Totox) value [10]. Degumming is a process of removing impurities including phosphate, protein, carbohydrate, water and resin from crude fish oil.

The quality of fish oil after purification process should be kept stable during storage. Capsulation is one of any method commonly used to maintain the stability of sardine fish oil for a longer shelf life [11]. This study aimed to evaluate the stability of sardine oil capsule at room (27-32°C), chilling (7-8°C) and freezing (-3°C) temperatures in addition to observe the degumming effect on its stability.

MATERIALS AND METHODS

Materials and Equipment: The main material used in this study was crude sardine (*Sardinella* sp.,) oil. The sardine oil was obtained from fish meal industry in Bali, Indonesia. The materials used for the qualitative analysis were ethanol 96%, phenolphthalein indicator, KOH 0.1 N,

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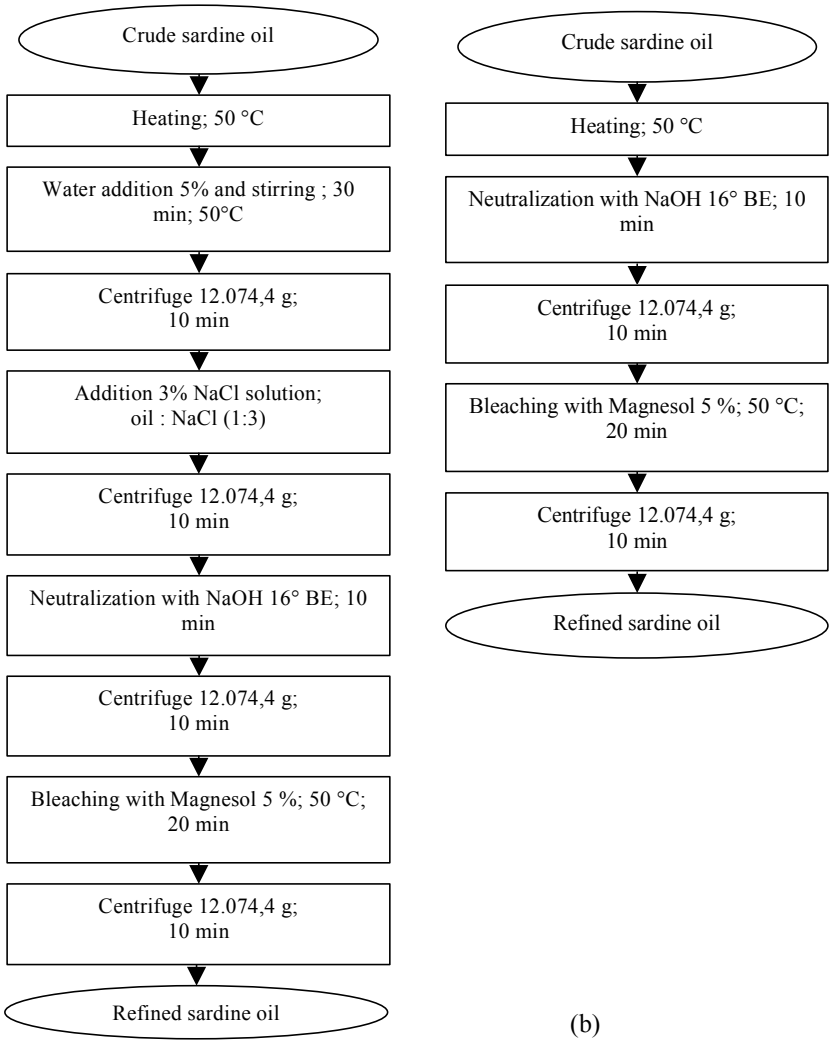

Fig. 1: Refining process flowchart of crude sardine oil with degumming (a) and without degumming (b)

chloroform, glacial acetic acid, saturated solution of KI, distilled water, starch solution 1%, $Na_2S_2O_3$ 0.1 N, isooctane, anisidine reagent and n-hexane. Tools that used were Erlenmeyer flask, aluminum foil, magnetic stirrer, magnetic stirring bar, stopwatch, digital scales, pipette, *water-bath*, electric stove, spectrophotometer UV-VIS (Agilent 8453), Centrifuge (Hitachi, Japan, R 10.80 cm, rotor type R21A) and *gas chromatography* (Shimadzu, Japan, GC2010 type, column dimension p=60 m, Ø=0.25 mm, 0.25 ⊚⊚m *film thickness*) for refining process.

Methods

Method of Refining Sardine Oil: There were two types refining methods used in this study; refining with degumming and refining without degumming process. The former method was carried out by weighing crude sardine oil needed. The oil then heated up to 50 °C for 15 min, subsequently added by 5% (v/v) of distilled water and stirred for another 15 min. The mixture then centrifuged for 10 min with centrifuge relative force of 12.074,4 g and the oil fraction was separated. Degumming process was performed adding 3% NaCl solution into the previously-separated-oil with ratio sardine oil: NaCl of 1:3. The mixture was stirred at 50°C for 30 min, centrifuged for 10 min (centrifugal force of 12.074,4 g) and separated for its oil fraction [16]. FFA value of sardine oil then measured to determine the amount of NaOH needed in neutralization step.

In neutralizing stage, NaOH solution was added into sardine oil and stirred at 50 °C for 20 min and centrifuged for 10 min; the oil fraction was separated. Further step was bleaching process by using 5% magnesol XL as an adsorbent. Magnesol was mixed with sardine oil by

stirring at 50°C for 10 min and centrifuged for 10 min and the oil fraction was separated.

In the latter method crude sardine oil only subjected to neutralization and bleaching process directly, without any degumming process. Flowchart of each refining method can be seen in Figure 1.

Capsulation Method: Encapsulation was done by dispensing of raw material (refined sardine oil), followed by preparation of gelatin mass and its capsule fill (add reference). Some test including density, viscosity, pH and appearance were performed to evaluate the quality of capsule fill.

Capsulated fish oil stored at room (27-32°C), chilling (7-8°C) and freezing (-3°C) and then followed by free fatty acid value (%) [12], peroxide value (meq/kg) [13], total oxidation value [14] and p-anisidine value (meq/kg) [15] analysis.

Fatty acid profile (AOAC 2005, method no. 969.33): A 20 mg of sardine oil was added by 1 mL NaOH (0.5 N in methanol) and heated up in water bath for 20 min. Then, 2 mL BF_3 solution (20%) and 5 mg/mL internal standard was added to the mixture and heated up for another 20 min. After cooled, a 2 mL saturated NaCl along with 1 mL isooctane were added to the mixture and shook well. The isooctane formed layer was pipetted into 0.1 g Na_2SO_4 anhydrate-filled tube and left for 15 min. Water phase formed was removed whilst oil phase was injected into GC instrument (1 iL) after a 1 iL FAME standard (Supelco 37 *component fatty acid methyl ester mix*) previously injected. N_2 and H_2 flow rate 20 and 30 mL/min with injector temperature of 200°C and 300°C respectively. Retention time and peak length for each component were measured and compared with standard retention time. Component quantity of the sample was calculated as follows.

$$\text{quantity of sample} = \frac{\frac{\text{V sample}}{100}\frac{\text{V sample}}{100}\times 100\%\ \text{Component}}{\frac{\text{Ax}}{\text{As}}\frac{\text{Ax}}{\text{As}}\times \text{C standard}} = \text{Sample weight}$$

where Ax = sample area; As = standard area; C standard = Standard concentration; V sample = sample volume

Statistical Analysis: All data obtained in this study was statistically analyzed by using descriptive analysis. Average value and standard deviation value of each stability parameter (FFA, PV, p-Anisidine dan Totox value) was determined in duplicate by using Microsoft Excel 2013 program.

RESULTS AND DISCUSSION

Oxidation parameters used to determine the stability of capsulated sardine oil in this study including FFA, PV, AnV, Totox and fatty acid profile. Analysis was conducted on crude oil, refined oil before capsulation and capsules during storage. The value of oxidation parameters of sardine oil can be seen in Table 1.

Table 1 showed that refined sardine oil without degumming and with degumming were appropriate to IFOS at all parameters, whereas crude sardine oil was not appropriate to IFOS standard in PV and FFA.

Free Fatty Acid (FFA): Referred to Figure 2, FFA values (%) of capsule with and without degumming that stored at room, chilling and freezing temperature was increasing during storage. FFA values of capsule with degumming (symbolized as D/ Degumming) and without degumming (symbolized as ND/ Not Degumming) which stored at freezing was lower than that of capsule stored at room and chilling temperatures.

The maximum standard of FFA value in fish oil by IFOS was 1.5% (Table 1). ND fish oil capsule stored at freezing temperature was appropriate to International Fish Oil Standard (IFOS) until the 5th day, while others remained under standard. D fish oil capsule stored at freezing temperature did not increase during storage until the 25th day. FFA values of D capsule stored at room were higher than that of chilling and freezing temperatures group. D capsule stored at freezing temperature has a lowest FFA value (1.03%) in the 30th day of storage. D capsule stored at room, chilling and freezing temperatures were appropriated to IFOS until the 30th day.

Refined catfish oil stored at 40°C for 4 weeks recorded FFA value of 2.32% [9]. Meanwhile for sardine oil combined with black seed oil (1:1) stored at 40 °C showed inappropriate FFA value on the 7th day of storage [17]. FFA values from these two studies were higher than that of current study on the 30th day of storage.

The FFA value of ND capsule was higher than that of D capsule. It happened due to the removal of impurities materials during degumming process [2] which led to the slower oxidation rate. FFA value in fish oil was also affected by temperature during storage [9]. Capsule sardine oil stored at freezing temperature has the lowest FFA value. This phenomenon implied that capsule stored

Table 1: Quality parameters of sardine oil before capsulated

	Sardine Fish Oil			
Parameters	Crude Sardine Oil[16]	Refined ND	Refined D[16]	*IFOS Allowable limit
FFA (%)	13.13±0.01	0.33±0.00	0.15±0.00	≤1.5
PV (meq/kg)	2.78±0.03	0.8±0.00	0.40±0.00	≤5.0
p-Anisidine (meq/kg)	9.48±0.01	3.62±0.07	1.43±0.02	≤20.0
Totox	15.03±0.19	5.22±0.03	2.22±0.01	≤26.0

ND = Refined sardine oil without degumming; D = Refined sardine oil with degumming
*International Fish Oil Standard (2011)

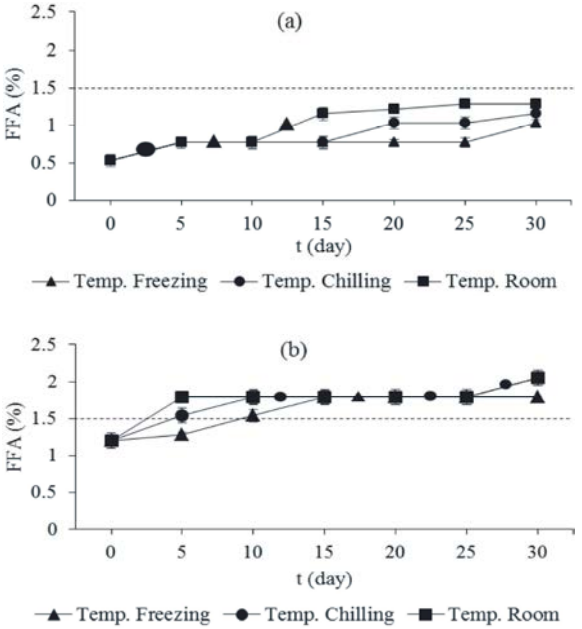

Fig. 2: FFA value of sardine oil capsule with (a) and without degumming (b)

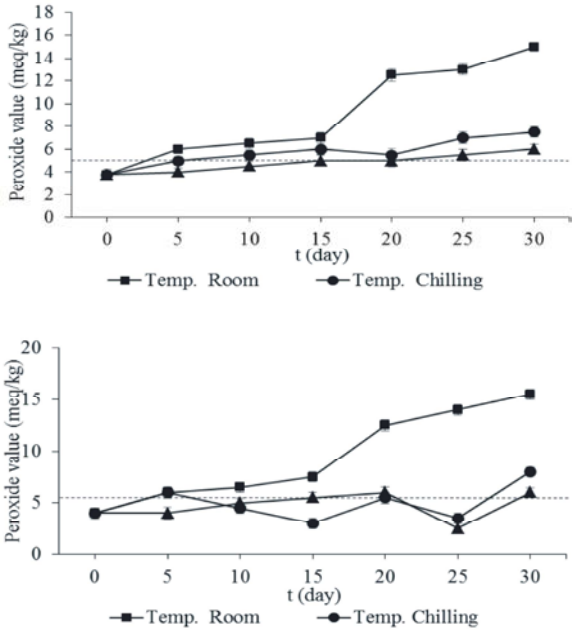

Fig. 3: Peroxide value of sardine oil capsule with (a) and without degumming (b) during storage

at freezing temperature was the best condition to keep the quality of fish oil. The lower temperature storage of fish oil the lower oxidation happened [11].

Peroxide Value (PV): The maximum standard of Peroxide in fish oil by IFOS was 5 meq/kg (Table 1). Figure 3 showed PV of D and ND sardine oil capsules. The PV values of D and ND capsules stored at room, chilling and freezing temperature were changes during storage. The PV of D capsule stored at freezing was lower than that of stored at room and chilling temperatures.

PV value of ND capsule was in coherence to IFOS limit on the 5th, 10th and 25th day for freezing temperature; on the 10th, 15th and 25th day chilling temperature and the 5th day for room temperature storage treatment. As for the ND capsule, PV value was appropriate to IFOS limit until 20th day for freezing temperature, 5th day for chilling temperature and around 3rd day for room temperature treatment.

PV of lemuru (*Sardinella lemuru*) oil was 11.14 meq/kg and 19.95 meq/kg stored at room temperature (27 °C); 10.33 meq/kg and 9.91 meq/kg at chilling temperature on the 15th and 30th day storage respectively [11]. Refined catfish oil stored at 40 °C showed PV of 7.27 meq/kg which inappropriate to IFOS standard on the 4th week of storage [9]. PV values from these two studies were higher than that of current study on the 30th day of storage.

According to Fig. 4, sardine capsules stored at freezing temperature showed the lowest PV than the other two groups (room and chilling). This phenomenon implied that freezing temperature had better performance in keeping PV compared to room or chilling temperature. The PV was in related to the oxidation happened in fish oil where peroxide formation was affected by temperature during its storage [18].

The PV was fluctuated during storage due to the peroxide decomposition into aldehyde and other derivatives. The higher number of peroxide in fish oil

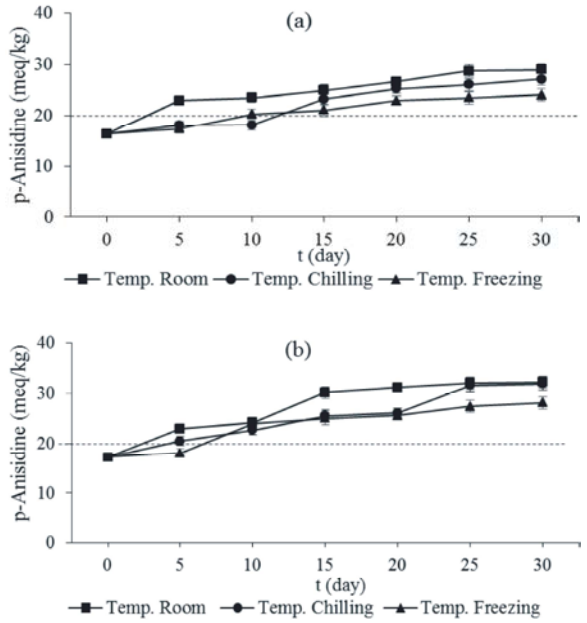

Fig. 4: p-Anisidine value of sardine oil capsule with (a) and without degumming (b) during storage

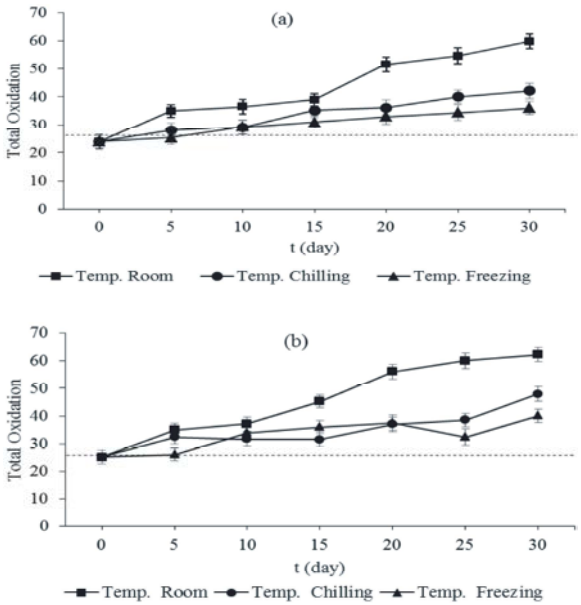

Fig. 5: Totox value of sardine oil capsule with (a) and without degumming (b) during storage

indicates the more oxidation take place. Nevertheless, the lower PV does not define a good condition of fish oil [12].

The PV of sardine oil capsule stored at freezing temperature was low. This condition was expected from a lesser contact between capsule and oxygen in freezing temperature. The higher storage temperature of fish oil caused more contact with oxygen [11]. The PV of ND capsule was higher than that of D group due to the impurities presence such as phosphatides, water, resin, protein and carbohydrate [2] and degumming process also able to decrease peroxide value in fish oil [10].

p-Anisidine Value (AnV): Figure 4 revealed that the p-Anisidine values of D and ND sardine oil capsule were increasing during storage. Both D and ND capsule stored at freezing temperature showed the best prevention from oxidation. The AnV of ND capsule stored at room and chilling temperatures excessed the allowable IFOS limit; in contrary to the capsule stored at freezing temperature (stable up to the 5th day). As for the D group, capsule that stored at chilling and freezing temperatures showed appropriate AnV with IFOS until the 10th day. D capsule stored at room temperature remained unacceptable since the 5th day.

The study of micro-encapsulated sardine oil revealed that AnV increased during 25 days of storage [19]. AnV of micro-encapsulated sardine oil stored at chilling temperature increased from 17.70 meq/kg (15th day) to 23.63 meq/kg (30th day) [11] which no longer suitable with IFOS allowable limit. Increasing AnV of ND capsules was in accordance to other similar study which stated that AnV of catfish oil increases during storage; from 5.09 meq/kg in the 1st week to 6.67 meq/kg in the 4th week [9]. However, AnV from current study was higher than that of study from catfish oil.

D and ND fish oil capsules stored at room temperature has a higher p-Anisidine value than that of chilling and freezing temperature groups. It happened due to the oxidation of fish oil which was affected by temperature. Lower storage temperature performs better quality of fish oil regarding to minimum contact between fish oil and oxygen [11].

D capsule showed lower p-Anisidine value compared to the ND capsule. Degumming process could decrease the p-Anisidine value in fish oil by removing impurities of fish oil during process [10].

Total Oxidation (Totox) Value: Figure 5 showed the Totox values of D and ND sardine oil capsule during storage. The Totox values of ND capsule stored at room and chilling temperature increased after the 0 day of storage. At freezing treatment, Totox value showed more stable result up to the 5th day storage and remained unstable for the other days. As for the D capsule, Totox values tend to increase at all temperature treatment. Totox value of D

Table 2: Fatty acids profile of sardine oil capsule without (ND) and with degumming (D)

		(% b/b)					
		Room		Chilling		Freezing	
Fatty acids (%)	Symbol	ND	D	ND	D	ND	D
Lauric acid	C12:0	0.08	0.07	0.08	0.07	0.08	0.07
Tridekanoic acid	C13:0	0.04	0.04	0.04	0.04	0.05	0.04
Myristic acid	C14:0	8.58	8.74	8.01	8.57	8.70	7.46
Pentadecanoic acid	C15:0	0.49	0.47	0.48	0.46	0.49	0.41
Palmitic acid	C16:0	16.51	15.98	15.75	16.41	16.94	14.97
Heptadecanoic acid	C17:0	0.59	0.67	0.55	0.56	0.61	0.51
Stearic acid	C18:0	3.03	3.40	3.08	3.35	3.15	3.30
Arachidic acid	C20:0	0.28	0.32	0.28	0.32	0.29	0.32
Heneicosanoic acid	C21:0	0.06	0.06	0.05	0.06	0.06	0.06
Behenic acid	C22:0	0.18	0.22	0.19	0.21	0.21	0.24
Tricosanoic acid	C23:0	0.04	0.05	0.04	0.04	0.04	0.04
Lignoseric acid	C24:0	0.13	0.16	0.14	0.18	0.15	0.16
Total SFA		30.01	30.18	28.69	30.27	30.77	27.58
Miristoleic acid	C14:1	0.03	0.02	0.02	0.02	0.02	0.02
Palmitoleic acid	C16:1	7.09	7.13	6.65	7.02	7.22	6.12
Elaidic acid	C18:1n9t	0.26	0.07	0.07	0.07	0.07	0.07
Oleic acid	C18:1n9c	4.50	5.85	5.18	5.87	4.63	6.94
Cis-11-eicosanoic acid	C20:1	2.21	1.57	1.57	1.61	2.34	1.40
Nervonic acid	C24:1	0.50	0.61	0.47	0.59	0.53	0.54
Total MUFA		14.59	15.25	13.96	15.18	14.81	15.09
Linolenic acid	C18:3n3	0.21	0.81	0.93	0.75	0.23	1.30
Linoleic acid	C18:2n6c	1.14	3.82	4.00	3.88	1.84	8.07
⍺-linolenic acid	C18:3n6	0.29	0.29	0.27	0.26	0.29	0.26
Linolelaidic acid,	C18:2n9t	0.18	0.05	0.08	0.05	0.08	0.06
Eicosedienoic acid	C20:2	0.15	0.16	0.15	0.16	0.16	0.17
Eicosetrienoic acid	C20:3n6	0.26	0.28	0.25	0.27	0.26	0.26
Arachidonic acid	C20:4n6	2.59	2.79	2.48	2.63	2.69	2.45
Dokosadienoic acid	C22:2	0.05	0.05	0.04	0.06	0.07	0.05
EPA	C20:5n3	15.40	14.27	14.59	13.77	15.85	12.67
DHA	C22:6n3	17.66	16.46	16.84	15.45	18.31	14.84
Total PUFA		37.93	38.98	39.63	37.28	39.78	40.13
Total Identified		82.53	84.41	82.28	82.73	85.36	82.80
Total Unidentified		17.47	15.59	17.72	17.27	14.64	17.2
PUFA/SFA		1.26/1	1.29/1	1.38/1	1.23/1	1.29/1	1.45/1

capsule stored at freezing temperature was appropriate to IFOS limit until the 5th, while the rest treatments remained inappropriate far before the 5th day.

Totox value of micro-encapsulated sardine oil stored at room temperature reached 24.76 in the 4th week of storage [20]. Totox value of refined sardine oil stored at 27°C was recorded as 22.32 (day 15th) and 31.95 (day 30th), as for the chilling (8°C) 20.7 (day 15th) and 19.86 (day 30th) [11]. The Totox value resulted from current study was higher than that of above mentioned studies.

To store D and ND fish oil capsule at room temperature resulted in a higher Totox values than that of chilling and freezing temperatures. Oxidation rate in fish oil was affected by temperature [8]. Despite of lower temperature, the presence of degumming process also contributed to the lower Totox value. Degumming process decreased Totox value of fish oil by removing its impurities [10] such as water, resin, phosphatides, protein and carbohydrate during process [2].

Fatty Acid Profile: Fatty acid profile of D and ND capsule can be seen in Table 2. According to the data, fatty acid profile of D and ND capsule were dominated by palmitic acid in SFA, palmitoleic acid and oleic acid in MUFA and eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) in PUFA. The highest PUFA of D and ND capsule were shown by freezing temperature treatment in the value of 40.13% and 39.78% respectively. Lemuru (*S. lemuru*) oil contained of 32.43% PUFA, 15.07% MUFA and 41.43% SFA [4]. Another study of sardine oil showed PUFA, EPA and DHA of 25.54%, 15.54% and 6.41% respectively [6].

Referred to Table 2, capsule stored at freezing temperature has a higher PUFA than that of stored at room and chilling temperatures. This condition was expected from the slower oxidation rate of sardine oil in freezing temperature. In concomitant to the lower number of FFA, PV and AnV, lower Totox value supporting the fact that freezing temperature provided better quality maintenance fish oil capsule. Oil or fat contained of lipase enzyme which able to hydrolyze triglycerides in which this enzyme activity was affected by temperature. Preserving fish oil in low temperature can reduce its enzyme activity so that fatty acid degradation potential could be reduced [21]. Lipase enzyme tends to be inactive in freezing temperature. Oxidation parameters (FFA, PV, AnV and Totox value) from present study showed that both D and ND capsules stored at freezing temperature experienced the lowest oxidation compared to those room and chilling temperature groups.

PUFA content was higher in D capsules compared to the ND group. It was in coherence to a study which stated that PUFA content increased along with impurities removal in fish oil. Refining process could remove impurities and residues (in fish oil) which led to the fatty acid decay [10].

In contra to the PUFA, EPA and DHA was higher in ND group than D group. It was expected from the oxidation against EPA and DHA throughout heating during degumming process. The heating process in degumming was 50 minutes in total (30 min. degumming by water and 20 min. by salt solution). EPA and DHA consisted of 5 and 6 double bonds which led to the easier and faster oxidation exposure [2] and heating process accelerated its oxidation.

PUFA/SFA ratio of ND and D capsules implied that PUFA content was higher than SFA for all temperature treatment; different from other study of lemuru oil which showed a higher SFA compared to the PUFA [4]. Fatty acid content in fish was affected by species, maturity, sex, season, feed, salinity and habitat temperature [22].

CONCLUSION

Degumming process resulted in high PUFA content of sardine oil capsules. Both D and ND capsules stored at freezing temperature (-3°C) experienced the lowest oxidation compared to room and chilling temperature groups. Degumming process combined with freezing storage temperature showed the best treatment to keep the stability of sardine oil capsule. The chosen capsules contained the highest PUFA content with PV, AnV and Totox value in accordance to IFOS standard up to the 5th day storage.

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