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Sardine (*Sardinella* Sp.) Oil Emulsion and its Stability during Storage

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Abstract: This study was aimed to make emulsion product of sardine oil, evaluate its stability against shaking and determine the best formula during storage. Sardine oil was purified by neutralizing 20°Be NaOH followed by bleaching process with 5% magnesol XL. The refined oil was then transformed into emulsion product in formulation of 5%, 10%, 15% sardine oil which combined by 0.5%, 1% and 1.5% whey protein. Emulsion's stability was evaluated by conducting shaking treatment with storage period of 21 days. The obtained refined oil contained 0.56% of FFA, 1.98 meq/kg of PV, 10.76 meq/kg of AnV with yield up to 75.64%. Dominant fatty acids were palmitic acid (17.28%), DHA (16.99%) and EPA (14.56%). Sardine oil emulsion was found stable against shaking treatment but decrease along with storage time. The best emulsion product was shown by treatment of 15% sardine oil combined with 0.5% whey protein where its viscosity, pH value and droplet size were recorded as 3671cP, 4.4 and 4.2µm respectively in the 18th day of storage at 27-30 °C.

Key words: Sardine Oil · Fatty Acid composition · Emulsion · Storage period

INTRODUCTION

Eicosapentaenoic (EPA) and *docosahexaenoic* (DHA) are dominant omega-3 compounds of fish oil [1] which hold important role for human body. Fish oil which was commonly produced from marine fish contained a high EPA and DHA due to their consumption against algae which comprised of these two compounds [2]. Omega-3 EPA was commonly found in lemuru (*Sardinella lemuru*), mackerel and shark (liver oil) with different amount up to 10.58%, 8.13% and 3.51% respectively [3]. In Indonesia, sardine (*Sardinella* sp.) is the most common source used for fish oil production.

Despite of many advantages for human health, in practice, fish oil could not dissolve in water and highly prone to oxidation. This oxidation's derivate compound caused unpleasant taste thus its application in food product extremely limited [4]. However, fish oil prepared in the emulsion form could easily be applied in food sector.

Protein and oil are considered as two main materials in emulsion system [5]. Whey protein has hydrophilic and hydrophobic properties that can form a fine protector film in oil droplets while decrease its surface tension [6,7]. At the same time, whey protein acts as proper antioxidant in material with oxidative properties.

There were no many studies found related to fish oil emulsion stability. A study of sardine oil emulsion which was produced by using lecithin and tween as emulsifier and carboxymethyl cellulose (CMC) as stabilizer showed a poor stability against oxidation [8]. The present study was aimed to prepare sardine (*Sardinella* sp.) oil emulsion with whey protein as emulsifier and guar gum as stabilizer to gain a better stability and longer shelf life of fish oil emulsion.

MATERIALS AND METHODS

Materials: Main material used in this study was crude sardine (*Sardinella* sp.) oil. Other materials used to measure sardine oil quality were etanol 96%, phenolphthalein indicator, potassium hydroxide (KOH), glacial acetic acid, chloroform ($CHCl_3$), saturated potassium iodide (KI), distilled water, sodium tiosulfat ($Na_2S_2O_3$) 0.1 N, starch indicator 1%, trimetil pentane (iso-octane), p-anisidine solution, natrium hydroxide

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(NaOH), magnesol XL (magnesium silicate hydrate), n-hexane and some materials for emulsion making including *whey protein*, guar gum, natrium benzoate and distilled water.

Sample Collection and Preparation: Ready-to-use sardine (*Sardinella* sp.) crude oil was in the form of by-product material collected from a fish processing plant in Bali, Indonesia. The samples were kept in the closed-dark glass bottle and stored at -4°C. The samples then subsequently thawed at room temperature prior to analysis. The quality of end product was determined according to the method of Permadi [8] for food and drugs including fatty acid (FA) composition (%), free fatty acid (FFA/%) value, peroxide value meq/kg (PV) and anisidine value (AnV). Appearance, odor and color evaluation [9] were also performed to determine the physical properties of raw materials.

Crude Oil Purification: Purification stage was divided into two steps namely neutralizing and bleaching process. In the former step, a precise amount (16.53 mL stated as *treat;*) of sodium hydroxide (0.143 N) was added into the crude oil. *Treat* value was calculated according to the following formulation

$$Treat = \frac{(0.142 \text{ x } \%FFA) + excess}{\%\ NaOG/100}$$

Treat (Σ NaOH added); 0.142 (constanta of NaOH at 20°Be); excess (excess amount of NaOH);%NaOH (NaOH concentration (w/v))

Crude oil was heated up to 50 °C and subsequently added by sodium hydroxide. The mixture was homogenized by using hot plate stirrer at 800 rpm for 10 mins [10]. Fish oil then centrifuged at 10,000 rpm for another 10 mins and the refined oil was separated. Bleaching process was performed by adding 8 gr magnesol XL (5% (w/w)) into refined oil [11]. The purified oil then subjected to some analysis such as FFA, PV, AV value, yield and physical properties evaluation including appearance, color and odor through organoleptic test [9].

Sardine Oil Emulsification: Sardine oil emulsification was done according to the method of [12] with some modifications. As many as 5%, 10%, 15% (w/w) refined oil from the previous process was combined with 0.5%, 1% and 1.5% (w/w) whey protein. Each formulation then added by 0.1% (w/w) sodium benzoate and 1.5% (w/w) guar gum which were considered as preservative and stabilizer agent respectively. A complete formulation's design of each treatment was shown by Table 1.

Sodium benzoate and whey protein were dispersed with distilled water and subsequently homogenized at 950 rpm for 1.5 mins. Guar gum was frequently added until the initial emulsion formed. Additional amount of sardine oil incorporated into the mixture and homogenized at 10000 rpm for 3 mins. Then, centrifugation test was conducted to evaluate emulsion's stability.

Emulsion Storage: Storage evaluation was performed to determine the emulsion stability of the chosen formula. The obtained emulsions were kept in the tightly closed container and stored at room temperature. Viscosity and pH value were measured in every 3 days for 21 days by using viscometer Brookfield TV-10. Viscosity measurement was performed by spining spindle 4 in emulsion at speed 100 rpm. Spindle 4 was inserted into emulsion and let it spin for 3-5 rotation until the result displayed on the screen [13]. The pH value was measured by dipping the sensor pen of pH meter (pen type pH meter, Gemmy pH-02) into the emulsion prepared at room temperatures (27-30 °C). As for the droplet size, measurement only conducted at 0 and 18th day of storage.

Chemical Properties Analysis of Emulsion: Obtained fish oil emulsion (before and after storage) was subjected to some analysis including fatty acid profile by using GC (Shimadzu Model GC 2010 Plus in column 19 *cyanopropil*

Table 1. Formulation and composition of sardine oil emulsions (%)

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Sardine oil	5	5	5	10	10	10	15	15	15
Whey protein	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Guar Gum	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Na. Benzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water	92.9	92.4	91.9	87.9	87.4	86.9	82.9	77.9	72.9
Total	100	100	100	100	100	100	100	100	100

methyl sil (*capilary column*), column dimension p = 60 m, Ø = 0,25 µm *film thickness*) [14], free fatty acid value (%) [15], peroxide value (meq/kg) [16], total oxidation value [17] and p-anisidine value [18] analysis.

Refined Oil Yield: The percentage of refined oil yielded was calculated by dividing amount of refined oil obtained (M2) to amount of crude oil before purification (M1). The calculation was done as following equation:

$$\text{Refined oil (\%)} = \frac{\text{M2M2M2M2M2}}{\text{M1M1M1M1M1}} \times 100$$

Emulsion Stability Test

Centrifuge Test: A 5 g emulsion sample was centrifuge at 3500rpm for 3 mins. Evaluation was performed by observed phase formed after centrifugation [12].

pH Value: pH value was measured by using pH meter (Gemmy pH-02) and measured at room temperature (27-30 °C) [13], which was calibrated prior to analysis. Measurement was done by dipping pH sensor directly to the sample prepared until a stabile value shown.

Viscosity: The relative flow time indicated emulsion viscosity was measured by using a Viscometer Brookfield TV-10 (TOKI SANGYO CO, LTD) equipped with spindle 4 at 100 rpm speed [19].

Droplet Size: Diameter size of emulsion droplets were observed by using a Primo Star (Zeiss) microscope equipped with camera (Axiocam ERc 5s). Sample was prepared by diluted emulsion with distilled water, pipetted the sample onto the object glass and observed it under microscope. Droplets under the same prepares were the measured [20]. Average diameter of droplet size was calculated as below.

$$\text{Average diameter} = \frac{\sum n.d \sum n.d \sum n.d \sum n.d \sum n.d}{\sum n \ \sum n \ \sum n \ \sum n \ \sum n}$$

where n = amount of the sample tested and d = diameter size of each droplet

Data Analysis: The experiment was run in duplicate with 3 x 3 factor combination. The data were analyzed descriptively by using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Refined Oil Characteristics: Sardine oil after refining process was having a clear brownish yellow color. Neutralizing and bleaching process improved color of fish oil due to the separation of degraded color at stacked fraction [21]. Magnesol XL used as bleaching agent in this experiment was able to improve fish oil color. Bleaching changed the ability of colored molecules in absorbed the light by changing unsaturation degree [22]. General characteristics of sardine oil before and after refining were shown in Table 2.

Fishy and rancid odor was caused by lipid oxidation in fatty acids. Hydrocarbon, furan, alcohols, aldehyde and ketone compound are formed by lipid oxidation [7]. In other study, 4-*Z*-heptenal, 2,6,-*E*-,*Z* nonadienal and 3,6-*E*,*E*-nonadienal compounds were reported as an important contributor in resulting undesired odor in fish oil [23].

High FFA value in unrefined sardine oil was likely expected from a high moisture content which caused hydrolysis. FFA forming could be led by hydrolysis process [24]. As for the high peroxide value, this happened due to excessive oxidation. Increasing temperature will increase peroxide value [25]. Factors such as handling, storage temperature, light exposure and metal contamination affected lipid oxidation [26].

Oxidation parameters such as FFA, PV and AV were decreased after refining process. Fish oil refining with alkali (NaOH) technically was a reaction form of fatty acids saponification. The quantity and concentration of alkali used was determined according to estimation of FFA contained in the raw sample. Saponified and unsaponified fraction was separated so that FFA in the sample decreased [27]. As for the PV, decreasing value likely possible due to the most peroxide from oxidation was absorbed by saponified fraction [28].

Yield of sardine oil was separated in centrifuge step after neutralizing and bleaching process. A high yield of refined oil was caused by low non-oil fraction included in saponified fraction after neutralizing [29]. The lower non-oil fraction content in fish oil implied that sample was still in a good condition without excessive oxidation.

Apart from yield, appearance and odor, other parameters including FFA, FA profile, PV and AV were also evaluated. According to FA profile result, the refined oil contained of 31.99% of saturated fatty acid (SFA), 15.38% of monounsaturated fatty acid (MUFA) and 36.71% of polyunsaturated fatty acid (PUFA). Dominant

Table 2: Physical properties of sardine oil

Parameter	Before refining	After refining	Decrement (%)	Standard
Appearance	Unclear brown	Yellowish brown	-	-
Odor	Fishy odor	Fishy odor		
FFA (%)	7.56±0,37	0.56±0,18	92.59	<1,13*
PV (meq/kg)	9.96±5,56	1.98±0,71	80.12	<5**
AnV (meq/kg)	25.27±4,35	10.76±1,41	57.41	<20**
Yield (%)	-	75.64	24.36	-

*International Fish Oil Standard (2011)

**International Fish Oil Standard (2014)

Table 3: Fatty acids composition of refined sardine oil

Fatty acid	Symbol	Amount (%b/b)
Lauric acid	C12:0	0.07
Tridecanoic acid	C13:0	0.04
Miristic acid	C14:0	9.43
Pentadecanoic acid	C15:0	0.49
Palmitic acid	C16:0	17.28
Heptadecanoic acid	C17:0	0.61
Stearic acid	C18:0	3.46
Arachidate acid	C20:0	0.32
Heneicosanoic acid	C21:0	0.06
Behenate acid	C22:0	0.20
Tricosanoic acid	C23:0	0.03
ΣSFA		31.99
Miristoleic acid	C14:1	0.02
Palmitoleic acid	C16:1	7.49
Cis-10-Heptadecanoic acid	C17:1	0.60
Elaidate acid	C18:1 ω9t	0.07
Oleic acid	C18:1ω9c	5.03
Cis-11-Eicosanoic acid	C20:1	1.55
Nervonic acid	C24:1	0.62
ΣMUFA		15.38
Linolelaidic acid	C18:2ω9t	0.05
Linoleic acid	C18:2ω6c	1.21
□-Linolenic acid	C18:3ω6	0.25
Linolenic acid	C18:3ω3	0.49
Cis-11,14-Eicosedienoic acid	C20:2	0.15
Cis-8,11,14 Eicosetrienoic acid	C20:3ω6	0.28
Arachidonic acid	C20:4ω6	2.68
Cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5ω3	14.56
Cis-13,16-Docosadienoic acid	C22:2	0.05
Cis-4,7,10,13,16,19-Docosahexaenoic acid,	C22:6ω3	16.99
ΣPUFA		36.71
□ ω6		3.21
□ ω3		32.04
Identified		84.08
Unidentified		15.92

Fas in the current study were recorded as palmitic acid (17.28%), DHA (16.99%) and EPA (14.56%) (Table 3). Other studies reported that the DHA and EPA contents from *lemuru* fish (*Sardinella lemuru*) were 10.14% and 7.31%, respectively [11] and from sardine (*Sardinella* sp.) were 17.48% and 8.03%, respectively [30]. The differences of omega-3 fatty acid amounts among obtained samples were expected as result of different feed consumption, genetic and environment factors. Generally, the differences in fatty acids composition between fish oils were caused by feeding habits, species, gender, sexual maturity, size, fishing location, temperature and seasonal condition [31].

Sardine Oil Emulsion

Phase Separation after Centrifuging: Centrifuge was conducted after emulsion preparation in order to evaluate its stability against shaking. Emulsion instability was possibly observed during centrifugation step which was indicated by phase separation in the sample. In the present study there was no phase separation found in the sample after centrifugation. This result implied that all formula studied were stable to shaking treatment.

Whey protein and guar gum were considered as effective emulsifier agents in obtaining a stable fish oil emulsion. This was likely caused by the ability of both emulsifiers at increasing the interface layer thickness which created a stable emulsion system. Interface layer thickness in an emulsion system affected its stability [32]. This thickness could resist the interaction between metal ion and hyper-peroxide lipid which was located next to the interface layer. In principal, interaction strength in emulsion system was defined as forces obtained from two adjacent droplets; steric force (repulsive) and van derwaals force power (attractive). This repulsion power among droplets refused coalescence which triggered phase separation [13].

Whey protein was commonly used as a protein emulsifier in food due to its hydrophobic and hydrophilic properties which adsorbed water and oil rapidly to form protector film. This film can structurally support the oil fraction through combination of electrostatic and steric interaction [6]. Protein as emulsifier was able to facilitate o/w emulsion transformation while increase its stability.

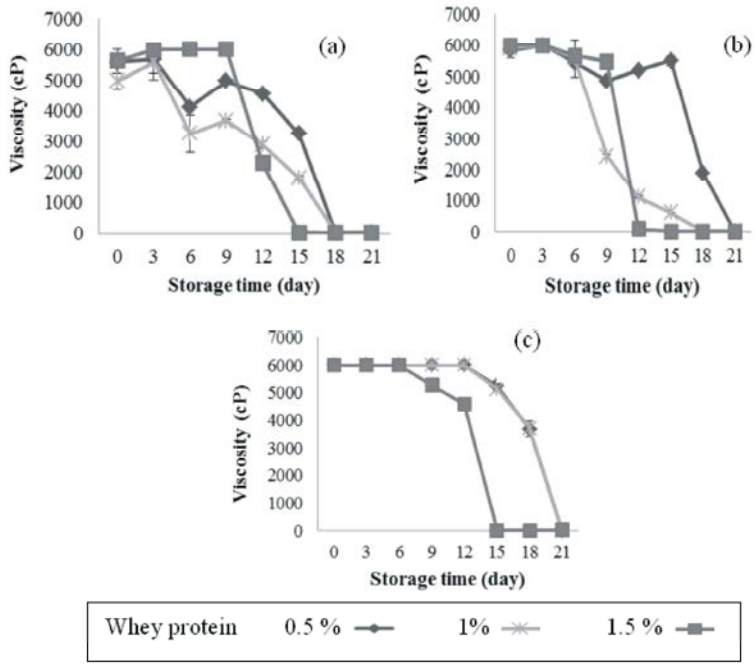

Fig. 1: Emulsion viscosity during storage; oil (a) 5% (b) 10% (c) 15%

Homogenization process helped protein in absorbing oil drop so that lowering surface tension and inhibit droplets to reunite by forming protector membrane around oil drops [7].

Emulsion Stability during Storage: Emulsion stability was evaluated subjectively and expressed as shelf life of the product. The stability of sardine oil was determined by looking after the viscosity, pH value and droplets size during storage.

Viscosity: The result showed that viscosity value of the sample increased along with increasing oil concentration; 5%, 10% and 15% respectively. Viscosity difference between samples tested was related to different droplets formed in the emulsion system. The more oil added to the sample, the more increased droplets formed. Emulsion prepared from 15% sardine oil was more stable compared to the other two; 5% and 10%. This formula showed emulsion stability up to the 12nd day of storage. Emulsion's viscosity values during storage can be seen in Fig. 1.

In this study, whey protein of 0.5% in 15% sardine oil was effective in obtaining a stabile emulsion system up to 18th day. Emulsion with a low whey protein concentration was allegedly able to completely adsorb the oil drops thus producing a high stability of emulsion. The ability of an emulsifier in adsorbing oil droplets was profoundly related to the available droplets amount in the emulsion system., The more oil drop adsorbed by emulsifier used which led to droplets increment. As the result, the viscosity of emulsion increased due to the attraction force amongst droplets.

Emulsion viscosity was also likely affected by the droplet's size. Small droplet gained through homogenization could increase the dispersed phase thus increasing the viscosity [33]. The high viscosity value the more stable emulsion system formed [34] since the droplet become more resistant to be dispersed in the outside phase. On the other side, guar gum as stabilizer also enhanced the emulsion viscosity which led to the high stability. Viscosity increment of emulsion could decrease oxygen diffusion coefficient. A high viscosity in an emulsion system could possibly decrease the diffusion of metal and other reactant thus inhibited the oxidation rate [7].

The fluctuation of viscosity value during storage showed that there were some changes in the emulsion system. These changes could be triggered by creaming, flocculation, coalescence and phase separation. This phase separation caused internal and external phase separated so that viscosity value decreased.

Emulsion with 5% sardine oil was containing the largest continuous phase (aqueous) due to the fewer droplet formed. This condition induced the dissolved oxidation initiator penetration to enter the emulsion

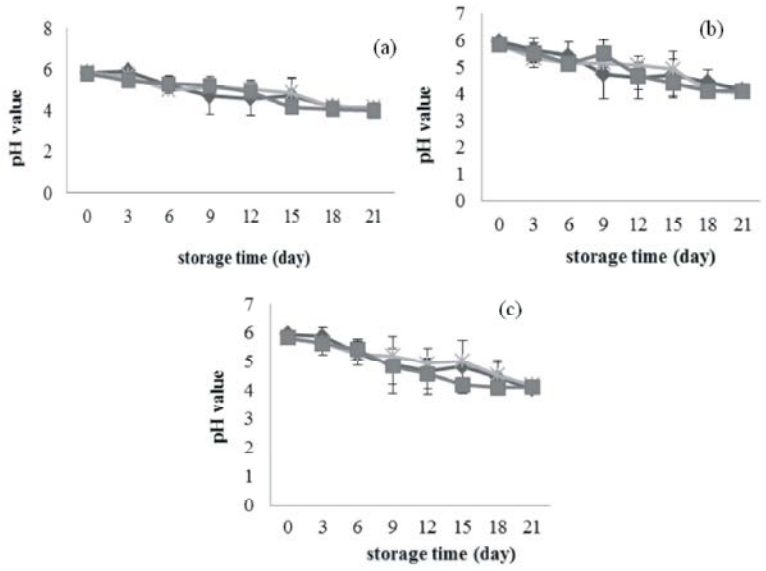

Fig. 2:

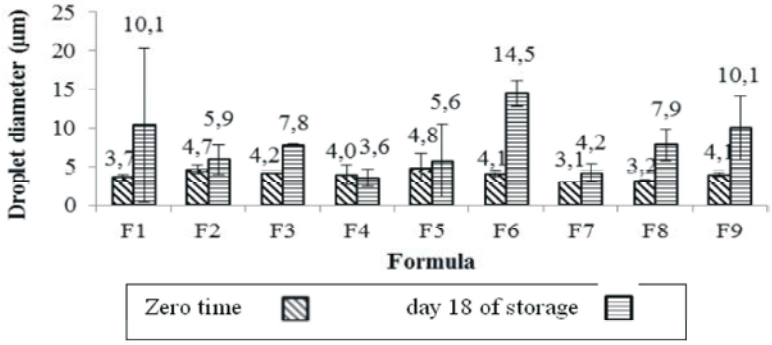

Fig. 3: Emulsion's droplet size

system which raised oxidation [34]. Oxidation affected fatty acid hydrolysis which caused emulsion instability and led to phase separation followed by viscosity decline.

pH Value: pH value ranged between 5.78 and 5.95 with declining trend during storage. The stability of pH was shown in Fig. 2. The decreasing trend of pH value during storage indicated that obtaining sardine oil emulsion was not in a good pH stability condition. The decrement was caused by fatty acids from fish oil added. When fatty acid content increased in the system, the large H^+ ion dissociated and this was responsible to the lower pH value of the obtaining emulsion.

A low pH value in the emulsion system could accelerate lipid oxidation process which caused pH stability decrease. A high pH value decreased lipid oxidation rate [7]. pH value decline in emulsion system were generally caused by fat hydrolysis, oxidation, light exposure and microorganism growth [35].

Droplet Size: Droplet size was evaluated at zero time and after 18 day of storage at room temperature (27-30°C). The average droplet size before storage (zero time) ranged between 3.1 and 4.7µm (Fig. 3). The smallest droplet size showed by emulsion with 15% sardine oil and 0.5% whey protein (F7), meanwhile the biggest one produced by 5% sardine oil combined with 0.5 and 1% whey protein (F2). The average droplet size of emulsion ranged between 0.1-100µm [36] and 0.5-50µm [35]. Small droplet size indicated a proper homogenization process. Homogenizing helped to distribute oil in the emulsion system. Intensity and homogenizing time depend on the required time to dissolve and distribute lipid globules in emulsion [34].

Droplet size of a dispersants was an important factor in evaluating physical stability of emulsion. Generally, a droplet with smaller size indicated a more stable emulsion product [37]. In accordance to Stokes law, droplets with

small diameter tend to separate in a slower way compared to the bigger one [38].

The droplet size change after storage indicated a damage to the emulsion system namely coalescence. This happened through small droplet unification to form a bigger droplet due to the collision between droplets caused by interface layer depletion [39].

CONCLUSION

Sardine oil emulsion stability did not changed against mixing but decreased along with storage period at room temperature. Viscosity, pH value and droplet size were changing during storage. Emulsion with 15% fish oil and 0.5% whey protein was found as the most stable formula up to 18th day of storage. Viscosity, pH value and droplet size of the chosen formula was 3671cP, 4.4 and 4.2µm respectively. Optimizing fish oil homogenization was profoundly suggested to obtain more stable emulsion. For further studies, extreme temperature changing during storage was also recommended to evaluate fish oil emulsion stability in various temperature conditions.

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