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THE EFFECT OF CITRIC ACID AND SODIUM CHLORIDE (NACL) TO QUALITY OF SARDINE OIL (*Sardinella Sp.*)

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ABSTRACT

The amount of sardine oil as by-product from a fish-meal processing industry is large and still contains high amount of omega-3 , but its quality is still for feed. Purification of fish oil is very important to improve the quality of fish oils for human consumption. This study aimed to determine citric acid and Sodium chloride (NaCl) treatment in the degumming process to improve the quality of fish oil. The Citric acid and NaCl used were 0.2% and 40%, respectively. The results showed that reduction of peroxide value, free fatty acid, p-ansidine, and TOTOX were 72.42-92.37%, 77.95-85.83%, 5.43-51.90%, and 49.59-76.99% respectively while transmission percentage range was 48.61-63.63%.

Keywords: Citric acid, degumming, NaCl, sardine oil.

INTRODUCTION

Sardine (*Sardinella* sp.) has a great potential as a source of fish oil (5-20%) and omega-3 (Suseno *et al*. 2014). Sardine is included to high fatty fish with a various fat content, especially in polyunsaturated fatty acids (PUFAs). These unsaturated fatty acids are highly susceptible to oxidation. Sardine oil which usually found in Indonesia is a by-product of fish-meal industry. Yunizal (2002) recorded that in 1996 the number of sardine oils as a by-product from fish processing industry was about 4,300 tons. The abundant amount of sardine oil by-product had not been optimized well, whereas this oil by-product has potential as omega-3 source. Suseno *et al.* 2014a; Suseno *et al.* 2014b; and Suseno *et al.* 2013b stated that omega-3 content of sardine oil by-product reached 23.34%b/b, 22.40%b/b, 28.81% b/b, respectively. Suseno *et al*. (2011) stated that the quality of fish oil by-product of fish-meal industry is for feed. Most of inventoried sardine oil had free fatty acid (FFA) content, peroxide value (PV), p-anisidine value (PAV), and total oxidation (TOTOX) value exceeded the International Fish Oil Standard (IFOS) (2011). Fish oil purification is very important to improve the quality of fish oils for human consumption. Purification is expected to increase the value added of fish oil as a by-product of fish-meal industry. The crude fish oil is a mixture of several compounds such as glycerides, free fatty acids, phospholipids, sterol, tocopherols, pigments, toxic substances and phosphatides (Cheryan 1998). Therefore, the purpose of oil purification is to remove impurities such as non-triglyceride compounds, colorants, flavor, and toxic substances to produce high-quality edible oils for human consumption.

The conventional fish oil purification practiced in the industry is usually chemical based. The chemical purification method involves the distinct stages such as degumming, neutralizing, bleaching, and deodorization. Degumming is able to remove phosphatides. Removal of phosphatides before alkali refining is important (Sharma *et al*. 1985). There are two types of phosphatides: hydratable (HPL) and nonhydratable (NHPL). Most of the phosphatides in crude fish oil can be removed by water degumming, but NHPL is not hydrated by water. Removing NHPL requires more complex process at increased temperature with the use of citric acid or other degumming substances. Citric acid is used not only for decomposition of metal salt but also as a chelating agent to keep the metals in water-soluble complex. In addition, Sodium chloride (NaCl) represents one of the chemicals which is able to dissociate the gum. Syakiroh (2012) stated that the addition of NaCl solution in degumming process is able to separate phosphatides, saturated fatty acids, and gum. This study aimed to determine citric acid and NaCl treatment in degumming process to improve quality of fish oil.

MATERIAL AND METHODS

Materials and equipments: The main material used in this study was crude sardine oil obtained from fish-meal industry in Bali, Indonesia. Furtermore, analysis material used were 96% ethanol, phenolphthalein indicator, Potassium hydroxide (KOH) 0.1N, chloroform ($CHCl_3$), acetic acid glacial (CH_3COOH), Potassium iodide (KI), aquades, 1% starch, Sodium thiosulfate ($Na_2S_2O_3$) 0.1N, isooctane (C_8H_{18}), p-anisidine (C_7H_9NO), Hexane (C_6H_{14}), Sodium hydroxide (NaOH), and Sodium chloride (NaCl). Some equipments used were were Erlenmeyer, beaker glasses, mohr pipette, digital scale burettes, UV-Vis spectrophotometer (Agilent 8453), water bath, magnetic stirrer, magnetic stirring bar, and high speed refrigerated centrifuge.

Fish oil purification: The degumming process was carried out by three treatments. The first treatment was degumming with 40% NaCl, the second treatment was degumming with 40% NaCl and 0.2% citric acid and the third treatment was degumming with 0.2% citric acid. Degumming was carried out by stirring the mixture continuously using magnetic stirrer for 15 minutes at several temperatures (50°C, 60°C, and 70°C), then the oil was separated by centrifugation at 10,000rpm for 10 minutes at a temperature of 10°C (Stansby 1981). Degummed oil then neutralized. Neutralization using NaOH was carried out by stirring the mixture continuously using magne tic stirrer for 15 minutes at several temperatures (50°C, 60°C, and 70°C), then a mixture was separated by centrifugation at 10,000 rpm for 10 minutes at a temperature of 10°C. After neutralization step, sardine oil then bleached with 5% Magnesol XL (Suseno *et al.* 2011). Bleaching was carried out by stirring the mixture continuously using magnetic stirrer for 15 minutes at several temperatures (50°C, 60°C, and 70°C), then a mixture was separated by centrifugation at 10,000 rpm for 10 minutes at a temperature of 10°C. The physicochemical properties of the fish oil samples were analyzed before and after purification.

Analysis of fish oil quality: The quality of fish oil was determined by several parameters. Determination the yield of fish oil after purification, free fatty acid (FFA) content (AOCS 1998) based on oleic acid molecular weight, peroxide value (PV) (AOAC 2000), p-anisidine value (PAV) (IUPAC 1987), total oxidation (TOTOX) (AOCS 1997) and oil clarity test (AOAC 1995).

RESULT AND DISCUSSION

Fish oil yield after purification: Fish oil which produced by fish-meal industry has lower quality from canned fish industry. The initial analysis showed that crude sardine oil contained FFA 2.54 %, PV 11.93 meq/kg, , PAV 11.60 meq/kg, TOTOX 35.47 meq/kg, and oil clarity ranged from 25.84-90.59%. The quality of crude sardine oil was not in accordance with IFOS. Fish oil with a high peroxide value indicated that the oil was not processed and stored well and caused oxidation. High level of free fatty acid may be due to the thermal process and high water content which allowed faster hydrolysis of fish oil (Irianto 2009). The raw material of fish-meal generally obtained from rejected fish which was not fresh.

The yield values of fish oil after purification can be seen in Table 1. The results showed that the highest fish oil yield was obtained by the treatment of citric acid at 60°C, while the lowest yield values obtained by the treatment of NaCl and citric acid at 70°C. Fish oil contained some impurities, such as free fatty acids, hydrocarbon, ketones, proteins, pigments, and phospholipid. Phospholipids caused many problems for the storage and processing of the crude oil and removed from oil during purification by a degumming process (Brekke 1975). There are two types of phospholipids: hydratable (HPL) and nonhydratable (NHPL), and they are removed from oil by degumming process. Most of the phospholipids in crude fish oil can be removed by the water degumming, but NHPL is not hydrated by water. Removing NHPL required more complex process at increased temperature with the utilization of citric acid or other degumming substances. Citric acid is used not only for decomposition of metal salt but also as a chelating agent to keep the metals in water-soluble complex.

Table -1: Yield of fish oil after purification

Treatments	Temperatures	Fish oil yield (%)
NaCl	50°C	54.43±0.60
	60°C	56.09±0.91
	70°C	54.66±1.27
NaCl + citric acid	50°C	55.43±1.23
	60°C	56.43±1.33
	70°C	53.09±1.09
Citric acid	50°C	54.86±1.54
	60°C	58.57±1.03
	70°C	53.23±1.51

Peroxide value: Oxidation of the fish oil made rancidity and flavors higher. An important stage in the oxidation is the addition of oxygen to the fatty acid molecules to form hydroperoxides, the amount of hydroperoxides can be used as measure -ment of the extent of oxidation in the early stages. Peroxide value indicates high amount of oil has been oxidized, but at a lower rate is not always indicate that the oxidation state is still early. Low peroxide value may be due to formation rate of new peroxide, smaller than the rate of degradation into other compounds, given the levels of peroxide degrades quickly and react with other substances (Raharjo 2008). Peroxide value in sardine oil after purification can be seen in Figure 1.

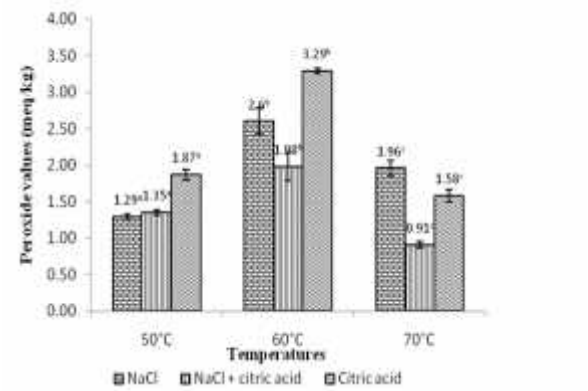

Figure 1. Peroxide value of sardine oil after purification

Sardine oil before purification contained PV 11.93 meq/kg. Figure 1 showed that all treatments effectively reduced peroxide value. The lowest peroxide value of fish oil shown in treatment NaCl and citric acid at 70°C. The result showed that fish oil from all treatments had peroxide value which was in accordance with the IFOMA (3-20 meq/kg) and IFOS (3.75 meq/kg for maximum limit). Citric acid is commonly used in vegetable oils as a metal chelator. Moreover, citric acid binds the metal ions that can otherwise contribute to rancidity as they catalyze free radical oxidation of lipids (Eastern Chemical Company 2003). The result showed a correlation between the efficiency of the citric acid as an antioxidant and the temperature which used in this study. The range of temperatures from 50-70°C was included in low temperature. The relative stability of the peroxide values at the lower temperatures is indicated of the efficiency of the citric acid when used as antioxidant for fish oil at low temperature. Akaranta and Akaho (2012) stated that citric acid and peanut skin extract that used as antioxidants at low temperature (60-80°C) caused the peroxide values in vegetable oil more stable than at high temperature (150-180°C). The high peroxide values at high temperatures indicated the inefficiency of the antioxidants at such temperatures.

Free fatty acid: Formation of free fatty acids is due to the hydrolysis and oxidation of oil caused by the presence of free radicals and decomposition of the double bond during heating (Paul dan Mittal 1997). FFA content in the crude oil depends on the fish species and season. Free fatty acids may constitute up to 12% of total lipids, but typical values are lower (Bimbo 1998). Free fatty acid levels tended to decrease after getting treatment of purification. It can be seen in Figure 2.

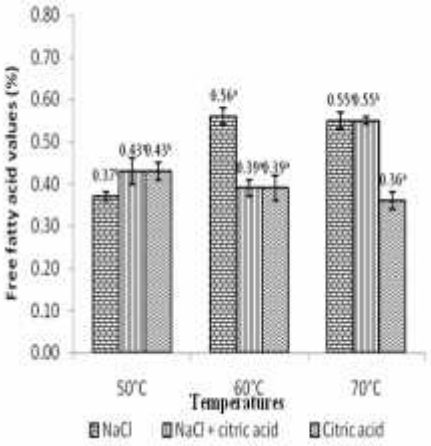

Figure -2: Free fatty acid value of sardine oil after purification

The free fatty acid value of sardine oil before purification was 2.54%. Figure 2 showed that the lowest value of free fatty acid was found in the treatment of citric acid at 70°C. The results showed that fish oil from all treatments had FFA content in accordance with the IFOMA (1-7%) and IFOS (1.13%) standards. All treatments effectively reduce FFA content. Common processes known to remove impurities in crude fish oil are degumming,deacidification or neutralization, bleaching, and deodorization. Degumming is designed to remove phospholipids and mucilaginous material, while deacidification is performed to remove free fatty acids (Subroto 2015).

p-Anisidine value: P-anisidine test is a method commonly used as a measure of the level of secondary oxidation products (carbonyl com-pounds). P-anisidine value reduction occurred after the puri-

fication. The result of p-anisidine value analysis can be seen in Figure 3.

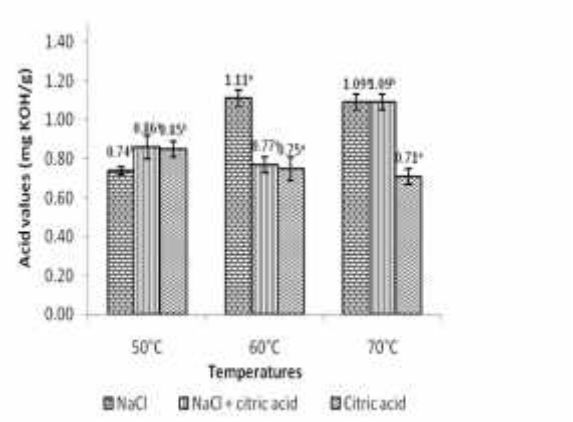

Figure-3: Acid value of sardine oil after purification

The highest p-anisidine value found in crude sample (11.60 meq/kg). The lowest p-anisidine value obtained by the treatment of NaCl at 50°C. P-anisidine values obtained from all treatments were still in the standard specified by IFOMA, it must be ranged from 4-60 meq/kg (IFOMA 1981). Fish oil from all treatments had anisidine values which were still below the maximum amount of anisidine values set by IFOS. Maximum value for anisidine according to the IFOS is 15 meq/kg[3]. Guillen and Cabo (2002) stated that p-anisidine value is not always in line with the high value of peroxide, but the high value of peroxide can cause high value of p-anisidine if the process given allowing fish oil to go further degradation.

Total oxidation: Totox value is the relationship of primary and secondary oxidation obtained by summing twice of peroxide value (2PV) and anisidine value (PAV) (Perrin 1996). Total oxidation value of each treatment can be seen in Figure 4.

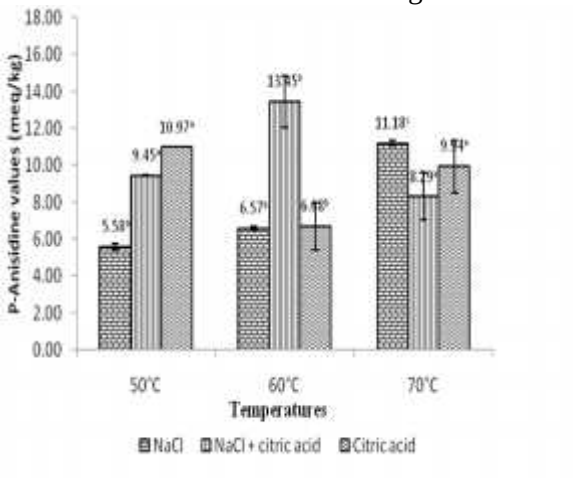

Figure-4: P-anisidine value of sardine oil after purification

Total oxidation value of sardine oil before purification was 35.47 meq/kg. Figure 5 showed that the lowest value of total oxidation obtained in the treatment of NaCl at 50°C. Fish oil from all treatments had total oxidation value which is in accordance with the IFOMA and IFOS standard. IFOMA standard assigned total oxidation for fish oil was at range of 10-60 meg/kg (IFOMA 1981). Maximum value for total oxidation according to IFOS is 20 meq/kg (IFOS 2011).

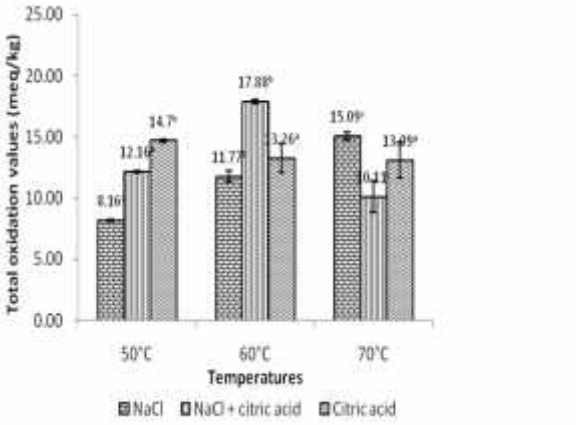

Figure 5. Total oxidation value of sardine oil after purification

Clarity value: Measurement of fish oil clarity performed at 3 wavelengths (450 nm, 550 nm, and 620 nm). Primary and secondary oxidation products will affect the color and turbidity of fish oil. If there is a higher content of primary and secondary oxidation products in fish oil, the appearance of the observed fish oil will be dark and level of clarity tends to decrease (Estiasih 2009). The clarity values of fish oil after purification can be seen in Table 2.

Table 2 clarity value of fish oil

Methods	Temperatures	% transmission		
		450 nm	550 nm	620 nm
Crude sardine oils	-	25.84±0.74	53.46±0.83	64.28±1.00
NaCl	50°C	68.38±0.85	88.32 ± 0.99	87.29 ± 0.96
	60°C	50.28 ± 1.57	75.69 ± 1.33	75.93 ± 0.96
	70°C	68.90 ± 1.09	90.04 ± 1.31	92.94 ±1.91
NaCl + citric acid	50°C	66.49 ± 1.51	93.64 ± 2.17	94.67 ± 2.21
	60°C	54.93 ± 1.05	75.95 ± 1.76	75.54 ± 1.36
	70°C	52.16 ± 1.04	73.21 ± 0.99	73.08 ± 1.96
Citric acid	50°C	42.09 ± 1,11	60.93 ± 1,25	60.95 ± 1,28
	60°C	54.01 ± 1,05	82.37 ± 1,28	86.80 ± 1,82
	70°C	71.05 ± 1,85	98.02 ± 1,41	96.07 ± 1,79

Table 2 showed that all treatments were able to increase the percentage of light transmission to the fish oil at various wavelengths tested. The highest percentage of light transmission of fish oil obtained in the treatment of citric acid at 70°C. Budiadnyani *et al*. (2015) stated that clarity value was increasing because of the elimination of non oil fraction and dark color causes of tuna meal processing's by-product oil. During refining using phosphoric acid and NaOH, heme structure would be damaged because Fe atom would dissolve by alkali. The red color intensity in fish oil was related with heme structure which was in myo-globin and hemoglobin protein (Sathivel *et al*. 2003). Decreasing red color intensity in fish oil caused the yellow color increase.

CONCLUSION

Crude sardine oil which produced by fish-meal industry had low quality. It can be concluded that purification can obtain a better quality. Citric acid and NaCl were used in degumming process. Reduction of peroxide value, free fatty acid, p-an sidine, and TOTOX were 72.42-92.37%, 77.95-85.83%, 5.43-51.90% and 49.59-76.99% respec-tively. Transmission percentage increased from 48.61-63.63%.

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