EFFECTS OF NEUTRALIZATION PERIOD AND TEMPERATURE IN ALKALI REFINING ON THE QUALITY OF SARDINE OIL

Hari Eko Irianto*), Sri Ningrum Pudjiati**), Pipih Suptijah***), Sri Purwaningsih***) and Rosmawati Peranginangin*)

Abstract

Most fish meal waste oil encountered in Indonesia has high free fatty acid (FFA) value, dark colour and strong fishy odour. Thus, the oil needs refining process for the quality improvement before further uses, such as by alkali refining application. This study investigated the effects of neutralization period and temperature during refining. The neutralization periods studied were 15, 30 and 45 minutes, and the neutralization temperatures observed were 60, 70 and 80°C respectively. Results showed that alkali refining was very effective to be used for reducing the FFA and absorbance values as well as improving sensory properties. Alkali refining insignificantly affected iodine and saponifiable values of the oil. With regard to the refined fish oil quality and stability, the suggested neutralization period and temperature were 15 minutes and 60°C respectively.

KEYWORDS: Alkali refining, neutralization period, neutralization temperature, sardine oil

Introduction

Fish oil in Indonesia is found as by-products of both canned fish and fish meal industries. Thus, the quality of fish oil encountered in the market varied chemically and physically as shown by Irianto (1995). In fact the fish oil quality is not only affected by processing practices but also by raw material qualities. All fish meal factories belonging to canneries also use fish wastes for raw material, such as heads, tails and guts.

In terms of fish oil origin, the fish oil resulted in fish meal processing is worse than the one obtained from canned fish factories. Fish meal waste oil has higher free fatty acid (FFA) values, darker and stronger fishy odour. Those facts indicate that the fish meal waste oil should be improved its properties before further uses, particularly for human consumption purposes.

One of the common methods applied for oil quality improvement is alkali refining which has been widely used in the production of vegetable oils. Alkali

*) Researcher at the Slipi Research Station of Research Institute for Marine Fisheries

**) Graduate Student of the Faculty of Fisheries, Bogor Agricultural University, Indonesia

***) Lecturer at the Faculty of Fisheries, Bogor Agricultural University, Indonesia

treatment is normally carried out after degumming by pouring caustic soda into the hot oil to neutralize free fatty acid and solubilize phospholipids, nitrogen, sulphur containing compounds and some pigments (Young 1982). The minimum amount of alkali required for neutralization can be calculated from the FFA value of the oil to be refined using the formula: %NaOH = %FFA x 0.142 (Gunstone and Norris 1983).

The objective of this study was to determine the effective condition of alkali neutralization by investigating the optimum neutralization period and temperature. The fish oil quality and stability resulted from tested neutralization conditions were also determined.

Materials and Methods

Fish Oil

Sardine oil used in this study was purchased from a fish oil collector in Muncar, East Java. The sardine oil was a by-product of fish meal processing wich used oil sardine (*Sardinella lemuru*) as raw material. The oil was packed in 25 ml-black plastic containers and then transported to the Slipi Research Station for Marine Fisheries, Jakarta.

Fish Oil Refining

Fish oil was refined through several steps, i.e. degumming, neutralization and bleaching. FFA content of fish oil was determined before refining.

Degumming was carried out by adding 80 ml 28% NaCl solution into 200 ml fish oil at 70°C. The fish oil was stirred during 15 minutes degumming period.

The degummed oil was then neutralized using 1N NaOH solution. The volume of neutralizing solution added was determined according to the FFA value of the oil using a formula described by Gunstone and Norris (1983). The neutralization temperature and time varied depending on the treatments. The neutralization periods studied were 15, 30 and 45 minutes. While, the temperature levels investigated were 60, 70 and 80°C. The mixture of oil and NaOH solution were stirred through the neutralization process.

The above mixture was centrifuged at 20°C and 10.000 rpm for 15 minutes. The oil fraction was separated from soap and water fractions. After that the oil was washed with warm water to liberate remaining soap and then centrifuged at the same condition as previous centrifugation. The oil fraction was separated again for further process.

The final step of fish oil refining was a bleaching process which was by mixing the oil with bentonite as much as 6% of fish oil weight. Bleaching was conducted at 60°C for 20 minutes. Bentonite was removed by centrifugation at 20°C and 10.000 rpm (20°C) for 15 minutes.

Stability Study of Fish Oil

Stability levels of fish oil were studied using Schaal oven method. The fish oil samples, each 150 ml contained in 250 ml beakers, and covered loosely with aluminum foil. The samples were stored in an oven at $\pm$63°C and withdrawn after 0, 3, 6, 9 and 12 days.

Quality Analysis of Fish Oil

The quality of refined fish oils were determined chemically, physically and organoleptically. Chemical analyses included FFA content (Fernandez 1986), iodine and peroxide values (Apriyantono *et al.* 1989), thiobarbituric acid (TBA) value (Fioriti *et al.* 1974) and saponifiable value (Sudarmadji *et al.* 1989). Physical analyses covered only absorbance value (wave length = 490 nm) measured using Spectronic 21. Descriptive and preference tests were carried out to determine the sensory properties of fish oil.

Data Analysis

Data were analyzed by analysis of variance (ANOVA) (Gaspersz 1991) to determine differences among the treatments. When F-values significant, honestly significant differences (HSD) were determined. Friedman test was used to analyze sensory data (Steel and Torrie 1980).

Results and Discussion

Quality of Refined Fish Oil

Quality analyses revealed that the crude oil had 2.71% (oleic acid) FFA content, 12.99 meq/kg peroxide value, 0.30 μmol malonaldehyde/g TBA value and 106.60 g/100g iodine value.

Results of experiment showed that refining process significantly improved fish oil quality indicated by pronounced decrease in FFA and absorbance values as well as better colour (Table 1).

The FFA value reduced from 2.711% to 0.229-0.306%. Refining also induced colour changes from brown to light yellow which was more attractive. Colour improvement was also exhibited by the sharp reduction of absorbance values

Table 1. Chemical and physical properties of refined and crude oils

Refined Oil		FFA Value	Iodine value	Peroxide value	Saponifiable value	Colour Absorbance	Colour
Neutralization Period (minutes)	Neutralization Temperature (°C)	(% oleic acid)	(g/100g)	(meq/kg)	(mg/g)	(590 nm)	
15	60	0.253	105.9	7.99	201.5	0.102	- light yellow
	70	0.229	106.1	10.99	196.8	0.108	- light yellow
	80	0.286	106.3	12.99	198.0	0.104	- light yellow
30	60	0.275	105.5	6.99	201.2	0.104	- light yellow
	70	0.235	105.5	9.49	198.6	0.105	- light yellow
	80	0.290	105.5	10.99	198.6	0.106	- light yellow
45	60	0.253	105.0	9.44	198.9	0.109	- light yellow
	70	0.299	105.8	11.49	198.3	0.138	- light yellow
	80	0.306	106.3	12.99	198.6	0.113	- light yellow
Crude oil		2.711	106.6	12.99	202.1	3.615	- brown

from 3.615 to 0.102-0.138. Most of those improvements probably occurred during neutralization process using NaOH as mentioned by Hendrix (1993) that caustic soda is very effective to be used in alkali refining to reduce FFA, phosphatides, metal ions, pigments, carbohydrate, protein, oxidation by-products and solid materials contents in crude oils. In terms of colour, further improvement was enhanced in bleaching process using bentonite as a pigment adsorber. Tested neutralization period and temperature ranges did not induce significant differences to both FFA and absorbance values of refined fish oils.

Iodine and saponifiable values were insignificantly affected by refining. This result revealed that oxidation did not severely attack unsaturated fatty acids of the fish oil during refining, in which the double bond numbers exhibited by iodine values were not pronouncedly affected. Meanwhile, the refining process, particularly neutralization treatment, did not apparently influence molecule weights of the oils reflected by insignificant change of saponifiable values.

In general, peroxide value of fish oil decreased after refining. The longer neutralization period resulted in the higher peroxide value. Obvious decreases were noted by the oil neutralized at 60°C. Many theories have been developed to explain hydroperoxide behaviour in the heating treatment of fat. The formation and destruction of hydroperoxide are extremely rapid at high temperature (Nawar 1985), since hydroperoxide are readily decomposed thermally (Hiatt and Irwin 1968). The decrease of peroxide value might be due to the decomposition of hydroperoxide into secondary products of oxidation. A similar result was noted by Irianto (1992) in the investigation of the effect of sterilization on the stabilization of fish oil.

Stability of Refined Fish Oils

Refined oils receiving various neutralization treatments during the process underwent stability study to investigate whether those treatments affected their stability or not. Parameters used to measure the stability were peroxide, TBA and absorbance values as well as sensory properties. In general, the results explained that refined oils were less stable than crude oil. Refining process may have induced the losses of natural antioxidant, particularly tocopherol, which then reduced the stability level of the oil. The occurrence of natural antioxidant losses during refining process have been reported for some oils, such as cod liver and sprat oils (Brzeska and Salmonowicz 1973) and menhaden oil (Scott and Catshaw 1991).

Chemical changes

Peroxide values of all tested fish oil increased during investigation (Table 2). Similar occurrences were also observed in the storage of groundnut oil (Narasimhan *et al.* 1986), canola oil (Hawrysh *et al.* 1989) and sardine oil (Irianto 1994). The increase was mainly due to the production of hydroperoxide as a result of oxidation process. The increase rate of peroxide value in refined oil was significantly higher than that in crude oil. The loss of natural anti-oxidant during refining may have enhanced oxidation process in refined fish oil.

Table 2. Peroxide value of refined and crude oils during stability study

Refined oil		**Storage Period at $\pm$63°C (days)**					
Neutralization Period (minutes)	**Neutralization Temperature (°C)**	0	3	6	9	12	15
15	60	7.99	18.98	40.95	48.45	95.89	127.86
	70	10.99	23.47	41.95	57.93	115.87	152.83
	80	12.99	24.97	42.95	60.93	119.86	170.81
30	60	6.99	18.98	36.96	46.95	103.88	156.82
	70	9.49	18.65	42.95	52.94	115.87	171.81
	80	0.99	25.97	44.45	71.92	124.86	225.78
45	60	9.44	22.33	33.96	47.45	85.90	116.37
	70	11.49	22.48	35.46	47.65	110.87	206.77
	80	12.99	22.62	35.96	62.93	125.86	249.72
Crude oil		12.99	19.98	21.98	24.97	37.96	43.95

Analyses of variance showed that only neutralization period obviously affected peroxide values of the oil during stability study. While Tukey test exhibited that peroxide values of fish oil neutralized at 60°C was significantly lower than those of the oil neutralized at 80°C. Peroxide values of the oil neutralized at both 60 and 70°C were insignicantly different. Those results showed that the refined oil obtained from the neutralization at 60°C was more stable than others.

Table 3 shows the TBA value changes, in which TBA value indicates the content of malonaldehyde forming as a secondary product of oxidation. All oils displayed an increasing trend of TBA value during storage and the increase rate in refined oil was faster than in crude oil. Malonaldehyde type compounds can react with amino acids, peptides and other compounds released from decomposition of protein (Kwon *et al.* 1965; Finley 1985). Malonaldehyde can cross link protein through a *Schiff* base reaction with the ϵ-NH_2 group of lysine (Belitz and Grosch 1987; Finley 1985; Gillat *et al.* 1988). These reactions may affect the increase rate of TBA values.

Analysis of variance revealed that TBA values of fish oil during storage were affected by neutralization period. Tukey test informed that neutralization at 70°C produced refined oil having insignificant different TBA value compared to the one obtained from the process applying neutralization at both 60° and 80°C. However, the TBA values of fish oil from the neutralization at 60°C were apparently higher than those from the neutralization at 80°C.

Table 3. TBA value of refined and crude oils during stability study

Refined Oil		Storage Period at ±63°C (days)					
Neutralization Period (minutes)	Neutralization Temperature (°C)	0	3	6	9	12	15
15	60	1.70	5.02	9.91	10.72	10.76	10.79
	70	0.74	7.06	9.35	9.79	10.69	10.33
	80	2.22	3.88	8.87	9.53	10.63	10.93
30	60	1.30	3.18	7.93	8.65	11.05	12.02
	70	1.36	3.45	8.07	9.67	10.83	12.02
	80	2.19	3.47	7.77	8.13	10.15	10.47
45	60	1.46	3.44	7.89	8.14	8.57	10.65
	70	1.08	2.94	7.97	8.46	8.95	10.46
	80	1.80	3.12	8.66	9.04	9.18	10.52
Crude oil		0.30	0.33	0.69	1.41	5.89	6.40

Physical changes

The only physical analysis conducted was absorbance value reflecting the colour intensity of the oil during storage and the results are displayed in Table 4. The absorbance values of refined oil through the storage were always lower than those of crude oil.

Analysis of variance showed that absorbance values of the oil were significantly influenced by neutralization temperature and period as well as the interaction of both treatments. The lower neutralization temperature and the shorter neutralization period, the lower absorbance value of the oil during storage would be. Carotenoids are the most common pigment composing fish oil colour (Brody 1965). The decomposition of carotenoids would result in loss of colour and form a more weakly coloured product (Emodi 1978). Neutralization treatment may have induced carotenoid instable and then decomposed during storage. The more obvious effects were noticed when neutralization was conducted at higher temperature and longer period.

Table 4. Absorbance value of refined and crude sardine oils during stability study

Refined oil		Storage Period at ±63°C (days)					
Neutralization Period (minutes)	Neutralization Temperature (°C)	0	3	6	9	12	15
15	60	0.102	0.100	0.075	0.070	0.059	0.057
	70	0.108	0.100	0.085	0.076	0.065	0.071
	80	0.104	0.100	0.084	0.069	0.058	0.053
30	60	0.104	0.101	0.079	0.076	0.064	0.060
	70	0.105	0.100	0.076	0.071	0.057	0.053
	80	0.107	0.103	0.096	0.089	0.063	0.060
45	60	0.109	0.106	0.075	0.077	0.064	0.051
	70	0.138	0.135	0.085	0.082	0.082	0.069
	80	0.113	0.113	0.084	0.075	0.054	0.047
Crude oil		3.615	3.156	2.423	2.146	1.950	1.458

Sensory changes

Descriptive and preference tests were used to evaluate the changes in sensory properties and acceptability of fish oil during stability study, especially on colour and odour. Panelists indicated that fish oil showed insignificant changes in colour during storage. These results, therefore, informed that the fish oil colour improvements detected by the changes in absorbance values were not apparently noticed using visual observation by panelists. Refined and crude oils colours were laid between yellowish-light yellow and light brown-brown respectively, which exhibited only slightly changes during storage (Appendix 1).

Preference test revealed that the panelists accepted the refined oil colour through the study, while the crude oil colour was unacceptable (Table 5). Those indications were supported by the result of friedman test. The acceptability of refined oil colour increased as the extention of strorage period.

In terms of odour, the odour of refined oil was more acceptable than the crude oil (Table 6). The refined and crude oils at the beginning of storage showed fishy and spoil odours respectively. The odour of refined oil was still accepted until 9 days storage. When the rancid odour appeared starting 12 days storage (Appendix 2), the panelists tended to reject the oil. It seems that the fishy odour was more acceptable than rancid odour. On the other hand, the odour of crude oil was unacceptable through the study.

Table 5. Acceptability changes of refined and crude oils colour scores*) during stability study

Refined oil		Storage Period at ±63°C (days)					
Neutralization Period (minutes)	**Neutralization Temperature (°C)**	**0**	**3**	**6**	**9**	**12**	**15**
15	60	7	7	7	7	8	8
	70	7	7	7	7	8	8
	80	7	7	7	7	8	8
30	60	7	7	7	7	8	8
	70	7	7	7	7	8	8
	80	7	7	7	7	8	8
45	60	7	7	7	7	8	8
	70	7	7	7	7	8	8
	80	7	7	7	7	8	8
Crude oil		3	3	3	3	3	3

*) Note: the score was in the range of 1-9, in which 1 = dislike very much and 9 = like very much

Friedman test revealed that neutralization temperature and period did not induce any different odour acceptability among refined oils obtained during storage and this result indicated that all treatments gave the same effect on the odour.

Table 6. Acceptability changes of refined and crude oils odour scores*) during stability study

Refined oil		Storage Period at ±63°C (days)					
Neutralization Period (minutes)	**Neutralization Temperature (°C)**	**0**	**3**	**6**	**9**	**12**	**15**
15	60	7	7	4	4	4	4
	70	6	5	3	4	4	4
	80	7	5	5	4	4	4
30	60	7	5	5	4	4	4
	70	6	5	5	4	4	4
	80	6	5	6	4	4	4
45	60	8	5	5	4	4	3
	70	6	5	6	4	4	4
	80	6	5	4	5	4	2
Crude oil		2	2	3	3	3	2

*) Note: the score was in the range of 1-9, in which 1 = dislike very much and 9 = like very much

Conclusion

Alkali refining was very effective to be used for the quality improvement of fish meal waste oil. FFA content and absorbance value could be reduced significantly. In addition, colour and odour of the oil could also be improved.

Neutralization should be carried out carefully, since the neutralization period and temperature did not only influence the quality, but also the stability of the refined oil. Suggested neutralization period and temperature for fish meal oil were 15 minutes and 60°C respectively.

References

Apriyantono, A., D.Fardiaz, N.L.Puspitasari, S.Budiyanto and Sedarnawati. 1989. Laboratory guide for food analyses. Inter University Center for Food and Nutrition, Bogor Agricultural University, Bogor (*in Indonesian*).

Belitz, H.D and W.Grosch. 1987. Food Chemistry. Springer-verlag, Berlin.

Brody, J. 1965. Fishery by-products technology, The AVI Publishing Co.Ltd. Westport. Connecticut.

Brzeska, Z. and J.Salmonowicz. 1973. The influence of tocopherol concentration in some fish oils upon their oxidation. Zesyty problemowe postepow nauk rolniczych. 136: 77-88. FSTA (8): 8n473.

Emodi, A. 1978. Carotenoids, properties and application. Food Tech. 32 (5): 38-48.

Fernandez, C.C. 1986. Refinement of fish oil for human consumption: engineering investigation. PhD Dissertation. University of Washington. Seattle.

Finley, J.W. 1985. Environmental effects on protein quality. *In* Chemical changes in food during processing. (T.Richardson and J.W.Finley, *eds*). The AVI Publishing Co.Inc. Westport. Connecticut.

Fioriti, J.A., M.J.Kanuk and R.J.Shin. 1974. Chemical and organoleptical properties of oxidized fats. JAOCS. 51: 219.

Gaspersz, V. 1991. Experimental design methods for agricultural, engineering and biological sciences. CV Armico. Bandung (*in Indonesian*).

Gillat, P.N., S.P.Kochhar and J.B.Rossel. 1988. A survey of the literature on the interaction of oxidized lipids with protein. No.163. Leatherhead Food RA, Surrey.

Gunstone, F.D. and F.A.Norris. 1983. Lipids in food. Chemistry, biochemistry and technology. Pergamon Press. Oxford.

Hawrysh, Z.J., P.J.Shand, B.Tokarska and C.Lin. 1989. Effects of tertiary butyl-hydroquinone on storage stability of canola oil. II. Practical Storage. Can.Ins.Fd. Sci.Technol.J. 22: 40-45.

Hendrix, B. 1993. Neutralization I: Theory and practice of conventional caustic (NaOH) refining *In* World conference proceedings: Edible fats and oils processing: Basic Principles and Modern Practices (Erickson, DR *ed*). American Oils Chemists' Society. Champaign. Illinois.

Hiatt, R. and K.C. Irwin. 1968. Homolytic decomposition of hydroperoxide. V. Thermal decomposition. J.Org.Chem 33: 1436-1441.

Irianto, H.E. 1992. Fish oil: refining, stability and its use in canned fish for the Indonesian market. PhD Thesis. Massey University. New Zealand.

Irianto, H.E. 1994. Effects of resin refining on the chemical and physical stability of sardine oils. Ind.Fd.and Nut.Prog. 1 (2): 22-27.

Irianto, H.E. 1995. Production and quality of Indonesian fish oil: case study in Muncar (East Java) and Bali. *In* FAO Fisheries Report No.514 Supplement. Working party on fish technology and marketing.p.243-250. FAO of the United Nation. Rome.

Kwon, T.W., D.B.Menzel and H.S.Olcott. 1965. Reactivity of malonaldehyde with food constituents. J.Fd.Sci. 30: 808-813.

Narasimhan, S., K.G.Raghuveer and C.Arumughan, K.K.Bhat and D.P.Shen. 1986. Oxidative rancidity in groundnut oil-evaluation by sensory and chemical indices and their correlation. J.Fd.Sci. and Tech. 16: 151-154.

Nawar, W.W. 1985. Thermal and radiolytic decomposition of lipids, *In* Chemical changes in food during processing (T.Richardson and J.W.Finley *eds*) p.79-105, The AVI Publishing Co Inc., Westport, Connecticut.

Scott, K.C. and J.D. Latshaw. 1991. Effects of commercial processing on the fat-soluble vitamin content of menhaden fish oil. JAOCS 68 (4): 234-236.

Steel, R.G.D. and J.H.Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, Inc. New York.

Sudarmadji, S., B. Haryono and Suhardi. 1989. Analysis of food and agricultural materials. Liberty. Yogyakarta (*In Indonesian*).

Young, F.V.K. 1982. The production and use of fish oils. *In* Nutritional evaluation of long-chain fatty acids in fish oil. (M.S.Barlow and M.E.Stansby, *eds*).p.1-24. Academic Press. London.

Appendix 1. Colour changes of refined and crude oil during stability study

Refined oil		Storage Period at $\pm 63^{\circ}C$ (days)					
Neutralization Period (minutes)	**Neutralization Temperature (°C)**	**0**	**3**	**6**	**9**	**12**	**15**
15	60	light yellow	light yellow	yellowish	light yellow	light yellow	light yellow
	70	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
	80	light yellow	light yellow	light yellow	light yellow	yellowish	light yellow
30	60	light yellow	light yellow	light yellow	light yellow	yellowish	light yellow
	70	light yellow	light yellow	light yellow	light yellow		light yellow
	80	light yellow	light yellow	light yellow	light yellow	light yellow yellowish	light yellow
45	60	light yellow	light yellow	yellowish	light yellow	yellowish	yellowish
	70	light yellow	light yellow	light yellow	light yellow		
	80	light yellow	light yellow	light yellow	light yellow	light yellow yellowish	light yellow light yellow
Crude oil		brown	brown	brown	light brown	light brown	brown

Appendix 2. Odour changes of refined and crude oil during stability study

Refined oil		**Storage Period at $\pm 63^{o}C$ (days)**					
Neutralization Period (minutes)	**Neutralization Temperature (^{o}C)**	**0**	**3**	**6**	**9**	**12**	**15**
15	60	slightly fishy	fishy	fishy	slightly fishy	slightly rancid	rancid
	70	slightly fishy	fishy	fishy	fishy	slightly rancid	rancid
	80	fishy	fishy	fishy	fishy	slightly rancid	strong rancid
30	60	fishy	fishy	strong fishy	fishy	slightly rancid	strong rancid
	70	fishy	fishy	strong fishy	fishy	rancid	rancid
	80	fishy	fishy	fishy	fishy	slightly rancid	rancid
45	60	fishy	fishy	fishy	strong fishy	slightly rancid	strong rancid
	70	fishy	fishy	fishy	fishy	slightly rancid	rancid
	80	slightly fishy	fishy	strong fishy	fishy	slightly rancid	slightly rancid
Crude oil		spoil	slightly spoil	slightly spoil	slightly spoil	strong rancid	strong rancid