Effects of Resin Refining on the Chemical and Physical Stability of Sardine Oils

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ABSTRACT

The effect of resin refining on the stability of sardine oil was studied. Fish canning waste oil and fish meal processing waste oil were used in the expriment. The oils were refined by passing through the resin packed column at fish oil and resin ratio of 1 : 1.

The fish oil stability was investigated using Schaal oven method by placing the oil in an oven at 63 ± 2°C and the sample was withdrawn after 0, 2, 4, 7 and 11 days. Resin refining improved fish oil quality as indicated by lower FFA value and brighter colour, this process reduced natural antioxidant content. Results of stability test indicated that refined oil had a lower stability than unrefined oil by showing a higher rate of peroxide value, TBA value, anisidine value and totox value increases as well as colour absorbance decrease. Meanwhile, canning waste oil exhibited a lower stability compared to fish meal processing waste oil.

INTRODUCTION

The use of resin for fish oil refining was originally developed by Fernandez (1986). Macroporous strong acid cation resin was found to be the most effective resin type used for this purpose and even proved to be superior over molecular distillation and freezing fractionation. This method is proposed as an alternative way for fish oil refinement requiring less heat involvement during the process.

Application of heat as encountered in the commercial process of fish oil refining should be avoided, since this operation would be detrimental to fish oil. Fish oil is the most polyunsaturated of all the oils in which polyunsaturated fatty acids are susceptible to heat and consequent oxidation. Fatty acids which are very labile to oxidation are mainly those with five and six double bonds, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Recently, both EPA and DHA are regarded as either nutritional supplements or as therapeutic agents inhibiting a variety of pathological conditions (Lundberg, 1965; Bank, 1967; Grigg, 1986; Kinsella, 1987; Lie and Regenstein, 1990; Fujimoto *et al*, 1990; Yongmanitchai and Ward, 1989).

Refining may also affect the stability of fish oil due to the loss of natural antioxidants, particularly tocopherols. The decrease of tocopherol content in refined fish oil was noted in cod liver oil and sprat oil refining using soda lye (Brzeska and Salmonowicz, 1973), and menhaden fish oil refining using different methods (Scott and Catshaw, 1991). The refining process also reduced tocopherol content in vegetable oils such as soya oil processed using degumming, neutralization, bleaching and deodorization (Gutfinger and Letan, 1974; Sleeter, 1981); and combined sunflower oil and rapeseed oil refined using acidification/ neutralization, washing, drying, decoloration and deodorization (Ludwiki *et al*, 1986).

The objective of this research was to investigate the effects of the application of macroporous strong acid cation resin for refining sardine oil on chemical and physical stability of the oil.

MATERIALS AND METHODS

Fish Oil

Two kinds of sardine oils, from fish meal processing and canning waste, were used in this study. Fish oil was supplied by P.T. Sumber Rejeki, Muncar (East Java). The canning waste oil came from P.T. Karya Manunggal Tri Sukses, Muncar (East Java).

Resin

Macroporous strong acid cation resin (Dowex xys 40032.007) used for fish oil refining was supplied by Dow Chemicals, USA. The resin, consisting of a

styrene/divinyl benzene matrix structure had sphere form, sodium ionic, 1 – 7 meq/ml/min total exchange capacity, 150 A pore size and 42 – 48% water retention capacity. The resin was packed using a column 39 cm in length and 1.65 cm in diameter.

Fish Oil Refining

Refining was performed with a fish oil-resin volume ratio of 1 : 1. The refined oil was defined as the oil freely flowing through the column. The resin packed column was cleaned using petroleum ether and methanol passed through the column alternately twice.

Canning waste oil was refined by passing the oil through the column once. While the fish meal oil was passed through the column twice, since the oil had a very undesirable odour.

Stability Test of Fish Oil

Fish oil stability was investigated using the Schaal oven method. The fish oil samples, each of 80 ml were contained in 100 ml beakers, and covered loosely with aluminium foil. The samples were stored in an oven at 63 ± 2°C and withdrawn after 0, 2, 4, 7 and 11 days. The experiment was run in two replication.

Analysis

Free fatty acid value. Analysis was carried out using the procedure described by Fernandez (1986).

Tocopherol And Tocotrienols Analysis. Quantitative analysis of tocopherol and tocotrienol was carried out before and after refining. Approximately 0.1 g of fish oil was accurately weighed into a 5 ml volumetric flask and then made up with hexane containing 200 ppm BHT antioxidant. Constituents were separated by using Maxima 820 high performance liquid chromatography (HPLC) Model 510 (Waters Associates, Milford, MA, USA) equipped with a Hitachi fluorescence spectrophotometer Model F1000 (Hitachi Ltd, Tokyo, Japan). The excitation was set at 295 nm and emission at 330 nm. The mobile phase was HPLC grade hexane containing 7% diisopropyl ether and 0.05% acetic acid at the rate of 2 ml per minute. Zorbax silica column (0.5μ, 30 cm × 3.6 mm) was used to provide the separation of α, β, γ and δ tocopherols, tocomonoenol and tocotrienol. The column temperature was maintained at room temperature ± 20°C. The volume injected was 50μl.

Peroxide value. Peroxide value analysis was based on the method described by Windsor and Barlow (1981).

Thiobarbituric acid (TBA) value. TBA values were determined using the method outlined by Fioriti *et al* (1974).

Anisidine value. The method described by Windsor and Barlow (1981) was used to determine anisidine value.

Total oxidation value (Totox value). Totox Value was calculated using the following equation (Patterson, 1989):

$$\text{Totox Value} = 2\,\text{PV} + \text{An.V}$$

Where: PV = Peroxide Value
An. V = Anisidine Value

Colour. The method described by Fernandez (1986) was used to measure the absorbance of fish oil using a Shimadzu Spectrophotometer Model UV-110-02 at 490 nm. The results were corrected using a petroleum ether as blank. Samples were equilibrated to 30°C in a water bath prior to measurement.

Refractive Index. Refractive Index (RI) values were determined using Bellingham Stanley Refractometer (Bellingham + Stanley Limited, England) at 25°C (Arya, 1969). The refractometer RI range was 1.30 – 1.74.

RESULTS AND DISCUSSION

Free Fatty Acid and Tocopherol Contents

Free fatty acid (FFA) values of both fish meal and canning waste oils decreased due to resin refining. This indicated quality improvement of the oils. The contents of tocopherol and tocotrienol as natural antioxidants found in the oil reduced after passing the oil through the resin packed column as shown in table 1. Canning waste oil contained only α-tocopherol and its amount was much lower than measured in fish meal oil. Fish meal oil also contained α-tocotrienol, γ-tocopherol and δ-tocopherol. Pokorny (1987) stated that α-tocotrienol possessed a slightly higher antioxidant activity than the corresponding tocopherol and this α-tocotrienol was present in fish meal oil at a higher level than other tocopherols.

Table 1. **Free fatty acid and tocopherol contents of fish meal and canning waste oils during refining process** *)

Analysis	Fish meal oil		Canning waste oil	
	Untreated oil	Refined oil	Untreated oil	Refined oil
FFA (% asam oleat)	0.20	0.17	0.27	0.19
α-tocopherol (ppm)	25.3	14.3	7.1	4.6
α-tocotrienol (ppm)	297.3	116.4	n.d	n.d
γ-tocipherol (ppm)	4.6	1.8	n.d	n.d
δ-tocopherol (ppm)	3.2	n.d	n.d	n.d

Note: n.d. = not detected

*) values are average of two determinations.

Chemical Changes

Peroxide values of both oils were relatively constant during the first two days of storage but values increased on the fourth day, except for the untreated fish meal oil (Figure 1). These PV values still showed an increase until the end of storage. The fastest PV increase was noted in the refined canning waste oil. The PV increase in the refined fish meal oil and unrefined canning waste oil occurred at approximately the same rate. The PV changes in unrefined fish meal oil were very slow compared to other tested oils. The fastest PV increase was found between the seventh and eleventh days for the refined canning waste oil showing the highest PV increase rate between the fourth and the seventh day.

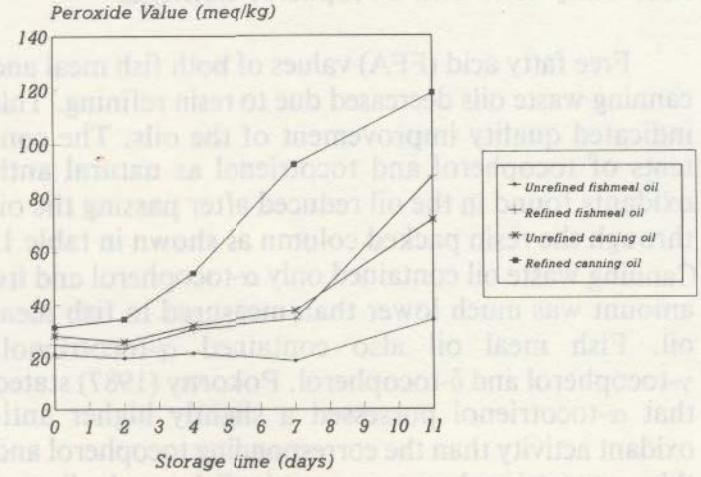

Figure 1. **Peroxide value changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values from duplicate samples and the values of each samples are mean values of two determinations)**

The availability of natural antioxidant in the oil as previously mentioned probably affected the production of hydroperoxide due to oxidation process. The progessive formation of peroxides due to oxidation process has also observed in the storage of groundnut oil (Narasimhan *et al*, 1986), and in canola oil (Hawrysh *et al*, 1989).

Figure 2 shows the TBA value changes, in which TBA value indicates the content of malonaldehyde forming as a secondary product of oxidation. All oils exhibited a very slow change in TBA values until the fourth day, except for refined canning waste oil where the TBA value increased significantly. Sharp increases in TBA values was observed starting on the fourth day for all oils. Refined canning waste oil showed the fastest TBA increase, while unrefined fish meal oil had the slowest increase in TBA value. Unrefined canning waste oil and refined fish meal oil displayed a similar increase rate in TBA value. 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 TBA values increase rate.

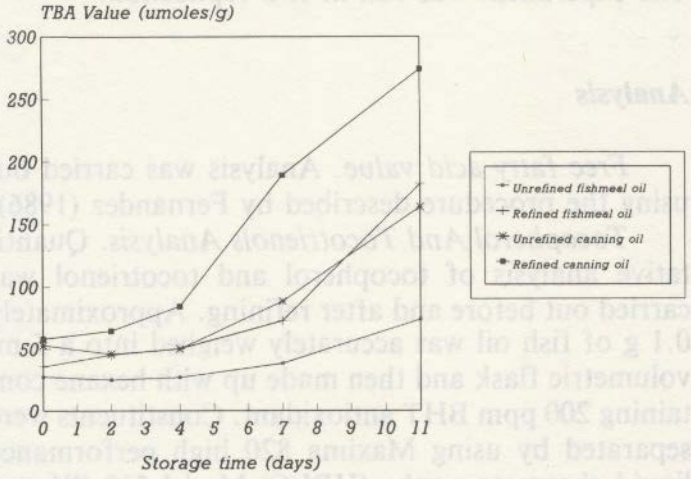

Figure 2. **TBA value changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values from duplicate samples and the values of each samples are mean values of two determinations)**

The changes in anisidine value measuring α/β-aldehydes as secondary products of oxidation are displayed in Figure 3. The linear increase in anisidine value was noted until the fourth day for refined canning

waste oil, the seventh day for both unrefined canning waste oil and refined fish meal oils, and the end of storage for unrefined fish meal oil as shown in Figure 3. After these periods, unrefined and refined canning waste oils, as well as refined fish meal oil, had a sharp increase in anisidine value. At the end of storage those oils had insignificant anisidine value differences. Unrefined fish meal oil showed the slowest anisidine value increase. The higher FFA value in fish meal oil might suppress the formation of aldehydes (Nair, 1979).

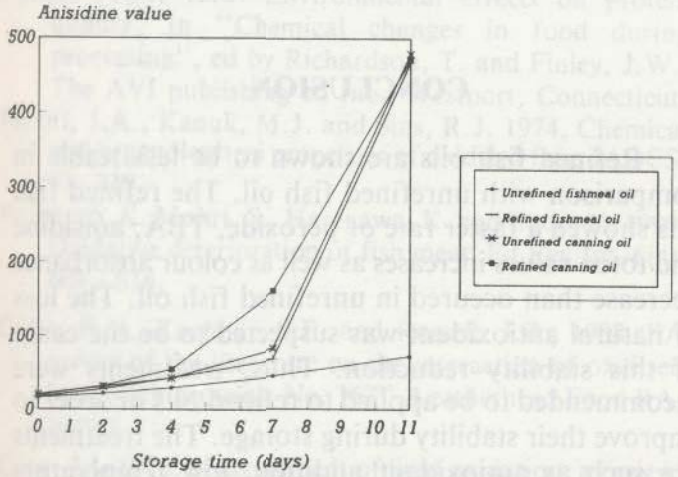

Figure 3. **Anisidine value changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values from duplicate samples and the values of each samples are mean values two determinations)**

The pattern of totox value change was similar to the pattern of anisidine value changes. However, as shown in Figure 4, at the end of storage the totox value of refined canning waste oil significantly higher in comparison with the values of unrefined canning waste oil, unrefined fish meal oil and refined fish meal oil. The unrefined fish meal oil exhibited the lowest totox value increase at all time during storage.

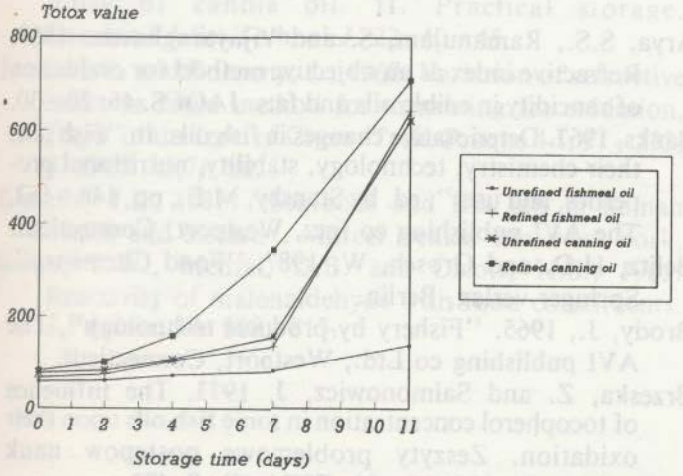

Figure 4. **Totox value changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values from duplicate samples and the values of each samples are mean values of two determinations)**

Physical Changes

The colour absorbance and refractive index (RI) values changes in both fish meal and canning waste oils during stability study are shown in Figures 5 and 6.

The colour absorbance value changes in fish meal oil showed a decreasing trend during storage. No significant change in absorbance value of unrefined oil was noted during the first two days of storage. The reduction of colour absorbance in refined oil occurred at a higher rate than in unrefined oil. Natural colour loss in fish oil during storage resulted in oil with pale and clear colour. Carotenoid are the most common pigment composing fish oil colour. The carotenoids, embracing the red, orange and yellow oil soluble pigments occur naturally in a number of different plan and animal fats (Brody, 1965; Clydesdale and Francis, 1976). The decomposition of carotenoids would result in loss of colour and form a more weakly coloured product (Emodi, 1978). The unsaturated portion of carotenoids is easily affected by oxygen (Struckey, 1972). Thus, the main cause of carotenoids degradation in foods is oxidation by reaction with atmospheric oxygen at rates dependent on light, heat and the presence of pro- and antioxi-

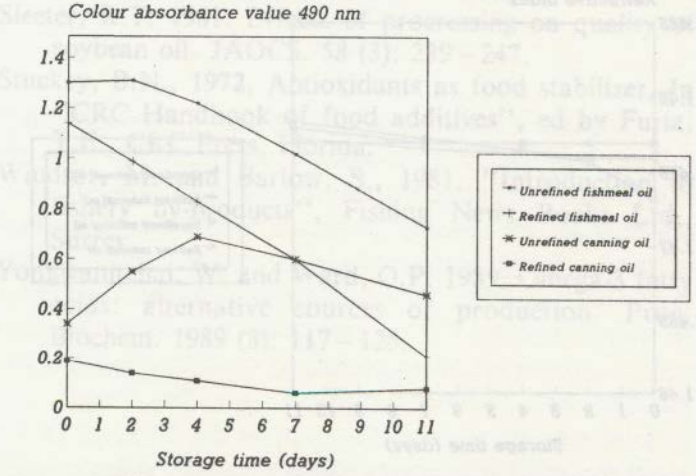

Figure 5. **Colour absorbance changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values from duplicate samples and the values of each samples are mean values of two determinations)**

dants (Clydesdale and Francis, 1976). Enzymatic degradation of carotenoids was also noted (Simpson *et al*, 1981.

Canning waste oil, however, exhibited a different trend in colour absorbance change when compared to the fish meal oil. Unrefined canning waste oil had a linear colour absorbance value increase until the fourth day. Then, colour absorbance value decreased gradually. The increasing colour absorbance value in the canning waste oil during the first four days might be due to the darkening process occurred at a higher rate than the carotenoids decolouration. The darkening process might be due to the reaction between proteins with hydroperoxides and their degradation products producing browning process (Belitz and Grosch, 1987). After the darkening process achieved its peak, carotenoids decolouration process became more abvious showing a reduction in colour absorbance value. Refined canning waste oil exhibited a gradual reduction pattern of colour absorbance value until the seventh day. Then, a relatively constant value was observed.

The refractive index (RI) values of unrefined fish meal oil was relatively unchange, but the refined fish meal oil showed an RI increase trend during storage. Sharp RI increases in fish meal oil occurred during the first two days but after that period, the RI value increased gradually. The RI increase was also recorded by Janick and Pokorny (1960) and Arya *et al* (1969) in edible oils and fats. The increase in RI with autoxidation is possibly attributable to conjugation known to proceed hydroperoxide formation in the

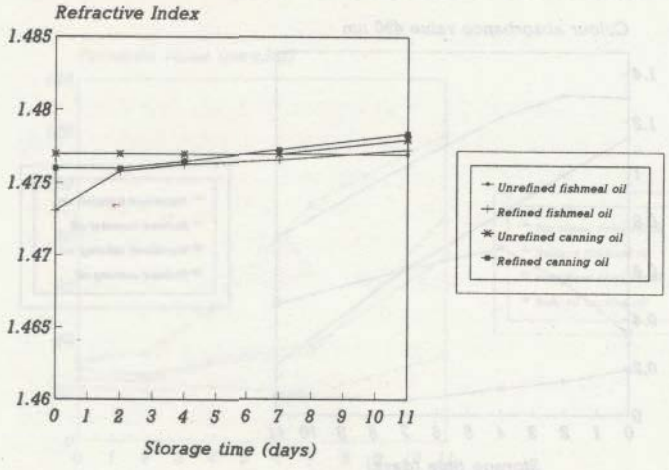

Figure 6. **Refractive index value changes in refined and unrefined sardine oils stored at 63 ± 2°C (Results are average of two values form duplicate samples and the values of each samples are mean values of two determinations)**

secondary stage and polymerization of partially oxidized fats in the tertiary stage of oxidation (Arya *et al*, 1969; Grey, 1978). At the end of storage, the RI value of refined oil was higher than unrefined oil.

The RI value of unrefined canning waste oil was relatively constant until the seventh day, but increased by the end of storage. The refined canning waste oil showed an increase trend of RI value starting at the second day. The RI value of this oil exceeded the RI value of unrefined canning waste oil starting at the seventh day.

CONCLUSION

Refined fish oils are shown to be less stable in comparison with unrefined fish oil. The refined fish oils showed a faster rate of peroxide, TBA, anisidine and totox values increases as well as colour absorbance decrease than occured in unrefined fish oil. The loss of natural antioxidant was suspected to be the cause of this stability reduction. Thus, treatments were recommended to be applied to refined oils in order to improve their stability during storage. The treatments are such as antioxidant addition, low temperature storage, vacuum packing and modified atmosphere storage.

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