*Sri Lanka J. Aquat. Sci.* **3** (1998): 109-117

# Evaluation of Natural Antioxidants used in the Preservation of Shark *(Carcharhinus falciformis)* Liver Oil

C.V.L. JAYASINGHE[1], W.M.K. PERERA[1] and A. BAMUNUARACHCHI[2]

[1] Institute of Post Harvest Technology
National Aquatic Resources Research and Development Agency
Crow Island, Colombo 15, Sri Lanka

[2] Department of Chemistry
University of Sri Jayawardanepura, Nugegoda, Sri Lanka

**Abstract**

An experimental storage trial was conducted with the ethanolic extracts of turmeric *(Curcuma domestica)*, tamarind *(Tamarindus indica)* seed and fruit, butylated hydroxy toluene and L-ascorbic acid to evaluate the antioxidant properties of shark *(Carcharhinus falciformis)* liver oil. Effectiveness of the treatments were determined by measuring the peroxide value, free fatty acid value, para anicidine value and iodine value at weekly intervals over a period of 112 days.

Turmeric treated oil samples showed the lowest hydrolytic rancidity and oxidative rancidity. Less than 250 ppm of ethanolic extract of turmeric (*Curcuma domestica)* was required to prevent the oxidation, which was comparable to using 200 ppm of butylated hydroxy toluene.

## Introduction

Total shark production in Sri Lanka was approximately 28477 MT in 1995 (Anon. 1997) and large quantity of livers of these fish were not made use of. Liver oil content of silky shark (*Carcharhinus falciformis)* which is the most dominant species in the catches, varies from 20% to 75% throughout the year. Fish liver oils contain n-3 fatty acids and are a rich source of vitamin A and D and therefore are nutritionally important in the human and animal diets (Gunstone & Herslof 1992).

Oxidative rancidity is the main problem faced in storing oil. It is responsible for the offensive odour, degradation of taste, consistency and appearance as well as the loss of nutritional value of most foods. To prevent or delay this autoxidation process, antioxidants have been used for over 50 years (Cuvelier et al. 1994). Use of synthetic antioxidants prevents formation of toxic substances in food and they are effective and less expensive than natural antioxidants. Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are the most commonly used antioxidants at present. They are added to a wide variety of items in the food industry. The newly developed tertiary butylhydroquinon (TBHQ) has an excellent ability to retard the absorption of oxygen (Chang et al. 1977). However, the recent consumer interest in natural products, requires

natural antioxidative substances to replace conventional antioxidants such as BHT and BHA (Cuvelier et al. 1994). A significant number of natural antioxidants have been identified by Loliger (1983). He discussed the effect of both tocopherol and spices (rosemary in particular), and pointed out that tocopherol is quite effective in stabilizing animal fats. Mehta et al. (1994) observed that ajowan as a source of natural antioxidant has a potential to decrease the rate of lipid oxidation. Hirahara et al. (1974), Bracco et al (1981), Osawa et al. (1992) and Masuda & Titoe (1994) have shown that Turmeric *(Curcuma domestica)* has some antioxidant properties. The present study was conducted to evaluate the antioxidant properties of several natural and artificial antioxidants when used with shark (*Carcharhinus falciformis)* liver oil.

## Materials and Methods

*Preparation of ethanolic extracts*

Turmeric, cleaned & dried tamarind fruits and seeds were cut into small pieces and oven dried at 50°C for 48 hours. One hundred grams of each sample were extracted by cold extraction method using ethanol as a solvent for 2 days. Concentrated crude extracts were obtained after rotary evaporation at room temperature (29°C). All extracts were stored in refrigerator (4-5°C) until tested.

*Extraction of liver oil*

Silky shark (*Carcharhinus falciformis)* livers were collected from Negombo landing site in the West Coast of Sri Lanka. Collected livers were stored in ice and transported to the laboratory of the National Aquatic Resources Research and Development Agency in Colombo. The livers were washed and cut to remove the blood vessels. Blended livers were steam-rendered for 90 minutes, centrifuged and filtered for separating the oil. Anhydrous sodium sulphate (5%) was added to crude oil and kept over-night. Crude oil was filtered to obtain anhydrous oil.

*Study 1*

Turmeric extract (2%), tamarind seed extract (2%), tamarind fruit extract (2%), butylated hydroxy toluene (0.02%) and L- ascorbic acid (0.05%) were added to shark liver oil. The mixtures were stirred thoroughly and poured into clean dry vials and kept at room temperature (29°C). Sampling was done at weekly intervals over a period of 112 days and treated for the determination of free fatty acid (FFA) (Anon. 1992a), peroxide value (PV) (Anon. 1992d), iodine value (IDV) (Anon. 1992c) and anisidine value (PAV) (Anon. 1992b). Triplicates of each treatment were prepared and evaluated along with the control.

*Study 2*

Turmeric extracts at levels of 250ppm, 500ppm, 1000ppm, 2000ppm, 3000ppm and BHT 200ppm were added to anhydrous shark liver oil. The mixtures were separately stirred thoroughly and poured in to clean dry vials. Samples were stored at controlled temperature of 30°C. Triplicates of each treatment along with controls were examined. A control sample was prepared each time under the same conditions without adding any antioxidant. Sampling was done at three day intervals in the beginning and then at one week intervals for the next 20 days as in study 1.

*Statistical analysis*

A one-way analysis of variance was carried out to study the effect of antioxidant treatments on the rancidity of shark liver oil by measuring FFA, PV, PAV, thiobarbaturic acid value (TBAV) & IDV in shark liver oil. Significance was accepted at a probability of 5% or less. Bonferroni's multiple comparison test (Zar 1984) was used to identify the means which were significantly different from each other.

## Results and Discussion

Changes of peroxide values obtained in shark liver oil with different treatments during the 16-week storage period are given in Fig. 1a. The lowest value (66.7±6.6) was recorded with turmeric treated oil sample followed by the oil with BHT (74.7±9.2). All means in this paper are presented with their standard errors (SEM). Liver oil treated with tamarind seeds and fruits showed highest peroxide values at the end of the storage period. A comparison of the variation of the peroxide value at the initial and the final stage in the oil samples treated with five different antioxidants, is shown in Fig. 1b. No significant differences were observed for peroxides in BHT and turmeric treated oil samples compared to others.

Variations of free fatty acid (FFA) values during storage period are given in Fig. 1c. At the end of the storage period, the FFA value (0.8 ±0.07) for the oil sample treated with 2% turmeric (TUR) extract was significantly lower than that of others treated with L- ascorbic acid (ASA), tamarind seed extract (TAS), tamarind fruit extract (TAF) and butylated hydroxy toluene (BHT) (Fig. 1d). Variation in para anisidine value during the storage period of the first month is presented in Fig. 1e. Oil sample treated with turmeric showed a slight decrease in the PA value with time and the final value was (9.5±2.6) significantly lower than the values of those treated with other antioxidants (Fig. 1f). The levels of iodine value of liver oil in all samples decreased with time (Fig. 1g). This is due to the breakdown of unsaturated fatty acids during the storage period. A rapid drop of the value was observed in all samples after 10 weeks of storage. The initial and final iodine values of liver oil treated with five different antioxidants are shown in Fig. 1h.

According to the results of the present study, turmeric showed the lowest free fatty acid value, peroxide value, para anicidine value and more or less similar iodine value compared to BHT, TAS, TAF and ASA. Results also showed that turmeric had markedly high preventive efficiencies on oil oxidation compared to the others. Many authors (Chipault et al 1956; Sethi & Aggarwal 1957) have reported that Curcumin which is one of the active components of turmeric, is responsible for the antioxidant

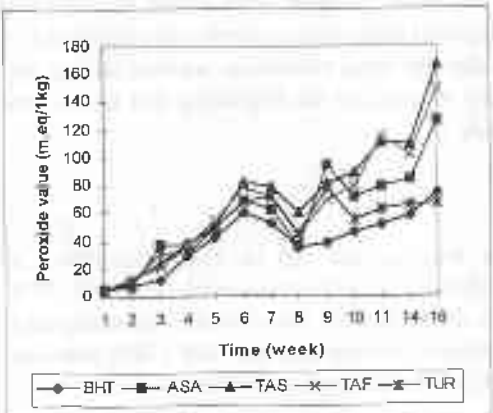


Figure 1a - Changes in the levels of peroxides of liver oil during storage at room temperature

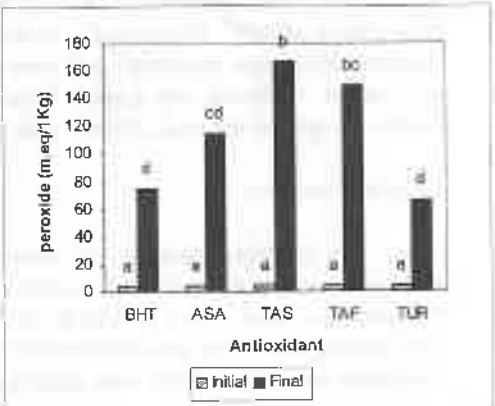


Figure 1b - The initial and final peroxide value of liver oil treated with five different antioxidants (values with similar letter are not statistically different at 5% level)

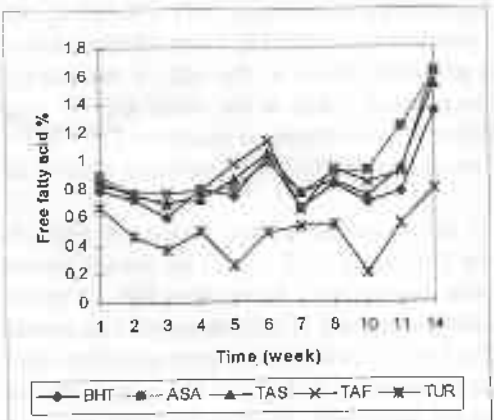


Figure 1c - Changes in the levels of free fatty acid percentage of liver oil during storage at room temperature

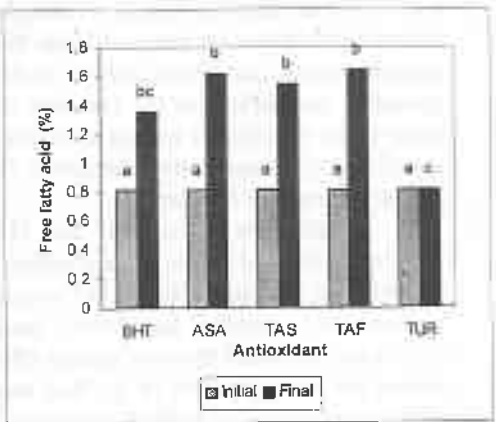


Figure 1d - The initial and final FFA% of liver oil treated with five different antioxidants (values with similar letter are not statistically different at 5% level)

properties as the antioxidant index of turmeric is greater than five. It protects oil in water emulsion and edible fats against oxygen absorption and peroxide development. The present results are in agreement with Hiraharra et al. (1974). They have confirmed that turmeric can increase shelf life of oils and fats. They observed that the ground turmeric showed significant antioxidative activity as measured by the active oxygen method in the methyl linoleate system when added to olive, soyabean, sesame or linseed oil.

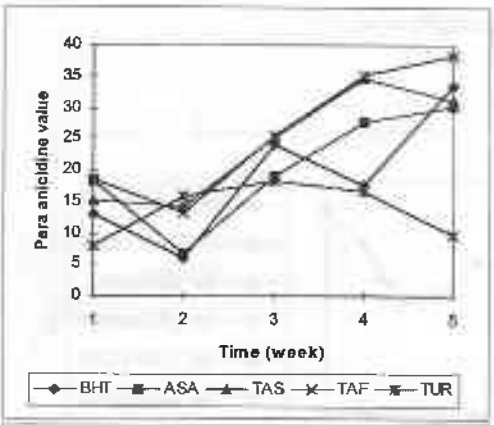


Figure 1e - Changes in the levels of para anicidine value of liver oil during storage at room temperature

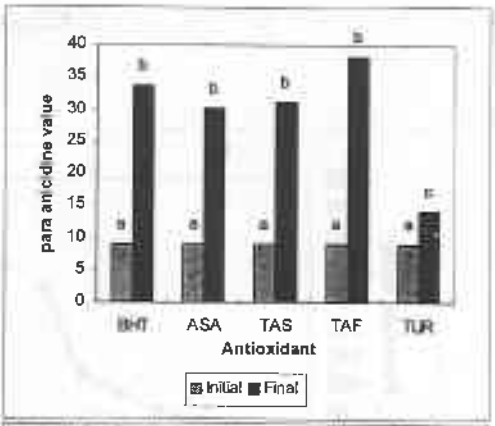


Figure 1f - The initial and final para anicidine value of liver oil treated with five different antioxidants (values with similar letter are not statistically different at 5% level)

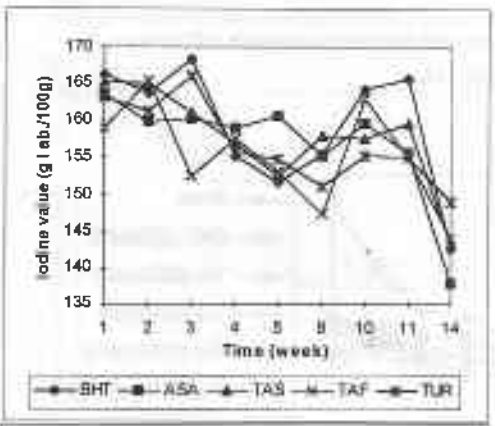


Figure 1g - Changes in the levels of iodine value of liver oil during storage at room temperature

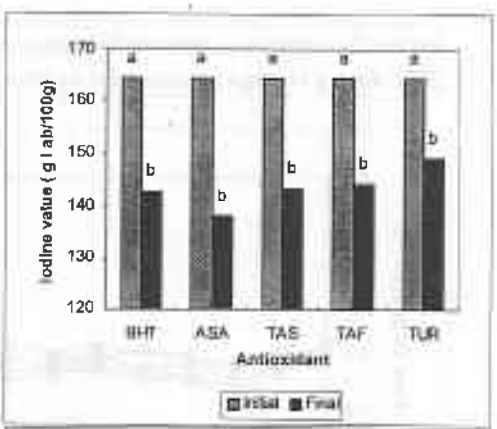


Figure 1h - The initial and final iodine value of liver oil treated with five different antioxidants (values with similar letter are not statistically different at 5% level)

The second study was conducted to determine the requirement of optimum turmeric extracts to prevent oxidation of shark liver oil. The average peroxide values of four replicates of each treatment were plotted against time (Fig. 2a). It was observed that the formation of peroxides in the samples which were treated with turmeric and BHT were lower than the control. Results indicated that the control sample was oxidized at a higher rate, as the sample did not contain antioxidative substances.

The variation of the FFA levels in all treatments is shown in Fig. 2b. The value of FFA were higher in the control sample compared to those of other treatments and the formation of FFA were more or less similar in the samples which were treated with turmeric (200ppm - 3000 ppm) and BHT (250 ppm).

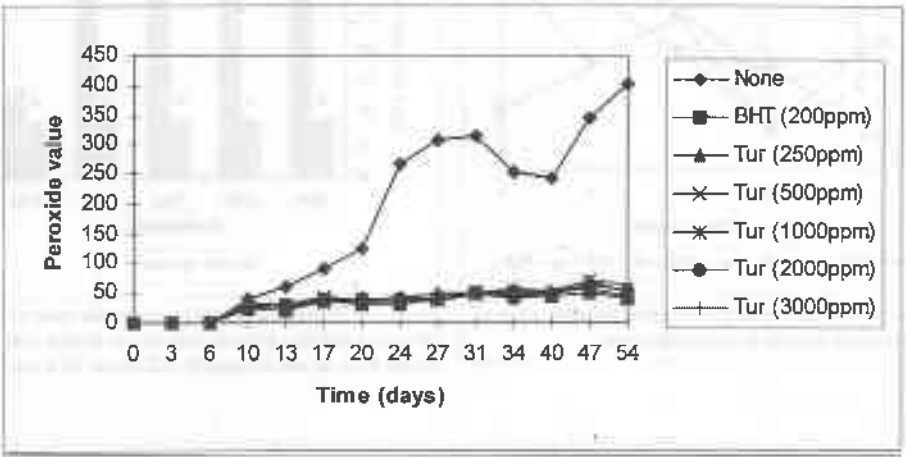


Figure 2a - Oxidation of shark liver oil treated with ethanolic extracts of turmeric & BHT during storage as measured by peroxide value

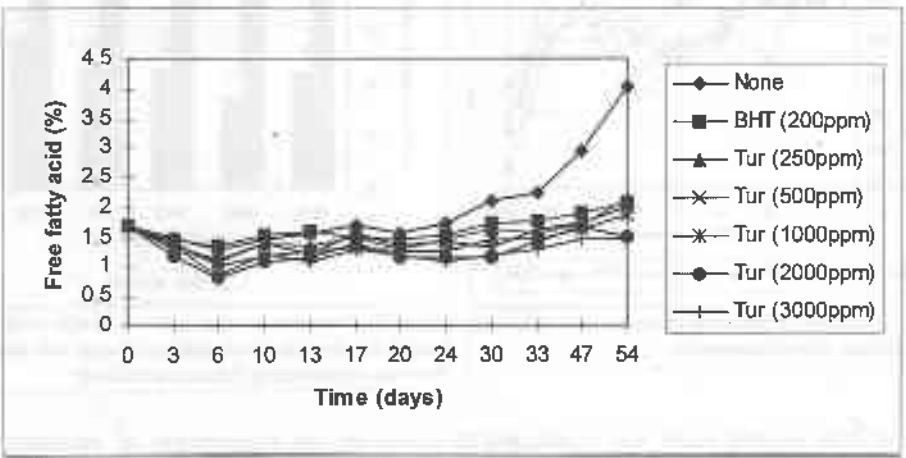


Figure 2b - Hydrolysis of shark liver oil treated with ethanolic extracts of turmeric & BHT during storage as measured by free fatty acid percentage.

Oxidation of shark liver oil treated with turmeric and BHT during storage at 30°C as measured by thiobarbaturic acid value is shown in Figure 2c. It indicated that the antioxidative capacity of control oil sample was lost after 20 days. However turmeric and BHT treated samples had an antioxidative capacity and did not show the oxidative rancidity after 40 days storage.

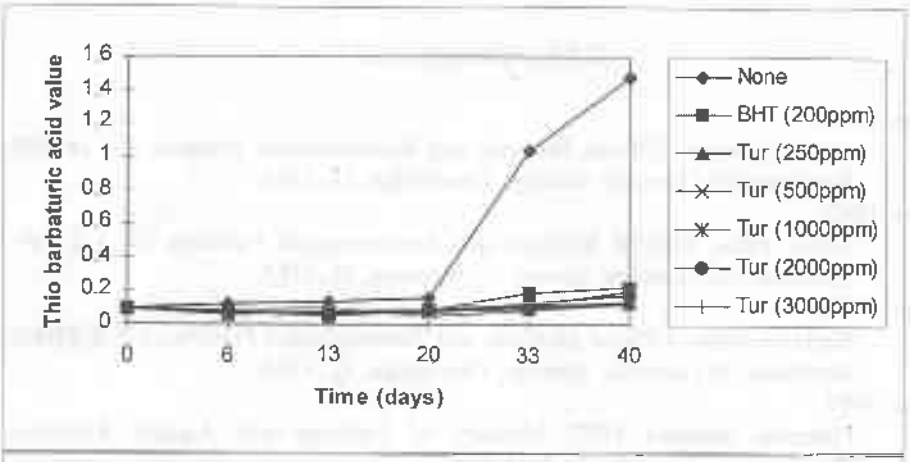


Figure 2c - Oxidation of shark liver oil treated with ethanolic extracts of turmeric & BHT during storage as measured by thio barbaturic acid value

Therefore the results indicate that oil quality deterioration factors such as oxidation and hydrolysis did not affect the shark liver oil treated with turmeric and BHT. The control sample was oxidized and hydrolyzed at the highest intensity, as indicated by highest peroxide, thiobarbaturic acid and FFA values. Oil with turmeric (200-3000 ppm) and BHT appears to be more stable compared to non treated samples during storage indicating the efficient antioxidative ability of the treatments.

## Conclusion

This study suggests that ethanolic extract of turmeric (*Curcuma domestica*) acts as an efficient antioxidant in shark liver oil when compared to tamarind (*Tamarindus indica*) fruit and seed, butylated hydroxy toluene and L-ascorbic acid. There is a possibility of using turmeric as an antioxidant in the commercial fish liver oil storage. A 250 ppm ethanolic extract of turmeric was found to be as efficient as a 200 ppm solution of butylated hydroxy toluene in preventing oxydation.

## Acknowledgements

This research was funded by a grant from the European Economic Commission funded STD-3 project and the National Aquatic Resources Research and Development Agency in Sri Lanka.

## References

Anon. 1992a.
Acid value, Official Methods and Recommended Practices Cd 3a 63(89), American Oil Chemists' Society , Champaign, IL, USA.

Anon. 1992b.
Anisidine value, Official Methods and Recommended Practices Cd 18 -90), American Oil Chemists' Society , Champaign, 1L, USA.

Anon. 1992c.
Iodine value, Official Methods and Recommended Practices Cd 1 25(89), American Oil Chemists' Society , Champaign, 1L, USA.

Anon. 1992d.
Peroxide value, Official Methods and Recommended Practices Cd 8 53(89), American Oil Chemists' Society , Champaign, 1L, USA.

Anon. 1997.
Fisheries statistics 1997, Ministry of Fisheries and Aquatic Resources Development, Colombo 10, Sri Lanka.

Bracco, U., J. Loliger & J.L. Viret 1981.
Production and Use of natural antioxidants. Journal of the Amarican Oil Chemists' Society 58: 686-690.

Chang, S.S., B.O. Matijasevic, O.A.L. Hsieh & C.L. Huang 1977.
Natural antioxidents from rosemery and sage. Journal of Food Science 42(4): 1102-1106.

Chipault, J.R., G.R. Mizuno & W.O. Lundberg 1956.
The antioxidant properties of spices in foods: Food Technology 10: 209-211.

Cuvelier, M.E., C. Berset & H. Richard 1994.
Antioxidant constituents in sage (*Salvia officinalis*), Journal of Agricultural and Food Chemistry 42: 665-669.

Govindarajan, V.S. 1980:
Critical reviews in food science and nutrition. Critical Reviews in Food and Nutrition 12: 199- 295.

Gunstone, F.D. & B.G. Herslof 1992.
A lipid glossary, The Oily press, New York.

Hiraharra, F., Y. Takai & H. Iwao 1974.
Antioxidant activity of various species on oils and fats. 1. Antioxidant activity for storage and heating. Japanese Journal of Nutrition (in Japanese) 32(1): 1-8.

Loliger, J. 1983.
Natural Antioxidants. In: Rancidity in Foods (J. Calten & R.J. Hamilton eds). pp. 100-107. Applied Science Publishers, London & New York.

Masuda, T. & A. Titoe 1994.
Antioxidative and antiflammatory compounds from tropical gingers; Isolation, Structure determination and activities of cassumins A, B and C, New complex Curcuinoids from Zingiber cassumunar. Journal of Agricultural and Food Chemistry 42: 1850- 1856.

Mehta, R.L., J.F. Zayas & S. Yang 1994.
Ajowan as a source of natural lipid antioxidant. Journal of Agricultural and Food Chemistry 42: 1420-1422.

Osawa, T., M. Inayoshi, T. Nakayama & S. Kawakishi 1992.
Antioxidative activity of Tetrahydrocurcumin, Oxygen Radicals. In: Oxygen Radicals ( K. Yagi, M.Kondo, E. Niki & T. Yoshikawa eds). pp. 801-804. Elsevier Publishers, Oxford.

Sethi, S.C. & J.S. Aggarwal 1957.
Stabilisation of edible fats by spices III. Journal of Science and Industrial Research (India) 16: 181-182.

Zar, J.H. 1984.
Biostatistical Analysis (2nd edition). Prentice Hall, London.