LIPID PEROXIDATION OF FISH OILS

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ABSTRACT

Fish and fish oils are the richest sources of ω -3 fatty acids. However, they are susceptible to lipid peroxidation due to their high degree of unsaturation. In the present study, the level of thiobarbituric acid reactive material in various fish oils available in the market with and without added Vitamin E was determined. The peroxide levels in fish oil heated to food frying temperature of 180°C and the effect of addition of vitamin E has also been studied. The results indicate that the peroxide levels in almost all the products available in the market were abnormally high irrespective of their Vitamin E content. This might be due to the inefficient methods used for processing and storage of fish oils. Addition of vitamin E was found to have a significant effect in lowering the rate of peroxidation of fish oil during thermal stress, showing that association of antioxidants with ω -3 fatty acids lowers the rate of lipid peroxidation.

KEY WORDS

Lipid peroxidation, Fish oils

INTRODUCTION

Diets with high polyunsaturated fatty acid to saturated fatty acid ratio (PUFA:SFA) lower serum cholesterol levels, particularly low density lipoprotein (LDL). This is considered to be beneficial in view of the relationship between serum cholesterol and coronary heart disease (1, 2). The lower incidence of heart disease, diminished platelet aggregation and prolonged clotting times in Greenland Eskimos have been attributed to their high intake of fish and fish oils (3) which contain relatively high concentrations of eicosapentaenoic acid (EPA : 20; 5 ω -3) and docosahexaenoic acid (DHA : 22 ; 6 ω -3). In addition, the plasma concentrations of cholesterol, triglyceride (TG) and LDL are all low in the Eskimos, whereas high density lipoprotein (HDL) concentration is raised. All these factors are considered to militate against atherosclerosis and myocardial infarction (1-4).

In the recent years, an increasing interest has developed in the long chain ω -3 fatty acids such as

EPA and DHA. So it has become a practice to consume ω -3 fatty acid rich fish oils in order to increase ω -3: ω -6 ratio of the diet (5). Several studies have indicated that consumption of ω -3 fatty acids can benefit persons with cardiovascular disease and rheumatoid arthritis. However, the PUFA's present in fish oils because of their high degree of unsaturation, have increased vulnerability to lipid peroxidation. To counteract this vulnerability, the practice of adding antioxidants to fish oils has come in vogue. It has been shown that supplementation of fish oil with antioxidant Vitamin E inhibits peroxidation of oil (5). Fish oils under different trade names are now available apparently to provide PUFA's with and without vitamin E supplementation. However, the safety of consumption of these products has not been established with reference to, particularly the level of peroxides contained in them.

Many humans are continually exposed to oxidized oils and fats in their diet which arise from either shallow or deep fat frying processes and the possibility that regular consumption of such products may be deleterious to human health, has attracted much interest (6-8). Based on the above reports, it was considered essential to determine the level of thiobarbituric acid (TBA) reactive material (Lipid peroxide level) present in the fish oils available in the market with and without Vitamin E supplementation. The extent of peroxidation has also been studied after heating the fish oil to 180°C (Food frying temperature) to different time durations, in order to assess the

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protective action of Vitamin E during thermal stress and the relevance of the TBA reaction method.

MATERIAL AND METHODS

All the chemicals used were the commercial products of highest analytical grade available in India. Fish oils with and without Vitamin E supplementation were collected from the drug houses of Mangalore, all within the expiry date mentioned on the labels. Thermal stressing of fish oil was done by adding 100 ml of fish oil (with and without Vitamin E) in a china dish with temperature regulated to 180°C and small aliquots were withdrawn at different time intervals.

The TBA reactivity in fish oils was determined by following the method of Ramachandra Prabhu with slight modifications (6). To 0.01 ml of fish oil was added 2 ml of 2-thiobarbituric acid (0.67% W/V) followed by 2 ml of trichloroacetic acid (10% W/V). After mixing, the tubes were covered with marble stones, kept in boiling water for 15 minutes, cooled to room temperature, and centrifuged (2000 g for 10 minutes). The pink TBA chromogen in the aqueous layer was measured at 532 nm against appropriate blanks.

RESULTS AND DISCUSSION

Table 1 summarizes the TBA reactivity of fish oil of various brands available in the market. It is significant to note that the level of lipid peroxidation products were highest in Product 1 mainly due to its exposure to atmospheric oxygen, since the product was marketed in bottles, whereas the other products in encapsulated form. Product 2, even though not supplemented with vitamin E, contained very comparable TBA reactivity to those oils supplemented with vitamin E: ω -3 fatty acid ratio of 4:3 or 4:6. The results do not indicate inverse relationship between the vitamin E content of the oil and TBA reactivity. The reasons proposed for such observations are the

inefficient method used for extraction of oil from fish and its storage and the delay in the supplementation of the oil with vitamin E.

Thiobarbituric acid reactivity of thermally stressed fish oil and the effect of Vitamin E addition are shown in Table 2. Vitamin E (2% W/V) significantly lowered the rate of peroxidation of fish oil subjected to thermal stress at 180°C at different time durations. It may be noted that the optical density values even at 0 min were significantly lower for the fish oil with vitamin E. These observations were attributed to inhibition of lipid peroxide formation during the assay of TBA reactivity, which requires boiling the assay mixture at 100°C for 15 min. This important and very significant observation to our knowledge has not been taken into consideration by the earlier workers who have measured the lipid peroxidation by TBA reaction.

It is evident from the data presented in Table 3 that the TBA reactivity of fish oil is about 26 times higher than the sunflower oil prior to thermal stress, 9 times higher than sunflower oil thermally stressed for 15 min during domestic frying and 5 times higher than sunflower oil thermally stressed for 2 to 3 hrs during commercial food frying. These observations clearly indicate the magnitude of increase of peroxidation of fish oils available in the market.

The toxic effects of PUFA -derived aldehydes are well described in animal studies (7). Dietary consumption of peroxidation products may represent an important risk factor for the induction and development of atherosclerosis in humans (8). In spite of substantial evidences of health benefits of ω -3 fatty acids, consumption of fish oil is unlikely to be suited to lifetime daily use. Considering their high level of peroxidation products, it is also very much necessary to refine the methods used for extraction and preservation of the fish oil before marketing them.

Table 1. TBA reactivity of fish oils

S.No	Product	Vitamin E : ω -3 fatty acid ratio	TBA reactivity, OD at 532 nm per 10 l oil
1.	Product 1	—	0.26 $\pm$ 0.03
2.	Product 2	—	0.10 $\pm$ 0.01
3.	Product 3	4 : 3	0.12 $\pm$ 0.03
4.	Product 4	4 : 3	0.16 $\pm$ 0.02
5.	Product 5	4 : 3	0.11 $\pm$ 0.02
6.	Product 6	4 : 6	0.10 $\pm$ 0.01

Products 1 and 2 were not supplemented with vitamin E. Product 1 was marketed in 120 ml bottles.

All the other products were in encapsulated form.

Results are $\pm$ SD of 5 separate experiments.

Table 2. TBA reactivity of fish oil: Effect of vitamin E and thermal stress

Vitamin E Content	TBA reactivity at different time durations of thermal stress per 10 l oil. Optical density at 532 nm					
	0 min	15 min	30 min	45 min	60 min	120 min
NIL	0.26±0.02	0.33±0.03	0.43±0.04	0.63±0.04	0.75±0.03	1.02±0.06
2 gm %	0.09±0.02	0.13±0.03	0.19±0.02	0.24±0.02	0.29±0.02	0.38±0.03

Results are mean ± SD of 4 separate experiments.

Table 3. TBA reactivity of sunflower oil, thermally stressed under different conditions and fish oil

	Prior thermal stress	Domestic frying	Commercial frying
Sunflower oil ⁵	0.01 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Fish oil (Product 1)	0.26 ± 0.03	-	-

OD at 532 nm

A better approach suggested to increase the dietary ratio of ω -3: ω -6 fatty acids is to consume ω -3 fatty acid rich foods such as fish, flaxseeds, walnuts, mustard and soybean rather than fish oils.

REFERENCES

1. Kinsella, J.E., Lokesh, B. and Stone, R.A. (1990). Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am. J. Clin. Nutr.* 52, 1-28.
2. Mori, T.A. (2004). Effect of fish and fish oil-derived omega-3 fatty acids on lipid oxidation. *Redox Rep.* 9, 193-197.
3. Nestel, P.J. (1990). Effects of N-3 fatty acids on lipid metabolism. *Annu. Rev. Nutr.* 10, 149-167.
4. Holman, R.T. (1998). The slow discovery of the importance of ω -3 essential fatty acids in human health. *Am. J. Nutr.* 128, 427-433.
5. Luotola, M.T. and Luotola, J.E.I. (1985). Effect of α -tocopherol on the peroxidation of cod liver oil. *Life chemistry reports* 3, 159-163.
6. Ramachandra Prabhu, H. (2000). Lipid peroxidation in culinary oils subjected to thermal stress. *Ind. J. Clin. Biochemistry* 15, 1-5.
7. Grootveld, M., Atherson, M.D., Sheerin, A.N., Hawkes, J., Blake, D.R., Richens, T.E., Silwood, C.J.L., Lynch, E. and Clarkson, A.W.D. (1998). *In vivo* absorption, metabolism and urinary excretion of α - β -unsaturated aldehydes in experimental animals. *J. Clin. Invest.* 102, 1216-1218.
8. Clarkson, A.W.D., Hawkes, G.E., Richardson, D.A., Naughton, D.P., Haywood, R.M., Chander, C.L., Atherton, M., Lynch, E.J. and Grootveld, M.C. (1994). Generation of lipid peroxide products in culinary oils and fats during episodes of thermal stressing: A high field HNMR study. *FEBS. Lett.* 355, 81-90.