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IN VITRO INHIBITION OF LIPID PEROXIDATION IN FISH BY TURMERIC (CURCUMA LONGA)

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ABSTRACT

The beneficial effects of ω-3 fatty acids on human health have been well documented. Fish and fish oils are the richest sources of ω-3 fatty acids. However, due to their high degree of unsaturation, they are highly susceptible to lipid peroxidation. Regular consumption of peroxidised oils may represent a risk factor for the induction and development of atherosclerosis. In view of the above reports, it was considered necessary to study the effects of turmeric on fish lipid peroxidation during standard cooking practices and on time-dependent changes in the peroxidation of fish homogenate. The antioxidant effect of α-tocopherol was also studied to confirm the relevance of the study. The results suggest that turmeric may be considered as a safe, cheap and readily usable antioxidant for food preparations.

KEY WORDS

Lipid peroxidation, fish homogenate, turmeric.

INTRODUCTION

Lipid peroxidation arising from the reaction between free radicals and lipids is considered as an important feature of cellular injury. Several studies suggest that lipid oxidation products ingested with food or produced endogenously represent a health risk (1). There is increasing interest in the role of diet and nutrition in human health, pathogenesis and possible prevention of disease. Endogenous antioxidant defense systems, though scavenge and minimize the formation of oxygen free radicals, are not fully effective especially in pathological conditions demanding use of exogenous antioxidants (2). Epidemiological studies indicate that a marine rich diet containing fish is beneficial due to presence of ω-3 fatty acids and is associated with reduced mortality from coronary heart diseases (3,4). The beneficial effects such as lowered plasma lipids, diminished thrombogenicity and decreased blood pressure have been attributed to the accumulation of ω-3 fatty acids in the individuals consuming fish regularly (5-7). The polyunsaturated fatty acids (PUFA) account for 30-50% of the total fatty acids in fishes from Indian waters (8).

Free radical mediated oxidation of lipids particularly affects the PUFA due to their high degree of unsaturation. Consumption of lipid peroxidation products are thought to be associated with various diseases such as atherosclerosis, cancer and myopathy (9,10). Haywood et al (11) have shown that heating of culinary oils to 180°C generated a variety of peroxidation products such as aldehydes and their conjugated hydroperoxy diene precursors. Such aldehydic products are found to be absorbed from the gut into the systemic circulation (12).

Several studies have been performed to ascertain the antioxidant potential of turmeric in diseases in which oxygen free radical plays a major role (13-16). Curcumin, (diferuloyl methane) is a natural antioxidant derived from turmeric, a spice used in Indian cooking and credited with therapeutic properties (17). Turmerin, the water soluble peptide present in turmeric, acts as a chain breaking antioxidant, in addition to being a quencher of reactive oxygen species (18). In view of the above reports, it was considered essential to determine the extent of antioxidant efficacy of turmeric towards lipid peroxidation in raw and cooked fish homogenate. The studies were extended to determine the time dependent lipid peroxidation changes with turmeric and α-tocopherol. The antioxidant effect

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of turmeric was studied along with a well- established lipoyl antioxidant α- tocopherol, to compare the relevance of the study.

MATERIALS AND METHODS

α-tocopherol was obtained from Fluka AG, Buchs, Switzerland. All other chemicals used were commercial products of highest analytical grade available in India.

Turmeric tubers were procured from local market and were finely powdered in a grinder.

Fish Processing: Fresh mackerels (Somberus Sombrus) were obtained from the local market, eviscerated, washed and fleshy muscular part was used.

Preparation of tissue homogenate and analysis of MDA:

(1) Raw and cooked fish muscle tissue (10%W/V) was homogenised in cold 10mM sodium phosphate buffer containing 0.15M sodium chloride, pH 7.2 using a tissue homogeniser.

(2) Raw and cooked fish muscle tissue (10%W/V) with turmeric powder (75mg) was homogenised as mentioned above. Cooking temperature was 100°C for 15 minutes. The unbroken cells and cell debris were removed by centrifugation at 2000g for 15minutes using Remi C-24 refrigerated centrifuge. The supernatant thus obtained was termed as homogenate and was used for the *in vitro* lipid peroxidation study. Lipid peroxidation studies were performed almost immediately after the homogenate preparation.

Thiobarbituric acid (TBA) reactivity in the homogenate was determined by following the method of Luotola (19). To 2ml of homogenate was added 3ml of 20% Trichloroacetic acid and mixed and centrifuged for 15min. To 2ml of supernatant, 2ml of 2-Thiobarbituric acid (0.67%W/V) was added, mixed and kept in boiling water bath for 10min, cooled to 20°C. The TBA chromogen was measured at 532nm against appropriate blanks. The amount of TBA-reactive material was expressed as nmoles of malondialdehyde (MDA)/gm of tissue taking the molar extinction coefficient of MDA as 1.56×10^5 (20).

Determination of TBA reactivity at different time intervals without thermal stress on fish homogenate treated with turmeric or α- tocopherol:

Raw homogenates prepared as above were divided into 3 groups: Group 1 represent control, Group 2 with Turmeric (75 mg%), Group 3 with 0.5ml of ethanol containing 5mg of α-tocopherol per 100ml of homogenate. These 3 groups were incubated at 37°C for 0,1, 2, 3, 4 hours. At the end of each incubation period, the extent of peroxidation in each groups were measured by thiobarbituric acid reactivity method and expressed as MDA equivalent/gm of tissue.

Statistical Analysis :The data are expressed as Mean ± SD. Statistical significance was determined by Mann-Whitney U test and Kruskal-Wallis test.

RESULTS

Effects of turmeric on lipid peroxidation on raw fish homogenate and cooked fish homogenate in saline phosphate buffer are summarized in Table 1. Homogenate when treated with turmeric and was cooked in phosphate buffer saline at 100°C for 15 minutes showed highly significant decrease ($P<0.01$) in peroxide level as compared to control.

TABLE 1
EFFECT OF TURMERIC ON LIPID PEROXIDATION IN FISH HOMOGENATE WITH AND WITHOUT THERMAL STRESS

Lipid peroxidation (nmoles of malondialdehyde/gm tissue) Mean ± SD			
Raw Fish Homogenate		Cooked Fish Homogenate	
Control n = 5	Treated with turmeric	Control	Treated with turmeric
19.86 ± 0.2	20.69 ± 0.2	27.17 ± 0.3	21.20 ± 0.5*

n is number of subjects; * $P < 0.01$

Comparative effects of turmeric and vitamin E on time-dependent lipid peroxidation of fish homogenate are summarized in Table 2. It is seen that fish homogenate treated with turmeric showed lipid peroxide level of 16.7±0.1nmole MDA/gm tissue to 17.9±0.1nmole MDA/gm tissue, while in

TABLE 2
EFFECTS OF TURMERIC AND α-TOCOPHEROL WITH CONTROL ON TIME COURSE OF LIPID PEROXIDATION IN FISH HOMOGENATE

LIPID PEROXIDATION(nmoles of malondialdehyde/gm tissue) Mean±SD					
HOUR	0	1	2	3	4
CONTROL	17.16±0.1	18.70±0.49	20.28±0.2	22.51±0.4	25.06±0.3
TURMERIC	16.77±0.1**	17.19±0.1*	17.44±0.1**	17.89±0.1**	17.9±0.1**
α- TOCO-PHEROL	16.50±0.0 **	17.19±0.1*	17.22±0.1**	18.25±0.2 **	18.67±0.3**

n = 4 in each group; * $P < 0.05$; ** $P < 0.01$

control the level of peroxides have increased from 17.16±0.1nmole MDA/gm tissue to 25.06±0.1nmoleMDA/gm tissue ($p<0.01$) over a four hour incubation period. α-tocopherol exhibited a similar pattern with 16.50±0.02nmole MDA/gm tissue to 18.67±0.3 nmole MDA/gm tissue as compared to control ($p <0.01$) over the four hour intervals.

Turmeric treated group exhibited profound reduction in lipid peroxides formed which was time-dependent becoming evident from the second hour onward. Turmeric reduced the lipid peroxides hourly by 2.2%, 8%, 13.6%, 20.5%, 28.5% respectively as compared to controls. Vitamin E treated homogenate group showed reduction of lipid peroxides hourly as 3.8%, 8%, 15%, 18.9%, 25.4% respectively as compared to controls.

DISCUSSION

Turmeric significantly inhibited the extent of lipid peroxidation in cooked fish homogenate. Gold et al (21) have reported that heating process generates mutagenic epoxides, hydroperoxides and unsaturated aldehydes, which are carcinogenic. Study done with the enzymes from different mackeral fish tissues (skin, gills, muscles) mackeral muscle lipoxygenase has the highest hydroperoxides forming ability at temperature more than 50ºC (22). Iron salts present in fish homogenate are known to decompose lipids to generate peroxyl and alkoxyl radicals, both can abstract $H^{\bullet}$ and propagate lipid peroxidation (23).

Turmeric contains lipid soluble curcumin and water soluble turmerin (17,18). Hence the additive effects are shown well by partitioning easily and spreading evenly well in favourable medium provided by cooking. Cooking helps the fish fat to get into the medium and solubilise the curcumin part of turmeric. Cooking also alters the physicochemical nature of the membranes thereby, the solubility and site of action is favoured by having more access to the radical and thus better activity (24). Antiperoxidant effects of curcumin on several natural lipids of microsomes, brain lipids and synthetic lipids on liposomes are well established (25). Studies have shown that ingestion of turmeric reduced the levels of lipid peroxides and resulted in higher activities of superoxide dismutase, catalase and glutathione peroxidase in liver homogenate (26, 27). Studies have shown that curcumin is a potent inhibitor of lipid peroxidation catalysed by iron and its chelates in rat brain homogenate and rat liver microsomes (28)

Curcuminoids are potent inhibitors of experimentally induced lipid peroxidation as that of α- tocopherol (17). Curcumin has been found to be more potent than α- tocopherol as an antioxidant in the inhibition of lipid peroxidation of rat liver microsomes (14). Antioxidant activity of turmeric and α-tocopherol is mainly attributed to the phenolic group. The phenolic group provides a labile hydrogen atom for abstraction by free radical like peroxyl or alkoxyl radicals and gets converted to phenoxyl radical. The antioxidant potency depends on the stability and reactivity of this phenoxyl radical (29). In α-tocopherol the phenoxyl radical is stabilized by steric and electronic factors (30). In curcumin, the resonance is stabilized due to several possible tautomeric forms, along the extended conjugated system of double bonds (31).

In conclusion, turmeric with the active curcuminoids and water soluble turmerin, have antioxidant properties and hence effectively inhibits the free radical damage to biomolecules. The fact that turmeric acts as an antioxidant by prevention and intervention processes makes it very unique as a natural antioxidant.

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