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Comparison between the effects of α-tocopherol and BHT on the lipid oxidation of Kilka fish

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ABSTRACT

Three species of kilka (Clupeonella spp.) in the Caspian Sea have a high commercial value. In Iran it is use to produce canned, marinated packs and paste in making sausage and nugget. Kilka is a nutritious fish with high level of iron and considerable level of polyunsaturated fatty acids making it prone to auto-oxidation. The quality of kilka is the most important factor affects on its products, therefore antioxidants can be used during kilka handling and storage. Since consumers tend to choose products with natural additives, in this study the effects of α -tocopherol on the kilka fish oxidation is compared with BHT. Kilka was immerged in 0.1, 0.5 and 1 perecent w/v basis concentrations of α -tocopherol and BHT for 15 minutes, and then treated samples were packed separately and stored at 4ºC. The inhibitory effects of α -tocopherol on the outooxidation of kilka was investigated every 24 hours over a period of 4 days by monitoring changes in peroxide value, free fatty acid content as well as thiobarbituric acid reactive substances. Results showed that there was no significant difference (p>0.05) between 1% levele of α -tocopherol and the same level of BHT. One percent of α -tocopherol is therefore regarded as the preferred concentration for controlling oxidation of kilka oil on the basis of efficacy.

Key words: kilka, Autoxidation, Antioxidant, α -tocopherol, BHT.

INTRODUCTION

Kilka is a family of small but common schooling saltwater plankton-feeding fish. They are found in scattered areas throughout the world's oceans. They are concentrated in temperate waters and rare or absent in very cold or very warm seas.[11]

Kilka is the most numerous fish in Caspian Sea. Three kilka species; Cluponella engraulifermis, Clupeonella grimmi, Clupeonella cultiventris are nutritionally and commercially important [11,12]. Fish oil has a high content of long-chain polyunsaturated fatty acid (PUFA) include all cis-5,8,11,14,17- eicosapentaenoic acid (EPA) and all cis-4,7,10,13,16,19-docosahexaenoic acid (DHA). [4]

These are particularly necessary for the development of the retina and brain; DHA plays an important role in infant growth during nervous development in early life. In addition, EPA decreases the risk of cardiovascular diseases some types of cancer, and immune disorders. The two are also important for cell growth, differentiation, and metabolism, as well as for improving resistance to stress and regulating gene expression. The feotus receives fatty acid supply from the mother via active placental transmission. Since PUFAs of the ω3 family are essential nutrients, dietary fatty acid intake must be sufficient to satisfy the needs of both the mother and the developing foetus during pregnancy.[5]

The high degree of insaturation makes fish oil very susceptible to auto-oxidation resulting in breakdown products such as various aldehydes and ketones that render unacceptable colours odours, and flavours. [3]

Due to the health benefits associated with intake of fish, stabilization of products containing fish against oxidation is required.[7,9,12] Kilka is consumed fresh fired, canned in oil, brine or tomato sauce, it is also marketed as paste for making nugget, sausage and fermented fish sauce.

Addition of antioxidants to kilka is done to protect it during handling and storage. BHT and tocopherols are primary antioxidants or chain breaking antioxidants. They are free radical scavengers that delay or inhibit the initiation step, or interrupt the propagation of auto-oxidation. Consumers prefer natural additives in foods, thus the addition of synthectic antioxidant such as BHT has become a sensitive marketing issue even though it is considered safe. Tocopherols are found in many plants and they are perceived as natural. The antioxidant activity of tocopherols is mainly due to their ability to donate their phoenolic hydrogens to lipid free radicals. Various researches have done on protective effect of α-tocopherol on fish oils. [8,9,10,15] The aim of the present study was to compare the inhibitory effect of α - tocopherol with BHT on auto-oxidation of kilka over a period of 4 days storage at $4^{\circ}C$. Peroxide values (PV), free fatty acid (FFA) content and thiobarbituric acid reactive substance (TBARS) ,of fish oil samples were determined to access the development of rancidity

MATERIALS AND METHODS

Sample preparation

The kilka samples used for the study were obtained from local fishery station (Babolsar,Iran) in the month of July. The amount of kilka lipid varies in different seasons. Kilka catches was made at night using a cone net with a 1500-kw light to attract the fish. Fresh kilka immediately were placed in chilled sea water (CSW)container filled with water and ice and delivered to the research station.

Fresh kilka were immerged in emulsified solutions contaning different level of antioxidants 0.1% ,0.5% and 1% w/v basis for 15 minutes. These treated samples were divided into groups of 150 grams and packed separately into polyethylene bags and kept at $4^{\circ}C$. The control group was packed without antioxidants. 25 ppm of polyoxyethylene sorbitan mono oleate (Twin 80) was used as an emulsifier to dissolve the antioxidants in water and prepare the oil in water emulsion. Fish oil was extracted from kilka tissues using a mixture of hexane/isopropanol (3:2, v/v) solvent system.[10,15] Freshly extracted kilka oils were also analyzed for PV, FFA and TBA values.

Chemicals

The following chemicals used in the study, α-Tocopherol, BHT, trichloroacetic acid (TCA) , thiobarbituric acid (TBA) and poly oxyethylene sorbitan mono oleate (Twin 80) were purchased from Fluka Co., Germany.

Peroxide value (PV)

5gr sampl was placed in a 250ml Erlenmeyer flask and 30ml mixture of acetic acid/chloroform 3:2 (v/v) was added to each flask while stirring to dissolve the sample. Aliquots of 0.5 ml of mixture 2% KI solution was added to the samples and the reaction mixtures were left to stand for 1 min at room temperature (about 25 ^{o}c) with occasional shaking. 30 ml of distilled water was added to the mixture and the resulting reaction mixtures were each titrated with 0.1 N standardized sodium thiosulphate to a pale yellow, then 2 ml of starch indictor was added and titration was continued to a colourless end-point. A blank was carried out using a sample containing all the above reagents except the fish oil. Triplicate measurements of peroxide values of each treatment concentration were carried out. PV was calculated as:

PV= (S-B)N*1000/mass of sample(g)

where S and B are the volumes of titrant for the sample and blank, respectively, and N is the normality of sodium thiosulphate solution. Proxide value determined according to the AOAC official method28.022. [2]

Free fatty acids (FFA)

0.1gr sample was dissolved in 50ml neutralized isopropanol in a 250ml flask . Then 5 drops of phenolphthalein solution, as an indictor was added.

The prepared solution was titrate with 0.1N standardized potassium hydroxide(KOH) to a pink end point. FFA was calculated as oleic acid percentage:

$$FFA\% = \frac{\text{ml KOH} \times N \times 28.8}{10 \times \text{g sample}}$$

N= normality of potassium hydroxide.

FFA determined by AOAC oficial method 28.029. [2]

Thiobarbituric acid (TBA value)
Oil samples were homogenized with 25 ml of 5% TCA in distilled water using a Polytron (model PT-MR 3000, Kinematica AG, Littau, Switzerland) at a setting of 3000 rpm for 30 s at room temperature (about 25^{o}C). Duplicates of 2 ml aliquots of the blended samples were mixed with 3 ml of 0.02 M TBA solution in screw capped test tubes and vortexed for 30 s. The tubes were incubated in boiling water for 20 min and cooled under running tap water. The absorbance of the samples were measured using spectrophotometer varian Australia AA20 at 532 nm against a blank made up of 2 ml 5% TCA solution and 3 ml 0.02 M TBA solution. The concentration of TBA reactive substance was determind from a standard curve which was obtained from 5 μl aliquot of 0.99 g/ml solution 1,1,3,3 tetraethoxypropane (TEP) with 5% TCA. Further dilutions were made over a concentration range of 9×10^{-8} to 7.9×10^{-7} g/ml. Each dilution was incubated, and absorbances were determined against a blank after cooling. The concentration was reported as meq malonaldehyde/kg of sample. This method has been described in detail by Egan.[3,12]

Statistical analysis
Data were analyzed by one-way analysis of variance (ANOVA) using the SPSS version11 to compare the mean values of each treatment. Significant differences between the means of parameters were determined using Duncan's test ($p<0.05$)

RESULTS AND DISCUSSION

Peroxide value (PV)
Perimery oxidation products (hydroperoxides) were determinde by PV measurment. PUFA autooxidation involves free radical generation through a reaction catalyzed by light heat or tracemetals and enzymes. The free radicals formed propagate autooxidation by reacting with oxygen to form hydroperoxides, which break down to generate other new free radicals. The reaction is terminated by the formation of stable compounds via dimerization of free radicals. The hydroperoxides formed can be measured titrimetrically and their concentration shows the extent of oxidation. However, the peroxides are transient and temperature dependent. [5,12,13]

This imposes limitations of peroxide value alone as indicator of oxidation and necessitates complimentary method such as thiobarbituric acid reactive substances.

The data presented in Table 1 shows that there was significant difference ($p \leq 0.05$) between the control and samples treated with α-tocopherol. There was no significant difference ($p>0.05$) between samples treated with 1% α-tocopherol and samples with same concentration of BHT.

α-tocopherol quenched peroxy radicals by donating a hydrogen atom. This rapid quenching of peroxy radicals apparently stabilized the carbon-carbon bond which prevented formation of trans-trans hydroperoxides. [10,15] This study reveals that time had significant effect ($p=0$) on oxidation in treated sample. The previous studies confirms this result. [9,10,15]

Free fatty acids (FFA)
The formation of FFA is an important measure of food rancidity. FFA formation is due to the hydrolysis of triglycerides; this process may be promoted by the reaction of oil with moisture Lipid rancidity gives rise to a number of effects such as hydrolyzed and oxidation rancidity. In particular, polyunsaturated acids easily oxidized by air, producing peroxide which breaks down into aldehydes and ketones. The production of aldehydes and ketones causes unpleasant taste.[5]Considering Table 2 the results of free fatty acid in presence of 0.1% and 0.5% BHT and α-tocopherol were very close. At 1% level tocopherol was more effective than BHT but the difference was not significant ($p<0.05$) α-tocopherol prevents formation of trans-trans hydroperoxides, also inhibits the formation of diperoxides though its H-donating mechanism. [7,14,15] A comparision of the data between BHT and α-tocopherol shows that there was no significant differences between them. The perevious studies confirms that α-tocopherol minimize auto-oxidation of fish oil. [5,10,15]

Thiobarbituric acid (TBA value)

The TBA test is based on the formation of malonaldehyde from unsaturated fatty acid decomposition of lipid peroxidation products. Malonaldehyde condenses with two molecules of TBA to form a fluorescent red chromogen with maximum absorbance at 532 nm. [5] TBA is employed as an indicator for monitoring secondary products of oxidation. [5,13,15] The data presented in Table 3 shows the effect of different concentrations of BHT and α-tocopherol antioxidants on the formation of thiobarbituric acid reactive substances in kilka oil.Control is referred to kilka without added antioxidant. Results demonstrate that there was significant difference (p=0) between the control and samples treated with 1% α-tocopherol and BHT was most effective for preventing lipid oxidation.[9,15]This experiment shows that time had no significant effect (p>0.05) on the formation of oxidation products in the sample treated with both α-tocopherol and BHT during 4 days monitoring. Diverse studies have a valuated the antioxidant properties of BHA and BHT comparing to herbal antioxidants confirms this result. [6,7,8]

Table 1: Peroxide value (meq o_2/kg) at different concentration of BHT and α-tocopherol

Time (day)	Control	BHT %			α-tocopherol%		
		0.1	0.5	1	0.1	0.5	1
1	1.3	0.5	0.2	0.1	0.1	0.11	0.12
2	26	8.3	5.69	6.47	20.6	10.8	9
3	23.1	7.52	4.1	7.68	20.8	15	12
4	17.5	7.35	3.9	9.86	20	19	13.9

** Values are means of triplicate readings.*
** Control is referred to kilka without added antioxidant.*

Table 2: Free fatty acid (mg/100g) at different concentration of BHT and α-tocopherol

Time (day)	Control	BHT %			α-tocopherol%		
		0.1	0.5	1	0.1	0.5	1
1	7.05	9.701	7.47	9.90	8.01	7.90	7.30
2	20.88	9.940	7.42	12.56	17.62	18.49	15.49
3	19.62	10.790	11.28	12.99	21.98	18.14	21.01
4	17.11	9.478	10.55	17.063	22.03	18.01	20

** Values are means of triplicate readings*
** Control is referred to kilka without added antioxidant.*

Table 3: Thiobarbituric acid value (meq mlonaldehyde/kg) at different concentration of BHT and α-tocopherol

Time (day)	Control	BHT %			α-tocopherol%		
		0.1	0.5	1	0.1	0.5	1
1	0.307	0.100	0.090	0.069	0.111	0.100	0.068
2	0.560	0.140	0.166	0.126	0.187	0.186	0.164
3	0.336	0.170	0.148	0.133	0.177	0.150	0.136
4	0.329	0.121	0.105	0.170	0.177	0.084	0.074

** Values are means of duplicate readings.*
** Control is referred to kilka without added antioxidant.*

CONCLUSION

Regarding to penetration of anti oxidant during conditioning into the fish's texture, and the interest of consumers to natural additives, α-tocopherol can be considered as a suitable replace for BHT to extend the shelf-life of kilka and it's products. This study confirms pervious ones that α-tocopherol could be used to minimize auto oxidation of fish oil.

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