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Effect of various antioxidants on solvent extracted Indian Mackerel (Rastrelliger Kanagurta) fish oil

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ABSTRACT

This paper presents a study on the extraction of Mackerel fish oil of Indian origin and to investigate the extent of oxidation of mackerel fish oil with added antioxidants. Production and characterization of Mackerel fish oil was carried out using Soxhlet apparatus and n-Hexane as the solvent for extraction. The extraction was carried out at the boiling point of the solvent. Antioxidant property was simulated by storing the fish oil at 4°C and 25°C. Four different antioxidants were used to evaluate oxidation during storage. The stability of fish oil was evaluated by measuring the peroxide value and the anisidine value. Little changes were observed in the peroxide values of the fish oil during the initial 7days.Addition of BHT and Citric acid had no significance for retarding autoxidation of the fish oil at both storage conditions. TBHQ and BHA gave good antioxidants effect for fish oil stored at 4°C and 25°C. They were effective to reduce the formation of peroxides in Fish oil. There was very little secondary oxidation of fish oil and no significant effects of all four antioxidants on changes of anisidine values during the storage period.

Keywords: BHA, BHT, TBHQ, antioxidants, stability.

INTRODUCTION

Fish has been recognized as an excellent food source for human beings and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids [1]. Fish are a rich source of polyunsaturated fatty acids (PUFAs), namely the n-3 and n-6 PUFAs, which are beneficial to human health. Fish meat and oils are good sources of unsaturated omega-3 fatty acids viz. eicosapentaenoic acid (EPA, 20:5; n-3) and docasahexaenoic acid (DHA, 22:6n-3) [2, 3]. Fish oil can be obtained from eating fish or by taking supplements. Farmed fish, especially oily fish such as salmonids fed on fish oil provide an excellent source of these acids. Fish oil can be used directly in a purified form (nutraceuticals) in a wide range of foods. The daily recommended intake of EPA and DHA of 0.25 to 0.50 g can then be met [4, 5].

Fish that are especially rich in the oils known as omega-3 fatty acids include mackerel, tuna, salmon, mullet, sardines, sturgeon, bluefish, anchovy, sardines, trout, menhaden, and trout. We get about 1 gram of omega-3 fatty acids in about 3.5 ounces of fish [6].

Fish oil has a wide range of application. It is widely used for conditions related to the heart and blood system. Fish oil is used to lower blood pressure or triglyceride levels (fats related to cholesterol). It has also been tried for preventing heart disease or stroke. The omega-3 fatty acids which are present in fish oil are thought to be beneficial in treating hypertriglyceridemia, and possibly beneficial in preventing heart disease [7].

This paper mainly focuses on the soxhlet extraction process of Indian Mackerel fish and investigates changes in the quality of fish oil due to time and storage temperature. Indian Mackerel (Rastrelliger kanagurta), locally known as Bangda, is the most popular marine fish in west and south coast of India due to its abundance, year-round availability and low cost. The body of the Indian mackerel is moderately deep, and the head is longer than the body depth. These fish have thin dark longitudinal bands on the upper part of the body, which may be golden on fresh specimen [8]. Indian mackerel reach a maximum fork length of 35 centimeters (14 in), but are generally around 25 centimeters (9.8 in) in length [9]. The total PUFA content in Indian mackerel body was 53.4 % [10].

MATERIALS AND METHODS

Materials

Fresh Indian Mackerels were purchased from the local fish market located in Sewri, Mumbai, India. Sodium Thiosulphate anhydrous, Starch soluble, Potassium iodide, BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole), TBHQ (tert-Butylhydroquinone), Citric Acid were purchased from Thomas Baker (Mumbai, India). Hexane, Acetic acid glacial and Chloroform were provided from Rankem (Haryana, India). Iso-octane and P-Anisidine were provided from Hi-Media (Mumbai, India). All chemicals used were of analytical grade.

Sample preparation

The Mackerel fish (Rastrelliger kanagurta) were bought fresh from the market. The internal organs were removed and the fish was washed to remove the residual blood by cold water. Fish fillet was obtained by cutting the fish into small pieces. The 10% in length of the anterior part of the fish is considered as the head. The body pieces and heads were stored at 4˚C separately. and then oven dried at a drying temperature 70˚C. The dried samples were kept in desiccators until use. For experimental analysis, the dried samples were ground into small particles.

Determination of moisture content of the fish

The Oven method was used for moisture content determination. The principle was that a test portion was heated at 105^0C until moisture and volatile substances are completely eliminated, and the loss in mass determine.

The fish oil extraction process

The fish oil was extracted using Soxhlet extractor and n-Hexane was used as the solvent. Weight of the sample taken and then sample placed in a porous thimble. Then thimble covered and placed in the inner tube of the apparatus. Apparatus then fitted to a round bottom flask that contains the solvent. To heat the solvent heat was applied to its boiling point for 1 hour. After some time the solvent in the flask started boiling and the water begins to drop from the top to the sample. The solvent siphoned over into the flask when it reached the top of the tube. During the process of refluxing the portion of oil has been extracted which removes in siphon cycle. The extracted oil was evaporated under vacuum at 45 ºC using an equitron roteva rotary evaporator (Germany). And solvent used was also recovered in this process. Oil extracted was collected and measured.

Chemical Analysis of extracted oil

Acid value (AV), Iodine value (IV), peroxide value (PV) and saponification value (SV), p-Anisidine values were determined according to procedures in AOCS method (AOCS, 1992). Totox value was calculated as 2PV + AV. All analyses were done in duplicate.

Storage conditions and Analysis of oxidative stability for oil with addition of Antioxidants

Stability at 4°C and at room temperature of Soxhlet extracted fish oil was evaluated by storage of oil sample in amber colored glass bottles. Four different antioxidants were used to determine oxidative stability of fish oil. Five sample bottles were stored at each storage condition. Among five sample bottles one was blank (without antioxidant) and other four samples were added with TBHQ, BHT, BHA and Citric acid individually. These five sample bottles were stored at 4°C and at room temperature.

AV, PV, p-Anisidine value and Totox were used to measure the extent of lipid oxidation at 2 days interval. All determinations were carried out in duplicates. Av, PV, p-Anisidine were measured using AOCS standard methods. Totox value was calculated as 2PV + AV. The oil sample without the addition of an antioxidant was employed as control.

RESULTS AND DISCUSSION

Moisture content in oil

The moisture content of Mackerel fish found to be 60.78%.

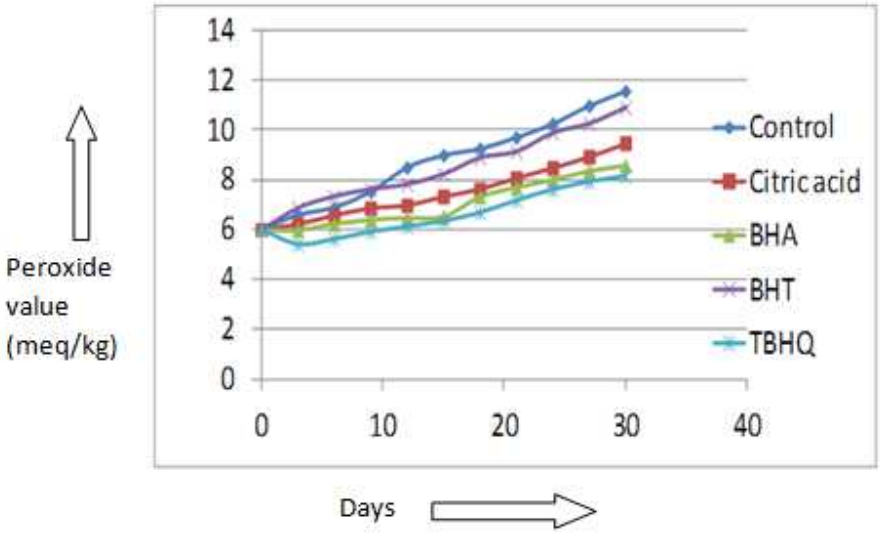
Table 1 Chemical analysis of extracted fish oil

Sample	F.F.A.	Acid Value (mg of KOH/kg)	Sap. Value	Iodine value (gl2/100gm)	Peroxide value (meq/kg)	p-Anisidine value	Totox number
Mackerel fish oil	2.79	5.16	198.38	185.37	5.985	15.625	27.595

Changes in the peroxide value of fish oil during storage

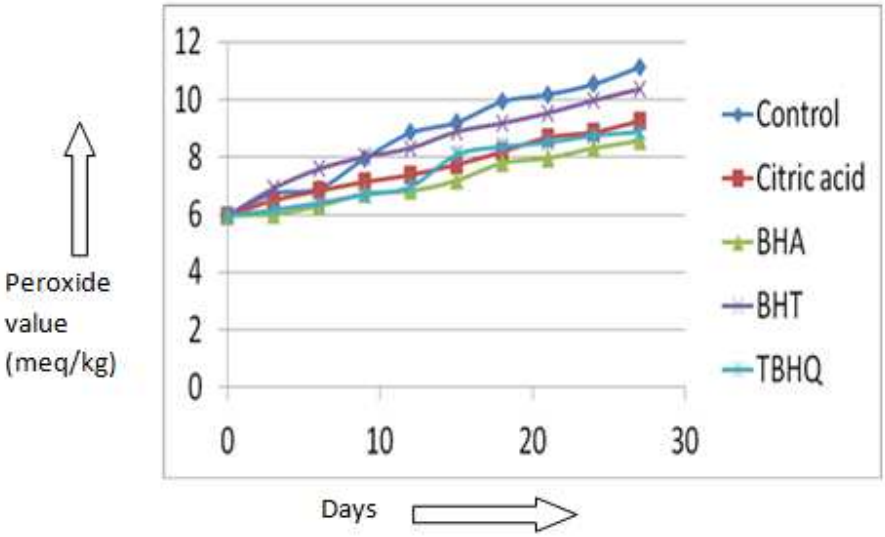
By measuring peroxide value primary oxidation of oils were determined. Changes in peroxide values of the fish oil with four different antioxidants and control sample stored at 4° C and 25°C are shown in Figure 1 and 2 respectively.

Figure 1 Changes in the peroxide value of fish oil stored at 4° C



There were very little changes in the peroxide values of samples with and without antioxidants until 1week of the experiment. Increasing storage time apparent differences were observed in the samples. In the case of TBHQ addition, the peroxide value was lower in comparison with other samples. At the end of the experiment the peroxide values of the control sample had the highest value of 11.5 meq/kg, followed by BHT (PV = 10.9 meq/kg), Citric acid (PV = 9.5 meq/kg), BHA (PV = 8.6 meq/kg) and TBHQ (PV = 8.1 meq/kg).

Figure 2 Changes in the peroxide value of fish oil stored at 25° C

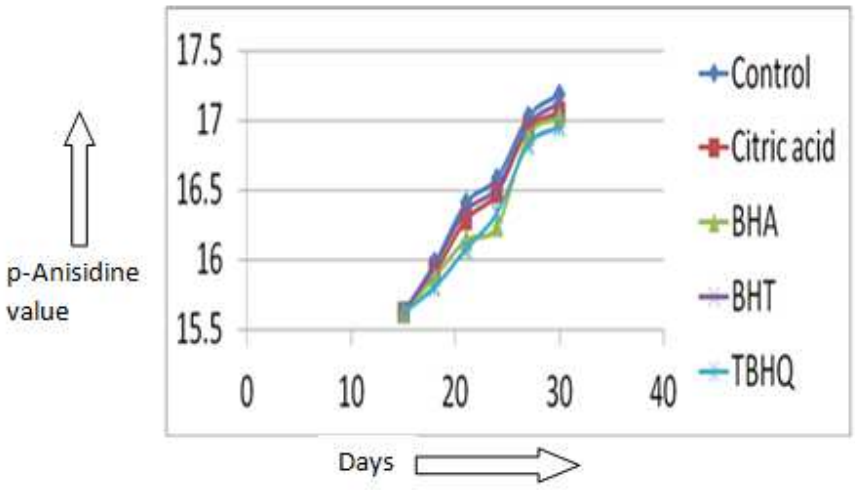


Increasing storage time apparent differences were observed in the samples. In the case of TBHQ addition, the peroxide value was lower in comparison with other samples. At the end of the experiment the peroxide values of the control sample had the highest value of 11.8 meq/kg, followed by BHT (PV = 11.2. meq/kg), Citric acid (PV = 9.8 meq/kg), BHA (PV = 8.9 meq/kg) and TBHQ (PV = 9 meq/kg).

Changes in the anisidine values of fish oil during storage

Secondary oxidation products formation was measured by p-anisidine values. Changes in anisidine values of the fish oil with and without antioxidants stored at 4°C and 25°C are shown in Figure 3 and 4 respectively.

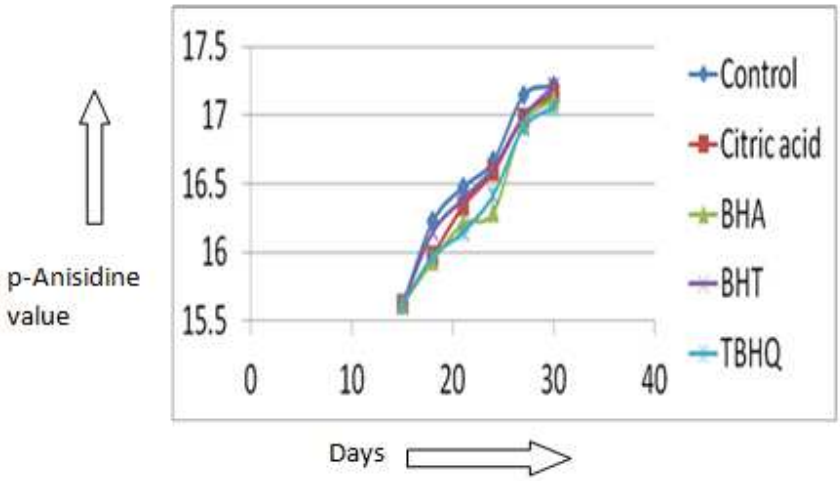
Figure 3: Changes in the anisidine values of fish oil with and without antioxidants stored at 4°C



As shown in Figure 3 there were little differences in anisidine value of the fish oil with and without antioxidants from the beginning to the end of the experiment.

At 4°C the anisidine value of samples that was 15.6 at beginning, increased slightly to 17.2(control), 17.1 (BHA), 17.1 (Citric acid), 17 (BHT) and 17 (TBHQ) at the end of the experiment.

Figure 4: Changes in the anisidine values of fish oil with and without antioxidants stored at 25°C



As shown in Figure 4 there were little differences in anisidine value of the fish oil with and without antioxidants from the beginning to the end of the experiment.

At 25°C the anisidine value of samples that was 15.6 at beginning, increased slightly to 17.2(control), 17.2 (BHA), 17.2 (Citric acid), 17.1 (BHT) and 17.1 (TBHQ) at the end of the experiment.

There was no significant difference in p-anisidine value for the samples with antioxidants and without antioxidants.

CONCLUSION

Under both conditions of storage of fish oil, little changes were observed in the peroxide values of the fish oil with and without antioxidants for the first 7 days. Anti-oxidative effects of TBHQ, BHA, BHT and Citric acid were observed in the fish oil. There was very little difference between the control and the sample with BHT and between samples of BHA and TBHQ. A significant difference was observed between the control and sample with TBHQ. The fish oil with TBHQ showed some difference from the other samples, which was apparent in lower peroxide values from the start of the experiment. TBHQ and BHA gave good antioxidants effect for fish oil stored at 4°C and 25°C. TBHQ and BHA were effective to reduce the formation of peroxides in Fish oil.

There was very little secondary oxidation of fish oil and no significant effects of all four antioxidants on changes of anisidine values during the storage period.

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