Pragasam A. et al., IJSRR 2019, 8(2), 2572-2585

Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

A Comparative Quality Assessment of Sardine Fish Oil Oxidation by Means of Sunlight, Uv Light and Heat Treatment

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ABSTRACT

Sardine fishes are good source of fish oil having rich nutritional value. Fish oil have high percent of unsaturated fatty acids and one of the conspicuous means of availability of omega-3 fattyacids. it is biologically active and plays significant role in preventing arterial hypertension, colon and prostate cancer, inflammatory diseases etc. The present work focused on characteristic deterioration sardine fish oil on heat treatment, irradiation of UV and sun light. On irradiation of the oil by sun light eight hours per day for nine days, recorded a maximum peroxide and para anis dine value of 335.6 meq O_2/kg and 50.2 respectively. Heat treatment and UV light irradiation records peroxide and para anis dine value 25.6, 101.5 meqO_2/kg and 24.7, 28.6 respectively. However, the peroxide and para anis dine values was controlled by using an antioxidant butylated hydroxyl toluene which reduce the peroxide and para anis dine values to148.8 meqO_2/kg and 34.8 respectively. A prominent FT-IR peaks at 3351 and 3374 cm $^{-1}$ validated the formation of new O-H stretching due to per oxidation of poly unsaturated fatty acids. The FT-IR peaks at 967 and 968 cm^{-1} indicate the C-H stretching frequencies of butadiene which has been formed due to oxidation of fish oil. The relative rate of per oxidation was maximum for the oil under the irradiation of sun light compared to UV light and heat treatment. It is cleared that the sun light is a strongest means of oxidation path way of sardine fish oil. Exposing of oil to sun light, the nutritional value significantly decreases compared to UV light and heat treatment. The comparative study correlates the effective energy source for the oxidation of sardine fish oil and concludes that sun light is most energetic source to oxidize the fish oil lipids.

KEY WORDS: UV light, Sunlight, Peroxide value, Para anisidine value, FT-IR, Peroxidation

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INTRODUTION

A large coastal population of India is depending on fisheries. Fish is a balanced food source and have a huge nutritional value. Fishes such as mackerel, salmon, sardines, tuna, etc. are some of the richest and cheapest sources of dietary supply of ω-3 fatty acids, including Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)[1,2,3,4]and Fish oil is an excellent dietary source, rich in essential fatty acids, especially Polyunsaturated Fatty Acid (PUFA) as (EPA) and (DHA). Researchers reported that consumption of fish oil containing PUFA prevents or cures arterial hypertension[5], colon and prostate cancer[6,7], inflammatory diseases[8] and disorders of the immune system[9]. Oil sardine (Indian oil sardine, Sardinella longiceps) is most landed fish in Yemen and the oil composed of many polyunsaturated fatty acids and more complex than land animal fat and vegetable oils as it contains a long chain polyunsaturated fatty acids[10]. Fish oils are unique in the variety of fatty acids of which they composed and their degree of unsaturation[11]. Refined fish oils are rich in polyunsaturated fatty acids of the linolenic acid family[12]. Fish oil is important not only for their application in food, but also for industrial applications, such as leather tanning, production of pharmaceuticals, cosmetics, paints, soap, glycerol and other products[13]. The consumption of omega3 fatty acids derived from fish oil, either in fish or as encapsulated fish oil has been shown to help maintain health, especially cardiovascular health[14]. Many of PUFA causes fish oil easily to hydrolytic spoilage especially oxidative deterioration[15]. Oxidation of lipids leads to some disadvantage including rancid odours and flavours, reducing nutritional quality and safety, which may cause health hazards[16]. The high nutritional benefits of fish consumption are mainly due to their proteins and lipids of high biological value, with long-chain polyunsaturated fatty acids (LC-PUFA) and certain minerals and vitamins[17]. Bahurmiz, O. M. et al. and Sugeng Heri Suseno et al.[18,19] evaluated the nutritional characteristics of the flesh of oil sardine (Sardinella Longiceps) and Indian mackerel (Rastrelliger Kanagurta) caught from Hadhramout coast of the Arabian Sea. The protein content was 21.6% and 18.1% (wet weight basis) for mackerel and sardine, respectively. The lipid content was much higher in sardine (10.1%) compared with mackerel (1.7%). The fatty acid composition showed that total saturated fatty acids had the highest relative value (37.5%) among other fatty acid groups in the flesh lipids of sardine, followed by polyunsaturated fatty acids (29.9%) and monounsaturated fatty acids (23.4%). In mackerel, polyunsaturated fatty acids were present at 37.4%, followed by saturated fatty acids (36.7%) and then monounsaturated fatty acids (14.3%). Most of polyunsaturated fatty acids in both fish were deposited as omega-3 (89.8% in sardine and 87.9% in mackerel), of which docosahexaenoic acid and eicosapentaenoic acid were the most abundant. Khoddami, A.et, al.[20] analyzed fish wastes for long-chain n-3 (omega-3) polyunsaturated (PUFA), particularly eicosapentaenoic acid (EPA) (C20:5 n-3) and docosahexaenoic acid (DHA)

(C22:6 n-3). These wastes include the head, liver and inestine. Sahana C Hiremath. Et, al.[21] characterized the fish oil by various analytical techniques such as High Performance Liquid chromatography (HPLC), thin layer chromatography (TLC), Fourier infrared spectroscopy (FTIR) in order to isolate omega 3 fatty acids from fish oil. Quality of fish oil was analysed in order to determine impurities, acid value and peroxide value. Ángela García Solaesa, et al.[22] reported that Triacylglycerols (TAG) of two different refined fish oils from sardine and a mixture of tuna and sardine oil were separated by reverse phase high performance liquid chromatography (RP-HPLC) with a binary solvent gradient of acetone/acetonitrile. The subsequent separation of 2-monoacylglycerol (2-MAG) by TLC (thin layer chromatography) analysis showed that ethanolysis system is effective for analysis of FA composition at the 2-position in oils containing PUFA.

Sardine (Sardinella lemuru) fish canning industries produce oil waste that can be potentially reused because of its omega-3 content. However, the majority of the omega-3 content are still bound to triacylglyceride form. Maria Goretti, et al.[23] reported hydrolysis process using lipase of whole-cell biocatalyst Mucor circinelloides immobilized in Poly-Urethane Foam (PUF). The optimum hydrolysis products were obtained at pH 7 and temperature 35 °C. The hydrolysis product at the optimum condition was fractionated using Thin Layer Chromatography (TLC). After hydrolysis, GC result showed an increase in omega3 (EPA and DHA) content as much as 12.56 % compared to the crude oil. Researchers reported the nutritional importance to the human body by reducing the risk of Alzheimer's disease, cardiovascular disease, cognitive decline and cancers, and by regulating inflammation, blood pressure and thickness, hormone production and the activities of the immune and central nervous systems[24,25,26,27,28,29,30]. Enrichment of omega-3 fatty acids in cod liver oil via alternate operation of solvent winterization and enzymatic inter esterification was attempted by Lei, Q.et al.[31]

The present study focuses the changes in the physicochemical characteristics of sardine fish oil extracted from fishes available in Arabian sea coastal region of Goa and Karnataka states, India. The rancidity due to primary and secondary oxidations were recorded with in terms of peroxide, para anis dine, iodine values. The rate of degradation of oil was studied under thermal and photolytic medium.

MATERIALS AND METHODS

1. Sample collection

I. 1kg of fresh variable sized sardine fish was collected from fish market, Karwar, Karnataka, India. The fishes were stored in freezer at -5^{0}C before used.

II. 1.5 Liters of fish oil was collected from fish oil extracting plant which was extracted by a wet process and stored in an air tight brown glass bottle.

2. Chemicals

The chemicals used are of analytical and HPLC grade which were purchased from Nice chemical supplier. Glacial acetic acid, Chloroform, Potassium iodide, Sodium thiosulfate, Potassium dichromate, Soluble starch, Isooctane and Anisidine.

3. Sampling

Fish oil samples were prepared as shown the table to determine the deterioration of the nutritional value of the oil.

Oil under heating	Fish oil heated to 80 -85^{0}C for eight hours before each determination
Oil under UV-light	Fish oil irradiated under UV- light for eight hours before each determination
Oil under sunlight	Fish oil exposed under sun light for eight hours before each determination
Control	Fish oil kept in moderately dark for eight hours before each determination
Oil + Butylated Hydroxyl Toluene(BHT)	Fish oil was added with BHT as an antioxidant and exposed under sunlight for eight hours before each determination

4.Extraction of fish oil

The, Fish was thoroughly washed with running water to remove sand and external debris. Scales, head, fins, spines, digestive system and excretory systems were removed and washed fleshy tissues of the fish thoroughly. The fleshy tissue was homogenized well and used for fish oil extraction. 100 g of homogenized tissue was taken into a 500 ml beaker. 20 ml of boiling water was added to the beaker mixed well and allowed to cool. Methanol: chloroform in 1:2 V/V was added and homogenized thoroughly. The homogenized mixture was centrifuged at 2000 rpm for 20 minutes at 25-27^{0}C. The supernatant liquid portion was taken in separation flask and the aqueous and organic layers were separated. The chloroform fraction was evaporated using flash evaporator and yield obtained was recorded.

Physical characterization

5. Moisture

Exactly five, grams of extracted oil were weighed into a beaker and heated in an oven at 100^{0}C for one hour. The oil was cooled outside the oven and then in the desiccator. Cooled oil was weighed for constant weight.

6. Density

Density of oil was determined specific gravity bottle method. The specific gravity bottle was washed well, rinsed with acetone and dried using a hair dryer. The bottle was cooled in desiccator and weighed for constant weight. The density of the sample was determined against distilled water as shown below.

$$\rho = \frac{Wo - W_e}{W_w - W_e}$$

ρ = Density of oil, Wo = Weight of specific gravity bottle with oil, W_e = Weight of an empty specific gravity bottle, W_w = Weight of specific gravity bottle with water

7. Refractive index

Refractive index of oil was determined by Abbe's refractometer at room temperature.

8. Acid value of oil (AV)

Fish oil was quizzed using muslin cloth from extracting plant to remove the suspended impurities and was used to determine the acid value of Acid value an important indicator of oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil. In this chapter, AV determination is based on the international standard method. The chemicals used are an analytical grade with 99% purity.

Exactly 1±0.05 grams of peanut oil was weighed into 250ml clean conical flask. To this, 25ml of neutralized ethanol was added and mixed well. The resulting solution was titrated against standard 0.1N KOH using phenolphthalein as an internal indicator to the end point colourless to pink colour. KOH solution was standardized by standard 0.1N oxalic acid solution.

$$\text{FFA or Acid Value} = \frac{V_{KOH} \times N_{KOH} \times \text{Eq Wt of KOH}}{W_{sample}}$$

$$\text{Acid Value} = \frac{V_{KOH} \times N_{KOH} \times 56}{W_{sample}}$$

8. Peroxide value(PV)

Exactly, 5gm fish oil was weighed into the Erlenmeyer flask with glass stopper. Acetic acid and chloroform mixture in the ratio 3:2 was added to the flask. To this 0.5 ml saturated KI solution was added, and peroxide value was determined by titrating against standard 0.01 M sodium thiosulfate solution using starch as an indicator. The procedure was followed by officially recommended method by AOCS.

$$PV = \frac{(S\text{-}B) \times N \times 1000}{\text{Weight of sample}}$$

B = Blank titre value. S = Sample titre value. N = Normality of sodium thiosulfate solution

9. p-anisidine value(p-AV)

The carbonyl content in oils was determined by the standard methods by AOCS. It measures the reactiveness of the aldehydes' carbonyl bond on the p-anisidine amine group forming a Schiff's base that absorbs at 350 nm. 2g (W) of sardine oil was dissolved in 25 ml isooctane and absorbance A_1 was measured at 350nm against a blank isooctane. An aliquot (5ml) of this solution, respectively 5 ml of isooctane (as blank) was transferred to each of the two test tubes of 10ml and 1ml anisidine solution (0.25% g/v glacial acetic acid) was added to each. After 10 minutes, the absorbance A_2 was measured at 350nm against isooctane containing p-anisidine. The p-AV is determined as; p-AV = 25 x 1.2 x $(A_2\text{-}A_1)$ / W[32].

RESULTS AND DISCUSSION

1.Proximate physical constants

Table1 gives the proximate values of moisture, density, refractive index, ash content and iodine. These values are the characteristics of oils that directly influence the nutritional value of the oil.

Table1: Proximate value of fish oil samples

Oil sample	Moisture percentage	Density g/ml	Refractive index	Ash Value	Iodine value
Fish oil extracted	0.24	0.9021-0. 9100	1.4772		166
Fish oil by wet process	0.38	0.8937- 0.9100	1.4780		164

2. Acid value

Acid Value is an important factor that decides the quality of fish oil and its nutritional value. It significantly evidences the lipid hydrolysis to form glycerol and free fatty acids. Table 2 correlates the acid values under thermal, UV- light, sunlight, darkness and with antioxidant in the presence of sunlight.

Table 2: Acid value versus time duration

Time duration(days)	1	2	3	4	5	6	7
Thermal activation	23.14	23.15	23.17	23.14	23.12	23.13	23.15
UV irradiation	23.15	23.14	23.15	23.15	23.15	23.13	23.12
Sun light	23.25	23.22	23.19	23.19	23.19	23.18	23.20
Control	23.14	23.14	23.14	23.15	23.15	23.14	23.14
Oil+ BHT in the presence of sunlight	23.14	23.15	23.15	23.16	23.17	23.16	23.18

A graph is plotted free fatty acid value versus time duration as shown in the figure 1 for different samples.

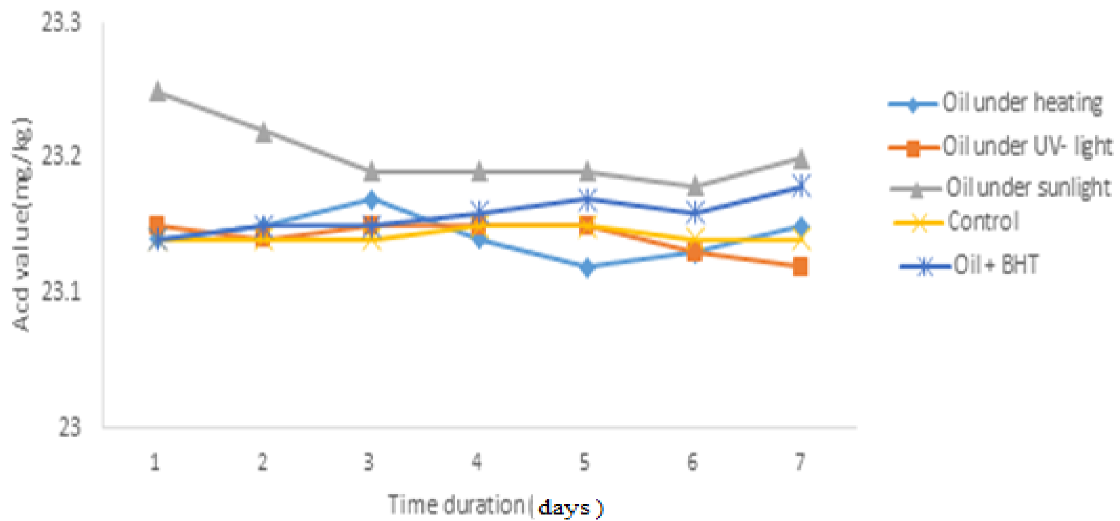


Figure 1: Free fatty acid value versus time duration

The plot clearly explains the trend of acid value on time duration. Fish oil on incubation temperature, 70^0C was allowed for eight hours and acid value was determined. This process was continued for seven days. Thermal activation is not much influenced the acid value and shows variation of 0.05. Up to three days, an increase in acid value was recorded with maximum from 23.14 to 23.17 and later, two day's AV decreased from23.17 to 23.12, but sixth and seventh days show an increase in the acid value. Irradiation of fish oil by UV radiation and sunlight for eight hours per and acid values were determined. The change in acid values remains almost a constant. However, when compared the AV of oil sample irradiated by sunlight, recorded maximum acid value of 23.25 and least of 23.18 than the other oil samples. The control was maintained in a moderately dark place whose acid value remains constant. The oil with BHT in the presence of sunlight show lesser acid values than oil without BHT. Overall, results of acid values of the sample correlates the hydrolysis of fish oil. That is, the hydrolysis is small.

3. Peroxide value

Peroxide values of different fish oil samples were recorded as shown in the Table 3 against time duration in days

Table 3: Peroxide value versus time duration

Time duration (days)	1	2	3	4	5	6	7	8	9
Thermal activation	6.40	8.0	9.5	9.6	11.8	13.4	16.8	17.6	25.6
UV irradiation	21.1	26.4	29.9	36.8	46.4	57.8	69.4	84.7	101.5
Sun light	49.6	75.1	107.1	142.3	201.5	248.9	276.8	307.5	335.2
Control	5.80	5.8	5.8	5.8	5.9	5.9	5.9	5.8	5.9
Oil+ BHT in the presence of sunlight	44.6	59.0	60.8	79.9	89.4	98.9	119.8	137.1	148.8

The graph was plotted peroxide values versus time duration of different fish oil samples as shown in the figure 2

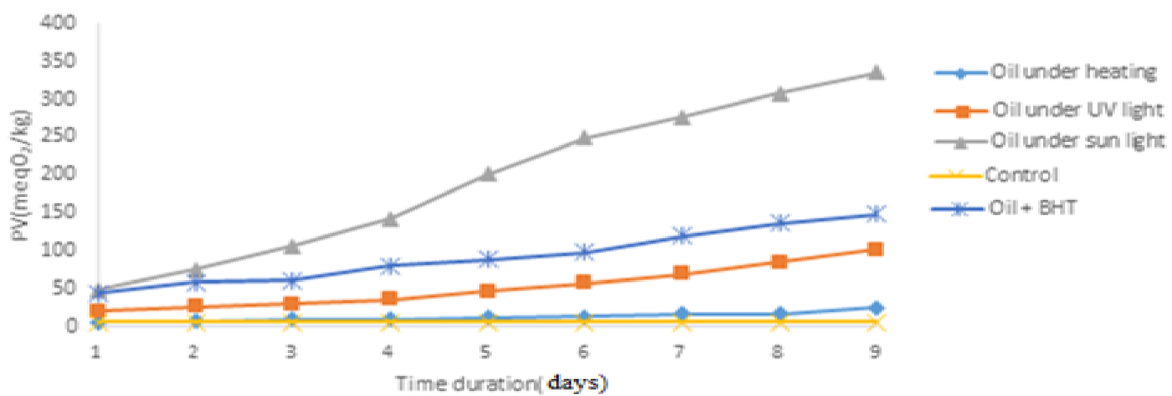
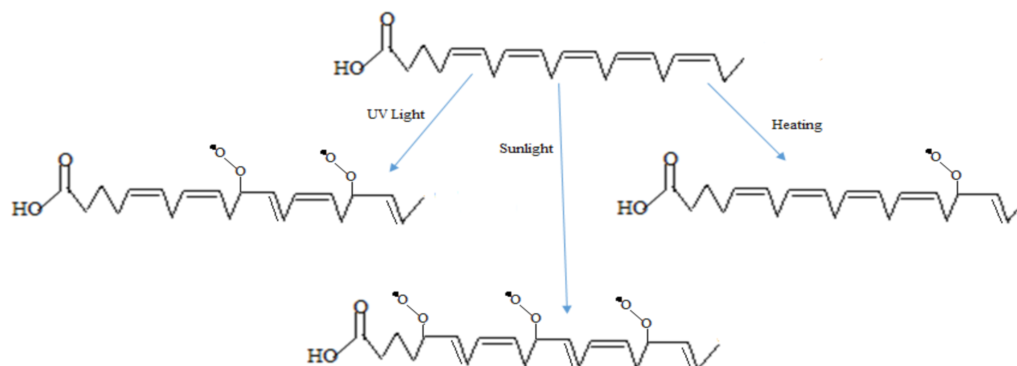


Figure 2: Plot of peroxide value against time duration

When fish oil heated at incubation temperature 70^0C for eight hours per day and peroxide value determined, the lowest peroxide value recorded was 6.4 meqO_2 for the first day and a maximum of 26.6 on the ninth day. Irradiation of oil by UV light for eight hours per day, and gives minimum peroxide value 21.1meqO_2 on first day and maximum of 101.5 meqO_2 on ninth day. When fish oil was exposed to sunlight for eight hours per day for nine days, minimum peroxide value of 49.6 meqO_2 was recorded on the first day and a maximum of 335.2meqO_2/kg on the ninth day in compared with controlled fish oil having BHT as an antioxidantrecording a peroxide of 44.6meqO_2/kg for the first day and 148.8 meqO_2 on the ninth day respectively. The control under dark doesn't change its peroxide value. It remains as constant from the first day to the ninth day (5.4 meqO_2/kg. Peroxide value of fish oil with sunlight is rapidly increased in comparison with the fish oil with UV light and heating. The rate of peroxidation of oil with sunlight is 12.6 times faster than the oil on heating and 3.3 times than that of oil with UV light irradiation. The peroxidation of with UV light is 3.8 times faster than that of the oil under heat treatment. The rate of peroxidation with sunlight has been reduced significantly 2.3 times with the use of antioxidant BHT. The experimental

discussion clearly indicates that the oil on exposing to sunlight, the nutritional values and quality of oil got deteriorated. This causes rancidity and reduces the lifetime of fish oil. Since, the fish oil consisted maximum amount of polyunsaturated fatty acids, rate of per oxidation was faster with sunlight. The schematic mechanism of per oxidation is as below



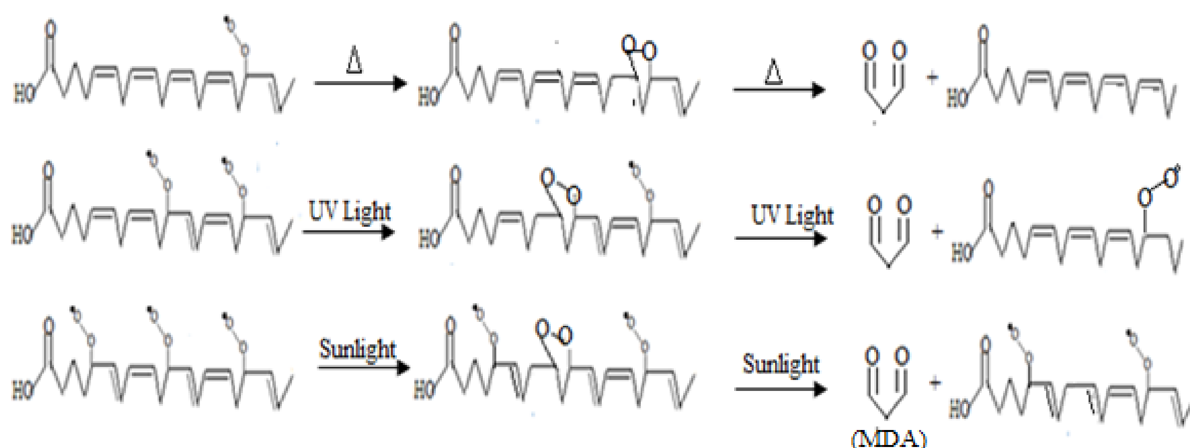
4. p-Anisidine value (p-AV)

p-Anisidine values of fish oil sample were determined against time duration at different conditions as shown in the Table 4

Table 4: p-Anis dine value versus time duration

Duration days	1	2	3	4	5	6	7	8	9
Thermal activation	15.2	15.7	16.8	17.7	18.9	20.2	21.8	23.1	24.7
UV irradiation	17.3	18.9	20.2	21.1	22.3	23.0	24.5	25.7	28.6
Sun light	24.3	26.5	28.6	31.4	34.6	36.2	39.7	45.8	50.2
Control	14.8	14.8	14.8	14.9	14.9	14.9	14.9	14.8	14.8
Oil+ BHT in the presence of sunlight	24.6	25.4	26.9	28.1	29.4	31.3	32.1	33.4	34.8

The p-AV under thermal activation was relatively lower compare to UV and sunlight irradiation. A maximum p-AV was recorded for the fish oil under sunlight as 50.2 to that UV light and thermal activation 28.6 and 24.4 respectively on the ninth day of the experiment. The rate of secondary oxidation of the fish oil under sunlight is 2.03 time faster to that of thermal activation and 1.76 times to that of UV irradiation. p-AV was significantly controlled by using an antioxidant BHT and records a value of 34.8 and the rate of secondary oxidation has been reduced 1.44 time to that without antioxidant. The secondary oxidative product ketones formed react with anisidine forming Schiff's base gives p-AV. The rate of formation of secondary product is directly related to polyunsaturated fatty acids of the oil. Fish oil has a maximum percent of EPA and DHA omega fatty acids. The schematic pathway of formation of secondary oxidative product is as shown below.



5. FT-IR spectrum of peroxidation

FT-IR ranging from 800- 3800 cm^{-1} was recorded for fish oil samples on the first and 9th day treatment. The different IR vibrations recorded were as shown in the table. The oxidation of lipids was related by observing weak peaks at 3351 and 3374 cm^{-1}. The prominent appearance of these peaks becomes conspicuous under irradiation with UV-light and sunlight on the ninth day of irradiation. The Figure 3 clearly indicates the absence of per oxidation by the missing of the peak at 3374 cm^{-1}on the first day. The appearance of peaks at 3351 and 3374 cm^{-1} indicates the hydroxyl stretching in peroxide as shown in the Figure 4. Peaks at 967 and 968 cm^{-1} confirm the stretching of C-H butadiene which has been formed due secondary oxidation of lipids. The other peaks related to triglycerides were as shown in the table.

IR Frequency(cm^{-1})	Mode of Vibration
3449	Overtone of the glyceride ester carbonyl
3374	Weak band of O-H stretching of hydro peroxide on oxidation
3351	The weak band associated with hydroxyl groups newly formed during the oxidation
3010	C-H stretching vibration of the cis-double bond (=CH)
2923	Asymmetric stretching vibrations of methyl groups
2852	Symmetric stretching vibrations of methyl groups
1742	The C=O group of triglycerides
1654	Stretching vibrations of carbon-carbon double bond cis
1464, 1375	Deformation vibrations of CH_2 and CH_3 groups
1250	Stretching vibration of the C-O ester group
1149	Bending vibration of the CH_2 group
968, 967	C-H stretching butadiene_

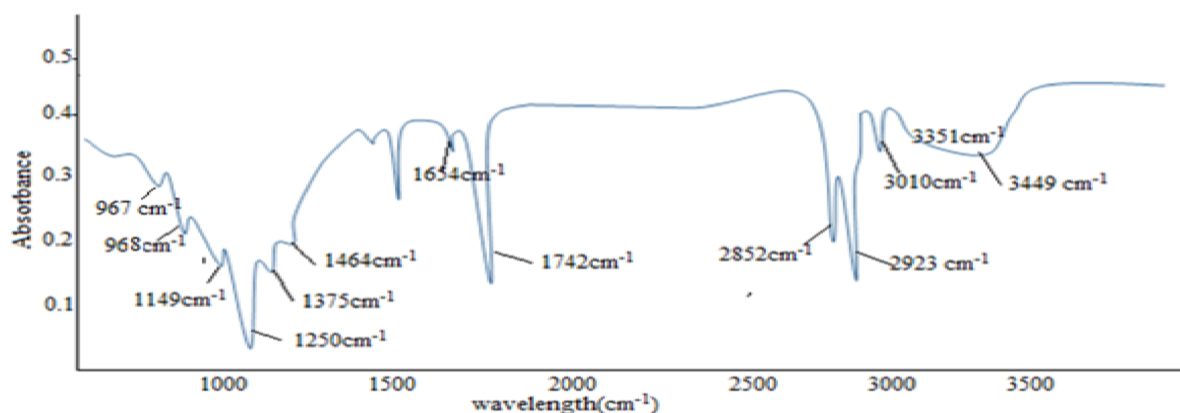


Figure 3: FT-IR of Fish oil before oxidation

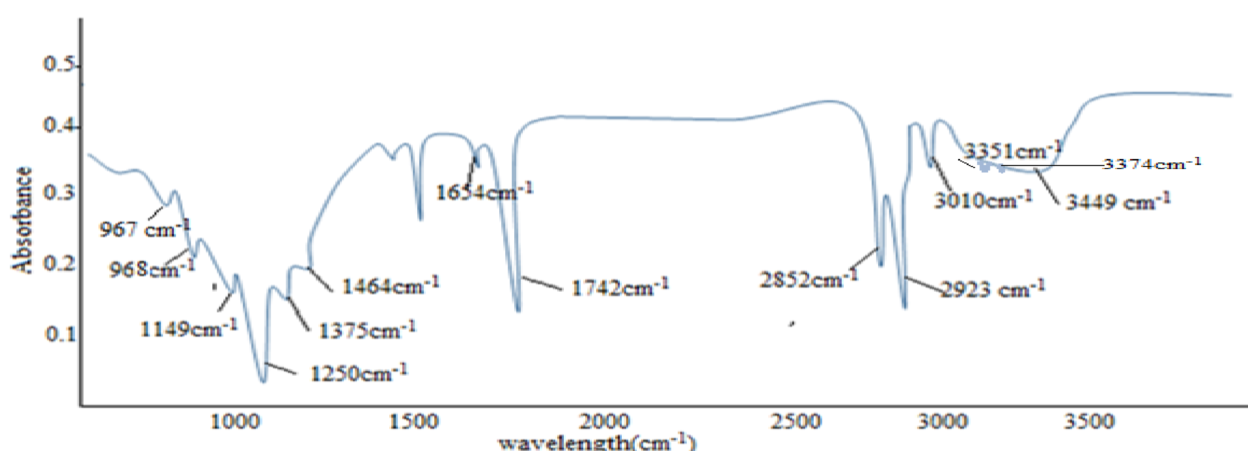


Figure 4: FT-IR of fish oil after oxidation

CONCLUSION

Nutritional value of fish oil depends on the mainly of physicochemical characteristics that decide the use of oil as a food application. Iodine value, peroxide value and para anis dine value are the quality parameters of oil in food industries. The present study correlates the primary and secondary oxidation of fish oil in terms of peroxide and para anis dine values. Fish oil on heat treatment is shown a controlled oxidation and it was enhanced with UV-light. However, in the presence of sunlight, the peroxide value was high. Oxidation of fish oil with sunlight is faster than with UV light and heat treatment .The rate of per oxidation of oil with sunlight is 12.6 times faster than the oil on heating and 3.3 times than that of oil with UV light irradiation. The per oxidation of oil with UV light is 3.8 times faster than that of the oil under heat treatment. The rate of per oxidation in the presence of sunlight has been reduced significantly 2.3 times with the use of antioxidant BHT. The quality deterioration of fish in the presence of sunlight is a maximum compared to the oil in the presence UV light and heat treatment. It was evidenced by the appearance

of FT-IR spectra at 3351 and 3374 cm^{-1}. The experimental discussions prove the quality deterioration of fish oil is maximum with sunlight.

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