IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 14, Issue 3 Ser. I (March. 2021), PP 39-44
www.iosrjournals.org

Soy Lecithin as an Antioxidant Enhancer for Butylated Hydroxyl Toluene in Controlling Rancidity of Sardine Fish Oil

Pragasam A[1, 2 *], Preeti N Tallur[1], V .M Naik[1, 2 *]
1(Research and development Centre, Bharathiar University, Coimbatore, Tamilnadu, India)
2(Government Arts and Science college, Karwar, Uttara Kannada, Karnataka, India)
**Corresponding Author*

Abstract:
Sardine fish oil has rich nutritional value as it composed of poly unsaturated fatty acid like omega-3 fatty acids. The present study has been focused on the characteristic deterioration of sardine fish oil conditional storage under irradiation by sun light. Oil on irradiation with sun light eight hour per day under goes rapid oxidation forming a peroxide value 49.6 meq/kg and 335.2meq/kg on first and ninth day in comparison with butylated hydroxyl toluene (BHT) as synthetic antioxidant and soy lecithin (SL) as natural antioxidant. Oil sample with BHT and SL recorded a minimum peroxide value 31.8 meq/kg and 94.8 meq/kg on the first and the ninth day. The combined anti-oxidative effect of BHT and SL to control the lipid oxidation is maximum as compared to individual anti-oxidative ability. The para asnisidine value (pAV) gives the secondary oxidation of fish lipids which is minimum for oil samples with BHT and SL. However, the oil with BHT in the presence of SL recoded a minimum pAV 31.9 on ninth day. The controlled peroxide value and pAV attributes to enhanced anti- oxidative effect of BHT by SL. Thus, SL enhance the anti-oxidative ability of BHT as secondary anti-oxidant.

Key words*: Sun light, peroxide value, para-anisidine value (pAV), butylated hydroxyl toluene (BHT), soy lecithin (SL).*

Date of Submission: 09-03-2021 Date of Acceptance: 23-03-2021

I. Introduction

Fish oil has a conspicuous level of nutritional value in food industries. Fishes are recognized as essential component daily diet to maintain important nutrients [1, 2]. Consumption of fish has emerging benefits mainly as it contains proteins and lipids of high biological value with long chain polyunsaturated fatty acids (LC- PUFA) along with vitamins and minerals [3, 4]. The lipid composition in fish is quite different from land animal lipids and vegetable oils due to the large quantity of two distinct n-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) that cannot be synthesized by human body [5]. Researchers reported that consumption of fish oil having polyunsaturated fatty acid prevents or cures arterial hypertension [6], colon and prostate cancer [7, 8], inflammatory diseases [9] and disorders of the immune system [10]. EPA and DHA play a vital role in development and functioning of the nervous system (brain), photoreception (vision), and the reproductive system [11, 12]. Sardine fish is one of the richest and cheapest sources of dietary supply of omega-3 fatty acids, including EPA and DHA [13-15]. Researchers 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 [16, 17]. The quality of fish oil is very important for its nutritional value. The main causes for quality deterioration fish oil is the oxidation by atmospheric oxygen which alter odour, flavour and other parameters. The sites of attack by oxygen are the unsaturated portions of the fatty acid moieties of triglycerides [18]. Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oils are highly susceptible to oxidation and the rate of fish oil oxidation is significantly different from that of other oils [19]. Normally with polyunsaturated oils, initiation of rancidity begins slowly, with the polyenoic ester (LH) giving the free-radical [20, 21]. Quality of fish oil will be decreased with increase in temperature and storage time. Over the last few years, many researches were carried out on numerous applications of the methods for concentration of omega-3-PUFAs from fish oil to improve the

quantity and quality [22-25]. The quality of the fish oil is an indicator for hygienic nutrition value of the oil. The literature survey clearly indicates the quality deterioration significantly retards its nutritional value and limits its biological application in food and other industries.

The present study focused on the quality deterioration of sardine fish oil on exposing to sunlight. The oxidation of sardine fish oil is determined in terms of peroxide value, para anisidine value and total oxidation number. The antioxidant effect of butylated hydroxyl toluene (BHT) as a synthetic antioxidant is evaluated in comparison with fish oil samples with and without BHT. Synergism of BHT is evaluated using soy lecithin (SL). The SL in fish oil without BHT is used to prove the pro-oxidative effect and with BHT is used to prove the secondary oxidative effect of SL in controlling the rancidity of fish oil. The enhanced anti-oxidative ability of BHT by SL is assessed by proposing a possible mechanism.

II. Material and Methods

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.

Extraction of fish oil: The Fishes were 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.

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

F_1	Control	Fish oil kept in moderately dark for eight hours before each determination
F_2	Oil + Sun light	Fish oil irradiated in sun light for eight hours per each determination
F_3	Oil +BHT	Fish oil was added with BHT as an antioxidant and exposed under sunlight for eight hours before each determination
F_4	Oil + SL	Fish oil was added with SL and exposed under sunlight for eight hours before each determination
F_5	Oil + BHT+ SL	Fish oil was added with (BHT+SL) and exposed under sunlight for eight hours before each determination

Peroxide Value(PV): Exactly, 5 gm 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.

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

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

pAnisidine value (pAV): The carbonyl content in oils was determined by the standard methods by AOCS. It measures the reactivity of the aldehydes' carbonyl bond on the pAnisidine 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 10 ml 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 pAnisidine. The pAV is determined as;

$$\mathrm{pAV} = \frac{25 \times 1.2 \times (\mathrm{A_2} - \mathrm{A_1})}{\mathrm{W}}$$

III. Result and Discussion

Proximate values of sardine fish oil: The proximate values for sardine extracted were listed in the Table 1. All these values were determined according to standard methods by AOCS.

Table 1 Proximate value of fish oil samples

Oil sample	Moisture (%)	Density(g/ml)	Refractive index	Ash Value	Iodine value
Fish oil extracted	0. 54	0.9023-0. 9100	1.4773	1.0- 1.1	164
Fish oil by wet process	0.41	0.8929- 0.9100	1.4779	0.9- 1.1	168

Peroxide value: Peroxide values of different fish oil samples were recorded as shown in the Table 2 against time duration in days

Table 2: Peroxide value versus time duration

Time duration (days)	1	2	3	4	5	6	7	8	9
Control	5.80	5.8	5.8	5.8	5.9	5.9	5.9	5.8	5.9
Oil + Sun light	49.6	75.1	107.1	142.3	201.5	248.9	276.8	307.5	335.2
Oil+ BHT	44.6	59.0	60.8	79.9	89.4	98.9	119.8	137.1	148.8
Oil + SL	35.1	78.4	100.7	134.4	175.7	203.3	233.4	256.7	279.2
Oil+BHT+SL	31.8	44.7	60.8	68.8	73.6	79.2	84.9	89.1	94.8

A graph of peroxide value of fish oil sample against time duration under irradiation by sunlight was plotted as shown in the Fig. 1.

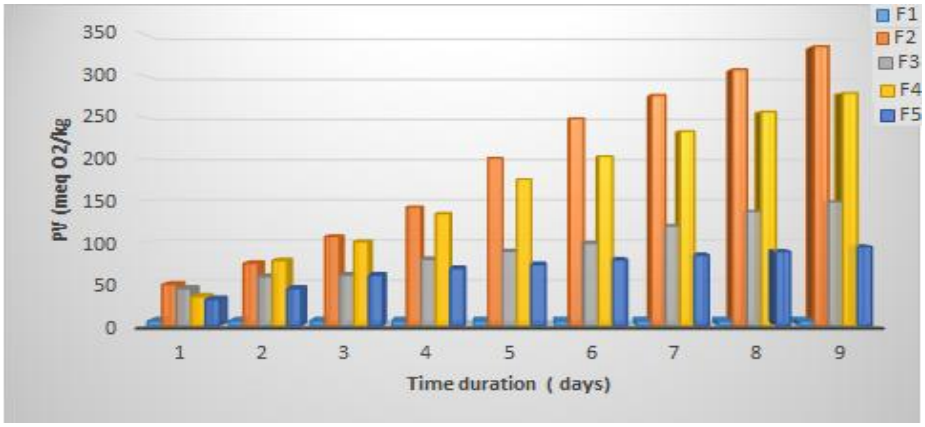


Fig. 1: Peroxide values of fish oil samples versus time duration

The plot of bar graph for peroxide values of fish oil samples against time duration under irradiation by sunlight eight hours per day explains the oxidative de-stability very significantly as shown in the Fig. 1. The control sample $F_1$was maintained in moderately dark place and the peroxide value was determined each day. It shows a constant value from first day of determination to the ninth day of determination of peroxide value 5.8 meq/kg. The peroxide values of F_2 were determined for each day after irradiating eight hours by sunlight. The PV on first day was 49.6 which was predominantly changed day by day and reaches a maximum value of 335.2 by ninth day. This indicates that the fish is highly susceptible for oxidation in presence of sunlight and the quality is get deteriorated vigorously. The oil sample F_4 containing soya lecithin(SL) shows a peroxide value of 35.1 meq/kg on the first day of irradiation and which was raised to a maximum of 279.2 on the ninth day of irradiation. A conspicuous difference was noted in peroxide values of samples F_2 and F_4. SL added control the oxidation of fish oil and acts as a moderate antioxidant to control the rancidity of fish oil. Oil sample F_3 records a peroxide value of 44.6 meq/kg on the first day of irradiation and that reaches a maximum of 148.8 meq/kg on ninth day of irradiation. F_3 containing butylated hydroxyl toluene (BHT) as a synthetic antioxidant reduces the PV three times to blank oil and made the oil more stable by suppressing the oxidation of the lipid. It was almost two times more stabilized the oil to that of sample F_4 and acts as a relatively strong antioxidant. When the sample F_5 containing BHT and SL was irradiated, the peroxide values significantly decreased. Maximum PV of 31.8 meq/kg was recorded on first day of irradiation and that of on ninth day was 94.8 meq /kg. The combined antioxidant effect of BHT and SL decreased the peroxide value 3.5 times to that of blank oil. This clearly indicates that the antioxidant property of BHT was enhanced by SL. Thus SL can act as a secondary antioxidant in presence of BHT and as a primary antioxidant in the absence of BHT.

pAnisidine value (pAV): pAnisidine values of fish oil sample were determined against time duration at different conditions as shown in the Table 3

Table 3 pAnisidine value versus time duration

Duration days	1	2	3	4	5	6	7	8	9
Control	14.8	14.8	14.8	14.9	14.9	14.9	14.9	14.8	14.8
Oil + Sun light	24.3	26.5	28.6	31.4	34.6	36.2	39.7	45.8	50.2
Oil+ BHT	24.6	25.4	26.9	28.1	29.4	31.3	32.1	33.4	34.8
Oil + SL	25.2	26.6	27.5	29.2	31.1	33.2	34.9	36.1	37.8
Oil + BHT + SL	18.3	19.8	22.3	23.9	25.2	27.4	28.8	30.5	31.9

A bar graph of peroxide value of fish oil samples against time duration under irradiation by sunlight was plotted as shown in the Figure 2.

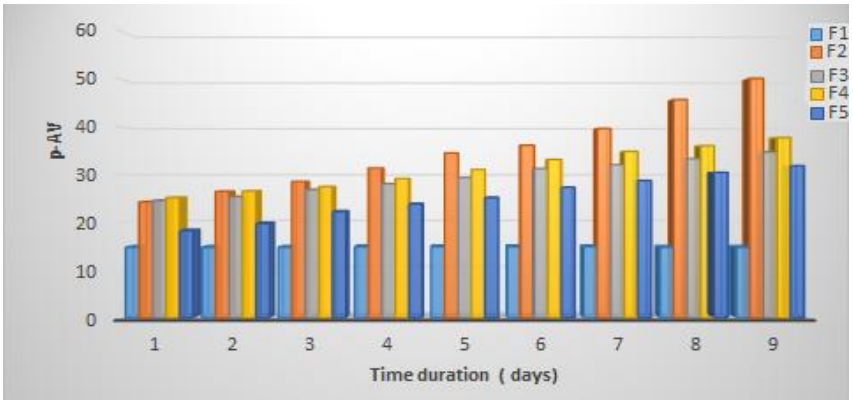


Fig. 2: pAnisidine values of fish oil samples versus time duration

The plot of bar graph for pAV values of fish oil samples against time duration under irradiation by sunlight eight hours per day explains the secondary oxidative de-stability very significantly as shown in the Figure 2. The control sample $F_1$was maintained in moderately dark place and the p-AV was determined each day. It shows a constant value from first day of determination to the ninth day of determination of pAV 14.8. The pAnisidine values of F_2 were determined for each day after irradiating eight hours by sunlight. The pAV on first day was 24.3 which was predominantly changed day by day and reaches a maximum value of 50.2 by ninth day. This indicates that the fish is highly susceptible for secondary oxidation in presence of sunlight and the quality is get deteriorated vigorously causing high rancidity. The oil sample F_4 containing soya lecithin (SL) shows a pAV of 25.2 on the first day of irradiation and which was raised to a maximum of 37.8 on the ninth day of irradiation. A conspicuous difference was noted in pAnisidine values of samples F_2 and F_4. SL added control the secondary oxidation of fish oil and acts as a moderate antioxidant to control the rancidity of fish oil. Oil sample F_3 records pAnisidine value of 24.6 on the first day of irradiation and that reaches a maximum of 34.8 on ninth day of irradiation. F_3 containing butylated hydroxyl toluene (BHT) as a synthetic antioxidant reduces the PV three time to blank oil and made the oil more stable by suppressing the secondary oxidation of the lipid. It was almost two time more stabilized the oil to that of sample F_4 and acts as a relatively strong antioxidant. When the sample F_5 containing BHT and SL was irradiated, the peroxide values significantly decreased. The maximum PV of 18.3 was recorded on first day of irradiation and that of on ninth day was 31.9. The combined antioxidant effect of BHT and SL decreased the pAV value 1.6times to that of blank oil. This clearly indicates that the antioxidant property of BHT was enhanced by SL. Thus SL can act as a secondary antioxidant in presence of BHT and as a primary antioxidant in the absence of BHT.

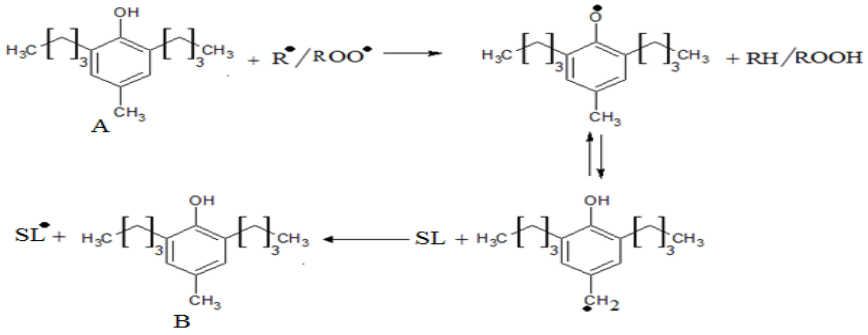
Mechanistic path way of lecithin as secondary antioxidant in the presence of BHT

The mechanistic path of soy lecithin as secondary antioxidant in the presence of BHT consists two stage

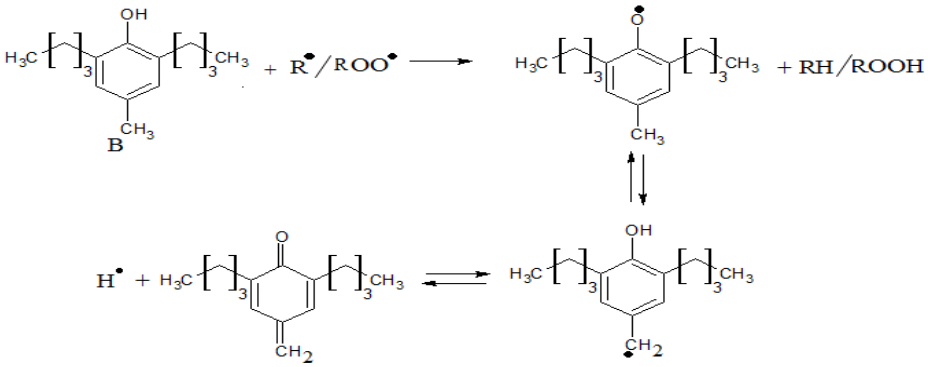
Stage I: Lipid alkyl radicle(R) or lipid peroxyl radicle abstract get reduced by BHT forming BHT radicle which on reaction with soy lecithin regenerates the BHT

Stage II: The regenerated BHT reacts with other lipid alkyl radicle(R) or lipid peroxyl radicle

Stage I



Stage II



Stage I explains the scavenges the lipid peroxyl free radical by BHT forming BHT free radical. The BHT regenerated from BHT free radical on interaction with SL resulting SL free radical. The BHT molecule can scavenge the next lipid peroxyl radical as in the stage II and the process is reversible.

IV. Conclusion

Sardine fish oil is a rich source of polyunsaturated fatty acids and has significant nutritional value in food industries. The physical characterization of sardine fish oil gives valuable information in the utilization of fish oil as food application. The fish oil is susceptible for moisture, oxygen, ions and under lipid oxidation. This deteriorates the quality of oil. Present work was focused on the determination of peroxide and para anisidine values sardine fish oil in the presence of sunlight for plain oil and with synthetic antioxidant BHT, soy lecithin and both BHT and SL. The peroxide of plain fish oil rapidly increases under eight-hour irradiation per day. Maximum peroxide and pAnisidine values of 335.2 meqO_2/kg and 50.2 were recorded on ninth day irradiation for the plain oil. The oil with SL has PV and pAV 279.2 meq O_2/kg and 37.8 respectively on ninth day irradiation to that of the oil containing BHT has 147.8meq O_2/kg and 34.8. This indicates that the PV and pAV were decreased in the presence of SL and BHT and controls the oxidation of lipids. BHT showed an efficient antioxidant property compare to SL. When SL used with BHT the oxidation of lipids was controlled significantly by reducing the PV and pAV to 94.8 meq O_2/kg and 31.9 respectively. It was very conspicuous that SL had enhanced the antioxidant ability of BHT to a very big margin by reducing the PV from 335.2 to 94.8 meq O_2/kg and pAV from 50.2 to 31.9. These values were relatively less compared to that of BHT alone. Thus, the mechanistic approach evidences that the SL could act as an enhancer of antioxidant property of BHT.

Acknowledgement

I would like to acknowledge the support of our beloved Principal and group members for their invaluable cooperation to work on this topic.

References

[1]. Pigott, G.M and Tucker, B. Seafood: Effects of technology of nutrition. New York: Marcel Dekker, Inc.1990.
[2]. Kinsella, J. E. Food component with potential benefits: The n-3 polyunsaturated fatty acids of fish oil. *Food Technology*., 1986, 40, 89-97.
[3]. Sidhu, K.S. Health benefits and potential risks related to consumption of fish or fish oil. *Regulatory Toxicology and Pharmacology*.,2003. 38(3), 336-344
[4]. Ackman RG. Seafood lipids, in sea food. Chem. Processing. Technol. Qual., London: Chaoman& Hall. 1994, p. 34.
[5]. Jittrepotch N, Ushio H, Ohshima T. Oxidative stabilities of triacylglycerol and phospholipids fractions of cooked Japanese sardine meat during low temperature storage. *Food. Sci.*, 2006, 99, 360-367.

[6]. Millar, J. A. and Wall-Manning, H. J. Fish oil in treatment of hypertension. *New Zealand Medical Journal.*, 1992, 105, 155.
[7]. Marchioli, R. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: Time course analysis of the result of gissi prevenzione. *Circulation.*, 2002, 105, 1897-1903.
[8]. Marchioli, R. Efficacy of n-3 polyunsaturated fatty acids after myocardial infarction: Results of Gissiprevenzione Trial. *Lipids*., 2001, 36, 119-126.
[9]. Belluzi, A., Campieri, M., Brignola, C., Gionchetti, P., Miglioli, M. and Barbara, L. Polyunsaturated fatty acid pattern and oil treatment in inflammatory bowel disease. *Gut*., 1993, l34, 1289-1290.
[10]. Levine, A. S. and Labuza, T. P. Food systems: The relationship between health and food science / technology. *Environment Health Perspective*., 1990, 86, 233-238.
[11]. Alasalvar, C., Taylor, K. D. A., Zubcov, E., Shahidi, F. and Alexis, M. Differentiation of cultured and wild sea bass (Dicentrarchuslabrax) total lipid content, fatty acid and trace mineral composition. *Food Chemistry*., 2002, 79, 145-150.
[12]. Skonberg, D. I. and Perkins, B. L. Nutrient composition of green crab (Carcinusmaenus) leg meat and claw meat. *Food Chemistry*., 2002, 77, 40-404.
[13]. Pettinello, G., Pallado, P. and Stassi, A. Production of EPA enriched mixtures by supercritical fluid chromatography: from the laboratory scale to the pilot plant. *Journal of Supercritical Fluids*., 2000, 19, 51-60.
[14]. Domingo, J. L. Omega-3 fatty acids and the benefits of fish consumption: Is all that glitters gold? *Environment International*., 2007, 33, 993-998.
[15]. Guil-Guerrero, J. L., Lopez-Martinez, J. C., RinconCervera, M. A. and Campra-Madrid, P. Onestep extraction and concentration of polyunsaturated fatty acids from fish liver. *Journal of American Oil Chemists' Society*., 2007, 84, 357-361.
[16]. Khoddami, A.1 Ariffin, A. A, Bakar, J, Ghazali, H. M. 2012. Quality and fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (Euthynnusaffinis). *African Journal of Biotechnology*., 2012, 11(7), 1683-1689.
[17]. SugengHeriSuseno, JenyErnawatiTambunan, Bustami Ibrahim, Saraswati. Inventory and Characterization of Sardine (Sardinella Sp.) Oil from Java Island-Indonesia. *Advance Journal of Food Science and Technology.*, 2014, 6(5), 588-592.
[18]. Stansby M. E. Fish oils: Their chemistry, technology, stability, nutritional properties and uses. The Avi Publishing Company, Inc, Westport 1967
[19]. *Boran, G. Karacam, H. Boran, M. Changes* in the *quality* of *fish oils due* to *storage temperature* and *time*. *Food Chemistry*., 2006, 98(4), 693-698.
[20]. Beddows C. G., Jagait C. and Kelly M. J. Effect of ascorbyl palmitate on the preservation of α -tocopherol in sunflower oil, alone and with herbs and spices. *Food Chemistry*., 2001, 73, 255-261.
[21]. Frankel E. N. Antioxidants in lipid food and their impact on food quality. Food Chemistry., 1996, 57, 51-55.
[22]. Haagsma, N., Gent, C. M., Luten, J. B., Jong, R. W., and Doorn, E. Preparation of an ω-3 fatty acid concentrate from cod liver oil. *Journal of American Oil Chemists' Society*., 1982, 59, 117-118.
[23]. Wanasundara, U. N. and Shahidi, F. Concentration of omega-3 polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. *Food Chemistry*., 1999, 65, 41-49.
[24]. Rubio-Rodriguez N., Diego, S. and Jaime, I. Supercritical fluid extraction of omega-3 rich oil contained in hake (Merlucciuscapensis - Merlucciusparadoxus) by-products: study of influence of process parameters on the extraction yield and oil quality. *Journal of Supercritical Fluids*., 2008, 47, 215-226.
[25]. Pragasam Antony, Nagaveni M. A, Preeti Nagesh Tallur, Vinayak M. Naik. Zanthoxylum rhetsa DC, a potential antioxidant preservative to control the rancidity of peanut oil, Journal of Ultra Chemistry., 2019, 15(2), 9-17.

Pragasam A, et. al. "Soy Lecithin as an Antioxidant Enhancer for Butylated Hydroxyl Toluene in Controlling Rancidity of Sardine Fish Oil." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 14(3), (2021): pp 39-44.