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Oxidative Quality Attributes of Commercially Available Fish Oils in India: A Novel Assessment

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Abstract

Fish oil supplements are widely accepted functional foods, valued for their array of health benefits, largely due to their high levels of polyunsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid. However, their high degree of unsaturation renders them prone to oxidation, which can make them unsuitable for dietary use. Accordingly, this study examined the oxidative quality attributes of twelve fish oil supplements procured from the Cochin local market and an e-commerce platform. The acid value, peroxide value, and anisidine value were measured according to ISO 660, ISO 3960, and ISO 6885, respectively, and the TOTOX value was calculated. All the tested samples complied with the acid value regulatory limit of 3 mg KOH/g. About 41.7% of the samples exceeded the recommended peroxide value of 5 meq O_2/kg fat, 8.3% exceeded the anisidine value of 20, and 16.7% of the fish oil samples recorded TOTOX values higher than the regulatory limit of 26. The results underline that utmost care should be taken during processing, packaging, transportation, and storage to minimise oxidative effects on fish oil. Further, heightened regulatory surveillance and inspection of fish oil supplements in the market are desirable.

Keywords: Fish oil, oxidation, acid value, peroxide value, anisidine value, TOTOX value

Introduction

The rise of non-communicable diseases due to unhealthy lifestyles has led to a change in consumer behaviour towards healthy eating and has reinforced the common perception about the linkage between food and health. Functional foods represent a novel approach to achieving optimal health by supporting wellness and reducing the recurrence of illness (Ghosh et al., 2022) through dietary means. Functional foods are present in every food category, but their presence varies in the market (Siró, Kápolna, Kápolna, & Lugasi, 2008). The global fish oil market is valued at $2.1 billion and is expected to grow significantly at a CAGR of 5.5% to $3.6 billion by 2031 (Fact.MR Food & Beverage Desk, 2024). The growth is driven by the expanding aquaculture industry and increasing health awareness among the population regarding health-based foods.

Fish oil has emerged as a functional food with diverse nutritional potential, including antioxidant, neuroprotective, cardioprotective, hepatoprotective, anti-inflammatory, and skin-protective properties (Chen, Jayachandran, Bai, & Xu, 2022). It is regarded as a significant dietary source of PUFAs (eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA), which confer various health benefits, as reviewed by Sarojnalini and Hei (2019). EPA has potent anti-inflammatory action. DHA plays a crucial role in the development of the brain and retina in foetuses and infants. Low levels of DHA may cause memory loss, dementia, depression, and vision issues. Furthermore, EPA and DHA are known to impede cancer progression (Sarojnalini & Hei, 2019) and protect against heart ailments, myocardial infarction, and sudden death (Mori, 2004). Considering the nutritional importance of EPA and DHA, various organisations have issued dietary guidelines. The National Heart Foundation of Australia (Heart Foundation) recommends consuming 250-500 mg/day of marine-sourced ù-3 fatty acids (EPA and DHA) (2-3 servings of fish per week) (Heart Foundation, 2015). The American Heart Association recommends consuming fish and fish oil, totalling 1 g/day of EPA and DHA, for

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patients with CHD. The Institute of Medicine, in collaboration with the National Institutes of Health, USA, recommends 1.1-1.6 g per day of ω-3 oils for adults (Venugopalan et al., 2021).

Lipid oxidation is one of the primary barriers to maintaining the quality of fish oil, rendering it sensorially unacceptable. An increase in unsaturation makes the oil more prone to oxidation (Remya, Sreelakshmi, Mohan, Basu, & Venkateshwarlu, 2024). Furthermore, the susceptibility of PUFAs to oxidation depends on the number of bis-allylic positions in the molecule. Fig. 1 illustrates the oxidation process of the fish oil as specified by Lembke and Schubert (2014). The decreasing order of oxidative susceptibility is- DHA (22:6, ω-3) > EPA (20:5, ω-3) > AA (20:4, ω-6) > LN (18:3, ω-3) > LA (18:2, ω-6) (Miyashita, 2019). The relative oxidation rate of oleic acid, linoleic acid, and linolenic acid is 1:40:96 (Frankel, 2005). In addition, heat, light, oxygen, and metals further enhance the oil's vulnerability to oxidation. Lipid oxidation leads to undesirable flavour and taste, and to nutrient loss (Cullere et al., 2019). Oxidation degrades the quality and safety of the fish oil, and the toxic chemicals generated may cause serious health implications, e.g., atherosclerosis, cancer, inflammation, thrombosis, and ageing. The primary oxidation product, hydroperoxides, exhibit cytotoxic behaviour, while others, such as aldehydes and oxysterols, show pro-inflammatory, cytotoxic, and mutagenic effects (Turner, McLean, & Silvers, 2006; Domínguez et al., 2019).

Quantifying individual lipid oxidation products to determine the extent of oxidation is challenging. Simpler analytical methods, such as peroxide value, anisidine value, free fatty acid content, and TOTOX value, are used to assess oxidation levels. Health-conscious consumers expect marketed fish oil supplements to comply with regulatory specifications. The quality standards prescribed by the Codex Alimentarius Commission (CXS 329-2017) are shown in Table 1.

As the market for fish oil supplements grows rapidly, these supplements must adhere to the food quality and safety guidelines of the relevant regulatory bodies to protect human health. However, various reports have noted the presence of oxidised fish oil supplements in the global market (Albert et al., 2015; Jairoun, Shahwan, & Zyoud, 2020; Suzan et al., 2022). However, data on the oxidation status of fish oils in the local Indian market are scarce, and this work appears to be the first of its kind to depict the oxidative status of fish oil supplements in the Indian context. With the rise of the fish oil nutraceutical market, evaluating fish oil quality against regulatory benchmarks is important to safeguard consumers' interests. In this context, the study was conducted to systematically investigate the oxidation status of commercially available fish oil supplements in the Indian retail market and to assess their compliance with national and international acceptable criteria. The work may provide initial exploratory insights into the quality of retail fish oil supplements in India and may help delineate the areas requiring heightened surveillance and attention.

Materials and Methods

Twelve fish oil supplement (Table 2) samples were procured from the local market in Cochin and from

Table 1. Quality parameters of fish oil

Parameters	Fish oil	
	Fish oils, fish liver oils, concentrated fish oils, and concentrated fish oils ethyl esters	Fish oils with a high phospholipid concentration of 30% or more such as krill oil
Acid value	≤ 3 mg KOH/g	≤ 45 mg KOH/g
Peroxide value	≤ 5 milliequivalents of active oxygen/kg oil	≤ 5 milliequivalents of active oxygen/kg oil
Anisidine value	≤ 20	-
Total oxidation value (TOTOX)	≤ 26	-

(CXS 329-2017; https://fssai.gov.in/upload/uploadfiles/files/Chapter%202_6_Fish%20and%20Fish%20products.pdf)

Table 2. Characteristics of the commercially available fish oil samples used in the study

Sample Code	Procurement Source	Fish Source (as Declared on Label)	Fish Origin
F1	Retail	Fish oil	Not mentioned
F2	E-commerce	Fish oil	
F3	E-commerce	Fish oil	
F4	E-commerce	Fish oil	
F5	E-commerce	Fish oil	
F6	E-commerce	Fish oil	
F7	E-commerce	Fish oil	
F8	E-commerce	Fish oil	
F9	E-commerce	Salmon fish oil	
F10	Retail	Cod liver oil	
F11	E-commerce	Sardine fish oil	
F12	E-commerce	Fish oil	

* *Storage instructions varied slightly among products and generally included statements such as "Store in a cool, dry place," "Protect from direct sunlight," "Store below 25 °C or 30 °C," and "Keep the container tightly closed after use," as specified on the product labels. The shelf life specified by the manufacturer was 18-24 months.*

** *All samples were purchased in soft gel capsule form.*

e-commerce platforms. The samples were kept in their original and unopened condition (manufacturer's packaging). Fish oil was extracted from the capsules immediately before the analysis (Heller, Gemming, Tung, & Grant, 2019). All the analyses were conducted in duplicate. The results are expressed as mean ± standard deviation (SD).

The oxidative quality parameters for the fish oil were determined according to standard protocol in duplicate. Acid value (AV) was determined according to ISO 660 (ISO, 2020). The acidity in the fats and oils can be expressed as AV or acidity as such. AV is the number of milligrams of potassium hydroxide required to neutralise the free fatty acids per gram of fat, while acidity represents the content of free fatty acids. AV was evaluated using the following equation in mg KOH/g fat.

$$AV\ (mg\ KOH/g\ fat) = \frac{56.1 \times c \times V}{m}$$

The peroxide value (PV) was determined by iodometric endpoint determination according to ISO 3960 (ISO, 2017). PV measures the amount of chemically bound oxygen in fat/oil as peroxides or hydroperoxides. The method is deemed valid for PV ranging between 0-30 meq O_2/kg oil. Briefly, 5-10 g of fish oil sample was taken in an Erlenmeyer flask and dissolved in 50 mL of glacial acetic acid/isooctane solution. 0.5 mL of saturated potassium iodide was added to the flask and mixed for exactly 60 s. 100 mL of distilled water was added for rinsing. The liberated iodine was titrated against 0.01 N sodium thiosulfate standard solution from yellow-orange to pale yellow and, after adding 0.5 mL starch indicator, from violet to colourless. The titration was stopped when colourlessness persisted for 30 s. The peroxide value was calculated using the following formula:

$$PV = \frac{(V - V_0) \times c_{thio} \times F \times 1000}{m}$$

where V is the volume of sodium thiosulfate solution used for the determination, V_o is the volume of the sodium thiosulfate standard solution used for the blank test, c_{thio} is the concentration of the sodium thiosulfate solution, m is the mass of the sample, and F is the factor of 0.001 N sodium thiosulfate solution.

p-Anisidine value (A_nV) measures the amount of aldehydes, particularly α- and β-unsaturated aldehydes, in fats and oils. Assessing secondary oxidation of oil reveals the oxidation history of oil. A_nV

was assessed following ISO 6885 (ISO, 2016). Briefly, about 0.4-4.0 g of melted fat sample was weighed carefully and dissolved in 5-10 mL of isooctane and made up to 25 mL. The unreacted test solution, reacted test solution, and blank sample were prepared separately as follows. For unreacted test samples, 5 mL of the sample was mixed with glacial acetic acid, incubated in the dark for 8 min at 23±3 °C, and then transferred to a cuvette within two minutes. For the reacted test solution, 5 mL of the sample was mixed with 1 mL of anisidine reagent, incubated in the dark for 8 min at 23±3 °C, and then, within two min, transferred to a cuvette. The blank was prepared by mixing five millilitres of isooctane with one millilitre of anisidine reagent, incubating in the dark for 8 min at 23±3 °C, and transferring the solution to the cuvette within two minutes. Before taking the spectrophotometric measurement at 350 nm, the zero absorbance was set up using an isooctane solution. The absorbance of the unreacted test solution, the reacted test solution, and the blank was estimated against isooctane. The A_nV was calculated using the following equation.

$$A_nV = \frac{100 \times Q \times V}{m} \times 1.2\ (A_1 - A_2 - A_0)$$

where V is the volume in which the test sample is dissolved, m is the mass of the sample, Q is the sample content of the measured solution, A_0, A_1, and A_2 are the absorbance of the unreacted, reacted, and blank, and 1.2 is the correction factor for dilution.

Further, the total oxidation value (TOTOX value) was calculated as per this equation:

$$TOTOX\ value = 2 \times PV + A_nV$$

Results and Discussion

Lipid oxidation is a complex phenomenon that commences with the formation of a free radical. Several factors, such as heat, oxidised compounds, metal ions, sunlight, free fatty acids, and mono- and diacylglycerols, contribute to the degradation of fats and oils by accelerating oxidative and chemical reactions (Lembke & Schubert, 2014). The oxidation of oil can be described in three steps (Fig. 2), i.e., the initiation phase, where free radical formation occurs; the propagation phase, which leads to the formation of hydroperoxides (primary oxidation products) in the presence of oxygen; and the termination phase, which results in the coupling of radicals to form secondary oxidation products (aldehydes, ketones, carboxylic acids), which may be volatile and contribute towards off-flavour. Oxidation of long-chain fatty acids with a high degree of unsaturation leads to greater free radical formation, which may further result in the formation of a large number of oxidation products.

The acid value (AV) indicates the quality of fatty acids present in the oil and is an indicator of hydrolytic rancidity in oils (Robards, Kerr, & Patsalides, 1988). A low AV is considered suitable for storing oil, while a higher value indicates rancidity (Ayeloja, Jimoh, & Garuba, 2024). Free fatty acids may convert into secondary oxidation metabolites, which may ruin the quality of the oil. The AV of the tested samples ranged from 0.321 to 1.558 (Fig. 3). All the samples had AV below the regulatory limit of ≤ 3 mg KOH/g. Previously, De Boer et al. (2018) found that 2.1% of fish oil exceeded the upper limit of 3.0 mg KOH/g, suggesting that hydrolytic rancidity was well controlled in global refined fish oil products. For the same fish oil products, 13.9%, 6.1%, and 8.8% of products exceeded the regulatory values for PV, A_nV, and TOTOX, respectively.

The peroxide value (PV) is the milliequivalents of peroxide oxygen (active oxygen) contained per kilogram of oil (Bako, Umogbai, & Awulu, 2017). A lower PV indicates a lower likelihood of rancidity, whereas a high PV indicates accumulated peroxides resulting from oxidation. The measurement of peroxides is considered suitable for determining primary oxidation products, namely peroxides and hydroperoxides, formed through a free radical chain reaction in the oil samples. A higher PV may indicate that the oil is under oxidative stress and may have turned rancid (CDRFoodLab, 2024). The PV of the fish oils tested varied from 2.950-9.204 meq O_2/kg fat (Fig. 4). Out of the twelve samples tested, the PV of five samples exceeded the allowable limit, i.e., 5 meq O_2/kg fat, as mentioned in CXS 329-2017 and FSSR Regulations 2011. Furthermore, three samples had a PV greater than 4.5, indicating that the oil is undergoing oxidation and contains hydroperoxides. Pasini et al. (2022) tested 20 fish oil supplements from the French market and found that all samples were safe for consumers, with only three samples on the borderline for PV. Ritter, Budge, and Jovica (2013) noted that 5 out of 16 fish oil preparations (3 fish body oils and 2 cod liver oils) were non-compliant with established regulatory criteria for PV in the United

States. Fierens and Corthout (2007) observed non-compliance in 46.7% of ω-3 food supplements/ preparations (7 out of 15) sourced from Belgium for PV and/or the labelled fatty acid composition. However, measuring PV alone can be misleading. During oxidation in fish oils, peroxides may form secondary oxidation products, which can lead to a decrease in PV. Highly rancid oils can have a reduced PV. Therefore, A_nV and TOTOX values are used to indicate the whole oxidation story of lipids (Eurofins Scientific, 2023).

A_nV is used to monitor the oil quality because it measures the presence of secondary oxidation products. These secondary lipid oxidation products form from the breakdown of peroxides and hydroperoxides. Flavour changes in the oil are linked to the formation of secondary oxidation products (aldehydes and ketones), which are detected via A_nV. A lower A_nV is generally associated with better oil quality, while a higher value indicates more secondary oxidation. A_nV may be considered a marker of the oxidation history of oil (Norwegian Fish Oil, 2025). The A_nV for the tested fish oil samples ranged from 1.449 to 26.402 (Fig. 5). The limit for A_nV in fish oils, as prescribed by FSSAI and Codex, is ≤20. Accordingly, only one sample exceeded the regulatory limit for A_nV. The A_nV of the tested fish oil samples that complied with the regulatory limit ranged from 1.449 to 15.988. Pasini et al. (2022) observed that all samples in the French market complied with the regulatory limit for A_nV in fish oils as prescribed by GOED (Global Organisation for EPA and DHA). Nichols, Dogan, and Sinclair (2016) found that three of eight fish oil supplements (37.5%) sourced in Australia exceeded the standard limit set for A_nV by GOED. The limits set by GOED are: PV < 5 meq O_2/kg fat; A_nV < 20 (Global Organization for EPA and DHA Omega-3, 2017).

The total oxidation value (TOTOX) provides a comprehensive assessment of the oxidation status of the oils, as it combines both primary and secondary oxidation products (early and later oxidation products). TOTOX covers both early and advanced oxidation stages of fish oil, and using TOTOX ensures that an oil is not simultaneously high in peroxides and aldehydes. The maximum TOTOX limit is lower than the sum of the maximal allowable limits for PV and A_nV. TOTOX values of the tested samples ranged from 14.383 to 36.337, and two samples exceeded the regulatory limit of 26 (Fig. 6). The TOTOX values for the fish oil samples deemed safe under the regulatory limit ranged from 14.383 to 24.514. Previously, Pasini et al. (2022) reported TOTOX values ranging from 19.4–25.9 in commercial ω-3 supplements sold in the French market. A TOTOX value approaching 26 suggests that, although the supplements may still fall within acceptable limits, they are considerably oxidised. In general, a TOTOX value below 10 indicates high quality, whereas values above 20–26 reflect substantial oxidation, rancidity, and overall degradation.

Oxidation is a serious problem in food oils, especially in fish oil, and is significantly accelerated by light, metal ions, oxygen, and high temperature.

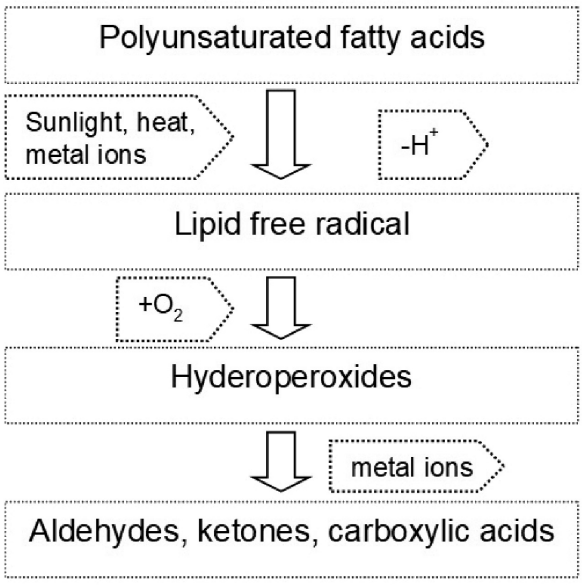

Fig. 1. Schematics describing the oxidation process of fish oil (Lembke & Schubert, 2014)

- Chain initiation:
  - $RH \rightarrow R^* + H^*$
- Chain propagation
  - $R^* + O_2 \rightarrow ROO^*$
  - $ROO^* + RH \rightarrow ROOH + R^*$
- Chain termination
  - $R^* + R^* \rightarrow R\text{-}R$
  - $R^* + ROO^* \rightarrow ROOR$
  - $ROO^* + ROO^* \rightarrow ROOR + O_2$

Fig. 2. Schematics showing various stages of lipid oxidation in foods

This leads to the formation of hydroperoxides, which are subsequently decomposed during oxidation into aldehydes, ketones and other off-flavour compounds. Oxidation degrades both the sensory and nutritional quality of the oil. It has also been reported that oxidative degradation of fats and oils may lead to food poisoning (Gotoh & Wada, 2006). Various studies from different parts of the world have investigated the oxidative quality parameters of fish oils. The results indicate that the fish oil samples on the market are more likely to be oxidised, with a range of 11-62%. Heller et al. (2019) observed that of the 26 fish oil supplements tested from the Australian market, 38%, 25%, and 33% of the samples failed to meet the regulatory requirements for PV, A_nV, and TOTOX value, respectively. Albert et al. (2015) observed that many fish oil supplements in New Zealand did not meet oxidative criteria set for fish oils. Around 83% of the supplements exceeded the recommended PV (primary oxidation) limit, 25% exceeded the A_nV limit (secondary oxidation), and 50% exceeded the TOTOX (total oxidation) levels. In a similar study on fish oil supplements in New Zealand, Bannenberg et al. (2017) reported that 72%, 86% and 77% of the tested supplements complied with maximum limits of PV, A_nV and TOTOX, respectively. All fish oil samples met the A_nV limit of 30, while 98% complied with the PV limit of 10 meq O_2/kg fat and 96% met the calculated TOTOX limit of 50. However, these limits are those used by the European and British Pharmacopoeia and the Australian authorities and are less stringent. Jairoun et al. (2020) analysed 44 commercially available fish oil dietary supplements in the United Arab Emirates and found that 40.9%, 6.8%, and 27.3% of the samples exceeded the maximum allowable limits for PV, A_nV, and TOTOX value, respectively. Although most products complied with oxidative quality standards, a notable proportion of these fish oil supplements failed to meet at least one parameter. Bannenberg et al. (2020) reported that the 48 most widely sold EPA/DHA ω-3 supplements in the U.S. originated from multiple countries, including 18 from the United States, 12 from Norway, and 12 from Peru. Although a high proportion met oxidative quality criteria (85.4% for PV and 95.7% for A_nV), nearly half of the fully tested products failed to meet at least one quality parameter.

The oxidation of fish oil depends on the type of manufacturing or processing it underwent. Dissolved oxygen/introduced oxygen (during processing)/oxygen from the headspace may oxidise oil during the storage period. The solubility of oxygen in marine triglyceride fish oils ranges from 1.22–2.44 mmol/kg oil at 20 °C (Phung et al., 2020) and varies with temperature. Ritter et al. (2013) suggested that factors such as exposure to air during manufacturing or packaging defects, including poorly fitted caps, may contribute to elevated oxidation levels in fish oil supplements. Furthermore, poor-quality, near-expiry, or mishandled ingredients may also cause oxidation. Heller et al. (2019) postulated that these supplements may have been within regulatory specifications at the time of manufacture, with oxidation occurring post-manufacture (during transportation and/or storage). Regardless of when the oxidation occurs, the consumer faces the risk of exposure to lipid oxidation products. Furthermore, there is a risk of oxidation during testing and sample storage. The entire supply chain needs to be investigated to determine the root cause of oxidation in fish oil supplements, and corrective measures must be implemented to ensure the production of safe supplements for consumers. Furthermore, the oxidation status of fish oil may be influenced by storage duration and environmental conditions prior to purchase, which were not considered in this study due to practical limitations. Since the study involved a limited number of fish oil supplements, which may not fully capture the variability in

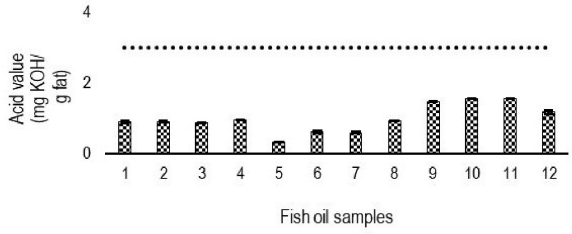

Fig. 3. Acid value of fish oil samples
*Regulatory limit for acid value: ≤ 3 mg KOH/g

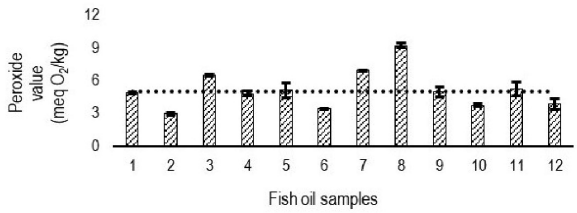

Fig. 4. Peroxide value of the fish oil samples
*Regulatory limit for peroxide value: 5 meq O_2/kg fat

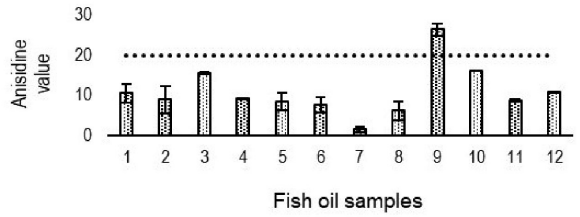

Fig. 5. Anisidine value of the fish oil samples
*Regulatory limit for anisidine value: ≤20

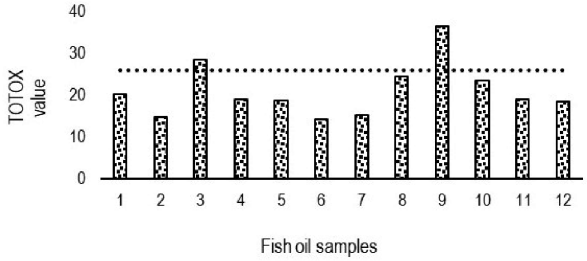

Fig. 6. Total oxidation (TOTOX) value of fish oil samples
*Regulatory limit for TOTOX value: 26

oxidation status among supplements in the Indian market, a larger, multiregional surveillance approach with a larger sample size across multiple brands and replicates seems desirable. Further, the oxidative parameters may also be correlated with other indicators – the content of EPA and DHA (strength), the presence of antioxidants, filler material, the form of fish oil (triglyceride or esterified), and whether it is encapsulated or not – to arrive at a comprehensive assessment of fish oil quality.

Fish oil oxidation is important from both food quality and food safety perspectives. Hence, it is essential to minimise the oil's contact with metals, oxygen, light, and high temperatures to limit lipid oxidation. Multiple factors govern the quality of fish oil, including the raw material quality, processing, packaging, storage and transportation. Furthermore, oxidation can be minimised by adding approved natural and synthetic antioxidants, thereby extending the oil's shelf life. Packaging also plays a crucial role in extending the shelf life of fish oils. The present survey provides initial insight, and the results offer an exploratory baseline assessment of fish oil in the retail market and should not be considered truly representative of the whole Indian market. A suitable sampling plan, with an expanded multiregional surveillance framework across regions/retail formats/brands, may be implemented to draw more suitable inferences.

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