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Influence of thermal degradation in the physicochemical properties of fish oil

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Abstract Physicochemical and thermal analyses were undertaken to evaluate the influence of the temperature on the oxidation of sea fish oil once its polyunsaturated fatty acids deteriorate rapidly. Fish oil displayed four decomposition steps in synthetic air atmosphere and only one step in nitrogen atmosphere. The first step started at 189 and 222 °C for oxidizing and inert atmospheres, respectively. An OIT value of 53 min was measured at 100 °C. After the degradation process the peroxide index and the iodine index reduced from 35.38 to 9.85 meq × 1000 g^{-1} and from 139.79 to 120.19 gI_2 × 100 g^{-1}, respectively. An increase of the free fatty acids amount from 0.07 to 0.17% was observed while viscosity increased from 57.2 to 58.0 cP. Absorption at 272 nm also increased. The thermogravimetric and spectroscopic techniques are reproducible and versatile being an option for characterization of edible oil oxidation.

Keywords Sea fish oil · Omega3-thermal oxidation · Thermal analysis

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Introduction

Fish oils are rich in the polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and are recognized by exercising an essential role in human nutrition being known by their beneficial health effects [1, 2]. These fatty acids compete with the arachidonic acid (AA) in the enzymatic reactions reducing the formation of proinflammatory mediators and favoring the production of mediators with reduced biological activity. As a consequence cardio protective effects are observed besides reduction in the risk of cancer, psoriasis, Parkinson's disease, Alzheimer's disease, schizophrenia, and joint problems [1–5].

However, the presence of long chain polyunsaturated fatty acids makes this oil susceptible to oxidation processes that can alter various properties such as sensory quality (flavor, aroma, texture, and color), nutritional value, functionality, and toxicity [6, 7]. This process is accelerated when oils are subjected to different temperatures during extraction, processing and storage, changing their acceptance, and causing damage to health due to prolonged and continuous intake [8].

Within this context, this study aims at evaluating the oxidative stress of fish oil subjected to thermal degradation, using thermogravimetry (TG), pressurized differential scanning calorimetry (PDSC), physicochemical methods, UV–vis spectroscopy and chromatography.

Experimental

The crude fish oil from different sea fishes, as sardine, tuna, and salmon (Campestre SA, São Paulo, Brazil) was subjected to a refining process in which it was degummed with

3% hot water at 60 °C and neutralized with 20% of sodium hydroxide. Then it was washed with hot water and dried under vacuum. The dried oil was bleached with adsorbents and filtered.

The thermogravimetric curves (TG) of the refined oil were obtained in a SDT 2960 equipment from TA Instruments, in the temperature range from 25 to 700 °C and heating rates of 10, 15, and 20 °C min^{-1}, under a flow rate of 100 mL min^{-1} of synthetic air (20% of O_2 and 80% of N_2) or nitrogen.

The calorimetric curves (PDSC) were obtained using a DSC 2920 differential scanning calorimeter coupled to a pressure cell (TA Instruments) using dynamic and isothermal conditions. The dynamic curves were obtained in alumina crucibles, under synthetic air atmosphere with a flow rate of 100 mL min^{-1}, 3.45 MPa of pressure and heating rate of 10 °C min^{-1}, in the temperature range from 25 to 500 °C. The isothermal curves were obtained in oxygen atmosphere, using the same conditions of pressure, flow rate and heating rate at the temperature of 100 °C. The oxidation induction time (OIT) values were obtained from the isothermal curves, considering the difference between the onset time and the time in which the sample reached the isothermal temperature.

The refined oil was subjected to a thermal degradation process at 190 °C (the first mass loss step observed in the dynamic thermogravimetric curve, under synthetic air atmosphere). The degradation process was carried out using 20 g of oil in porcelain crucibles in a muffle, with heating rate of 10 °C min^{-1}, coupled to a synthetic air flow system, to simulate the conditions of thermogravimetry.

Free fatty acids and peroxide indices were measured according to the standards of the American Oil Chemists' Society (AOCS) [9] while the iodine index was determined according to the methodology developed in the Adolfo Lutz Institute [10]. Viscosity was measured in a LV-DVII Brookfield equipment at 25 °C. The oil samples were also characterized by ultraviolet–visible spectroscopy (UV–vis) (UV-2550 spectrometer, Shimadzu), at the wavelength ranging from 200 to 400 nm, after dilution in dichloromethane (1:5000).

The composition of the oil was determined by chromatography. In this measurement, the methyl esters obtained from the oil were evaluated according to the methodology described by Hartman and Lago [11]. This analysis was performed in a gas chromatograph coupled to a mass spectrometer GCMS-QP 2010 (Shimadzu) with Durabond DB-23 column (J & W Scientific). Helium was used as the carrier gas (3 mL min^{-1}), operating under the following conditions: injector and detector at 250 °C and temperature program of 170 °C for 16 min, rising to 210 °C at 2 °C min^{-1}, and injection of 1 mL of the sample with the split ratio of 1:50. The fatty acids were identified by comparison with standards of the detector library, and quantified by the area of each peak as a function of the total area of the peaks.

Results and discussion

In the thermogravimetrics curves of the fish oil in synthetic air and in nitrogen atmospheres (Fig. 1), it was noticed that the increase in the heating rate shifted the curves toward higher temperatures. This behavior was already observed in the literature [12, 13] being attributed to variations in the heat transfer rate and in the exposure time to a given temperature as higher heating rates lead to less exposure time.

Thermogravimetric results (Table 1) indicated that the refine oil was stable up to 188 and 222 °C in nitrogen and in synthetic air atmospheres, respectively, suggesting that the oxidizing atmosphere favored the thermal decomposition process. In synthetic air, four mass loss steps were observed that can be attributed to volatilization and/or combustion of the triacylglycerides. Conversely, in inert atmosphere only one step was observed, probably due to volatilization process besides pyrolysis.

According to Hellín and Clausell [14], thermal polymerization reactions occur in oils in temperature range from 200 to 300 °C in absence of oxygen. In the presence of oxygen, thermal oxidation process or oxy-polymerization occurs at high temperatures. In the present case, the

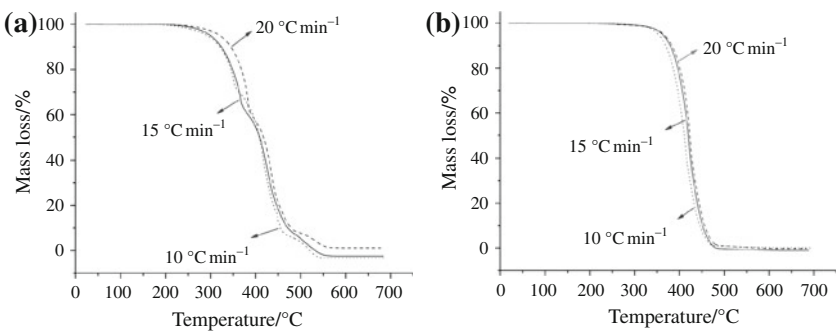

Fig. 1 TG curves of the refined fish oil at different heating rates, in the atmospheres of **a** synthetic air and **b** nitrogen

Table 1 TG data from refined fish oil in synthetic air and nitrogen atmospheres at heating rates of 10 °C min^{-1}

Sample	Step	$T_{initial}$/°C	T_{final}/°C	Δmass/%
Refined fish oil (synthetic air atmosphere)	1st	188	356	27.9
	2nd	356	442	53.3
	3rd	442	477	12.4
	4th	477	561	6.3
Refined fish oil (N_2 atmosphere)	1st	222	484	100.0

fish oil displayed a lower thermal decomposition temperature than other oils [15–17]. This was probably due to its composition richer in long chain polyunsaturated fatty acids that are more susceptible to oxidation.

The OIT of the fish oil was determined from the isothermal PDSC curve in oxygen atmosphere at 100 °C. This temperature was chosen considering that the first exothermic peak observed in the dynamic curve took place near this temperature (Fig. 2a). The refined fish oil showed an OIT of 53 min (Fig. 2b).

The physicochemical properties of the fish oil before and after degradation at 190 °C are shown in Table 2. An increase in the percentage of free fatty acids from 0.07 to 0.17 was observed being assigned to a typical process of hydrolytic rancidity that was favored by heating at temperature in which free fatty acids were released. Reda [18] studied the behavior of vegetable oils submitted to a high temperature for different exposition times and observed the same behavior with percentages of free fatty acids varying from 0.13 to 1.63%.

The oxidation of polyunsaturated fatty acids occurred with the formation of hydroperoxides and displacement of double bonds, with the consequent formation of conjugated dienes that absorb at 232 nm. Unsaturated ketones are also formed as secondary oxidation products having an absorption maximum at 270 nm. As a consequence of these reactions different spectra are obtained after oxidation allowing to evaluate the oxidative process. In this sense a higher absorbance at 232 nm indicates that peroxides were formed corresponding to the beginning of the oxidation process. Conversely, a higher absorbance at 270 nm is normally associated to higher amounts of secondary products [19–21].

Figure 3 shows similar absorption intensities at 232 nm for the refined and degraded oils, while lower absorption intensity was observed by the degraded oil at 270 nm, probably due to the formation of stable polymers. These observations are consistent with literature reports for ultraviolet spectra of unsaturated compounds that show a pronounced bathochromic effect due to the progressive thermal oxidation of oils as a consequence of the formation of peroxides and conjugated *trans* isomers [22]. These results indicate that UV–vis spectroscopy is an important tool for monitoring the oil quality [20].

A high value of peroxide index was noticed for the refined oil (35.38 meq × 1000 g^{-1}) pointing out its degradation as confirmed by the reduced amounts of their characteristic eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (Table 3) and by the iodine index of 139.79 gI_2 × 100 g^{-1}, which was lower than the value found in the literature for fish oil (155 gI_2 × 100 g^{-1},

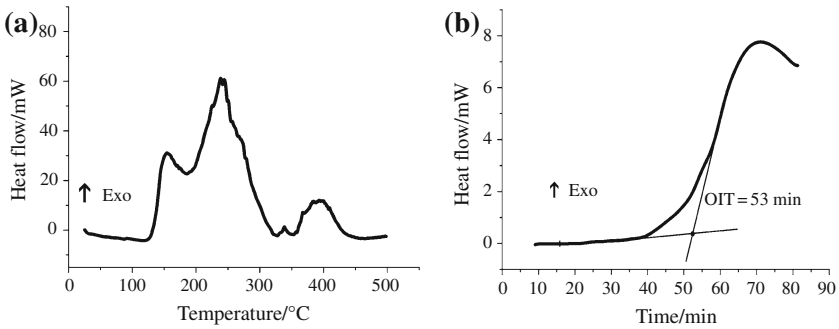

Fig. 2 Dynamic (**a**) and isothermal (**b**) PDSC curves of fish oil

Table 2 Physicochemical analysis of fish oils crude, refined and thermally degraded

Physicochemical analysis	Crude fish oil	Refined fish oil	Oil degraded at 190 °C
Free fatty acids/%	1.29 ± 0.12	0.07 ± 0.01	0.17 ± 0.04
Iodine index/g I_2 × 100 g^{-1}	119.23 ± 0.10	139.79 ± 0.17	120.19 ± 0.62
Peroxide index/meq × 1000 g^{-1}	53.28 ± 0.12	35.38 ± 0.16	9.85 ± 0.04
Viscosity/mPa s	57.64 ± 0.09	57.2 ± 0.15	58.0 ± 0.16

Average values followed by standard deviation obtained from three measurements

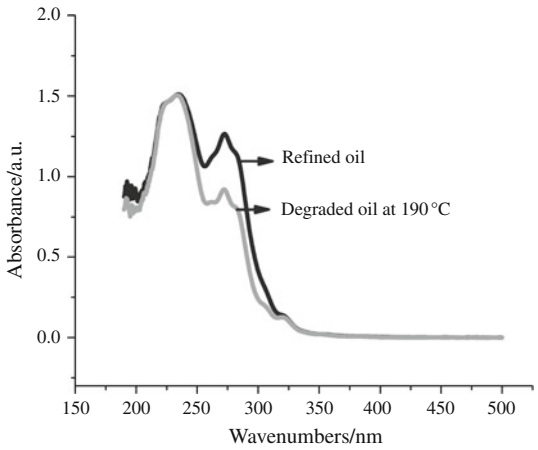

Fig. 3 UV–vis absorbance spectra of refined of degraded fish oils

according to Barlow and Yong [23]). After degradation at 190 °C, a reduction in the peroxide index was observed indicating that an advanced stage of oxidation was reached since peroxides are very unstable, reactive and easily degradable and should not be considered in isolation as they lead to the formation of more stable products as dimers, trimers, and polymers [18]. Thus, these results suggest that the refined fish oil was already well oxidized and the degradation process increased such oxidation and finally led to the formation of stable polymers that did not show an important absorption at the wavelength of 270 nm (Fig. 3).

Table 3 Fatty acid chromatographic profile of the refined and degraded fish oil

Fatty acids	Refined fish oil/%	Fish oil degraded at 190 °C/%
Palmitic 16:0	13.43	12.88
Stearic 18:0	4.65	4.59
Arachidic 20:0	0.40	0.45
Behenic 22:0	0.50	0.36
Lignoceric 24:0	0.17	0.10
Total saturated	19.15	18.38
Palmitoleic 16:1n-7	0.57	0.54
Oleic 18:1n-9	29.93	29.73
Docosenoic 22:1 n-9	0.16	0.17
Eicosenoic 20:1 n-9	0.30	0.29
Total monounsaturated	30.96	30.73
Linoleic 18:2n-6	47.91	48.95
Alfa-linolenic 18:3 n-3	0.73	0.78
Eicosapentaenoic 20:5 n-3	0.29	0.25
Docosahexaenoic 22:6 n-3	0.96	0.91
Total polyunsaturated	49.89	51.38

Reda [18] reported a direct correlation between the values of peroxide index and absorptivity at 232 nm in the ultraviolet spectra only at the beginning of oxidation. In this study, during the advanced oxidation stages the absorption intensity at 232 nm did not change while a reduction in peroxide index was observed. On the other hand, the absorption intensity at 270 nm decreased indicating that secondary intermediate products reacted leading to polymer formation. This fact can demonstrate the validity of spectrophotometric analysis method for oils in all stages of oxidation, as far as comparative analyses are done.

Table 3 shows the fatty acid chromatographic profile of the refined fish oil and of the fish oil after the degradation process. The oil degraded at 190 °C achieved a reduction of saturated and monounsaturated fatty acids from 19.15 to 18.38% and from 30.96 to 30.73%, respectively, while an increase in the amount of polyunsaturated fatty acids was observed being attributed to the formation of linoleic acid.

The contents of fatty acids characteristic of sea fish oil observed in present work, 0.29% eicosapentaenoic acid and 0.96% of docosahexaenoic acid, are smaller than the amounts reported by Shimada et al. [24] of 6.5 and 22.9%, respectively. These results indicated that the refine fish oil already showed a loss of polyunsaturated fatty acids due to oxidation reactions.

Besides oxidation, several factors influence the corporal composition of a fish such as feeding type, maturation, age, sex, geographical habitat, and season, leading to different characteristics from one species to another and within the same species [25]. Other factors of influence can be cited such as the whole sea food processing, the fishing method, the period between capture and discharge, the time and type of storage in fishing ships and in industries [26].

Conclusions

The results indicated that the refined fish oil was already oxidized. The thermal stresses undertaken by the sample during the degradation process increased such oxidation, as demonstrated by the physicochemical properties, the UV–vis spectra, the chromatograms and the thermogravimetric curves. These instrumental analytical techniques are reproducible and versatiles being a good option to the evaluation of the oxidative characteristics of oils by the food industry, as compared with the traditional analytical techniques.

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