J Am Oil Chem Soc
DOI 10.1007/s11746-015-2612-9

ORIGINAL PAPER

Oxidation Rates of Triacylglycerol and Ethyl Ester Fish Oils

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Received: 13 May 2014 / Revised: 4 January 2015 / Accepted: 29 January 2015

Abstract Fish oil is available primarily as triacylglycerols (TAG) or ethyl esters (EE). Anecdotal evidence suggests that TAG have superior bioavailability and oxidative stability compared to EE. In this work, peroxide value (PV) and *p*-anisidine value (AV) were used to monitor oxidation in commercially available TAG and EE fish oils incubated at temperatures from 5 to 60 °C. Pseudo first-order kinetics were assumed and rate constants were calculated for each temperature. At all temperatures, the rates of oxidation were higher for EE oils than TAG oils. For PV and AV measured in both oils, non-linear Arrhenius models were plotted, generating activation energies that ranged from 7 to 103 and 2 to 159 kJ/mol for PV and AV, respectively. Although TAG were more resistant to oxidation than EE, they had lower activation energies (E_a) at ≤15 °C for reactions measured with PV and AV. The E_a for EE was negative at temperatures ≥45 °C, indicating that reaction rate was influenced by factors in addition to temperature.

Keywords Lipid oxidation · Reaction rates · Kinetics · Arrhenius model · Shelf life · Activation energy

Electronic supplementary material The online version of this article (doi:10.1007/s11746-015-2612-9) contains supplementary material, which is available to authorized users.

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Introduction

Fish oil is a popular dietary supplement due to the high levels of polyunsaturated fatty acids (PUFA) it contains. The major PUFA found in fish oil are the omega-3 fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), both of which have demonstrated efficacy in improving cardiovascular health (e.g., [1–3]). Fish oil supplements are most commonly found encapsulated in gelatin, but liquid fish oil supplements are also popular.

Fish oil is found naturally in the form of triacylglycerols (TAG). During the refining of fish oil, TAG are often transesterified with ethanol to form ethyl esters (EE). EE can be fractionated, removing specific fatty acids and allowing the levels of EPA and DHA to be adjusted to required concentrations using molecular distillation, supercritical extraction or other techniques [4]. In some cases, the final product can contain >90 % EPA and DHA. An additional, but costly, processing step converts EE back to TAG. The high cost of the re-esterification process means that the price of TAG oil can be considerably greater than an EE product with the same levels of EPA and DHA. As a result, many fish oil supplements are sold as EE rather than TAG; however, the form of the supplement is rarely specified on the package, making it difficult for consumers to know the type of product they are purchasing. This is particularly important because there is evidence that suggests that omega-3 fatty acids have greater bioavailability in TAG form than EE [5, 6].

Polyunsaturated fatty acids, including EPA and DHA, are prone to oxidation due to the high number of double bonds they contain, which can produce fishy flavors and odors that make fish oil supplements unappealing to some customers. According to classic lipid oxidation pathways, oxidation is initiated when singlet oxygen reacts with fatty acids, generating free radicals and starting a chain

Published online: 14 February 2015

reaction of free radical mediated oxidation [7]. The primary products of lipid oxidation are lipid hydroperoxides, usually monitored using the peroxide value (PV), which do not contribute to the sensory properties of oil, but can be viewed as an indicator of the taste that an oil may acquire in the future. They form directly from lipids and thus are related to the amount of oxidized lipid present early in oxidation. Hydroperoxides undergo further breakdown to form volatile secondary oxidation products, including aldehydes, ketones and alcohols. It is these secondary oxidation products that are responsible for the off-flavors and odors found in rancid oils. Relative amounts of secondary oxidation products, specifically aldehydes with 2,4-unsaturation, are commonly measured using the *p*-anisidine value (AV). Both PV and AV are popular tests used by fish oil manufacturers to assess oxidation in oils.

The oxidation kinetics of TAG fish oils have been modeled in few papers. Our previous work investigated the kinetics of primary oxidation of two commercial TAG fish oil preparations containing different levels of PUFA using PV and found that oil containing lower levels of PUFA followed a pseudo first-order model at temperatures $\geq$20 and $\leq$40 °C, while the fish oil with higher levels of PUFA followed pseudo first-order kinetics at temperatures $\geq$4 and $\leq$40 °C [8]. Alternatively, Bórquez et al. [9] monitored concentrations of EPA, DHA and stearidonic acid (SDA, 18:4n-3) in mackerel oil at temperatures from 80 to 100 °C and found that degradation of these fatty acids could be used to model kinetics of fish oil. Studies that compare oxidation of TAG and EE are also rare, and most focus only on specific fatty acids (e.g., [10–12]). A recent study [13] did report greater oxidation rates of EE compared to TAG fish oils; however, that work employed oils with differing EPA and DHA proportions, confounding interpretation and introducing the possibility that the increased rate of oxidation of the EE oil was due to higher PUFA levels, rather than inherent instability associated with EE oils. Similar to other kinetic studies, that study also used elevated temperatures (50–90 °C), generating results that could not be reliably extrapolated to lower temperatures.

To ensure that fish oil supplements are of the highest quality, it is essential to understand the oxidation kinetics of TAG and EE fish oils. Without this knowledge, it will be impossible to predict shelf life at ambient temperature from data acquired from experiments using accelerated oxidation at elevated temperatures. This study evaluates the oxidative stability of fish oils in TAG and EE form, containing practically identical concentrations of EPA and DHA. Oils were selected to be representative of those typically available as a raw material for food and dietary supplement manufacturers, containing EPA and DHA proportions of approximately 65 % of total FA. Samples were incubated at temperatures between 5 and 60 °C, and PV, AV and fatty acid profiles, parameters particularly relevant to the fish oil industry, were monitored. The ultimate goal was to determine if the rates of oxidation vary between the two products.

Experimental Procedures

Materials

Optima chloroform was obtained from VWR (Oakville, ON). Methyl tricosanoate, methyl eicosapentaenoate and methyl docosahexaenoate were purchased from Nu-Chek Prep (Elysian, MN). All other chemicals and glassware were obtained from Fisher Scientific (Ottawa, ON). Molecularly distilled, steam deodorized TAG and EE fish oil containing a blend of mixed natural tocopherols were obtained from Ocean Nutrition Canada Ltd. (Dartmouth, NS). These oils are available to commercial manufacturers of foods and dietary supplements. Both oils had similar fatty acid profiles and were blends of anchovy and sardine oil sourced from Peru.

Fatty Acid Concentrations

Eicosapentaenoic acid and DHA in both oils were quantified as mg/g of free fatty acid at the initial and final time points for each temperature by conversion to methyl esters (ME) and analysis with GC-FID, following the method described by the Global Organization for EPA and DHA (GOED) in their Voluntary Monograph [14], modified as described by Sullivan et al. [15]. ME were separated on a DB-23 column (30 m × 0.25 mm × 0.25 µm film thickness) using helium as the carrier gas (1.0 mL/min). The oven temperature was initially held for 2 min at 153 °C then increased at 2.3 °C/min to 174 °C and held for 0.2 min. The temperature was then increased at a rate of 2.5 °C/min to 205 °C and held for 8.3 min, resulting in a total run time that was approximately 32 min. The FID was maintained at 270 °C, and the injector (split mode 1:100, 250 °C, 4 mm liner) at 250 °C. The full fatty acid profile was reported as mass percentages of the total fatty acids identified in the initial samples. All analyses were conducted in triplicate.

Tocopherol Analysis

Levels of α, γ and δ-tocopherols were measured using normal phase HPLC with evaporative light scattering detection (ELSD) following a modified version of the method presented by Carpenter [16]. Oil samples were diluted in 1.5 % isopropanol (IPA) in hexane to a final concentration of approximately 0.2 g/mL and filtered through a 0.45 µm filter. An injection volume of 10 µL was used on a 5 µm silica column (250 mm × 4.6 mm, Supelco Inc.). The

Table 1 Fatty acid proportions (mass percent of total fatty acid, mean ± SD, $n = 3$) of triacylglycerol (TAG) and ethyl ester (EE) fish oils at the initial sampling point

Fatty acid	TAG	EE	Fatty acid	TAG	EE
14:0	0.3 ± 0.0	0.2 ± 0.0	20:4n-6	2.4 ± 0.0	2.4 ± 0.0
16:0	0.9 ± 0.0	0.7 ± 0.0	20:3n-3	0.2 ± 0.0	0.2 ± 0.0
16:1n-7	0.7 ± 0.0	0.5 ± 0.0	20:4n-3	1.6 ± 0.0	1.7 ± 0.0
16:3n-4	0.1 ± 0.0	0.0 ± 0.0	20:5n-3 (EPA)	38.0 ± 0.2	37.7 ± 0.1
16:4n-1	0.2 ± 0.0	0.0 ± 0.1	22:0	0.4 ± 0.0	0.2 ± 0.0
18:0	2.3 ± 0.0	2.9 ± 0.0	22:1n-11	1.3 ± 0.0	1.2 ± 0.1
18:1n-9	3.9 ± 0.0	5.3 ± 0.1	22:1n-9	0.4 ± 0.1	0.5 ± 0.0
18:1n-7	1.4 ± 0.1	2.0 ± 0.1	22:1n-7	0.3 ± 0.0	0.3 ± 0.0
18:2n-6	0.5 ± 0.0	0.6 ± 0.0	21:5n-3	2.1 ± 0.0	1.8 ± 0.0
18:2n-4	0.2 ± 0.0	0.3 ± 0.0	22:4n-6	0.4 ± 0.0	0.3 ± 0.0
18:3n-6	0.1 ± 0.1	0.1 ± 0.0	22:5n-6	0.8 ± 0.0	0.9 ± 0.0
18:3n-4	0.1 ± 0.1	0.1 ± 0.1	22:4n-3	0.2 ± 0.0	0.2 ± 0.0
18:3n-3	0.3 ± 0.0	0.4 ± 0.0	22:5n-3	6.9 ± 0.1	5.2 ± 0.0
18:4n-3	0.8 ± 0.0	1.2 ± 0.0	22:6n-3 (DHA)	28.0 ± 0.3	28.1 ± 0.1
18:4n-1	0.1 ± 0.0	0.1 ± 0.0	24:1	1.1 ± 0.0	1.0 ± 0.1
20:0	0.5 ± 0.0	0.5 ± 0.0	Total EPA + DHA	66.0 ± 0.4	65.8 ± 0.1
20:1n-11	0.2 ± 0.0	0.2 ± 0.0	Total omega-3 FA	78.1 ± 0.4	76.5 ± 0.1
20:1n-9	1.8 ± 0.0	1.8 ± 0.0	Total omega-6 FA	4.2 ± 0.1	4.3 ± 0.1
20:1n-7	0.7 ± 0.0	0.7 ± 0.0	Total PUFA	83.8 ± 0.4	82.2 ± 0.2
20:2n-6	0.4 ± 0.0	0.5 ± 0.0	Total MUFA	11.8 ± 0.2	13.4 ± 0.2
20:3n-6	0.5 ± 0.0	0.4 ± 0.0	Total SFA	4.4 ± 0.9	4.5 ± 1.0

Also included are total EPA + DHA, omega-3 fatty acids (FA), omega-6 FA, polyunsaturated FA (PUFA), monounsaturated FA (MUFA) and saturated FA (SFA)

mobile phase consisted of 1.5 % IPA in hexane (2 mL/min) and the run time was 10 min. Tocopherol concentrations in each sample were determined using external standard calibration. All analyses were conducted in triplicate.

Stability Study

Oils were divided into 25-mL aliquots in glass scintillation vials. Open vials were incubated in the dark at 5, 15, 30, 45 and 60 °C. Samples were collected at different time intervals for analysis of PV, AV and EPA and DHA, ensuring five time points for each incubation temperature. The duration of incubation varied due to temperature. Samples stored at 5 °C were incubated for 21 days while samples stored at 15 °C were incubated for 9 days. All other incubation periods were 4 days in duration. Different incubation temperatures required different sampling periods in order to ensure that a range of PV and AV were captured. Samples stored at high temperatures were sampled more frequently than those stored at low temperatures because they oxidize more rapidly. PV and AV were measured at each time point using American Oil Chemists' Society (AOCS) Official Method Cd 8-53 and AOCS Official Method Cd 18-90, respectively [17, 18]. To ensure that all samples were incubated under the same conditions, three separate stability studies were carried out concurrently at each temperature, with three 25-mL aliquots being sampled at each time point.

Determination of Rate Constants and Shelf Life Prediction

Kinetic analysis of data was conducted using methods described by Labuza [19], Labuza and Berquist [20] and Gómez-Alonso et al. [21]. Pseudo first order reaction conditions were assumed, with oxygen being in excess, so that

$$\ln C = \ln C_o + kt \quad (1)$$

where C_o is the initial concentration of oxidation products, C is the concentration of oxidation products at a given time, t, and k is the rate constant. Plots of ln C versus time were linear with slopes of k. The rate constants can then be fitted to an Arrhenius model:

$$\ln k = \ln A - E_a/RT \quad (2)$$

where A is the pre-exponential factor, R is the universal gas constant, T is the absolute temperature and E_a is the activation energy in J mol^{-1}. Shelf life prediction is generally done by extrapolating rate constants at high temperatures to predict shelf life at lower temperatures, so the validity of that method was investigated here by calculating t_{rej}, the amount of time required to reach the upper limit of product quality, measured in this case, with either PV or AV,

$$\ln t_{rej} = \ln a - b\mathrm{T} \quad (3)$$

where a and b are constants and T is absolute temperature. The upper limit was 5 mequiv/kg for PV and 20 for AV, as specified by GOED.

Significant changes in amounts of EPA and DHA were identified using one-way analysis of variance (ANOVA; SPSS 11.0, SPSS Inc., Chicago IL, USA).

Results and Discussion

Fatty Acid Concentrations

The fatty acid composition of the TAG and EE samples indicates that the proportions of EPA and DHA comprise 66.0 ± 0.4 % and 65.8 ± 0.1 %, respectively, of the oils tested (Table 1). Individual proportions of EPA and DHA were also practically identical, at 38 % EPA and 28 % DHA in the oils. The total proportion of PUFA varied only slightly in the TAG and EE oil with 83.8 ± 0.4 and 82.2 ± 0.2 %, respectively. This demonstrates that the EPA and DHA levels were effectively identical in the TAG and EE samples, and comprised a large proportion of the fatty acids in the oils. The EPA and DHA concentrations did vary over the course of the experiments, with slight but statistically significant differences with initial concentrations at several time points (Table 2); however, the differences were within the expected amount of variance associated with the method [14] and, therefore, could not be attributed to changes due to oxidation. This indicates that fatty acid analysis using GC-FID as done here is not sensitive enough to detect minute changes in concentrations of EPA and DHA that could be occurring as a result of oxidation. The lack of change in EPA and DHA concentrations is not unexpected. Oxidation of one fatty acid molecule can result in the formation of many different primary and secondary oxidation products, so a very small change in the concentration of a specific fatty acid can result in large amounts of oxidation products. For the purpose of this study, the change in concentrations of other fatty acids were not monitored. This is because EPA and DHA represent 66 % of the total fatty acids and are thus most likely to contribute to oxidation. The levels of other PUFA are much lower than these FA and were deemed to play an insignificant role in oxidation in comparison to EPA and DHA.

Table 2 EPA and DHA concentrations (mg g^{-1} FFA, mean ± SD, $n = 3$) at initial and final time points at each incubation condition in triacylglycerol (TAG) and ethyl ester (EE) fish oils

Time point	TAG		EE	
	EPA	DHA	EPA	DHA
Initial	308 ± 4	225 ± 1	308 ± 3	219 ± 1
5 °C final (Day 22)	307 ± 2	223 ± 1	299 ± 5	215 ± 3
15 °C final (Day 10)	304 ± 1	221 ± 1[a]	299 ± 4[a]	216 ± 2
30 °C final (Day 4)	304 ± 1	220 ± 1[a]	300 ± 3[a]	214 ± 1[a]
45 °C final (Day 4)	311 ± 3	224 ± 1	304 ± 0	217 ± 0[a]
60 °C final (Day 4)	312 ± 2	225 ± 1	303 ± 3	215 ± 1[a]

[a] Significantly different from the initial value (ANOVA; $p < 0.05$). Differences were within the acceptable amount of variance associated with the analytical method [14] and therefore could not be attributed to oxidation

Tocopherol Analysis

The levels of α, γ and δ-tocopherols were statistically different ($p < 0.05$) between TAG and EE oils (Supplementary Table 1). β-Tocopherol was not detected in either sample. The TAG fish oil had higher levels of all three tocopherols than EE, containing 2,669 ± 57 mg/g, compared to 2,037 ± 53 in the EE but both had tocopherol levels consistent with the manufacturer's specifications of a minimum of 2,000 ppm. With higher levels of tocopherols, it would seem that TAG oil would be more resistant to oxidation. However, literature pertaining to the optimal levels of tocopherol usage in fish oil is minimal, with no published studies found on the use of tocopherols to stabilize EE fish oils. Of the scant information available, it seems that more is not necessarily better [22, 23]. For instance, Kulås et al. [23] found that the efficacy of both α- and γ-tocopherol were decreased at concentrations >500 ppm while Hamilton et al. [22] found that the addition of 2,000 ppm of α-tocopherol to Chilean fish oil resulted in a pro-oxidant effect. It therefore seems unlikely that the slower rate of oxidation (Figs. 1, 2) and the superior stability seen in the TAG oil is simply due to the difference in tocopherol content.

Stability Study

At all temperatures, PV and AV of both oils increased with time. EE samples also oxidized more rapidly than TAG samples at all temperatures (Figs. 1, 2; Table 3). Both TAG and EE samples had very low and similar PV at the start of the study (0.00 ± 0.00 and 0.53 ± 0.06 mequiv/kg, respectively), indicating that samples were not highly oxidized and were of similar quality. The rate of hydroperoxide formation increased with storage temperature at conditions ≥5 and ≤45 °C; however, the pseudo first order reaction rate at 60 °C was slower than at 45 °C for both TAG and EE. This is consistent with our previous work [8], where oxidation, as measured using PV, was found to follow a different kinetic model at 60 °C than at 40 °C. It is likely that oxygen solubility is substantially lower at 60 than 45 °C and may be beginning to limit the rate of oxidation. The initial AV of both oils was also fairly low and similar, at 12.17 ± 0.07 for TAG and 12.18 ± 0.03 for EE, well below the limit of 20 specified in the GOED Voluntary Monograph for EPA and DHA [14], commonly followed by fish

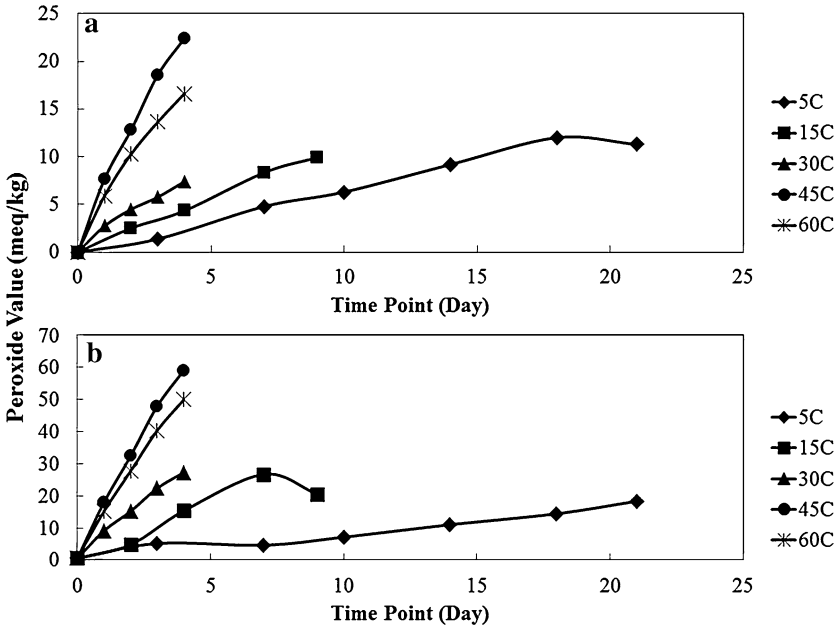

Fig. 1 Variation in hydroperoxide value (PV, mean ± SD, $n = 3$) over time for fish oil **a** triacylglycerols (TAG) and **b** ethyl esters (EE)

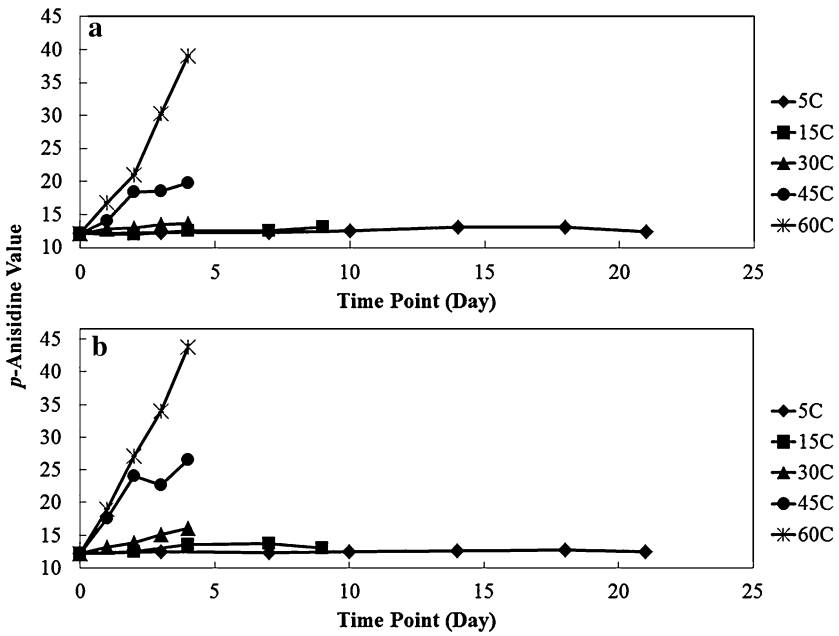

Fig. 2 Variation in *p*-anisidine value (AV, mean ± SD, $n = 3$) over time for fish oil **a** triacylglycerols (TAG) and **b** ethyl esters (EE)

oil manufacturers. The rate of formation of secondary oxidation products, as measured using AV, increased with temperature and showed no decline at 60 °C (Fig. 2). Thus, rate constants (k) were calculated for data from the entire temperature range (Table 3). Because PV seemed to follow a different model at 60 °C, data from that temperature were not included the Arrhenius model. In contrast, the increase in reaction rate for AV with increasing temperature was expected because AV measures the formation of secondary oxidation products that are derived from the breakdown of hydroperoxides. It is likely that hydroperoxides were decomposing at a faster rate than they were generated at the higher temperatures, resulting in increasing production of secondary oxidation products with increasing temperature.

A number of kinetic models were considered in this work. Zero-order kinetics were deemed inappropriate because such models are independent of substrate concentration and we have previously shown that the rate of oxidation increases as the concentrations of EPA and DHA increase [8]. With oxygen present in the headspace at concentrations far in excess of substrate in the experimental design, pseudo first order kinetics seemed more

Table 3 First-order rate constants (k, mean ± SD), activation energies (E_a) and pre-exponential factors (A) at different temperatures determined using Arrhenius plots and the slope of the curve at each temperature

Temp (°C)	TAG			EE		
	k (days^{-1})	E_a (kJ mol^{-1})	A (days^{-1})	k (days^{-1})	E_a (kJ mol^{-1})	A (days^{-1})
PV						
5	0.10 ± 0.02	64	1.2×10^{11}	0.11 ± 0.02	103	2.1×10^{18}
15	0.25 ± 0.02	49	1.6×1^{8}	0.38 ± 0.06	66	3.0×10^{11}
30	0.47 ± 0.04	27	2.0×10^{4}	0.88 ± 0.14	15	2.89×10^{2}
45	0.71 ± 0.10	7	1.0×10^{1}	1.04 ± 0.18	NAp	NAp
60	0.65 ± 0.09	NAp	NAp	1.01 ± 0.17	NAp	NAp
R^{2a}	0.88	–	–	0.85	–	–
R^{2b}	0.95	–	–	0.88	–	–
AV						
5	0.00 ± 0.00	81	3.3×10^{12}	0.00 ± 0.00	159	5.9×10^{26}
15	0.01 ± 0.00	76	4.3×10^{11}	0.01 ± 0.00	126	6.1×10^{20}
30	0.03 ± 0.00	68	1.8×10^{10}	0.07 ± 0.00	80	4.5×10^{12}
45	0.12 ± 0.01	62	1.9×10^{9}	0.18 ± 0.02	39	4.6×10^{5}
60	0.29 ± 0.01	56	1.9×10^{8}	0.31 ± 0.01	2	5.4×10^{1}
R^2	0.84	–	–	0.94	–	–

Rates were calculated using peroxide value (PV) and *p*-anisidine value (AV) for fish oil triacylglycerols (TAG) and ethyl esters (EE). Coefficients of determination for the fit of the data to the Arrhenius plots are also given

[a] Coefficient calculated for all data

[b] Coefficient calculated excluding data where E_a was not determined

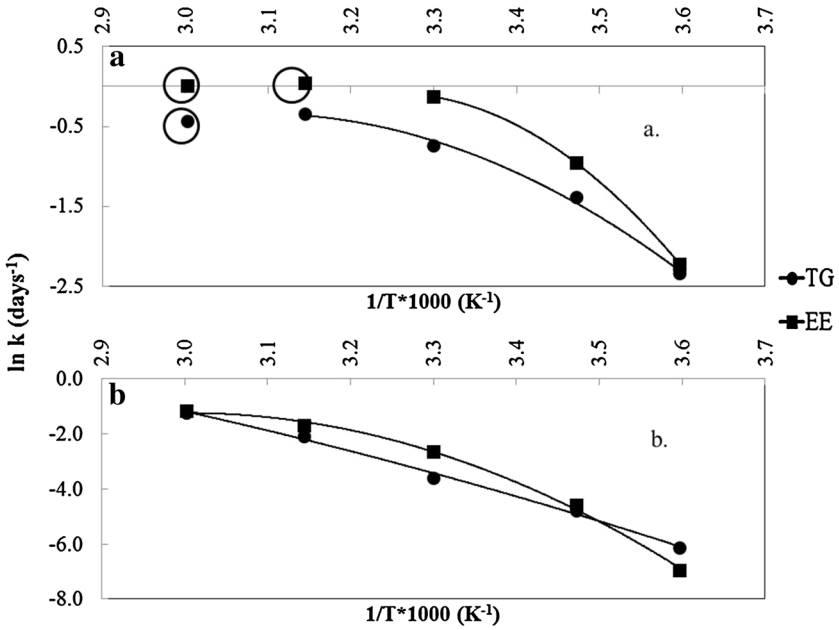

Fig. 3 Arrhenius plots of **a** peroxide value (PV) and **b** *p*-anisidine value (AV) data for fish oil **a** triacylglycerols (TAG) and **b** ethyl esters (EE). *Circled points* had negative activation energies (E_a)

appropriate. However, our data at 60 °C suggest that oxygen solubility may in fact be limiting at that temperature, so data were also fit to a second-order kinetics model. Coefficients of determination were used to assess the fit and it was determined that, despite the unusual result at 60 °C, the first-order model remained most appropriate for both PV and AV.

The original intention of this paper was to determine the activation energies (E_a) for reactions monitored using PV and AV; however, the Arrhenius plots of ln k *vs* 1/T for both oxidation markers were non-linear (Fig. 3). The Arrhenius model assumes that all variables except for temperature remain constant; however, oxygen solubility in oil decreases with increasing temperatures [24]. Non-linear Arrhenius plots prevented simple calculation of a single E_a over the temperature range, derived from a constant slope. Instead, E_a was calculated by fitting plots of ln k *vs* 1/T to the PV and AV data with polynomial curves and taking the instantaneous slope of the line at each temperature [25], resulting in a different E_a for each temperature (Table 3). At temperatures ≤15 °C, the E_a of hydroperoxide formation in TAG was lower than that of EE despite the fact

that the rate of hydroperoxide formation was greater in EE oils. At 30 °C, the pattern was reversed with higher E_a for EE than TAG oil. It was not possible to compare E_a of hydroperoxide formation at temperatures >30 °C because the E_a values calculated for EE were negative, due to the lower rate constant at 60 °C, compared to 45 °C. A similar unexpected relationship of E_a with temperature was found with the AV data where E_a was higher for EE than TAG at temperatures ≤ 30 °C. However, this contradicts our data (Fig. 2; Table 3) that show identical rate constants derived from AV for both EE and TAG samples at low temperatures. When considering variation in the reaction rate with temperature, the pre-exponential factor, *A*, in the Arrhenius equation must also be taken into account. This term represents the rate at which collisions occur between reactants. The higher value of *A* in fish oil EE than TAG at each temperature indicates that there were more collisions of molecules per second that might lead to a reaction in fish oil EE than TAG at each temperature (Table 3). Intuitively, this is to be expected because EE molecules are smaller in size than TAG and there would be more molecules in a given volume. Fish oil EE are also less viscous than TAG allowing molecules to move more freely and participate in more collisions.

Often, the main purpose in modeling kinetic data is to apply it to the prediction of product shelf life under realistic conditions using data collected at elevated temperatures. Our primary interest here was prediction of shelf life at room temperature (20 °C) using PV data; because we did not measure PV at 20 °C, our predictions were validated by comparing the actual shelf life to the calculated shelf life at 15 °C (Supplementary Table 2; Fig. 4). An upper limit of 5 mequiv/kg for PV and 20 for AV were used, as these are the quality limits that are specified in the GOED Voluntary Monograph [14] and the limits that are used by many fish oil manufacturers. For practicality, the shelf lives were rounded to the nearest day. This was done using shelf life plots (Fig. 4) as described in Eq. 3. The calculated shelf life based on PV at 15 °C matched the actual shelf life (4 days for TAG and one day for EE), demonstrating good fit of the shelf life plot. In the present study, AV only reached 14 at temperatures ≤30 °C; therefore, data were insufficient to calculate shelf life based on this value.

The results here agree well with those of our previous work with TAG oil where we found that the first order kinetic model might be invalid for hydroperoxide formation at temperatures ≥40 °C. An E_a of 55 kJ mol^{-1}, which fits within the range of those reported here, was also found. We attempted to follow the approach of Bórquez et al. [9] using fatty acid degradation to investigate the kinetics of oxidation but the decrease in EPA and DHA over time was not great enough to model the data. Perhaps if this study had continued for a longer period of time, greater changes in EPA and DHA concentrations would have been noted; however, the PV exceeded quality limits at all temperatures, indicating that the oil was already very oxidized and further extension of the experiment would have resulted in levels of oxidation far beyond that typically encountered in industry. Additionally, Bórquez et al. [9] used temperatures of 80–100 °C that were much higher than the recommended temperature of 40 °C for accelerated stability studies of fish oil [24]; therefore, the kinetic models developed are not likely applicable at lower temperatures [26].

There are a variety of other methods available to determine oxidation and, as Shahidi and Zhong [27] point out in their review of methodology for lipid oxidation methods, it is important to select a technique appropriate to the application being considered. We chose PV and AV to monitor

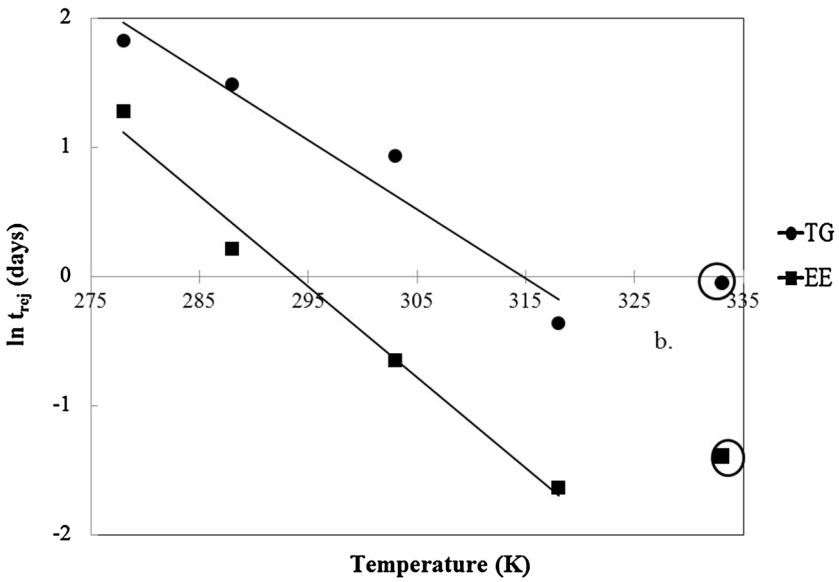

Fig. 4 Shelf life plots based on peroxide value (PV) for fish oil triacylglycerols (TAG) and ethyl esters (EE). A t_{rej} of 5 mequiv/kg was used. R^2 for TAG = 0.96, R^2 for EE = 0.99. *Circled points* are not included in the regression.

oxidation in fish oils because these are the tests recommended in the GOED monograph and are typically used by the fish oil industry to assess oxidative quality. Thus, from an industry perspective, the rates of increase of these parameters are most appropriate when investigating oxidation in fish oils. However, other studies of fish oil oxidation, with a more academic focus, have chosen different methods to monitor oxidation, including analysis of head space volatiles [10] or the Rancimat test [13], making it difficult to find comparable studies in the literature. Of the few studies that did compare oxidation rates of TAG and EE fish oils using different methods to assess oxidation, the overall results generally agree with ours. For instance, Lee et al. [10] monitored oxidation of EPA and DHA in DHA-enriched fish oil TAG and EE, incubated at 80 °C with air bubbling through and found that EE oxidized more rapidly than TAG. Because oil was only oxidized at one temperature, no kinetic modeling was attempted. Yoshii et al. [11] examined the kinetics of autoxidation of DHA-enriched TAG and EE oils by monitoring oxygen concentration in the headspace of samples stored at 35, 50 and 70 °C. They found that oxidations rates for EE were greater than for TAG oils at all temperatures and they reported E_a for TAG and EE oils of 62 and 50 kJ mol^{-1}, respectively. These values are within the range of E_a seen in this study; however, in that study, oils of different DHA concentrations were used and different methodologies were applied, making it difficult to compare their results to ours. Finally, Martin et al. [13] used the Rancimat test to investigate the relationship between temperature and rates of oxidation in TAG and EE oils containing >80 % EPA + DHA at temperatures of 50–90 °C. In contrast to our results, they did not find a difference in rates of oxidation; however, when they monitored oxidation at a single lower temperature of 15 °C using PV and AV, they did note decreased stability of the EE oil, agreeing with our findings. Given the overall pattern of results reported in these diverse studies, using a variety of different methodologies and oils with differing PUFA concentrations, we expect that our results demonstrating decreased oxidation stability of EE oils will hold for other fish oils having different EPA and DHA concentrations.

Conclusions

Oxidation of fish oil TAG and EE was monitored using PV and AV. The rate of oxidation, as measured by changes in PV and AV, was lower in TAG fish oil than in EE. The Arrhenius model predicted the relationship of PV with temperature at ≤45 °C, while AV data from both oils fit the Arrhenius model over the full range of temperatures assessed. TAG was more resistant to oxidation than EE, but had lower E_a than EE at temperatures ≤15 °C for PV, and ≤30 °C for AV formation. At temperatures ≥45 °C, the E_a for PV formation in EE was negative, suggesting that reaction rate was no longer dependent solely on temperature. The same was true for PV formation in TAG at 60 °C. This study clearly shows that EE fish oil oxidizes more rapidly than TAG fish oil containing near identical levels of EPA and DHA. We have also demonstrated that PV data acquired at temperatures ≤45 °C, sufficient to accelerate oxidation, can be extrapolated to predict shelf life at lower temperatures, but we were unable to assess the usefulness of AV as a predictor with our experimental design.

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