Food Chemistry 119 (2010) 1578–1583

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Storage stability of liver oil from two ray (*Rhinoptera bonasus* and *Aetobatus narinari*) species from the Gulf of Mexico

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ARTICLE INFO

Article history:
Received 3 October 2008
Received in revised form 6 August 2009
Accepted 14 September 2009

Keywords:
Rajiform
Liver oil
DHA
Lipid oxidation

ABSTRACT

The effect of storage time on the quality of liver oil from two commercial rajiform species (*Rhinoptera bonasus* and *Aetobatus narinari*) captured in the Gulf of Mexico (State of Campeche coast line) was evaluated. Oil characterisation was conducted by physical (specific gravity, saponification index and water content) and chemical analyses (fatty acid content, carotenes and tocopherols) whilst storage stability (peroxide value, free fatty acids, conjugated dienes, anisidine value and changes in docosahexaenoic acid, DHA and content) was studied for 87 days at 25 °C. Increases ($p < 0.05$) in free fatty acids, conjugated dienes, peroxide value and anisidine value, and a decrease ($p < 0.05$) in DHA were observed during the storage time for both oil species as oxidation proceeded. It was concluded that *R. bonasus* and *A. narinari* oils lasted for 52 and 66 days under these storage conditions, respectively.

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1. Introduction

At present, fish oil constitutes the major source of omega-3 and 6 PUFAs, and particularly eicosapentaenoic (EPA) and docosahexaenoic acids (DHAs). Some living organisms obtain their EPA and DHA by a series of successive de-saturations and elongations of the α-linolenic acid (α-LNA). A study by Williams and Burdge (2006) showed that only 8% of α-LNA is transformed to EPA and less than 0.1% to DHA in adult humans. Although their high unsaturation number offers important health properties, unsaturated oils are vulnerable to oxidation. Amongst the factors that promote their oxidation, oxygen, metals, temperature, water and light are the most common. In the same way, the more PUFAs present in the oil, the faster is the oxidation (Shiota, Konishi, & Tatsumi, 1999), especially without antioxidant protection. Lipid oxidation products (free radicals, peroxides, aldehydes and ketones) can react with vitamins, proteins and other lipids, thus reducing their biological availability and nutritional value. On the other hand, this lipid oxidation can be avoided by the use of antioxidants such as tocopherols. These antioxidants can be either naturally present in the oils, protecting them from becoming oxidised by sequestering the oxygen and inhibiting the free radical formation, or they can be added to the oils (Ranken, 1993).

Nowadays, omega-3 PUFAs are in high demand due to their health benefits. Thus, new sources are valuable, especially if these sources are currently underutilised. Such is the case with the livers from rajiform species in Mexican waters. As an example, the Mexican ray fishery, more specifically in Campeche, a state located in the south-east part of the Mexican Republic, represents 50% (1266 tonnes) of the total capture in the Gulf of Mexico and Caribbean region (SAGARPA, 2004). Fishermen use only its muscle, discarding the rest of the body, including the liver. Ray liver represents an important source of EPA- and DHA-rich oil (Navarro-García, Bringas-Alvarado, Pacheco-Aguilar, & Ortega-García, 2004; Navarro-García, Pacheco-Aguilar, Bringas-Alvarado, & Ortega-García, 2004). The former authors showed that livers in ray species represented around 5–11% of the total fish weight, with an oil content of approximately 50% of its weight. EPA and DHA represented 16–18% of the fatty acids present in the oil (similar levels to those of high PUFA content species).

With the aim of making use of this important underutilised resource, basic information is needed such as lipid content and composition variability and/or stability (Wetherbee & Nichols, 2000). Unfortunately, available literature on liver oil studies from ray species in Mexico and in the world is scarce (Navarro-García, Bringas-Alvarado, et al., 2004; Navarro-García, Pacheco-Aguilar, et al., 2004; Pal, Banerjee, Patra, Patra, & Ghosh, 1998; Val Ould El Kebir, Barnathan, Siau, Miralles, & Gaydou, 2003). Knowing the oil storage stability (amongst other parameters) is very important for its scale-up production since it mostly defines the quality of the

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doi:10.1016/j.foodchem.2009.09.045

product. Hence, the objective of the present study was to characterise the physical, chemical and storage stability characteristics of liver oil from two ray species, *Rhinoptera bonasus* and *Aetobatus narinari*, from the Gulf of Mexico.

2. Materials and methods

2.1. Sampling and oil extraction

Ray specimens of *R. bonasus* and *A. narinari* were captured in the Gulf of Mexico near Ceybaplaya shore in Campeche. Ten specimens from each species and their livers were measured and weighed for calculating their liver and oil yield. Dissected livers were placed in polyethylene bags and frozen at −20 °C for their transportation in coolers to Hermosillo, Sonora, Mexico, to the Seafood Laboratory at CIAD. Elapsed time, from their capture to final destination, was not more than 15 days. At the Seafood Laboratory, livers were left at −80 °C prior to oil extraction. For this procedure, livers were previously thawed and homogenised for 2 min, using a 14-507-7M cutter (Fisher Scientific, Pittsburgh, PA). For oil extraction, the procedure described by Kang, Timmins, and Ackman (1998) was used with a small modification. Homogenised liver was heated at 45 °C for 1 h and centrifuged at 11,300×*g* for 20 min at room temperature in a model J2-21 Beckman centrifuge (Fullerton, CA). Fifty millilitres of liver oil from each individual fish (for both ray species) were pooled, constituting the sample from which physicochemical characterisation was conducted. For the oil storage stability, the sample was placed in a 40 ml polypropylene tube covered with aluminium foil and stored for 87 days at 25 °C. The oxidation process of stored samples was monitored by measuring peroxide value, free fatty acids, anisidine value, conjugated dienes, EPA and DHA contents of each oil species.

2.2. Characterisation of liver oil

2.2.1. Physical characteristics

Specific gravity (AOAC method, No. 920.213, 1995), saponification index and water content (AOCS, 1998; Cd 3-25 and Ca 2c-25, respectively) were measured for the oil of each species.

2.2.2. Chemical characteristics

2.2.2.1. Fatty acids content. Fatty acids were derivatized to their correspondent methyl esters using 7% BF_3–MeOH, (method Ce 2-66; AOCS, 1998). Identification and quantification of fatty acid methyl esters (FAME) were achieved by capillary gas chromatography in a Varian 3800 gas chromatographer (Varian Inc., Walnut Creek, CA) fitted with a 30 m × 0.25 mm i.d. CP-Wax 52CB capillary column (Varian Inc., Walnut Creek, CA, USA) and equipped with a flame ionisation detector. Initial oven temperature was 140 °C. After 1 min it was raised to 190 °C with a rate of 9 °C/min; then it remained constant for 1.5 min; again it was raised to 230 °C with a rate of 3 °C/min and finally remained constant for 2 min. Individual components were identified by comparing retention times with those obtained from the FAME mixture standard (Supelco-Sigma cat. No. 4-7885, Aldrich Química, Mexico). Heptadecanoic acid (C17:0) was used as an internal standard.

2.2.2.2. Carotenes and tocopherols. The total carotene analysis in the oil was carried out according to Simpson and Haard (1985). The oil samples (10 ml) were dissolved in petroleum ether and the mixture absorbance at 468 nm recorded using a UV–visible spectrophotometer Perkin Elmer Lambda 2S (Wellesley, MA). The tocopherol determination was carried out according to Medina-Juárez, Gámez-Meza, Ortega-García, Noriega-Rodríguez, and Angulo-Guerrero (2000) in a Varian 9050 HPLC chromatograph (Walnut Creek, CA), equipped with a Lychrosorb column Si 60 (25 cm × 4 mm × 5 μm) (Supelco-Sigma, Aldrich Química, Mexico) and a Varian 3400 ultraviolet light detector (Walnut Creek, CA). Readings were measured at a wavelength of 292 nm. The mobile phase used for the analysis was a mixture of hexane/isopropanol, 99.5:0.5 (v/v), at a flow rate of 1.6 ml/min. Before use, the mobile phase was filtered through a 0.45 μm filter. Two grams of oil were diluted in 25 ml of hexane and filtered at low pressure through a 0.45 μm nylon Whatman membrane filter. The samples (10 μl) were injected in triplicate into the HPLC. The chromatographic peaks were identified and quantified by the comparison of the retention times and the areas of standards of α-tocopherol, γ-tocopherol and δ-tocopherol (Sigma, Aldrich Química, Mexico).

2.2.2.3. Accelerated oxidation test (Rancimat). For the Rancimat test, 3.00 ± 0.05 g of oil were placed in a model 679 Rancimat apparatus (MetrohmAG, Herisau, Switzerland). An air flux (20 l/h) was bubbled through the oil heated at 90 °C. The induction period was automatically determined by the equipment (Navarro-García, Bringas-Alvarado, et al., 2004).

2.3. Monitoring the oxidation process during oil storage

Free fatty acids, peroxide value (PV), anisidine value (AV) and conjugated dienes (CD) of oil samples, stored for 87 days at 25 °C, were determined according to AOCS (1998) (Ca 5a-40, Cd 8b-90, Cd 18-90, Ti 1a-64 methods, respectively).

2.4. Statistical analysis

Descriptive statistics (means ± standard deviation) were calculated from two sampling trips. A *t*-test analysis was carried out for physicochemical characteristics and fatty acids composition. For the oil storage stability study, a one-way analysis of variance was carried out in order to identify mean differences for species. When differences were found, Duncan's multiple test analysis was conducted. Linear regression analyses were performed for peroxide value whilst a 2nd-order polynomic regression analysis for peroxide value changes was used to observe the behaviour of this parameter during the storage time. A linear regression was performed in order to evaluate the relationship between peroxide value and DHA concentration. All analyses were performed using an $\alpha = 5\%$ and a Statgraphic Extra V 3.3″ package for Windows.

3. Results and discussion

3.1. Physical and chemical characteristics of the liver oils

Table 1 shows the physical and chemical characteristics of the liver oil extracted from the two species in the present study. As expected, similar specific gravity values for both oil species were detected. Results agree with values found for *Clupea harengus* oil (Adeniyi, 2006) and for liver oil of *Hilsa ilisha* (Salam, Motahar Hossain, Khurshid, Pervin, & Absar, 2005). Water contents of oils were very low (<0.1%) (Table 1); however, values fell within the established limits for crude fish oil (1%) (Bimbo, 1989). Rajiforms from the Campeche fishing area showed higher saponification indices than did the ones from the Gulf of California area (Navarro-García, Bringas-Alvarado, et al., 2004; Navarro-García, Pacheco-Aguilar, et al., 2004). According to Hazra, Ghosh, Banerjee, and Mukherjee (1998), there is a relationship between saponification index and short chain saturated fatty acids content, as palmitic acid. In the present study, the high saponification values found for *R. bonasus* and *A. narinari* were in agreement with the high palmitic acid (16:0) levels found for both species.

Table 1
Physical and chemical properties of the liver oils from *Rhinoptera bonasus* and *Aetobatus narinari*.

Species	Specific gravity[A] ($g\ cm^{-3}$)	Water Content (%)	Induction period[B] (h)	Saponification index (per g of oil)	Carotenes (mg/100 mg of oil)	Tocopherols (mg/100 mg of oil)
Rhinoptera bonasus	0.9391 ± 0.00[a]	0.07 ± 0.0[a]	8.3 ± 0.0[b]	219 ± 4.5[a]	0.9 ± 0.0[a]	48.7 ± 0.0[b]
Aetobatus narinari	0.9227 ± 0.00[b]	0.03 ± 0.0[b]	24.5 ± 1.3[a]	221 ± 0.2[a]	0.6 ± 0.0[b]	69.1 ± 0.0[a]

Values with the same letters in each column are statistically equal ($p \geqslant 0.05$).
[A] Density was measured at 25 °C. Data are the means ± standard deviation from two reps.
[B] Rancimat test.

As expected, both oils showed the presence of carotenes and tocopherols (Table 1); however, tocopherol concentrations were higher than those in the oil of rajiforms from the Gulf of California area (Navarro-García, Bringas-Alvarado, et al., 2004; Navarro-García, Pacheco-Aguilar, et al., 2004). Tocopherol is found in the liver, mainly as an α-isomer (Parazo, Lall, Castell, & Ackman, 1998), a reason why the rest of the isomers were not found in the oils. Besides, Cowey et al. (1981) reported that α-tocopherol can be stored in the mitochondria of fish hepatic tissue, and used according to metabolic requirements.

Oxidation was measured by the Rancimat test. This measures the induction period, which is the time needed to decompose the hydroperoxides produced during the accelerated oxidation of the oil (Läublii & Bruttel, 1986). This test showed that *A. narinari* oil was three times more stable than was *R. bonasus* oil (Table 1) which can be explained by the fact that *A. narinari* oil had a lower EPA + DHA content and higher α-tocopherol content than had *R. bonasus* oil. Nevertheless, *R. bonasus* oil showed a higher induction period (8.3 h) than that reported for *R. steindechneri* ray (3.4 h) by Navarro-García, Bringas-Alvarado, et al. (2004). Although the liver oils from both species (*R. steindechneri* and *R. bonasus*) had similar EPA + DHA contents, their α-tocopherol content varied to a great extent with *R. bonasus* having almost six times higher concentration than that reported for *R. steindechneri* (48.7 vs. 8.3 mg/100 g of oil, respectively), which can explain this oxidation stability difference in these oils.

Major fatty acids found in the oil of both species showed that six out of the 14 fatty acids were statistically different (Fig. 1). This analysis showed that the major fatty acid found in both oils (from *R. bonasus* and *A. narinari*) was palmitic acid (16:0) (Fig. 1). This result is comparable with others found for liver oils from different rajiforms (Navarro-García, Bringas-Alvarado, et al., 2004; Navarro-García, Pacheco-Aguilar, et al., 2004; Pal et al., 1998; Val Ould El Kebir et al., 2003) and sharks (Bakes & Nichols, 1995; Deprez, Volkman, & Davenport, 1990; Jayasinghe, Gotho, Tokairin, Ehara, & Wada, 2003; Kang et al., 1998; Navarro-Garcia, Pacheco-Aguilar, Vallejo-Cordova, Ramirez-Suarez, & Bolaños, 2000; Nichols, Bakes, & Elliott, 1998). *R. bonasus* showed a higher total unsaturation level than did *A. narinari* (Fig. 1). One special characteristic of *R. bonasus* oil is that it practically doubled the EPA + DHA concentration of *A. narinari* oil (Fig. 1), thus showing better nutritional quality.

3.2. Storage stability of the liver oils

The storage stability of each oil showed no significant differences ($p \geqslant 0.05$) in free fatty acids (FFAs) content throughout the storage, fluctuating from 1.47 to 1.73 mg of KOH/g of oil for *R. bonasus* and 0.59 to 0.76 mg of KOH/g of oil for *A. narinari* (Table 2). However, values were lower than the 7–8 mg of KOH/g of oil, which is the recommended limit for human consumption (Bimbo, 1998). This low level of FFA is very convenient if it is going to be used for human consumption since FFAs are easier to oxidise than

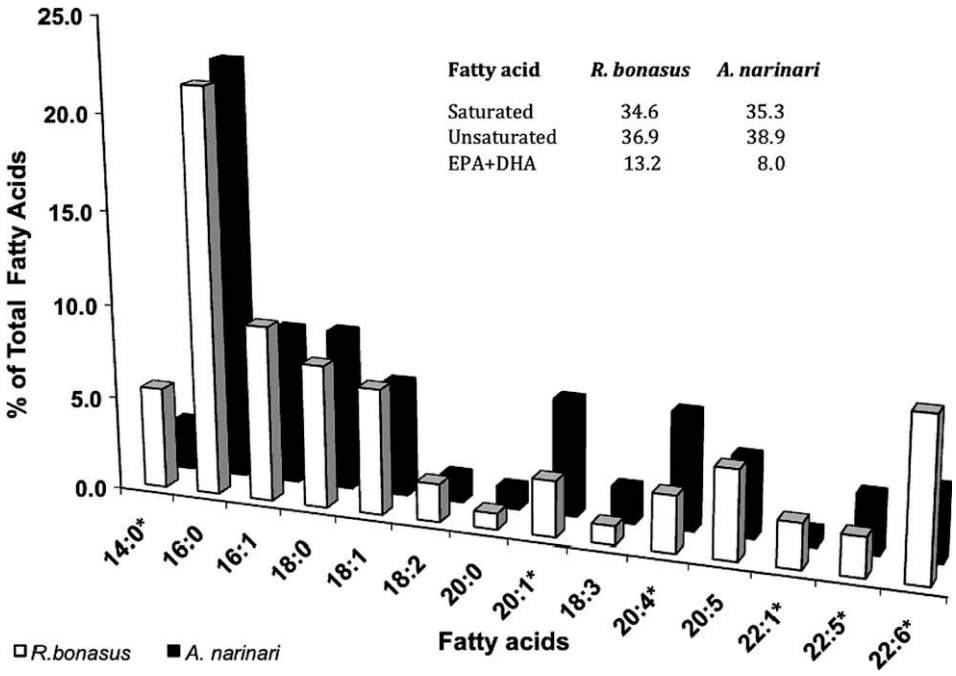

Fig. 1. Fatty acids compositions of liver oil from *Rhinoptera bonasus* and *Aetobatus narinari*.

Table 2
Quality changes in liver oils from *Rhinoptera bonasus* and *Aetobatus narinari* during storage at 25 °C.

Days	Peroxide value (meq O_2/kg of oil)		Free fatty acids (mg KOH/g of oil)		Conjugated dienes (specific extinction at 233 nm)		Anisidine value (mmol/kg)		DHA (% of total fatty acids)	
	R. bonasus	*A. narinari*	*R. bonasus*	*A. narinari*	*R. bonasus*	*A. narinari*	*R. bonasus*	*A. narinari*	*R. bonasus*	*A. narinari*
0	n.d.	n.d.	n.d.	n.d.	2.98^{c}	3.24^{c}	n.d.	n.d.	11.1^{a}	3.75^{a}
16	1.17^{i}	n.d.	1.48^{d}	0.64^{de}	3.28^{cb}	3.51^{c}	n.d.	n.d.	11.2^{a}	3.70^{a}
31	3.93^{h}	n.d.	1.58^{c}	0.68^{bcd}	3.60^{b}	3.86^{b}	n.d.	n.d.	10.4^{b}	3.50^{bc}
38	5.34^{g}	1.17^{e}	1.67^{b}	0.68^{bcd}	3.62^{b}	3.43^{c}	n.d.	n.d.	10.5^{b}	3.48^{bc}
45	8.56^{e}	2.17^{e}	1.69^{ab}	0.66^{de}	4.73^{a}	4.17^{b}	n.d.	n.d.	10.4^{b}	3.30^{c}
52	7.07^{e}	3.80^{d}	1.73^{a}	0.74^{ab}	4.64^{a}	4.96^{a}	n.d.	n.d.	9.80^{bc}	3.28^{c}
59	10.90^{c}	7.41^{c}	1.49^{d}	0.59^{e}	4.73^{a}	5.05^{a}	19.4^{b}	3.79^{d}	9.60^{cd}	3.00^{a}
66	12.95^{b}	9.05^{b}	1.51^{d}	0.67^{cd}	4.85^{a}	5.09^{a}	16.8^{d}	4.45^{d}	9.01^{d}	3.06^{d}
73	15.25^{a}	13.26^{a}	1.60^{c}	0.66^{cd}	4.94^{a}	5.23^{a}	20.5^{a}	4.56^{c}	8.31^{e}	2.69^{ef}
80	10.32^{cd}	13.46^{a}	1.59^{c}	0.73^{abc}	4.34^{a}	5.14^{a}	18.1^{c}	6.78^{b}	7.90^{ef}	2.55^{e}
87	9.39^{de}	13.97^{a}	1.47^{d}	0.76^{a}	4.85^{a}	5.28^{a}	17.3^{d}	9.33^{a}	7.37^{f}	2.38^{f}

Values with the same letters in each column are statistically equal ($p \geqslant 0.05$). n.d., non-detected.

are the esterified ones. Aidos, Lourenço, van der Padt, Luten, and Boom (2002) reported that oil extraction temperature can have a denaturing effect in the oil lipases and phospholipases, thus inhibiting the hydrolytic process, as could have happened in the present study.

When the unsaturated fatty acids are oxidised, conjugated fatty acids can be produced. These compounds can absorb UV light at 232–234 nm (Kulås & Ackman, 2001). As expected, an increase in these primary products of oxidation during storage was found ($p < 0.05$) for both oils (Table 2). However, although significant differences were found amongst storage days, these values are still low. These last results agree with the peroxide values found for both oils which never surpassed the 15.3 and 14.0 meq O_2/kg of oil for *R. bonasus* and *A. narinari*, respectively. Nonetheless, the acceptability limit for crude oil is 10–20 meq O_2/kg of oil (Huss, 1998) which, in the present study, was reached at days 52 and 66 for *R. bonasus* and *A. narinari*, respectively. Fig. 2 shows the polynomial relationship between PV and storage days for both oils tested. The polynomial regression analysis reflects the natural behaviour of these primary oxidation products, reaching a maximum and then a decrease, especially for *R. bonasus* oil. This oil, being more polyunsaturated (see Fig. 1) and presenting a lower tocopherol content (Table 1), was less stable to oxidation throughout the storage, giving a PV at earlier testing days (day 16 against day 38 for *A. narinari* oil, Table 2). The highest PV for *R. bonasus* was reached at day 73. Thereafter, PV decreased due to the decomposition of the primary oxidation products. In contrast, and probably due to the lower unsaturation and/or higher stability, the *A. narinari* oil did not reach the maximum peroxide value until the end of the storage period.

Secondary oxidation products generated by hydroperoxide decomposition were measured by the anisidine value technique. For both oils, it was only after day 59 when anisidine values became detectable (Table 2), which is in agreement with the results found for PV (primary products of oxidation). A greater increase in this value was noticed for *R. bonasus* oil, which agrees with its greater susceptibility to oxidation, due to its high EPA + DHA and low α-tocopherol contents.

The polyene index is a measure of the oxidation degree in oil that has been used by several authors (Bragadóttir, Pálmadóttir, & Kristbergsson, 2002; Márquez-Ruiz, Velasco, & Dobarganes, 2000; Rodríguez et al., 2005). This index relates the EPA and DHA contents to palmitic acid. In our study, no significant difference was found for this index (data not shown). However, DHA content decreased significantly ($p < 0.05$) throughout the storage time for both species (Table 2). DHA decrease resulted as a consequence of its oxidation, as shown in Figs. 3 and 4, which reveals the linear relationship given by the DHA content and the primary products of oxidation (PV). Both rajiform liver oils showed an inverse relationship between PV and DHA, with high coefficients of determination (R^2). Although *A. narinari* oil started its oxidation at later storage stages (Table 2), once it started, its rate was higher than that of

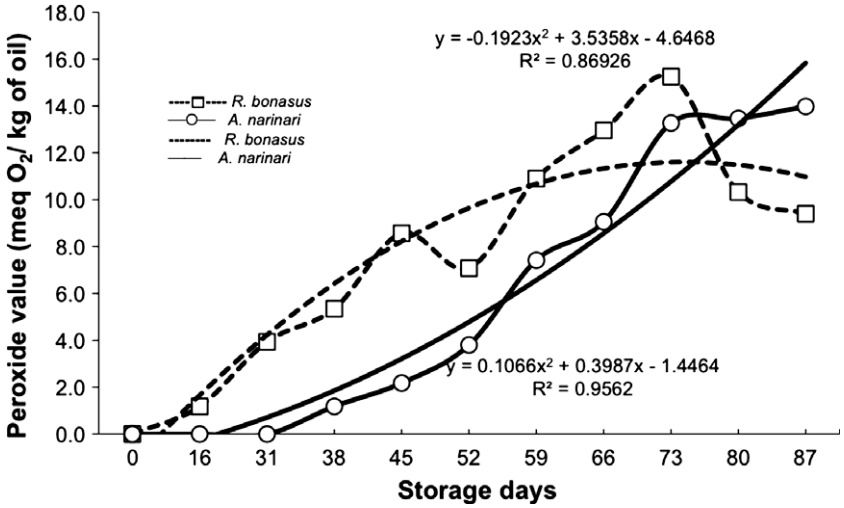

Fig. 2. Peroxide value changes in liver oils from *Rhinoptera bonasus* and *Aetobatus narinari* through storage time at 25 °C.

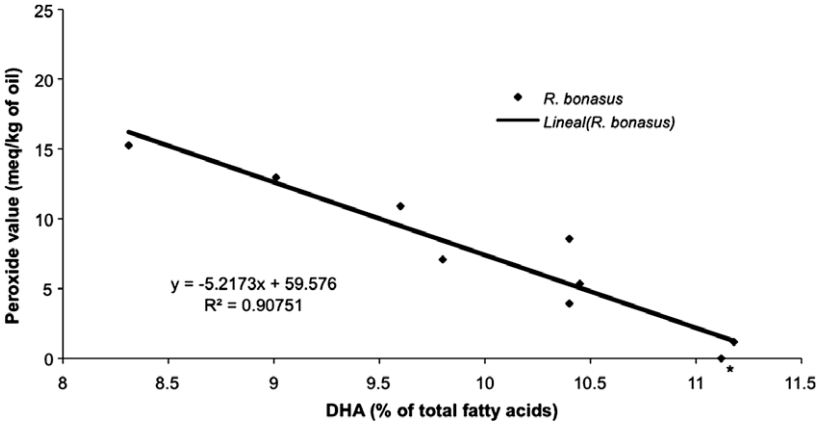

Fig. 3. Relationship between peroxide value and DHA content for liver oil of *Rhinoptera bonasus* during the storage (days 0–73). *Day 0.

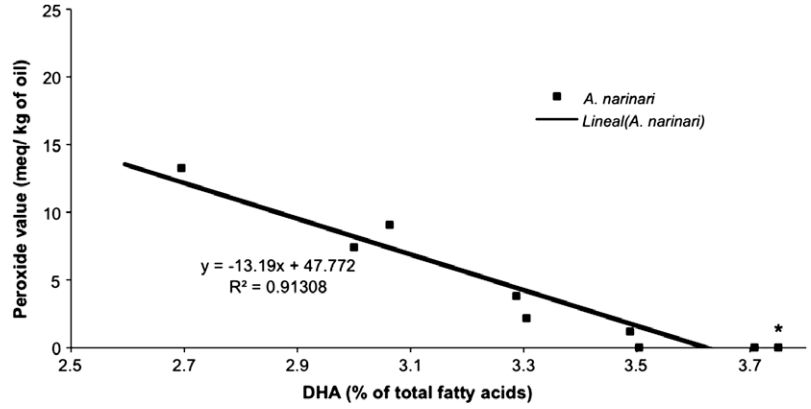

Fig. 4. Relationship between peroxide value and DHA content for liver oil of *Aetobatus narinari* during the storage (days 0–73). *Day 0.

R. bonasus oil. These results can be corroborated when comparing their slopes (−13.2 vs. −5.2, respectively) given in the respective equations in Figs. 3 and 4.

4. Conclusions

Results in the present study showed that liver oil from *A. narinari* had higher oxidation stability than had *R. bonasus*, due to its lower omega-3 polyunsaturated fatty acids and higher α-tocopherol contents. Analyses adequately described the lipid oxidation changes produced in both oils during their storage, which resulted in good oil quality estimators. Finally, and based on the suggested acceptability limits for the studied chemical indices, it can be concluded that *R. bonasus* and *A. narinari* oils lasted, with good quality, for 52 and 66 days, respectively.

Acknowledgements

Authors wish to thank Salomón Ortega Hernández, Sonia América Arellano Cahuich (owners of "Pescados y Mariscos, S.A.") for their extraordinary support for the samplings in the present study and also Manuel Huchín Maturel (from Centro Regional de Investigaciones Pesqueras de Lerma) for his support in Campeche. This study was part of the Project "Aprovechamiento del hígado de raya para la obtención de aceite rico en ácidos grasos poliinsaturados omega-3" (#CAMP-2005-C01-058) supported by the Fondo Mixto del Consejo Nacional de Ciencias y Tecnología (CONACyT)–Gobierno del Estado de Campeche.

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