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Analysis of the thermal and physicochemical properties of unsaturated fatty acid concentrates from cobia (*Rachycentron canadum*) and Argentine croaker (*Umbrina canosai*) waste

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SUMMARY: Several studies have been carried out to obtain unsaturated fatty acid (UFA) concentrates, due to their nutritional importance in food applications. The aim of this work was to obtain UFA concentrates from bleached cobia (*Rachycentron canadum*) and Argentine croaker (*Umbine canosai*) oil by complexation with urea, and to evaluate their physicochemical and thermal properties during processing. The fatty acids found in high amounts in the crude and bleached oils of cobia and Argentine crocker were palmitic, oleic and linoleic acids. Higher percentages of UFA were present in the oils extracted from the visceras, around 69 and 63% for cobia and Argentine croaker, respectively, and after complexation with urea, the percentages of UFA present in both concentrates were around 88%. Through the thermograms it was possible to observe that the UFA concentrates showed a 50% decrease in their maximum degradation temperature.

KEYWORDS: *Fish oil; UFA; Urea complexation*

RESUMEN: ***Análisis de las propiedades térmicas y fisicoquímicas de concentrados de ácidos grasos insaturados de residuos de cobia (*** **Rachycentron canadum*****) y corvina argentina*** **(Umbrina canosai)**. Se han realizado varios estudios para obtener concentrados de ácidos grasos insaturados (UFA), debido a su importancia nutricional para su posterior aplicación en alimentos. El objetivo de este trabajo fue obtener concentrados de UFA de aceite de cobia decolorado (*Rachycentron canadum*) y de corvina argentina (*Umbine canosai*) por complejación con urea, y evaluar sus propiedades fisicoquímicas y térmicas durante el procesamiento. Los ácidos grasos que se encontraron en mayores cantidades en los aceites crudos y decolorados de cobia y corvina argentina fueron palmítico, oleico y linoleico. Los porcentajes más altos de UFA estaban presentes en los aceites extraídos de las visceras, alrededor del 69% y 63% para la cobia y la corvina argentina, respectivamente, y después de la formación de complejos con urea, los porcentajes de UFA presentes en ambos concentrados fueron alrededor del 88%. A través de los termogramas se pudo observar que los concentrados de UFA tuvieron una disminución del 50% de su temperatura máxima de degradación.

PALABRAS CLAVE: *Aceite de pescado; Complejación con urea; UFA*

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1. INTRODUCTION

Food supplements produced from unsaturated fatty acids (MUFA and PUFA) have aroused the interest of consumers who are searching for ways to lead a healthier life. Studies have reported methods to obtain unsaturated fatty acid concentrates, UFA (Chakraborty *et al.*, 2009), and their incorporation into elaborated foods has increased. Fish oil is an important source of unsaturated fatty acids (UFA), and it is used as raw material for the preparation of UFA concentrates (Crexi *et al.*, 2012). The consumption of these fatty acids has shown effects on child growth, reducing risks of cardiovascular disease, and inflammations, and Alzheimer's disease, among other benefits to human health. (Wanasundara and Shahidi, 1999; Ghasemian *et al.*, 2015).

The per capita consumption of fish has increased greatly in recent decades. According to a study conducted by the Food and Agriculture Organization (FAO/UN), the world's fish consumption reached 20 kilograms per capita per year, up from 12 kilograms recommended by the World Health Organization (FAO, 2016). The residues generated in the fishery industry represent about 60% of the raw material, and are composed of the head, spine, scales and fish viscera. The use of these wastes is economically important for the fishery industry for obtaining fishmeal andto minimize the problems of environmental pollution (Cretton *et al.*, 2016). Due to the extension of its water resources, Brazil is considered one of the countries with the greatest potential for aquaculture expansion. Among the various segments of aquaculture, marine fish farming is the one that has seen the greatest growth worldwide. The cobia (*Rachycentron canadum*) presents high nutritional and medicinal value due to its balanced composition of essential amino acids and its richness in unsaturated fatty acids. Cobia fishing is relatively limited in Brazil because it is a fish that does not form shoals. However, cobia can be easily raised in captivity because it presents rapid growth and resistance, in addition to being a high-quality product. The Argentine croaker (*Umbrina canosai*) is a demersal species of the most abundant and heavily exploited in the southern Brazil continental shelf, from Rio de Janeiro to the province of Buenos Aires, Argentina. This species is considered an important fishing resource which is exploited mainly in southern Brazil, accounting for about 20% of the total fish production in the State of Rio Grande do Sul (Liao; Leaño, 2007; Taheri *et al.*, 2012; Militelli *el al.*, 2012). The increase in fish production and consumption is directly linked to the need to provide feasible technologies for the reuse of waste generated by the fishery industry. In this way, there is potential to produce oil from cobia and argentine croaker processing waste. The use of these residues presents an alternative for treating waste and preventing environmental contamination.

Several studies have been developed to determine the best conditions and methods for obtaining fatty acid concentrates, including complexing with urea, complexing with enzymes, high performance liquid chromatography, and supercritical fluid extraction. Coelho *et al.*, (2013) performed vegetable oil complexation by an enzymatic method, obtaining a yield of around 84%. Among these methods, urea complexation is a favored process due to its high separation capacity and simple manipulations (Liang *et al.*, 2018; Paim *et al.*, 2012). This separation method, also called the inclusion method, is based on separation by the degree of instauration, where the most unsaturated acids are less complex with urea (Carvalho *et al.*, 2003, Crexi *et al.*, 2012). The aim of this work was to obtain UFA concentrates from bleached cobia (*Rachycentron canadum*) and Argentine croaker (*Umbine canosai*) oil by complexation with urea, and to evaluate their physicochemical and thermal properties during the processing.

2. MATERIALS AND METHODS

2.1. Raw materials

The raw materials used in the experiments were viscera of cobia (*Rachycentron canadum*), acquired from the Aquaculture Marine Station of the Federal University of Rio Grande (FURG), and Argentine croaker (*Umbrina canosai*), which was obtained from a fishery processing plant located in the city of Rio Grande, Brazil. The fish viscera samples were immediately refrigerated and transported in coolers to the Industrial Technology Laboratory of the Federal University of Rio Grande, and stored in a freezer at −18 °C.

2.2. Experimental procedure

2.2.1. Obtainment of crude fish oil

The crude fish oil was obtained from the thermomechanical process using the conditions described by Crexi *et al.*, (2010), which include the steps of cooking, pressing and centrifugation. The extraction yield was expressed in percentage of recovered oil in relation to the oil present in the viscera of cobia and of Argentine croaker, according to Equation 1:

$$\%Yield = \frac{W_{BO}}{W_{BOD}} \tag{1}$$

where W_{BO} is the mass of recovered crude oil in the thermomechanical process (g) and W_{BOD} is the mass of oil extracted by the Bligh and Dyer (1959) method.

2.2.2. Obtainment of the bleached oil and free fatty acids

The crude oil refinement was conducted according to the methodology described by Crexi *et al*., (2010), following the degumming, neutralization, washing and bleaching steps. The free fatty acids were obtained from the bleached oil of cobia and argentine croaker through the chemical hydrolysis reaction, using the conditions described by Crexi *et al*., (2012). The separation process of the free fatty acids was performed according to the methodology proposed by Wanasundara and Shahidi (1999).

2.2.3. Obtainment of polyunsaturated fatty acid concentrates

The UFA concentrates were obtained by complexation with urea according to the methodology of Paim *et al*., (2012), under conditions of fatty acid:urea ratio of 6:1 and crystallization temperature of −12 °C for a time of 14 h. A thermostated bath (Quimis, Q-304-264, Brazil) was used to obtain the UFA with a mixture of alcohol and water to achieve the desired crystallization temperature. In complexing with urea, the mixture of the fatty acids and the urea solution in 95% aqueous ethanol was heated between 60 and 70 °C under vacuum and stirring until a homogeneous and clear mixture was obtained, then crystallization was performed.

The separation of the formed crystals (complex fraction containing saturated fatty acids) from the liquid fraction (non-complexed fraction containing the UFA) was performed by vacuum filtration. The liquid fraction was diluted with equal volume of distilled water and acidified to pH 4–5 with 6 M HCl, after an equal volume of hexane was added and the mixture was stirred for 1 h, then transferred to a separatory funnel. The hexane layer was washed with distilled water, and the wash water was separated and discarded. An anhydrous sodium sulfate was added, and the remaining solvent was removed in a rotary evaporator (Heidolph, Laborota 4000, Germany) at 40 °C. The non-complexed fraction was weighed and separated, and the recovery percentage was calculated according to Wanasundara and Shahidi (1999).

2.3. Analytical analysis

The viscera of cobia and argentine croaker were characterized for moisture content (method 925.10), ash content (method 945.46) and protein content (method 960.52), according to the AOAC (1995), and for the determination of lipid contents the method of Bligh and Dyer (1959) was used.

The raw and bleached oils of cobia and Argentine croaker were characterized in relation to free fatty acids (FFA) (Ca 5a - 40), iodine value (IV) (Cd 1-25), peroxide value (PV) (Cd 8-53), saponification value (SV) (Cd 36-76), refractive index (RI) (7-25 cc) and density (CC 10a-25) according to AOCS (2017) methods. The determination of TBA (thiobarbituric acid) was carried out according to the methodology described by Vyncke (1970). The oil color was determined using the Lovibond method (Lovibond Color Staler Tintometer, F model, United Kingdom), according to methodology proposed by Windsor and Barlow (1984).

To identify the functional groups present in the crude oil, bleached oil and UFA concentrates, the attenuated total reflectance (FT-IR-ATR) infrared (Prestige 21, 210045, Japan) was used. The samples were subjected to spectroscopic determination in the infrared region (600–4000 cm^{-1}) with a resolution of 4 cm^{-1}. The thermogravimetric curves (TGA) were obtained in a thermobalance (Shimadzu TGA-60, Japan), using a nitrogen flow of 50 $mL\cdot min^{-1}$ and heating rate of 10 $°C\cdot min^{-1}$.

The fatty acid profiles of crude oil, bleached oil and UFA concentrates of cobia and argentine croaker were determined by gas chromatography. The preparation of samples was carried out by AOAC (2002) methodology. This method allows injection of the samples on the device in the methyl ester form. The fatty acid methyl esters were identified by gas chromatography (GC) in a chromatograph (Agilent, 6890N GC, USA) fitted with a fused silica capillary column DB-23 (30 m × 0.32 mm ID × 0.25 mM). The analysis of the methyl esters of fatty acids was performed in duplicate by injecting 1.0 µL reason SPLIT 1: 200. Chromatograph conditions were: injector temperature of 225 °C; flame ionization detector temperature of 285 °C. Helium was used as the carrier gas at a flow rate of 0.75 $mL\cdot min^{-1}$ with linear velocity of 18 $cm\cdot s^{-1}$. The initial column temperature was 100 °C, which was increased to 240 °C at 3 °C min^{-1}. The software used for the quantification of the fatty acids of the samples was Chemstation.

The characteristics of the crude and bleached oils, as well as the fatty acid profiles of the non-complexed fractions (UFA) from cobia and Argentine croaker were compared by analysis of variance and Tukey test at the 95% confidence level ($p < 0.05$) (Box *et al*., 2005), using the Statistic 7.0 software (StatSoft, USA). The assays were performed in triplicate.

3. RESULTS AND DISCUSSIONS

The centesimal composition of the cobia viscera showed values for moisture content of 63.0 ± 1.0%, ash content of 1.5 ± 0.2%, protein content of 10.1 ± 0.2% and lipid content of 24.0 ± 0.1%. The values for the chemical composition obtained in this study were similar to those found in the literature, especially regarding the lipid content. Liu *et al*., (2009) studied the cholesterol content, lipid content and fatty acid profile of different tissues of cobia, and the authors found a lipid content extracted from viscera of around 16%. For the Argentine croaker viscera, the centesimal composition was moisture content of 73.0 ± 1.0%, ash content of 2.0 ± 0.6%, protein content of 16.0 ± 1.5% and lipid 9.0 ± 0.5%. Contreras-Guzmán (1994) found values for lipid content between 1.5 and 4% for Argentine croaker, although these authors studied the whole fish, while in our study only the viscera was used, which contains structures that store fat, such as liver and gonads (Amorim, 2014).

The higher oil content in the cobia viscera was due to the fact that it is considered a fatty fish, while the Argentine croaker is considered a semi-fatty fish. Therefore, the variations in the chemical composition, especially in lipid content and fatty acids, can be explained by differences in environmental conditions (Druzian *et al.*, 2007).

The cobia and the Argentine croaker showed extraction yields of 86 and 83%, respectively. Crexi *et al.*, (2010) studied a production and refinement of oil from carp (*Cyprinus carpio*) viscera and obtained a similar result for extraction yield (85%) by the thermomechanical process. Table 1 shows the extraction yields (% in mass) and the free fatty acids (%FFA in oleic acid) obtained from the solid (complexed) and liquid fractions (non-complexed with urea).

For both fish samples, the highest yields were found for the non-complexed fraction, which was enriched in UFA. As can be observed in Table 1, the non-complexed fraction presented a % FFA higher than the complexed fraction for both samples, which could mean a higher UFA content. Similar results were found by Paim *et al.*, (2012), who studied the concentration of unsaturated fatty acids obtained from carp oil (*Cyprinus carpio*) using the urea complexation method, and found the yields (%) and FFA (% oleic acid) of the complexed fraction of 34.6% and 35.9% oleic acid, respectively, and for the non-complexed fraction of 65.4%, and 35.8% oleic acid.

3.1. Physicochemical analysis

The characteristics of raw and bleached oils obtained from cobia and Argentine croaker are shown in Table 2.

The crude oils obtained from cobia and Argentine croaker showed significant differences ($p < 0.05$) in

TABLE 1. Complexation yields and free fatty acids of the cobia and Argentine croaker oils.

Fish	Fractions	Yields (%)	FFA (% oleic acid)
Cobia (*Rachycentron canadum)*	Solid fractions (complexed)	32.0 ± 0.5^{b}	34.0 ± 0.5^{b}
	Liquid fractions (non-complexed)	68.0 ± 0.5^{a}	39.0 ± 0.6^{a}
Argentine croaker (*Umbrina canosai*)	Solid fractions (complexed)	33.0 ± 0.4^{b}	33.0 ± 0.5^{b}
	Liquid fractions (non-complexed)	67.0 ± 0.6^{a}	38.0 ± 0.5^{a}

Mean value ± standard deviation (n = 3). FFA: free fatty acids. Different lowercase letters in the same column are significantly different according to Tukey's test at 95% significance ($p < 0.05$).

TABLE 2. Characteristics of crude and bleached oils from cobia and Argentine croaker viscera

	Crude cobia oil	Crude Argentine croaker oil	Bleached cobia oil	Bleached Argentine croaker oil
FFA (% oleic acid)	1.46 ± 0.03^{b}	$2.\ 21 \pm 0.15^{a}$	0.16 ± 0.01^{c}	0.11 ± 0.03^{d}
PV (meqO_2·kg^{-1} oil)	3.06 ± 0.02^{b}	3.77 ± 0.01^{a}	2.41 ± 0.06^{c}	2.46 ± 0.03^{c}
TBA (mg malondialdehyde kg^{-1} oil)	$0.\ 675 \pm 0.015^{b}$	0.831 ± 0.010^{a}	0.444 ± 0.020^{d}	0.605 ± 0.020^{c}
Color Lovibond (20 Y) R	1.16 ± 0.01^{a}	1.06 ± 0.01^{b}	0.10 ± 0.01^{d}	0.20 ± 0.01^{c}
IV (cgI2 g^{-1})	128 ± 1^{a}	125 ± 1^{b}	128 ± 1^{a}	125 ± 1^{b}
SV (mgKOH·g^{-1})	198 ± 1^{a}	185 ± 1^{b}	198 ± 1^{a}	186 ± 1^{b}
Refractive index at 40°C	$1.\ 4649 \pm 0.0001^{a}$	$1.\ 4662 \pm 0.0001^{b}$	$1.\ 4648 \pm 0.0001^{a}$	$1.\ 4650 \pm 0.0001^{a}$
Density (g·cm^{-3})	905.7 ± 0.2^{d}	925.7 ± 0.1^{a}	912.5 ± 0.5^{c}	914.6 ± 0.5^{b}

Mean value ± standard deviation (n = 3). FFA: free fatty acids; PV: peroxide value; TBA: thiobarbituric acid; Y: yellow; R: red; IV: iodine value; SV: saponification value. Lowercase letters with different superscripts represent significant differences according to Tukey's test at 95% significance ($p < 0.05$).

the physicochemical analysis, demonstrating that the type of fish used directly influenced the characteristics of the obtained oil. The FFA of the Argentine croaker crude oil were higher compared to crude cobia oil, which can be associated with the fact that the Argentine croaker viscera presented a higher moisture content, leading to the hydrolysis reactions of the ester bonds, and consequently increasing the FFA.

In relation to the bleached oils, the process reduced the levels of FFA for both cobia and Argentine croaker oils. This reduction can be explained because the neutralization step has the main objective of reducing the FFA present in the crude oil through the formation of soap.

As can be seen in Table 2, peroxide values for the crude oils are below the accepted values for the quality and acceptability established for human consumption, which is 10 meq $O_2 \cdot kg^{-1}$ oil (FDA, 2002). This index represents the initial stage of lipid oxidation, and it is important to define the degree of deterioration of oils and fats (Boran *et al.*, 2006). Crexi *et al.*, (2010) studied the thermomechanical process to produce fishmeal from common carp (*Cyprinus carpio*) and found the peroxide value from crude oil of 3.38 meq$O_2 \cdot kg^{-1}$oil, which is similar to those obtained in our study.

Similar to peroxide value, thiobarbituric acid (TBA) is used as an indicator of the degree of lipid oxidation the decomposition of hydroperoxide and the formation of secondary oxidation products, such as aldehydes, ketones and alcohols. In this study, the amount of TBA showed significant differences ($p < 0.05$) between crude cobia oil and Argentine croaker (Table 2). The TBA value was higher for crude Argentine croaker oil than the crude cobia oil; and, the FFA also presented a higher value and can act as a precursor in the secondary oxidation reaction (Boran *et al.*, 2006).

In both bleached fish oils, it was verified that the peroxide value and the TBA value were decreased significantly ($p < 0.05$) compared to the crude oils, due to the degumming and neutralization steps which remove phospholipids, free fatty acids, impurities and other compounds (Monte *et al.*, 2015). In relation to the color, there was a reduction of 91% in the bleached cobia oil and of 81% in the bleached Argentine croaker oil. This difference can be associated with the fact that the Argentine croaker oil presented a larger amount of initial oxidation compounds, which may have interfered with the adsorbent ability of the adsorbate. In the bleaching step, the adsorbents remove pigments and other impurities such as trace metals, phospholipids and oxidation products (Pohndorf *et al.*, 2016).

The iodine value is related to the unsaturation degree of the oil, and the saponification value is related to the molecular weight of the fatty acids esterified to glycerol. Both parameters did not show significance differences ($p > 0.05$) due to the refinement steps, and only differences between the types of fish were observed, demonstrating that there is a difference in the fatty acid composition. Similarly, as the iodine value, the refractive index refers to the oil unsaturation degree, and it is widely used in identification and quality control because this parameter increases with the iodine value (Costa-Singh *et al.*, 2012).

Regarding the values obtained for the bleached oils (iodine, saponification, refractive index, and density), there were no significant differences ($p > 0.05$) compared to the crude oils because the refinement process does not alter the fatty acid composition of the triacylglycerols. Table 3 shows the fatty acid profiles of the crude and bleached oils and fatty acid concentrates obtained from cobia and Argentine croaker.

Significant differences ($p < 0.05$) were observed in relation to the contents of SFA and UFA. The SFA found in higher quantities were palmitic acid (C16:0) and stearic acid (C18:0) in both crude fish oils. On the other hand, the UFA present in higher quantities in the cobia oil were oleic acid (C18:1) and linoleic acid (C18:2), but for Argentine croaker oil, the linoleic acid was the lowest. The Argentine croaker oil showed about 2.2 time more ΣEPA + DHA than the cobia oil, which can be explained by the fact that the Argentine croaker is a cold water fish while the cobia is a warm water fish. (Greene and Selivonchick, 1987). When the crude oil was compared with the bleached oil of each fish species significant differences ($p < 0.05$) were observed in the SFA and UFA contents.

After complexation, increases in PUFA, arachidonic acid (C20:4), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) were found for both species. However, the highest increase was observed for linoleic acid (C18:2), especially in the Argentine croaker concentrates, which quadrupled its value in relation to the crude oil. In contrast, there was a strong decrease in palmitic acid (C16: 0), confirming that complexation with urea was efficient to concentrate the UFA. Moreover, it can be affirmed that the sum of SFA decreased by approximately three times from the crude oil to the concentrates, and that for both fish species, the sum of UFA was around 89% in the concentrates. Similar results were obtained in studies carried out by Crexi *et al.*, (2012) and Esquerdo *et al.*, (2017), who complexed carp oil with urea, obtaining percentages of MUFA and PUFA of 88.9 and 88.5%, respectively.

In Figure 1 the vibrational spectra (FT-IR) of the crude oil and UFA concentrates from cobia can be observed. In these spectra, it can be seen that

Table 3. Percentage of saturated and unsaturated fatty acids present in the crude, bleached and concentrates oils from cobia and Argentine croaker viscera

Fatty acids	COC	COA	BOC	BOA	NCC	NCA
C14: 0	2.89 ± 0.01[a]	3.72 ± 0.01[b]	2.82 ± 0.05[c]	3.65 ± 0.05[d]	2.84 ± 0.01[c]	3.14 ± 0.01[e]
C16: 1	8.02 ± 0.02[a]	12.60 ± 0.01[b]	7.98 ± 0.02[a]	12.94 ± 0.06[c]	9.00 ± 0.01[d]	21.01 ± 0.01[e]
C16: 0	23.11 ± 0.05[a]	26.36 ± 0.08[b]	23.16 ± 0.03[a]	25.80 ± 0.08[c]	8.88 ± 0.03[d]	7.84 ± 0.01[e]
C18: 3	1.65 ± 0.03[a]	1.30 ± 0.07[b]	1.68 ± 0.03[a]	1.46 ± 0.06[c]	2.65 ± 0.01[d]	3.29 ± 0.01[e]
C18: 2	15.85 ± 0.03[a]	3.61 ± 0.01[b]	15.80 ± 0.04[a]	3.64 ± 0.03[b]	23.41 ± 0.01[c]	13.74 ± 0.01[d]
C18: 1	31.96 ± 0.06[a]	28.11 ± 0.08[b]	31.91 ± 0.03[a]	28.22 ± 0.07[b]	34.64 ± 0.01[c]	22.58 ± 0.02[d]
C18: 0	4.24 ± 0.01[a]	6.65 ± 0.01[b]	4.20 ± 0.03[a]	6.70 ± 0.05[b]	0.85 ± 0.02[c]	0.40 ± 0.01[d]
C20: 5 (EPA)	0.16 ± 0.01[a]	0.19 ± 0.02[b]	0.15 ± 0.01[a]	0.21 ± 0.03[b]	0.17 ± 0.01[a]	0.41 ± 0.03[c]
C20: 4 (AA)	1.24 ± 0.02[a]	2.08 ± 0.02[b]	1.22 ± 0.01[a]	2.05 ± 0.03[b]	1.70 ± 0.01[c]	3.83 ± 0.01[d]
C20: 3	3.25 ± 0.01[a]	3.41 ± 0.08[b]	3.29 ± 0.03[a]	3.51 ± 0.07[b]	4.66 ± 0.03[c]	7.73 ± 0.02[d]
C20: 1	1.28 ± 0.01[a]	1.26 ± 0.03[a]	1.29 ± 0.01[a]	1.29 ± 0.03[a]	0.60 ± 0.01[b]	0.98 ± 0.01[c]
C22: 6 (DHA)	0.67 ± 0.01[a]	1.66 ± 0.01[b]	0.65 ± 0.01[a]	1.63 ± 0.04[b]	0.86 ± 0.02[c]	1.69 ± 0.02[d]
C22: 2	4.36 ± 0.01[a]	5.30 ± 0.05[b]	4.38 ± 0.01[a]	5.40 ± 0.06[b]	7.39 ± 0.01[c]	12.76 ± 0.02[d]
ΣunFA	2.00 ± 0.05[a]	3.65 ± 0.01[b]	2.00 ± 0.01[a]	4.03 ± 0.01[c]	2.35 ± 0.02[d]	2.64 ± 0.02[e]
Total (%)	100.00 ± 0.06	100.00 ± 0.02	100.00 ± 0.05	100.00 ± 0.05	100.00 ± 0.03	100.00 ± 0.01
Σ SFA	30.24 ± 0.03[a]	36.73 ± 0.05[b]	30.18 ± 0.05[a]	36.55 ± 0.08[b]	12.57 ± 0.03[c]	11.38 ± 0.04[d]
Σ(MUFA+PUFA)	69.76 ± 0.03[a]	63.27 ± 0.06[b]	69.82 ± 0.08[a]	63.45 ± 0.02[b]	87.43 ± 0.02[c]	88.62 ± 0.02[d]

Mean value ± standard deviation (n = 3). Lowercase letters with different superscripts represent significant differences according to Tukey's test at 95% significance ($p < 0.05$). ΣunFA: sum of unidentified fatty acids; ΣSFA: sum of saturated fatty acids; Σ (MUFA + PUFA): sum of the mono and polyunsaturated fatty acids; (COC: crude oil of cobia; COA: crude oil of Argentine croaker; BOC: bleached oil of cobia; BOA: bleached oil of Argentine croaker; NCC: non- complexed fraction of cobia oil; NCA: non-complexed fraction of Argentine croaker oil).

the samples showed characteristic bands for oils, such as asymmetric and symmetric axial stretches of CH_2 observed in 2926 and 2855 cm^{-1}, respectively. The band around the 1745 cm^{-1} is attributable to the axial vibration of the carbonyl group (C=O). The band at 1460 cm^{-1} refers to the angular deformation of the CH_2 and CH_3 groups. Around 1238 and 1099 cm^{-1} axial stretchings of C-O groups regarding the triacylglycerols were identified. Further, the bands referring to unsaturated fatty acids in 3008 cm^{-1} were determined, corresponding to stretching (C-H) of cis double bands (CH=CH). In 1653 cm^{-1} stretching of the C=C bond was observed and around the 750 cm^{-1} bands the angular deformations of the same *rocking* type groups -HC = CH- (cis) were observed. The main changes were identified in the non-complexed fractions (enriched with UFA) obtained from cobia oil (Figure 1c). In this spectrum an increase in all the bands related to unsaturation was observed. In addition, there was a reduction in the bands related to the C-O bonds of the triacylglycerols, along with a tendency toward the formation of a single band at around 1250 cm^{-1} which was related to the C-O bond of the fatty acid esters formed after hydrolysis. Moreover, the band at around 950 cm^{-1} is associated with the overlapping of the angular vibrations outside the plane of the groups -HC = CH- (cis and trans) (Guillén, 2000). The spectra related to the crude and bleached oils and the UFA concentrates from Argentine croaker viscera showed a similar behavior to that from cobia viscera.

3.2. Thermal characteristics

Figures 2 and 3 show the thermogravimetric and derivative thermogravimetric curves (TG and DTG) of crude oil, bleached oils and non-complexed fractions (UFA) obtained from cobia and Argentine croaker, respectively.

In both fish oils, it can be observed that the decomposition of triacylglycerols occurred in the temperature range from 220 to 500 °C (Figures 2a and 3a). According to Santos *et al*., (2004), the process begins with the decomposition of PUFA, followed by MUFA and finally SFA.

The crude and bleached oils from cobia (Figure 2b) had a maximum decomposition temperature (DTG peak) of around 410 °C, however, the crude and bleached oil from Argentine

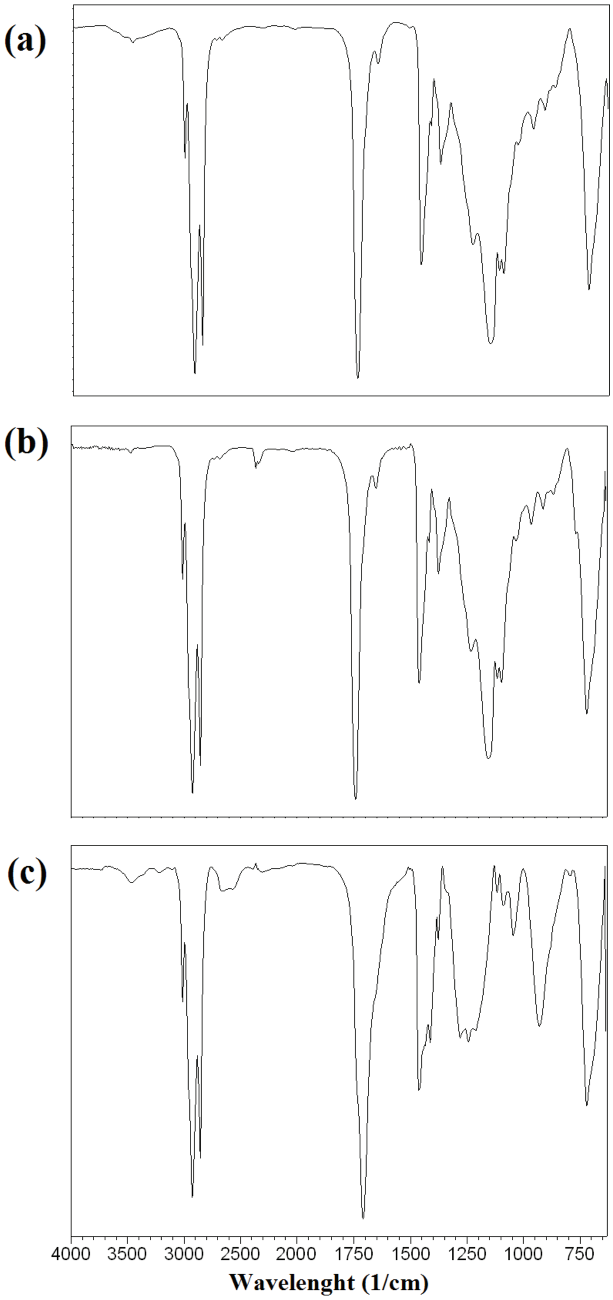

Figure 1. FTIR spectra of (a) crude cobia oil, (b) bleached cobia oil and (c) UFA concentrates from cobia viscera.

croaker (Figure 3b) had a maximum decomposition temperature of around 403 °C. Up to 300 °C, mass losses of 3.9% and 6.1%, respectively, were observed in the thermogravimetric curves for the crude oils from cobia and Argentine croaker. This may be attributed to the presence of non-glyceride components in the oil. The crude fish oil obtained through the thermomechanical process can present phospholipids, sterols, pigments and primary and secondary oxidation compounds in their compositions, as well as impurities, such as protein residues from the oil extraction, which may be removed during refinement.

The bleached oils extracted from cobia and Argentine croaker (Figures 2a and 3a) showed a tendency toward initial mass loss at around 300 °C, volatilizing entirety at 470 °C. The crude and bleached oils of Argentine croaker showed lower degradation temperatures than the cobia oils. This fact indicates that even with a lower content of UFA, the content of PUFA from Argentine croaker was higher than the cobia oil, which decreased its degradation temperature because the volatilization process was initiated by PUFA.

The maximum decomposition temperatures for bleached oils from Argentine croaker and cobia were slightly higher when compared to the values for the crude oil. This difference can be explained because the fish crude oil presents impurities, such as protein residues from the extraction, which absorb part of the energy provided to the sample, leading to a decrease in the degradation temperature (Huang and Shativel, 2008). Araújo *et al.*, (2011) observed that the refined fish oil has only one decomposition stage, with initial volatilization occurring at 220 °C and total mass loss at 484 °C. These values were similar to the values found in our study. In relation to the UFA concentrates obtained from the bleached oils of cobia and Argentine croaker, it can be observed that the maximum decomposition temperature rates (DTG-peak) were 248 and 255 °C, respectively (Figures 2b and 3b), indicating that the amounts of UFA between the samples were similar.

4. CONCLUSIONS

The crude and bleached oils from cobia and Argentine croaker presented significant differences ($p < 0.05$) in relation to free fatty acids, iodine value, saponification value, peroxide value, thiobarbituric acid value, color, refractive index and density. For the fatty acid profile, a significant difference ($p < 0.05$) was observed, with a higher percentage of UFA present in the oils extracted from cobia. The fatty acid profile of the liquid fractions showed an increase in the percentages of UFA and a reduction in the percentages of SFA. By the melting thermograms, it was possible to observe that the fatty acid concentrates had lower degradation temperatures when compared to the crude and bleached oils. This decrease in degradation temperature demonstrated that complexation with urea was efficient, since the liquid fraction was enriched in MUFA and PUFA. Based on these results, the cobia and Argentine croaker oils can be considered as rich sources of ω-3 and ω-6 series fatty acids.

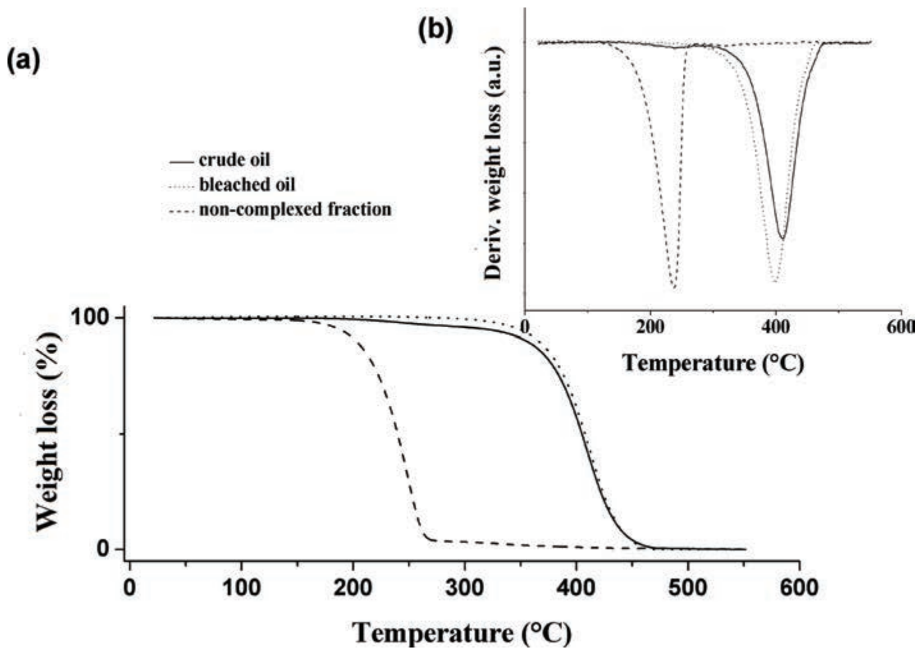

FIGURE 2. Thermogravimetric curves (TG and DTG) of crude oil, bleached oil and non-complexed fraction from cobia viscera.

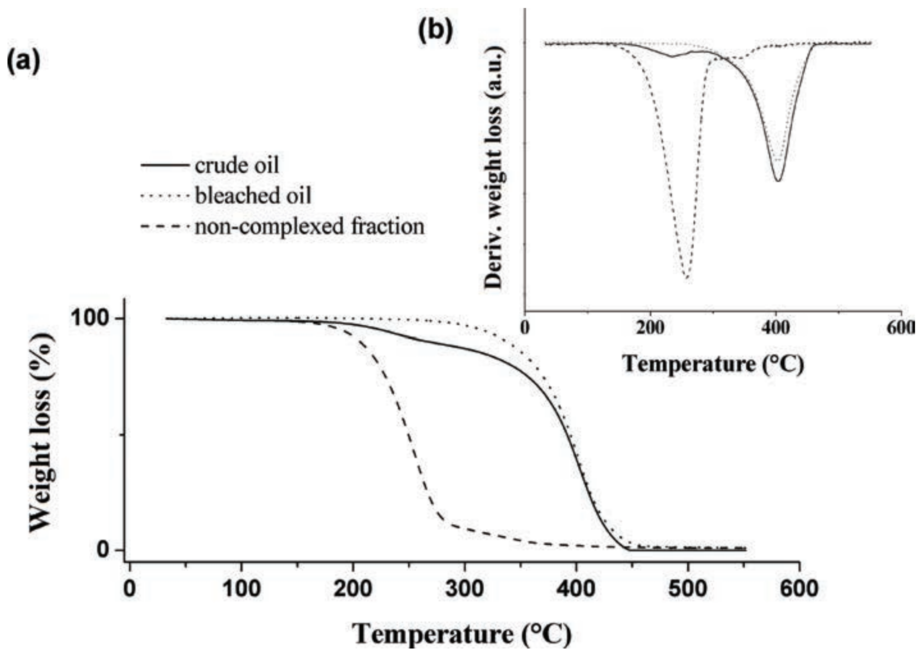

FIGURE 3. Thermogravimetric curves (TG and DTG) of crude oil, bleached oil and non-complexed fraction from Argentine croaker viscera.

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