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Research article

Quality of red tilapia viscera oil (*Oreochromis sp.)* as a function of extraction methods

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ABSTRACT

This study aimed to propose a simple and efficient heating-freezing method for oil recovery from red tilapia (Oreochromis sp.) viscera, suitable for industrial application and that does not affect its composition. Three methodologies for oil extraction were studied: a) direct heating (69 °C and 29 min) of samples followed by separation of the oil by decantation, b) direct heating with subsequent freezing and c) solvent extraction assisted by ultrasound. For the oil obtained by each methodology, the following factors were determined: peroxide and iodine values, oxidative stability index, yield percentages and fatty acid profile and, to evaluate the changes thereof, a thermal analysis by differential scanning calorimetry was performed. An oil extracted by centrifugation from fresh viscera was used as control. Results showed yields of 92,126%, 60,99% and 55,36% for the oil obtained by heating and freezing, heating and decanting and solvent extraction, respectively, the other evaluated parameters were similar among each other. The content of PUFA was not affected by heating when compared to the control oil, although a decrease was observed in the solvent extracted oil. This behavior was corroborated with the thermal analysis, which showed that the higher PUFA content, the lower the melting temperatures of the oils and the energy required for phase change. A principal component analysis allowed determining that while there are no differences in the abundance of fatty acids C20:1, 14:0, 18:0, 16:1 and C16:0, there are differences for fatty acids C18:1 and C18:2 depending on the method of extraction used in the oil obtention. The results of this study show that the heating-freezing extraction method is a good alternative for acquiring value-added products and facilitates their implementation in rural areas. Furthermore, allows obtaining a product with high content of polyunsaturated fatty acids (at least a third of the total content).

1. Introduction

Among the most consumed fish, tilapia (*Oreochromis* sp.*)* represents the fourth most important freshwater species with an approximate production of 4.2 million tons in 2016. Freshwater tilapia, catfish and carp species are projected to account for about 62% of total global aquaculture production in 2030, compared to 58% in 2016 [1]. In Colombia, red tilapia production was 41,732 tons in 2015, constituting 51.77% of the products obtained nationally by fish farming [2] and in 2017 the aquaculture production was 100,000 tons, of which 97,000 tons corresponded to freshwater fish [3].

Traditionally, tilapia is marketed whole and the post-harvest handling is limited to gill removal, gutting, and scaling [4]. As a result, large amounts of by-products are generated, which are usually discarded causing numerous environmental problems. These by-products contain considerable amounts of protein with high nutritional value in relation to essential fatty acids composition and protein content, which varies from 15 to 60% [5]. In addition, it has been found that one of the by-products of increasing interest is viscera, which represents about 15% of the weight of each individual tilapia and contains approximately 35% of oil rich in unsaturated fatty acids [6, 7]. These features explain why in the last 60 years, viscera went from being a waste to a highly valued product in the food industry [8], for which its recovery is a relevant aspect.

Different methods have been used for extracting oil from the viscera and, in general, from fish farming waste, such as: enzymatic hydrolysis [9], biological silage [10], pressing of fish waste, wet pressing [11], supercritical fluid extraction [12], solvent extraction [13] among others [14]. Most of these methods have high investment costs or long process

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times, which has prevented their implementation at small or moderate scale production systems. Moreover, the extreme temperature and pressure conditions used in some methods, and the subsequent release of the oil, can partially modify the polyunsatured fatty acids (PUFA), due to degradation reactions such as hydrolysis and oxidation that can be caused by these demanding conditions [15]. As a result, it has become evident that there is a need for methods that improve the efficiency of the extraction process, reduce costs and facilitate implementation conditions in rural areas and at different production scales.

One of the methods that has been used with fair success at different scales of production consists of heating the substrate to relatively high temperatures (around 90 °C), for a certain time, and allow it to stand to separate the fat that rises to the surface of the mixture [16]. Despite the efficiency of the process, the high temperatures used can cause degradation of unsaturated fatty acids and oxidation of the oil [11], causing losses of nutritional quality, undesirable flavors and rancidity [17].

Thus, this study aimed to propose a simple and efficient heating-freezing method based, as early step in oil recovery from red tilapia (Oreochromis sp.) viscera, whithout affecting its composition.

2. Materials and methods

2.1. Handling of raw materials

The fish were not specifically slaughtered for use in this experiment, and were instead from a commercial fish factory. In that sense, viscera of red tilapia (Oreochromis sp.) were collected at the Gaitero fish farm located in the Municipality of Sopetrán (Antioquia, Colombia) and transported in expanded polystyrene coolers with refrigerant gels to the laboratory of the Grupo de Nutrición y Tecnología de Alimentos of the Universidad de Antioquia. The samples were processed the same day to avoid their emulsification and potential difficulties with phase separation.

2.2. Characterization of viscera and lipids

The analyses in triplicate of the fresh and defatted viscera samples included the moisture determination using the official method of analysis AOAC 950.46 and a moisture balance with halogen lamp MOC63 (Shimadzu, Japan). The protein content analysis was made with the micro kjeldahl technique [18], in a DK 12 heating digester (Velp Scientifica, USA). The ethereal extract was determined according to the official solvent extraction method [18] using Starfish (Radleys, United Kingdom) soxhlet extractor equipment, with petroleum benzine as solvent. To determine the ash content, samples were calcined at 550 °C in a D8 muffle (Terrigen, Colombia) [18]. The iodine and peroxide values were analyzed according to the Colombian technical standards (NTC) 283 and 236, respectively [19].

2.3. Oil extraction methods

In each of the three extraction methods, were evaluated the yield of the extracted oil according to Eq. (1), the iodine value (Y) (g iodine absorbed/100 g of sample), the peroxide value (P) (meq-g of O2 consumed per kg of fat), the induction time (OSI) in h, the lipids content (G), (g extracted lipids/g total lipids in initial sample*100) and the lipid profile. Additionally, an oil extraction process not subjected to thermal processes, nor mixed with solvents was performed by centrifugation, and it was used as control oil (CO). The conditions used in each extraction method were defined in a prior study [16].

$$\text{Yield of the extracted oil } (\%) = \frac{RL}{TL} * 100 \quad (1)$$

Where RL is the recovered lipid content (g) and TL is the total lipid content (g).

2.3.1. Obtaining control oil (CO)

An unprocessed viscera sample (100 g) was centrifuged for 15 min/6860 *g* at 4 °C in a U-320R centrifuge (Boeco, Germany). The obtained oil was used as control for using its features as reference for comparison with those obtained with the other methods, and thus detect whether there were significant changes. The obtained oil was stored at -20 °C until analysis.

2.3.2. Oil extraction by heating and decanting (OEHD)

Viscera samples (35 g) were placed in 50 mL falcon tubes and placed in an AccuBlock dry bath model D1200 (Labnet, United States) at a temperature of 67 °C for 29 min. The samples were allowed to stand until the precipitation of the densest phase, the oil was then separated by decantation and the samples were stored at -20 °C until further analysis.

2.3.3. Oil extraction by heating and freezing (OEHF)

This extraction process used the same previously described heating conditions for the heating and decanting process. The samples were allowed to stand until the temperature dropped to 45 °C, then they were frozen at -18 °C for 24 h to obtain a better separation of the oil since the phases separate as they solidify [16]. The lipid phases were then detached from the still-frozen aqueous phases by means of a cross section and stored until analysis.

2.3.4. Oil extraction with organic solvent (OES)

This process takes advantage of the solubility of lipids in specific organic solvents and their insolubility in water, which facilitates their separation from other components with different polarity. This method is widely used for oil extraction for analytical purposes [11], and was therefore used as a reference method in this study. To obtain the oil, 600 g of viscera were triturated and homogenized for 1 min in a food processor model FP1336 (Black & Decker, United States), then divided into three containers in equal amounts (200 g) and immersed for 2 h at 20 °C in a dichloromethane-methanol mixture (2:1), in a 2:1 solvent-viscera ratio, in order to prevent oxidation of the tissues by the action of endogenous enzymes [20]. Subsequently, they were subjected to ultrasound in a Branson 5510R-DTH (Bransonic, United States) for 20 min and suction filtration was performed with gauze and Whatman No. 100 filter paper. The procedure was repeated two more times and the filtrate, collected from the three extractions, was dried in a Laborota 4001 rotary evaporator (Heidolph, Germany) at a temperature lower than 45 °C, with reduced pressure and constant agitation. The oil-water mixture was partitioned with dichloromethane and the organic phase was filtered over anhydrous Na2SO4 to remove traces of moisture from the oil. The sample was kept in the dark at -18 °C until different analyses were performed.

2.4. Oxidative stability index determination (OSI)

In this method, a constant flow of air is passed through the sample while temperature is controlled at a high level to accelerate its oxidation and reduce the time of analysis [21]. The oxidation process is driven by radical reactions involving unsaturated fatty acid structures. During an initial induction phase, almost no secondary products are formed, and it is abruptly followed by an oxidation phase characterized by a rapid increase in the peroxide value and the formation of volatile products, which, depending on the basis of the method, could correspond to formic acid [22].

OSI determination was carried out on a Rancimat 892 (Metrohm, Switzerland). Each oil sample contained in a reaction vessel was heated and air was continuously passed through at a rate of 20 L/h at 120 °C, after which the fatty acids decomposed and volatile organic acids were formed and transported by an air stream to a measuring beaker containing distilled water. A continuous measurement of electrical conductivity was performed and its increase indicated the presence of organic

acids. The elapsed time until formation of these oxidation products is known as the OSI [22]. This procedure was repeated for all samples.

2.5. Chromatographic analysis of lipid fractions, fatty acids and their derivatives

2.5.1. Thin layer chromatography analysis (TLC)

This technique was used to separate the different lipid groups present in each oil sample and to monitor the reactions during the obtention of the methyl ester and pyrrolidide derivatives of the fatty acids. For this analysis, silica gel 60 F254 was used as stationary phase, a 4:1 ratio hexane/ethyl acetate mixture as mobile phase and 5% phosphomolybdic acid in ethanol as developer, with subsequent heating. Standards of a methyl ester and linoleic acid with >99% purity (Nu-Chec Prep, Inc, USA) and cholesterol (PanReac, Germany) were used as references to determine the presence of the fractions of interest by comparison with their retardation factors (Rfs).

2.5.2. Column chromatography analysis (CC)

This technique was used to separate and purify the obtained methyl ester fractions. Silica gel 40 (0.063–0.200 mm) (Merck, Germany) was used as stationary phase and hexane/ethyl acetate (4:1) as mobile phase.

2.6. Obtaining fatty acids and their derivatives

Fatty acids were obtained by basic hydrolysis by adding a NaOH 2N solution in methanol (1 g NaOH in 12.5 mL MeOH) to each oil sample (10 mg oil/mL solution). The mixture was placed in a bain-marie at 55 °C for 15 min.

2.6.1. Preparation of methyl ester derivatives

The methyl esters were obtained by adding 5% HCl in MeOH (1 mL of solution per each 8–10 mg of sample) to the mixtures previously obtained in the basic hydrolysis. The new solutions were heated in a bain-marie at 55 °C for 15 min, concentrated at a temperature lower than 45 °C, with reduced pressure and constant agitation to eliminate the organic solvent, then liquid-liquid extraction was carried out with dichloromethane and later with hexane, adding water each time, to facilitate the separation of the phases. The obtained organic phases were mixed and filtered over anhydrous Na_2SO_4 in order to eliminate the traces of water, and the obtained fractions were concentrated again until evaporation of the solvent. The formation of the methyl ester derivatives and the separation of the components of each fraction was verified with TLC using the previously described conditions.

Following that, each fraction was purified by CC, according to the above-mentioned conditions and through a new TLC analysis, the subfractions containing the methyl esters were identified. These were unified, concentrated and then each sample was divided into two parts: one part was stored, protected from light, air and temperature, for subsequent GC-MS analysis, and the other part was used for the preparation of the pyrrolidide derivatives of the fatty acids.

2.6.2. Preparation of pyrrolidine derivatives

These derivatives were obtained by adding 1000 μL of pyrrolidine and 100 μL of glacial acetic acid to 10 mg of each of the previously prepared methyl ester subfractions. The mixtures were boiled at 100 °C under open reflux for 90 min and then washed five times using a mixture of water: dichloromethane (1:1), the organic phases were filtered over anhydrous Na_2SO_4. They were concentrated and the formation of the pyrrolidine derivatives was verified with TLC according to the conditions of section 2.5.1.

2.6.3. Fatty acid derivatives GC-MS analysis

GC-MS analysis of the methyl ester and pyrrolidine derivatives allowed identifiying the fatty acids present in each fraction. The analyses were performed on a model 6890N gas chromatograph (Agilent, USA) coupled to a model 5973N mass selective detector (Agilent, USA), on a DB-5MS column, using helium as carrier gas. The injection volume was 0.2 μL and temperatures of 175 °C and 250 °C were used for the injector and detector, respectively. The oven was programmed in three steps: it was started at a temperature of 50 °C for 1 min, then increased at a rate of 5 °C/min to 150 °C, where it remained for 3 min, and then increased at 10 °C/min to 300 °C and held for 16 min.

The mass spectra were compared with the NIST02.L and NIST5a.L databases and the information was generated with MSD ChemStation software. The relative abundance of the fatty acids was calculated with the area under the curve of each chromatographic peak of the methyl ester derivatives. Each analysis was performed in triplicate.

2.7. Thermal analysis of the oils by differential scanning calorimetry (DSC)

The analysis was conducted on a DSC Q20 V24.11 Build 124 (TA Instruments, USA), which was calibrated with indium (ΔHf = 28.71 J/g and melting point = 156.6 °C) using nitrogen as purge gas at a flow rate of 50.0 mL/min. The oil samples were weighed (6–7 mg) in aluminum dishes that were hermetically sealed. An empty aluminum dish was used as a reference.

The heat treatment of the oils was carried out by heating from -80 °C to 200 °C with a ramp of 10 °C/min, in nitrogen atmosphere. Heat flux data (W/g) were recorded as a function of temperature, from which the melting profile, enthalpy of fusion and the onset and offset temperatures of the phase changes were obtained. The enthalpies were calculated by integrating the melting curves with the universal V4.5A software (TA Instruments®, United States).

2.8. Statistical analysis

A principal component analysis was performed with the abundance of the fatty acids identified in the oils obtained by different extraction methods (CO, OEHF and OES). The free software PAST 3.0 was used for the analyses. The difference in means was made using Fisher's LSD (Least significant difference) test with statgraphics centurion XVI software.

3. Results and discussion

3.1. Substrate characterization

Table 1 shows the physiochemical characterization of the red tilapia (*Oreochromis* sp.) viscera. A lipid percentage of 33.602% can be observed, which is greater than that reported for rainbow trout (*Oncorhynchus mykiss*), of 13% [23], and for red tilapia, of 15.75% [24]. This variation on the results for the same species may be caused by factors such as sex, size, diet, location, temperature and season [25]. As per this study, such increment is favorable due to the interest in lipid content.

3.2. Physiochemical paramenters of the oil obtained by different extractions methods

Table 2 shows the analysis results of the oil obtained by each of the extraction methods (CO, OEHD, OEHF and OES). It is evidenced that in

Table 1. Physicochemical composition of red tilapia (*Oreochromis* sp.) viscera.

Component	Viscera (% ± SD)[a]
Moisture	62.69 ± 1.9
Protein	4.57 ± 0.23
Lipid	33.06 ± 1.6
Ash	0.73 ± 0.04

[a] correspond to average percentage ±standard deviation (SD) of triplicate viscera samples.

all treatments the peroxide value (P) is above the values obtained for the control oil, but much below the maximum values allowed for oils (up to 10 meq of active oxygen/kg of oil) [26]. The iodine value (Y) showed that all treatments reduce the number of double bonds, being the solvent extraction the treatment that generates the greatest effect. Furthermore, it is noted that the oxidative stability index (OSI) values are lower for every oil with respect to the control oil, although with few differences, except for the solvent-extracted oil. Generally, it is observed that the tested parameters are better in the heated and decanted extracted oils, with or without freezing, respect to the solvent extracted oils. In heating and decanting processes, values for (Y) and (OSI) were greater and for (P) were lower, in comparison to those obtained by solvent extraction.

The values of the tested parameters are very similar for the heating-decanting and heating-freezing processes since the applied thermal treatments are the same and the cold does not restore any of the properties of the oil, as it only conserves them in the state in which they are prior to the freezing. Moreover, there are differences in the yields of the oil obtained by these methods, being greater in the heating-freezing method, which is explained by the technological problems caused by the difficulty in separating the supernatant in the heating-decanting method. The heating-freezing method also allows obtaining the oil with acceptable physiochemical features (Table 2), it is above the solvent extraction, method used as reference in this study, and it is competitive if compared to other methods reported in the literature, as in enzymatic hydrolysis crude oil extraction from fresh anchovy waste, in which 69.7% was recovered [27] or in the extraction with supercritical fluids in lipids from common carp viscera [28], wherein a yield of 74.845% was obtained. On the other hand, despite the yield values previously reported are above those obtained for OEHD (60.989% ± 0.845) in this study, OEHF, showed a greater yield (92.283% ± 0.327) for the oil, and the other reported methods do not compete with it as for costs, ease for scale-up and implementation in rural areas [16] considering that oils with acceptable physiochemical features were obtained (Table 2).

The methods highlighted herein regarding physiochemical features are the heating-decanting and heating-freezing methods. In both of them the strict control of the temperature is important since treatments with elevated temperatures promote reaction between fatty acids and species derived from molecular oxygen (O_2), resulting from the double bond breaking in which an oxygen molecule O_2 is fixed with the formation of peroxides [29]. It should be considered that the addition of oxygen radicals to fatty acids is produced preferably at double bonds between carbon atoms, following first order temperature dependent kinetics [21].

Furthermore, in general OSI values for all OEHD, OEHF and OES methods were lower than for CO, which means that the products of the lipid oxidation are formed faster in the tested methods than in the control. This is due to the effect that temperature exerts on the rate of chemical reactions, in the case of the methods comprising heating [17] and specifically in the oxidative processes of fats [30], in which, increases in temperature accelerate the decomposition of lipid hydroperoxides [31], so that at higher working temperatures there is less resistance to oxidation, i.e. lower OSI values [32].

Table 2. Physicochemical parameters of the oil extracted with the different methods and the CO.

Sample	P	I	OSI	Yield (%)
CO	0.004 ± 0.002^{a}	173.341 ± 2.742^{a}	0.590 ± 0.013^{a}	—
OEHD	0.014 ± 0.003^{b}	161.151 ± 0.816^{b}	0.295 ± 0.020^{b}	60.989 ± 0.845^{a}
OEHF	0.014 ± 0.001^{b}	161.671 ± 1.304^{b}	0.290 ± 0.031^{b}	92.283 ± 0.327^{b}
OES	0.039 ± 0.006^{c}	151.708 ± 3.269^{c}	0.080 ± 0.046^{c}	54.349 ± 31.378^{c}

P: peroxide value; I: iodine value; OSI: oxidative stability index. Means on the same row having different superscripts are significantly different ($p < 0.05$).

Regarding the OES method, further to presenting grater P, lower Y and lower lipid extraction yield, it also has lower OSI value in comparison to other methods tested in this study. These results are comparable with Fiori et al. [33] who obtained extraction yields of 57% in trout (*Oncorhynchus mykiss*) viscera using a modified Soxhlet and also observed higher fatty acid oxidation with the use of solvents. Additionally, the extraction by Soxhlet technique has the disadvantages that it requires a relative dry sample, it is destructive, it involves a high time consumption and it generates large amounts of residual solvent [10].

OSI values obtained in the evaluated methods in this study are lower than those reported for other vegetable oils like hazelnut (OSI 1.39 h) [34] and moringa (OSI 7.237 h) [35] at 140 °C, possibly because the analyzed oils were not refined and because they comprise a higher content of unsaturated fatty acids (UFA) than the vegetable oils.

3.3. Chromatographic analysis of fatty acids

3.3.1. TLC analysis

TLC analysis of the tested samples showed the presence of various components. For all samples, characteristic bands corresponding to triglycerides (Rf: 0.90), free fatty acids (Rf: 0.58) and sterols (Rf: 0.52) were observed, compared to the standards.

Rfs of 0.83 and 0.13 were observed in the chromatographic analysis of the methyl ester and pyrrolidide derivatives, respectively. The standard of a methyl ester of a fatty acid was used for comparison, and it was observed a marked difference between the Rfs of both types of compounds, consistent with the change from lower to higher polarity of the obtained derivatives, which confirmed, at this stage of the process, the formation of pyrrolidides from methyl esters.

3.3.2. GC-MS analysis

GC-MS analysis allowed determining the relative abundance (%) of the fatty acids present in the oils obtained from different methods; in decreasing order: C18:1 (oleic acid), C16:0 (palmitic acid), C18:2 (linoleic acid) and C16:1 (palmitoleic acid) (Table 2). It was found that the principal components are the UFAs with a total percentage of 66.74% for the CO, 62.04% for the OEHF and 61.87% for the OES. Despite a reduction in the abundance of UFA is observed when using OEHF and OES methods, they are higher than those found in carp (*Rohu and Catla*) [36], bocachico (*Prochilodus magdalenae*) and nile tilapia (*Oreochromis niloticus*) [37] visceras, proving that fish viscera are a good source of UFA.

Furthermore, CO and OEFH show a PUFA content higher and similar when compared to OES (Table 3), which demonstrates that the heating extraction, under optimum conditions, is a proper process for obtaining an oil with high content of these fatty acids without affecting their original composition. In addition, it is a method that, as the supercritical fluid extraction, reduces the fish oil oxidation and produces solvent free extracts, which is of great importance in food and pharmaceutical industries [38]. The results obtained suggest that at least a third of the total extract yield is represented by PUFA, which makes the red tilapia viscera a potential source of these bioactive components [28].

Figure 1 shows the results of the principal component analysis performed with the abundance data of the fatty acids identified in the oils obtained by CO, OEHF and OES (Table 3). Principal components 1 and 2 explain the 97.45% and the 2.19% of the total observed variation, respectively. Component 1 separates fatty acids by their abundance so that when moving towards the left of x-axis those that present lower abundance can be observed, and when moving towards the right of that axis, all those with higher abundance are shown. Component 2 separates the fatty acids by their abundance and according to the extraction method used for obtaining the oil.

For the most abundant fatty acids, C20:1, 14:0, 18:0, 16:1 and C16:0, no differences were observed among extraction methods, while 18:1 and 18:2 showed differences in their abundance depending on the extraction method utilized for obtaining the oil.

Table 3. Fatty acids profile of the CO extracted by centrifugation, OEHF and OES.

Fatty acids		Short name	Relative abundance (%)		
			CO	OEHF	OES
SFA (saturated fatty acid)					
1	Dodecanoic	C12:0	0.09 ± 0.01[a]	0.14 ± 0.01[a]	0.15 ± 0.01[a]
2	Tetradecanoic	C14:0	5.34 ± 0.05[a]	6.16 ± 0.00[b]	5.94 ± 0.06[c]
3	Pentadecanoic	C15:0	0.70 ± 0.03[a]	0.93 ± 0.01[b]	1.00 ± 0.01[c]
4	Hexadecanoic	C16:0	18.4 ± 0.07[a]	19.49 ± 0.04[b]	19.86 ± 0.03[c]
5	Heptadecanoic	C17:0	1.47 ± 0.01[a]	2.00 ± 0.00[b]	1.92 ± 0.00[b]
6	Octadecanoic	C18:0	5.94 ± 0.00[a]	6.88 ± 0.00[b]	6.86 ± 0.00[b]
7	Nonadecanoic	C19:0	0.18 ± 0.01[a]	0.35 ± 0.01[b]	0.33 ± 0.03[b]
8	Eicosanoic	C20:0	0.37 ± 0.08[a]	0.56 ± 0.02[b]	0.56 ± 0.00[b]
9	Docosanoic	C22:0	0.15 ± 0.01[a]	0.29 ± 0.01[b]	0.27 ± 0.00[b]
10	Tricosanoic	C23:0	0.07 ± 0.01[a]	0.14 ± 0.03[b]	0.14 ± 0.00[b]
11	Tetracosanoic	C24:0	0.07 ± 0.02[a]	0.14 ± 0.08[b]	0.14 ± 0.00[b]
Total SFA			**32.78 ± 0.44[a]**	**37.08 ± 0.52[b]**	**37.17 ± 0.23[b]**
MUFA (monounsaturated fatty acid)					
13	6-tetradecenoic	C14:1	0.42 ± 0.05[a]	0.48 ± 0.02[b]	0.46 ± 0.02[b]
14	5-hexadecenoic	C16:1	0.79 ± 0.00[a]	0.25 ± 0.00[b]	0.25 ± 0.06[b]
15	6-hexadecenoic	C16:1	9.05 ± 0.04[a]	8.85 ± 0.03[b]	8.79 ± 0.00[b]
16	8-heptadecenoic	C17:1	0.09 ± 0.00[a]	0.17 ± 0.00[b]	ND
17	9-octadecenoic	C18:1	22.25 ± 0.00[a]	16.06 ± 0.03[b]	23.93 ± 0.00[c]
18	7-nonadecenoic	C19:1	0.20 ± 0.01[a]	0.31 ± 0.07[a]	0.24 ± 0.01[a]
19	8-eicosaenoic	C20:1	0.21 ± 0.00	ND	ND
20	10-eicosaenoic	C20:1	3.45 ± 0.01[a]	3.58 ± 0.05[a]	3.45 ± 0.08[a]
21	13-docosaenoic	C22:1	0.25 ± 0.00[a]	0.34 ± 0.00[a]	0.33 ± 0.07[a]
22	16-tricosaenoic	C23:1	0.05 ± 0.00[a]	0.06 ± 0.01[a]	0.07 ± 0.01[a]
23	9-tetracosaenoic	C24:1	0.18 ± 0.01[a]	0.16 ± 0.02[a]	0.18 ± 0.02[a]
Total MUFA			**36.76 ± 0.41[a]**	**30.26 ± 0.40[b]**	**37.70 ± 0.36[c]**
PUFA					
24	3,6,9-hexadecatrienoic	C16:3	0.19 ± 0.02[a]	0.19 ± 0.00[a]	0.15 ± 0.00[b]
25	3,6-hexadecadienoic	C16:2	0.41 ± 0.04[a]	0.42 ± 0.08[a]	0.43 ± 0.01[a]
26	5,9,12- octadecatrienoic	C18:3	2.09 ± 0.08[a]	2.09 ± 0.02[a]	2.07 ± 0.00[b]
27	5,8-octadecadienoic	C18:2	1.15 ± 0.01[a]	0.98 ± 0.04[b]	1.05 ± 0.01[c]
28	4,9-octadecadienoic	C18:2	15.65 ± 0.01[a]	16.45 ± 0.03[a]	8.49 ± 0.00[c]
29	5,8,11,14-eicosatetraenoic	C20:4	1.58 ± 0.07[a]	1.63 ± 0.03[a]	1.67 ± 0.03[a]
30	5,9,13-eicosatrienoic	C20:3	0.50 ± 0.01[a]	0.41 ± 0.01[b]	0.53 ± 0.03[a]
31	5,8,11-eicosatrienoic	C20:3	2.06 ± 0.25[a]	2.54 ± 0.00[a]	2.08 ± 0.07[a]
32	5,8-eicosadienoic	C20:2	0.70 ± 0.01[a]	0.73 ± 0.01[a]	0.72 ± 0.01[a]
33	7,10- eicosadienoic	C20:2	1.39 ± 0.08[a]	1.57 ± 0.01[a]	1.52 ± 0.01[a]
34	4,7,10,13,16,19 docosahexaenoic	C22:6	0.82 ± 0.11[a]	0.92 ± 0.00[a]	0.91 ± 0.00[a]
36	4,7,10,13,16- docosapentaenoic	C22:5	1.09 ± 0.02[a]	1.32 ± 0.02[b]	1.42 ± 0.03[c]
37	5,8,11,14-docosatetraenoic	C22:4	1.33 ± 0.01[a]	1.42 ± 0.07[a]	1.37 ± 0.04[a]
38	6,9,12-docosatrienoic	C22:3	1.31 ± 0.01[a]	1.49 ± 0.01[b]	1.51 ± 0.02[b]
39	6,9-docosadienoic	C22:2	0.13 ± 0.05[a]	0.18 ± 0.04[b]	0.27 ± 0.08[c]
Total PUFA			**30.40 ± 0.29[a]**	**32.03 ± 0.42[b]**	**24.19 ± 0.52[c]**

Means on the same row having different superscripts are significantly different ($p < 0.05$); ND: not determined.

Moreover, similarities in the SFA were found when comparing CO, OEHF and OES extraction methods, based on the abundance and types of fatty acids obtained. When analyzing the MUFA, it was found that the CO and OES methods extracted a higher content of fatty acid C18:1 than OEHF, wherein the percentage obtained was lower. As for the PUFA, CO and OEHF methods extracted the greatest amount of C18:2 with respect to the OES method, in which was observed the lowest percentage of this fatty acid. Additionally, in the oil obtained by OES there was an absence of fatty acids C17:1 (8-heptadecenoic) and C20:1 (8-eicosaenoic), the latter also absent in the oil extracted by OEHF. Both MUFA showed low abundance in the obtained oils (8-heptadecenoic <0.18% in OEHF and CO; 8-eicosaenoic, 0.21% in CO), which does not significantly affect the total value of their abundance obtained in OEHF and CO (Table 2).

3.4. DSC analysis

Figures 2a, 2b and 2c show melting curves of the different red tilapia oils (CO, OEHF and OES) analyzed by DSC, in which different transition temperature peaks are observed, indicating that the oil has a complex composition of different types of fatty acids. The transition for CO starts at -55.83 °C and ends at 24.81 °C, with a maximum peak at 6.32 °C (Figure 2a), which is a lower temperature compared to the other oils that presented maximum peaks of 10.21 °C (Figure 2b) and

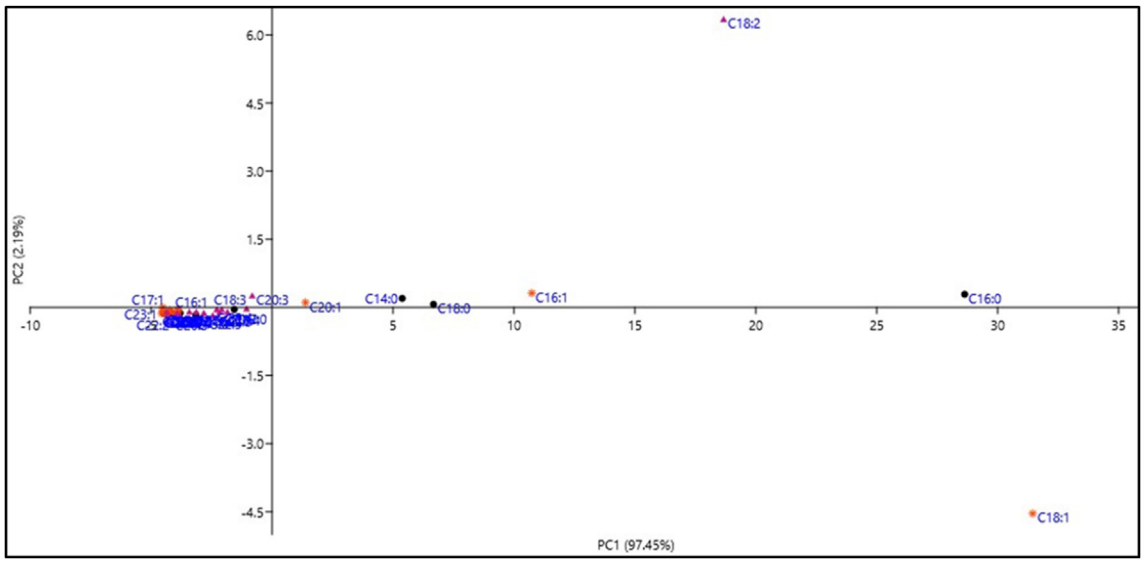

Figure 1. Principal component analysis of fatty acids content of oil extracted by CO, OEHF and OES methods (dark circle: CO; red star: OEHF; purple triangle: OES).

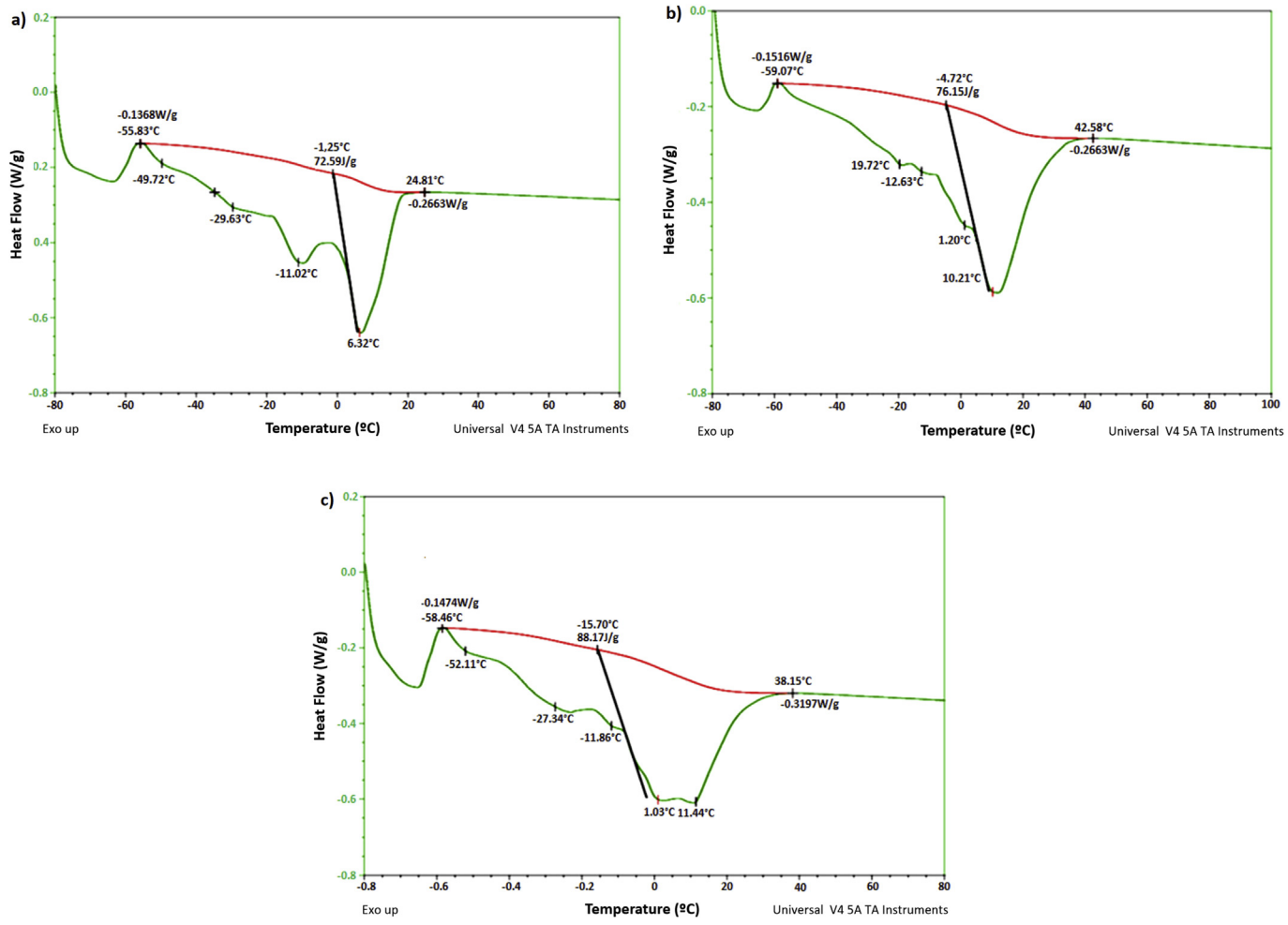

Figure 2. DSC melting curves for the a) CO, b) OEHF and c) OES.

11.44 °C (Figure 2c) for OEHF and OES, respectively. This suggests that OC changes phase at a lower temperature than the other oils because it has a lower content of saturated fatty acids (SFA) as evidenced in Table 4. Therefore, a high UFA content results in low melting temperatures, while a high SFA content results in high melting temperatures [39].

On the other hand, the enthalpy of the oil explains whether it changes from one state to another, either by absorption (endothermic)

Table 4. Thermodynamic parameters of DSC analysis performed on oils obtained by different extraction methods.

Sample	T_{on} (°C)	T_{off} (°C)	Transition Temperatures	Enthalpy (J/g)
CO	-55.83	24.81	6.32	72.59
			-11.02	
			-29.63	
			-49.72	
OEHF	-59.07	42.58	10.21	76.15
			1.20	
			-12.63	
			-19.72	
OES	-58.46	38.15	11.44	88.17
			1.03	
			-11.86	
			-27.34	
			-52.11	

or by release (exothermic) of heat [40]. In this case, OC has a lower enthalpy of fusion (72.59 J/g) than OEHF and OES (76.15 J/g and 88.17 J/g, respectively), which is consistent with the fact that less energy is required for changing phases (Table 3). Sathivel et al. [30] reported enthalpy values very similar to those obtained in this study (84.7–73.9 kJ/kg) for catfish (*Ictalarus punctatus*) oils, and Huang et al [39] reported lower values for salmon (*Oncorhynchus gorbuscha*) oil (58.7 kJ/kg), in line with those reported by Adeoti et al. [41] for unrefined salmon (*Salmo salar*) oil (33.43 kJ/kg), herring (*Clupea*) (52.92 kJ/kg), mackerel (*Scomber scombrus*) (53.6 kJ/kg) and north cod (*Gadus morhua*) (41.45 kJ/kg). These low values in the enthalpy of fusion may be due to the fatty acid composition present in the different oils since they have a lower SFA content.

4. Conclusions

The results of this study allowed determining that red tilapia (*Oreochromis sp.*) viscera has significant levels of PUFA and essential fatty acids. Moreover, the extraction methods used for obtaining the oil from the fish viscera, by heating and subsequent freezing and by heating and subsequent decanting, have the following advantages: they allow obtaining high yields of the oil, they are simple methods of easy implementation, free of chemical contaminants, do not require the use of toxic solvents, expensive enzymes or robust equipment and preserve the PUFA percentage. The heating and decanting method is low cost and easy to apply at different production scales and it further allows recovering the oil as a first stage so that it can be then processed according to the given application.The results of this study showed as well that the higher the PUFA content, the lower the melting temperatures of the oils and the energy required for the phase change. Furthermore, this study also showed that there are differences in the abundance of fatty acids C18:1 and C18:2 depending on the extraction method used for obtaining the oil.

The results obtained herein show that the oil extraction from *Oreochromis sp* viscera through the OEHF method allows obtaining a product with good physiochemical features and high PUFA content, which makes it a value-added product with potential use at industrial level.

Declarations

Author contribution statement

Lorena ARIAS: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Diana M. MARQUEZ: Analyzed and interpreted the data; Wrote the paper.

José E. ZAPATA: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] FAO (Food and Agriculture Organization of the United Nations), El estado mundial de la pesca y la acuicultura 2018. Cumplir los objetivos de desarrollo sostenible, 2018, p. 250. Rome, IT, http://www.fao.org/3/I9540es/i9540es.pdf. (Accessed 24 January 2020).

[2] Boletín Tecnológico, Trucha y Tilapia. Aprovechamiento de Subproductos. CIGEPI-Centro de Información Tecnológica y Apoyo a la Gestión de la Propiedad Intelectual. Superintendencia de Industria y Comercio, 2018. URL: https://www.sic.gov.co/sites/default/files/files/Propiedad%20Industrial/Boletines_Tecnologicos/Trucha_tilapia_boletin.pdf. (Accessed 24 January 2020).

[3] FAO (Food and Agriculture Organization of the United Nations), Fisheries & Aquaculture. Visión general del sector acuícola nacional. Colombia, 2019. URL: http://www.fao.org/fishery/countrysector/naso_colombia/es. (Accessed 24 January 2020).

[4] M.C. Merino, S.P. Bonilla, F. Bages, Diagnóstico del estado de la acuicultura en Colombia. Plan Nacional de Desarrollo de la Acuicultura Sostenible en Colombia AUNAP-FAO. Bogotá, Colombia, Ministerio de Agricultura y Desarrollo Rural, 2013. https://www.aunap.gov.co/wp-content/uploads/2016/04/25-Diagn%C3%B3stico-del-estado-de-la-acuicultura-en-Colombia.pdf.

[5] J. Roslan, K.F. Yunos, N. Abdullahb, S.M. Mustapa, Characterization of fish protein hydrolysate from Tilapia (Oreochromis niloticus) by-product, Agriculture and Agricultural Science Procedia 2 (2014) 312–319.

[6] S.C. Hathwar, B. Bijinu, A.K. Rai, B. Narayan, Simultaneous recovery of lipids and proteins by enzymatic hydrolysis of fish industry waste using different commercial proteases, Appl. Biochem. Biotechnol. 164 (1) (2011) 115–124.

[7] E.D. Braga, J.D. Malveira, M.A. Milhome, M.D. de Aquino, R.F. Nascimento, Characterization of the fatty acids present in wastewaters from production of biodiesel Tilapia, J. Chem. (2015) 1–6.

[8] A. Valenzuela, J. Sanhueza, S. Nieto, Docosahexae-noic acid (DHA), essentiality and requirements: why and how to provide supplementation, Grasas Aceites 57 (2) (2006) 229–237.

[9] L. Qi-yuan, Q. Jun-qing, W. Xiao-ge, Optimization of enzymatic fish oil extraction from mackerel viscera by response surface methodology, Int. Food Res. J. 23 (3) (2016) 992–997. https://www.proquest.com/openview/7d5cb2d95168e65d2c7f10bd9486729c/1?cbl=816390&parentSessionId=ywPaKTSsKAISpM6VyYUElDfuHvs26o7T8jzVL7mGWf4%3D&pq-origsite=gscholar&accountid=26276.

[10] I.A. Adeoti, K. Hawboldt, A review of lipid extraction from fish processing by-product for use as a biofuel, Biomass Bioenergy 63 (2014) 330–340.

[11] J.R. Bonilla-Méndez, J.L. Hoyos-Concha, Methods of extraction, refining and concentration of fish oil as a source of omega-3 fatty acids, Ciencia y Tecnología Agropecuaria 19 (3) (2018) 645–668.

[12] D. Nascimento Santos, E. Takahashi, A. Barros, A. Lopes de Oliveira, Supercritical extraction of cobia (Rachycentron canadum) liver oil as a new source of squalene, Food Publ. Health 6 (6) (2016) 157–164.

[13] S. de Jesus Sérgio, R. Maciel Filho, Recent advances in lipid extraction using green solvents, Renew. Sustain. Energy Rev. 133 (2020) 110289.

[14] S. Hao, Y. Wei, L. Li, X. Yang, J. Cen, H. Huang, X. Yuan, The effects of different extraction methods on composition and storage stability of sturgeon oil, Food Chem. 173 (2015) 274–282.
[15] B. Mbatia, D. Adlercreutz, P. Adlercreutz, A. Mahadhy, F. Mulaa, B. Mattiasson, Enzymatic oil extraction and positional analysis of Omega 3 fatty acids in Nile perch and salmon heads, Process Biochem. 45 (5) (2010) 815–819.
[16] L. Arias, L.J. Gómez, J.E. Zapata, Efecto de Temperatura-Tiempo Sobre los Lípidos Extraídos de Vísceras de Tilapia Roja (*Oreochromis sp.)* Utilizando un Proceso de Calentamiento-Congelación, Inf. Tecnol. 28 (5) (2017) 131–142.
[17] P. Navas, Componentes minoritarios y propiedades antioxidantes de aceites vírgenes y tortas residuales obtenidos por presión en frío a partir de fuentes vegetales convencionales y no convencionales, Universidad de Castilla La Mancha, Facultad de Ciencias Químicas. España, 2010, p. 302 [Tesis Doctoral], https://ruidera.uclm.es/xmlui/handle/10578/2824. (Accessed 24 January 2020).
[18] AOAC (Association of Official Analytical Chemists), Official Methods of Analysis of AOAC International, fourteenth ed., 1984. Washington, D.C., U.S.A.
[19] NTC 218:2011. Grasas y aceites vegetales y animals. Determinación del índice de ácidez y de la acidez, Bogotá, Colombia, 2011.
[20] M.A. Rincón-Cervera, M.B. Villarreal-Rubio, R. Valenzuela, A. Valenzuela, Comparison of fatty acid profiles of dried and raw by-products from cultured and wild fishes, Eur. J. Lipid Sci. Technol. 119 (9) (2017) 1600516.
[21] E. Villanueva, D. Castillo, G. Rodríguez, Influencia de los parámetros Rancimat sobre la determinación del índice de estabilidad oxidativa del aceite de Sesamum indicum L, Scientia Agropecuaria 4 (3) (2013) 173–180.
[22] W.W. Focke, I. Van der Westhuizen, X. Oosthuysen, Biodiesel oxidative stability from Rancimat data, Thermochim. Acta 633 (2016) 116–121.
[23] O. Villamil, H. Váquiro, J.F. Solanilla, Fish viscera protein hydrolysates: production, potential applications and functional and bioactive properties, Food Chem. 224 (2017) 160–171.
[24] N.L. Pinzón, J.C. Sánchez, Aprovechamiento de las vísceras de pescado como fuente de energía para minimizar el problema de contaminación ambiental del sector piscícola, Revista de Investigación Agraria y Ambiental 7 (2) (2016).
[25] T.C. Torres, L.J. Londoño, A.S. Hincapié, R.C.A. David, Extracción y caracterización de aceite de pescado derivado de subproductos de trucha arco iris (Oncorhynchus mykiss), J. Agric. Animal Sci. 2 (2) (2013) 34–41, https://web.p.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=22563342&AN=102149489&h=qVgGyF1Kexa7804XEu%2fuXpyI7gK3vGzQ89weDnPUGfDJSRVyZDeWJHptb03AlEF8wccgeFG9VnsFseNFZcWkZA%3d%3d&crl=c&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3fdirect%3dtrue%26profile%3dehost%26scope%3dsite%26authtype%3dcrawler%26jrnl%3d22563342%26AN%3d102149489.
[26] CODEX STAN 019-1981, Norma del Codex para Grasas y Aceites Comestibles no Regulados por Normas Individuales. FAO and WHO, Roma, Italia, 1981, pp. 1–5.
[27] D.T. Llerena, P.D. Arand, Extracción y caracterización del aceite crudo obtenido de un hidrolizado enzimático de residuos frescos de anchoveta (Engraulis ringens), Anales Científicos Universidad Nacional Agraria La Molina – Perú. 78 (1) (2017) 34–42.
[28] K. Lisichkov, S. Kuvendziev, Z. Zeković, M. Marinkovski, Influence of operating parameters on the supercritical carbon dioxide extraction of bioactive components from common carp (Cyprinus carpio L.) viscera, Separ. Purif. Technol. 138 (2014) 191–197.
[29] S. Rodríguez, A. Escobar, E. Ortiz, Desarrollo de una Prueba de Estabilidad Oxidativa del Aceite de Pescado, Comparando Medidas de Técnicas Bromatológicas con las de Calorimetría Diferencial de Barrido, Rev. Colomb. Fisica 42 (2) (2010) 191–195. http://fisica.udea.edu.co/ojs/ojs/index.php/rcf/article/download/420227/88. (Accessed 24 January 2020).
[30] S. Sathivel, W. Prinyawiwatkul, I.I. Negulescu, J.M. King, Determination of melting points, specific heat capacity and enthalpy of catfish oil during different purification steps, J. Am. Oil Chem. Soc. 85 (3) (2008) 291–296.
[31] P.J. García-Moreno, R. Pérez-Gálvez, A. Guadix, E.M. Guadix, Influence of the parameters of the Rancimat test on the determination of the oxidative stability index of cod liver oil, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 51 (1) (2013) 303–308.
[32] J. Velasco, C. Dobarganes, F. Holgado, G. Marquez-Ruiz, A follow-up oxidation study in dried microencapsulated oils under the accelerated conditions of the Rancimat test, Food Res. Int. 42 (1) (2009) 56–62.
[33] L. Fiori, M. Solana, P. Tosi, M. Manfrini, C. Strim, G. Guella, Lipid profiles of oil from trout (Oncorhynchus mykiss) heads, spines and viscera: trout by-products as a possible source of omega-3 lipids? Food Chem. 134 (2) (2012) 1088–1095.
[34] H. Ciemniewska-Żytkiewicz, K. Ratusz, J. Bryś, M. Reder, P. Koczoń, Determination of the oxidative stability of hazelnut oils by PDSC and Rancimat methods, J. Therm. Anal. Calorim. 118 (2) (2014) 875–881.
[35] S. Alberca, M. Huanca, Evaluación del Índice de Estabilidad Oxidativa del Aceite De Moringa (Moringa oleífera) por el Método Rancimat, Revista Científica Ingenieria: Ciencia, Tecnología e Innovación 2 (2) (2015) 59–69. http://revistas.uss.edu.pe/index.php/ING/article/view/261/272.
[36] A.K. Rai, H.C. Swapna, N. Bhaskar, P.M. Halami, N.M. Sachindra, Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera, Enzym. Microb. Technol. 46 (1) (2010) 9–13.
[37] G. Rodríguez, E. Villanueva, P. Glorio, M. Baquerizo, Estabilidad oxidativa y estimación de la vida útil del aceite de sacha inchi (Plukenetia volubilis L.), Scientia Agropecuaria 6 (3) (2015) 155–163.
[38] S. Kuvendziev, K. Lisichkov, Z. Zeković, M. Marinkovski, Artificial neural network modelling of supercritical fluid CO 2 extraction of polyunsaturated fatty acids from common carp (Cyprinus carpio L.) viscera, J. Supercrit. Fluids 92 (2014) 242–248.
[39] J. Huang, S. Sathivel, Thermal and rheological properties and the effects of temperature on the viscosity and oxidation rate of unpurified salmon oil, J. Food Eng. 89 (2) (2008) 105–111.
[40] L. Zhao, S.H. Yalkowsky, A combined group contribution and molecular geometry approach for predicting melting points of aliphatic compounds, Ind. Eng. Chem. Res. 38 (9) (1999) 3581–3584.
[41] I.A. Adeoti, K. Hawboldt, M.R. Santos, Thermal and flow properties of fish oil blends with bunker fuel oil, Fuel 158 (2015) 641–649.