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ORIGINAL PAPER

Polyunsaturated Fatty Acid Concentrates of Carp Oil: Chemical Hydrolysis and Urea Complexation

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Abstract The aims of this study were to compare three treatments in the chemical hydrolysis reaction of bleached oil from carp (*Cyprinus carpio*) heads and to obtain polyunsaturated fatty acid concentrates by urea complexation. The three treatments were carried out with different oil:ethanol molar ratios. In the treatment with a 1:39 molar ratio, a higher yield of free fatty acids was found. These fatty acids were submitted to urea complexation (−10 °C for 20 h, and urea–fatty acid ratio of 4.5–1). There was a 31.4% increase in monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) content and a 75% decrease in saturated fatty acids (SAF) content. An increase of 85.4% in the EPA + DHA content was found. The non-urea complexing fraction can be considered a rich source of MUFA and PUFA with a total amount of 88.9%.

Keywords Chemical hydrolysis · Fish oil · Polyunsaturated fatty acids

Introduction

Methods to obtain polyunsaturated fatty acid (PUFA) concentrates have been reported in studies due to the fact that the market provides a great potential in food supplements and in products in which they are incorporated. Fish oil constitutes an important source and it is therefore used as raw material for to prepare PUFA concentrates [1, 2]. The common carp (*Cyprinus carpio*) is an exotic species native of Asia. The waste found during carp processing may total 60% of raw material, and the waste is mainly skins, heads and collars [3]. Thus, there is potential to produce oil from carp waste, such as the heads.

The simplest and most efficient technique to obtain PUFA concentrates through free fatty acids (FFA) is the urea complexation [2, 4]. Firstly, a chemical or enzymatic hydrolysis of fish oils is carried out to prepare PUFA concentrates, obtaining the FFA for subsequent complexation. Enzyme-catalyzed procedures, using lipase as the catalyst, do not produce side reactions, but the lipases are very expensive for industrial scale production and a three-step process is required to achieve a 95% conversion [5]. In the chemical hydrolysis, which is a reaction between triacylglycerol and alcohol, the esters are obtained through the displacement of glycerol by the alcohol in the presence of catalysts [6]. The urea complexation (inclusion) method is based on separation by the degree of unsaturation, and the more unsaturated fatty acids complex less with urea [7].

The molar ratio between alcohol and triacylglycerol is one of the most important variables that affect the conversion of triacylglycerol into esters. The stoichiometric ratio for hydrolysis requires three moles of alcohol and one of triacylglycerol to produce three moles of esters and one of glycerol. However, the hydrolysis reaction has a reversible character and therefore requires an excess of alcohol in order to have a displacement of the reaction to the right [6]. Consequently, in the chemical hydrolysis reaction, the study of the molar ratio between alcohol and triacylglycerol is necessary in order to obtain higher yields of FFA for urea complexation. The aims of this study were to compare three different treatments used in the chemical

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hydrolysis reaction of bleached oil from carp (*C. carpio*) heads as well as produce PUFA concentrates by urea complexation.

Experimental Procedures

Materials

Carp (*C. carpio*) heads, obtained from fish of approximately the same size (average weight 1.30 kg) and age (over 2 years old) from a commercial fish-processing plant were utilized as raw material for oil extraction using a fishmeal process; these residues were transported and immediately frozen in plastic containers at the laboratory.

Procedures

In the extraction of crude oil, 35 kg of carp heads were used. The heads were processed in the laboratory through the following steps: grinding, cooking, screening and centrifugation; with similar conditions to the ones used in a fish processing plant. The fish heads were defrosted at room temperature during the night and ground in a meat grinder. Afterwards, the raw material was cooked at 95–100 °C for 30 min and was later sifted in a Tyler sieve 14 to remove the solid fraction.

In order to separate the oil, the liquid fraction was centrifuged for 20 min at 7,000×g (model SIGMA 6-15, D-37250, Sigma, Osterode, Germany). The product included three fractions: the bottom solid cake, an aqueous middle layer and the upper oil layer. The oil fractions were separated and stored in an amber bottle at −20 °C for further analysis and refinement.

The steps of crude oil refinement (degumming, neutralization, washing, drying and bleaching) were carried out under conditions described by Crexi et al. [8]. The degumming step was carried out for 30 min at 80 °C and 500 rpm agitation, with the addition of 1.0% of phosphoric acid (85% v/v) in relation to the oil mass. The neutralization step occurred during 20 min, at 40 °C and agitation of 500 rpm, with addition of sodium hydroxide solution 20% w/w (using 4.0% of excess in relation to the acidity value after the degumming step). After each step, material was centrifuged for 20 min at 7,000×g for oil separation. The washing step consisted of adding 10% water in relation to the oil mass, at 95 °C, for a contact time of 10 min, with 500 rpm agitation and oil temperature maintained at 50 °C. This step was repeated three times. The drying step lasted for 20 min with temperature at 90–95 °C and 500 rpm agitation. The bleaching step was carried out at 70 °C and 40 rpm, with the addition of 5% of adsorbents (mixture of activated earth and activated coal at a 9:1 ratio), with contact time of 20 min. Filtration was carried out in a Büchner funnel with a pre-layer of diatomaceous earth. The refining steps were carried out in vacuum (720 mmHg).

The yield of the crude oil was expressed as a percentage of recovered crude oil in relation to the one present in the carp heads obtained by the Bligh and Dyer method [9]. The yield was calculated according to Eq. (1):

$$\%\ Y_{\mathrm{Oil}} = \frac{W_{\mathrm{RCO}}}{W_{\mathrm{OBD}}} \times 100 \qquad (1)$$

where W_{RCO} is the weight of recovered crude oil (kg) and W_{OBD} is the carp heads crude oil (kg) extracted by the Bligh and Dyer method.

The molecular weight of the carp oil was calculated by the saponification value (SV) according to Eq. (2) [10]:

$$M_{\mathrm{W}} = \frac{3000 \times 56.1}{\mathrm{SV}} \qquad (2)$$

where M_{W} is the molecular weight of the carp oil and SV is the saponification value.

Preliminary tests were performed in order to determine the conditions of the chemical hydrolysis reaction. The oil:ethanol molar ratios were tested according to references of vegetable and fish oils, and the molar ratio of 1:39 was used in order to test the type and catalyst concentration. The catalysts were KOH and NaOH, whose concentrations were fixed in relation to the initial oil mass (2, 5 and 22%) according to literature [4, 6]. The chemical hydrolysis reactions were carried out in a reactor with agitation at 600 rpm under a vacuum (400 mmHg) at 60 °C for 60 min.

Three treatments were carried out by the chemical hydrolysis reaction (at 60 °C for 60 min) with the purpose of comparing three oil:ethanol molar ratios. The treatments were carried out with a KOH catalyst in a concentration of 22% in relation to the oil mass, and the oil:ethanol molar ratios were of 1:21, 1:27, and 1:39. For each treatment, 50 g of bleached carp oil was used with the addition of antioxidant BHT (400 ppm) and catalyst solution in anhydrous ethyl alcohol. The FFA were obtained through chemical hydrolysis after the end of the reaction, according to the procedure described by Wanasundara and Shahidi [4], with modifications. The equal volume of distilled water was added to the saponified mixture in relation to the initial oil mass. Hexane was also added to the reaction mixture (twice the volume of hexane in relation to the initial oil mass) in order to extract the unsaponifiable matter and discard it. This procedure was performed twice.

The saponifiable matter was acidified (pH = 1.0) with 3 mol/L HCl, and the mixture was transferred into a separation funnel. The fatty acids were extracted with hexane (equal volume in relation to the initial amount of oil). The hexane layer, which contained the FFA. was dried with anhydrous sodium sulfate. The excess of solvent was

removed at 40 °C under a vacuum and FFA were stored at a freezing temperature (−18 °C).

The yield (% *Y*) of the hydrolysis reaction was calculated according to Eq. (3):

$$\% Y_{\text{reaction}} = \frac{W_{\text{FFA}}}{W_{\text{OR}}} \times 100 \quad (3)$$

where W_{FFA} is the weight of free fatty acids and W_{OR} is the weight of oil used in the chemical hydrolysis reaction.

The urea complexation was carried out in a thermostat bath using a crystallization temperature of −10 °C. In this reaction, the FFA resulting from the chemical hydrolysis reaction were mixed with urea (20%, w/v) in alcohol solution (95% v/v aqueous ethanol). The urea-to-fatty acid ratio of 4.5 was used, and the mixture remained at −10 °C for 20 h ([2, 4, 11, 12]).

The separation of the crystals formed was performed by vacuum filtration. The liquid phase (non-urea complexing fraction) was diluted with an equal volume of water and acidified at pH 4–5 with 6 mol/L HCl. An equal volume of hexane was added to the mixture and it was stirred for 1 h, and then transferred to a separation funnel. The hexane layer, containing liberated fatty acids, was separated from the aqueous layer containing urea. The hexane layer was washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulfate and the solvent was then removed at 40 °C under a vacuum. The fatty acids of the complexing fraction (crystals) were recovered in a similar way to the non-urea complexing fraction. The two fractions were weighed separately and the percentage recovery of each was calculated.

The variation in the percentages of unsaturated and polyunsaturated fatty acids, and the variations in the percentage of saturated fatty acids were calculated by Eq. (4):

$$\% \text{ fatty acid variation} = \frac{\left(\sum \text{pfaC} - \sum \text{pfaBO}\right) \times 100}{\sum \text{pfaBO}} \quad (4)$$

where $\sum$pfaC is the percentage of fatty acids after urea complexation. $\sum$pfaBO is the percentage of fatty acids in the bleached oil.

Analytic Methodology

According to the methods of the AOAC [13], the carp heads were characterized in duplicate for moisture, protein and ash contents. The Bligh and Dyer method [9] was used to determine the lipids content. The crude and bleached oils were characterized considering the FFA, PV (peroxide value), IV (iodine value) and SV according to the methodologies of the AOCS [14]. FFA found were analyzed for lipid oxidation by PV analysis.

The crude and bleached oils and non-urea complexing fraction were analyzed in relation to the profiles of fatty acids by GC. The samples were prepared according to the methodology described by Metcalfe and Schmitz [15]. The methyl esters of fatty acids were identified in a chromatograph (VARIAN model CX-3400, Palo Alto, CA, USA) equipped with DB-17 J&W Scientific capillary column (50% phenyl methylpolysiloxane). The analysis of the ethyl esters of fatty acid was performed in duplicate by injecting 1.0 µL ratio (1:50 SPLIT) into the capillary column (30 m × 0.25 mm, 0.25 µm film in thickness). The conditions of the chromatograph were: temperature of the injector 250 °C, temperature of the flame ionization detector 300 °C, the carrier gas was helium with flow of 1.00 mL/min, linear velocity 24 cm/s, and initial temperature of the column 100 °C. This temperature was kept for 1 min and later increased at 6 °C/min to 160 °C The oven temperature was held at 160 °C for 5 min, then increased to 230 °C at 6 °C/min and held at 230 °C for 8 min The fatty acids of methyl esters were identified through direct comparison of the retention times with standards (SUPELCO TM 37, Bellefonte, Palo Alto, USA), and quantified by normalization of the areas.

Statistical Analysis

Statistical analysis was performed using Tukey's HSD test of mean differences [16] and using the software Statistica for Windows 6.0 (Statsoft Inc., Tulsa, Okla, EUA). Characteristics of the crude and bleached oils, FFA from chemical hydrolysis, fatty acid profiles of the crude oil, bleached oil and non-urea complexing fraction were compared. Values were considered significant at a level of $p < 0.05$.

Results and Discussion

The raw material (carp heads) had a moisture content of 75.3 ± 1.4%, protein content of 11.4 ± 1.0%, lipid content of 3.6 ± 0.3%, and ash content of 9.7 ± 1.2%. In this study, 0.03 kg of crude oil per 1 kg of carp heads were recovered, a yield according to Eq. (1) of approximately 83.3% in recovered crude oil was found. Crexi et al. [17] found a yield of 85% of carp oil obtained from viscera through the fishmeal process and the acid ensilage process. FFA, PV, IV and SV of crude and bleached oils from carp heads obtained are shown in Table 1.

Table 1 shows significant difference ($p < 0.\ 05$) for FFA and PV in the crude and bleached oils. After the steps of degumming and neutralization, the bleached oil had a decrease in the amounts of FFA and PV. In the bleaching step, the removal of pigments occurs, as well as the

Table 1 Characterization of the crude and bleached oils from carp heads

Index	Crude oil[A]	Bleached oil[A]
FFA (% oleic acid)	1.76 ± 0.02[a]	0.30 ± 0.02[b]
PV (mequiv peroxide/kg)	4.35 ± 0.05[a]	2.73 ± 0.03[b]
IV (cg I_2/g)	112 ± 1[a]	111 ± 2[a]
SV (mg KOH/g)	202 ± 2[a]	203 ± 1[a]

FFA free fatty acids, *PV* peroxide value, *IV* iodine value, *SV* saponification value

[A] Mean values ± standard error ($n = 3$)

Different superscript letters in the same line are significantly different ($p < 0.05$)

Table 2 Mass yield (%), free fatty acids (FFA) and peroxide value (PV) for the treatments used in the chemical hydrolysis reaction

	Treatments (oil:ethanol molar ratios)		
	(1:21)[A]	(1:27)[A]	(1:39)[A]
Mass yield (%)	70.0 ± 1.5[a]	81.4 ± 1.3[b]	84.5 ± 1.0[c]
FFA (% oleic acid)	34.0 ± 1.0[a]	40.5 ± 1.0[b]	44.4 ± 1.0[c]
PV (mequiv peroxide/kg)	3.5 ± 0.1[a]	3.7 ± 0.1[a]	3.5 ± 0.2[a]

[A] Mean values ± standard error ($n = 3$)

Different superscript letters in the same line show that the values are significantly different ($p < 0.05$)

removal of the neutralization sludge and oxidation products [17].

In Table 1, the content of FFA for crude oil remained below the one found in literature for crude oil from carp viscera of 3.35% [17], which is probably due to the body part from which the oil is obtained. Higher amounts of endogenous enzymes responsible for the degradation process of protein and lipids are found in the fish viscera, thus, the oil from the fish heads has a lower content of FFA [18]. According to Meher et al. [6], the FFA value of the oil used as a raw material in the chemical hydrolysis reaction must be inferior down to 3%. High amounts of FFA cause the formation of soap that, besides consuming part of the catalyst during its formation, create emulsions and make the segregation of the products (esters and glycerol) difficult in the end of the reaction. in Table 1, the values for FFA of the bleached carp oil remained below 3%. The IV and SV for crude and bleached oils were not significantly different ($p > 0.05$). That is due to the fact that the refining steps (degumming, neutralization and bleaching) do not affect the composition of the fatty acids of the triacylglycerols. The SV was used in order to determine the average molecular weight of the carp oil through the Eq. (3), and the calculated value was of 833 g/mol.

Chemical Hydrolysis Reaction

In preliminary tests, the soap formation occurred in the step after the reaction when NaOH was used as catalyst, causing emulsification and difficult separation of the glycerol. According to Meher et al. [6], the insufficient amount of catalyst as well as an excess of catalyst may cause soap formation. The potassium and sodium soaps behave differently. The first one remains suspended on the ester layer and does not mix with the glycerol, however the sodium soap decants and facilitates the solubility of the esters in the glycerol, favoring the formation of emulsions. Nevertheless, the use of 2% KOH provided low mass yield of the reaction. So, 5% and 22% concentrations of KOH catalysts were tested. The use of 5% of the catalyst resulted in a lower yield of FFA than the use of 22% of the catalyst. The yield of FFA in relation to the initial mass was of 16% and 84%, respectively, for 5% and 22% KOH.

In the preliminary test in which the amount of alcohol in the hydrolysis reaction was studied, the separation of the fraction of FFA did not occur in oil:ethanol molar ratios lower than 1:12 due to the emulsion formation. The emulsions are caused by the formation of reaction intermediates, such as monoacylglycerols and diacylglycerols which have polar hydroxyls that are grouped in non-polar hydrocarbon chains. Hence, probably the reaction was not complete with the use of 1:12 oil:ethanol molar ratio resulting in the formation of mono and diacylglycerol intermediates, which caused the emulsion formation and precluded separation of the methyl esters from the glycerol. Nonetheless, the separation of FFA from glycerol occurred in higher oil:ethanol molar ratio. The treatments were carried out with a KOH catalyst in the concentration of 22% in relation to the oil mass and oil:ethanol molar ratios of 1:21, 1:27 and 1:39.

Table 2 shows that the three treatments performed for the hydrolysis reaction had a significant difference ($p < 0.05$) for the mass yield and FFA. The use of more alcohol (1:39 oil:ethanol ratio) led to a higher yield of FFA. Regarding lipid oxidation, the amount of alcohol did not significantly influence the PV, as there was no significant difference ($p > 0.05$) for between in the treatments. Hydrolysis caused an increase in PV compared to the bleached oil (Table 1). This increase may have occurred because of the process conditions, which are a temperature of 60 °C, a time of 60 min and a vacuum of 400 mmHg. The PV for the treatments are within the values required for oils for human consumption of 8 mequiv/kg [19], thus, such index is within the acceptable quality standards in all treatments.

Urea Complexation

The urea complexation was carried out in the fatty acids resulting from the chemical hydrolysis reaction of the 1:39

oil:ethanol molar ratio. The percentage recovery of non-urea complexing fraction in relation to bleached carp oil was around 34%. Fatty acids profiles (%) of the crude and bleached oils and the non-urea complexing fraction are shown in Table 3.

Table 3 shows that the crude and bleached oils were not significantly different ($p < 0.05$). The SFA content remained around 26.2% and the MUFA + PUFA content in the oil remained around 67.7%. The fatty acids C18:1ω-9 (oleic) and C18:2ω-6 (linoleic) were the ones found in greater quantity. The linoleic acid (ω-6) and the linolenic acid (ω-3) are essential PUFA, as the double bounds located in the third and sixth carbon atoms cannot be produced by the human body, and as a result they need to be acquired through a diet [1]. Other essential PUFA are synthesized in the organism: the arachidonic acid (AA) from linoleic acid, and the eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) from linolenic acid [20].

Table 3 shows that there is significant difference ($p < 0.05$) between the bleached oil and the non-urea complexing fraction. There was an increase in the percentage of the MUFA + PUFA and a decrease in the SFA in the non-urea complexing fraction. The formation of the urea inclusion compounds depends on the degree of unsaturation of the fatty acids. The presence of double bonds in the carbon chain increases the volume of the molecule and reduces the probability of its urea complex [21].

Lipid classes in bleached oil and non-urea complexing fraction are shown in Table 4. The variation in the percentage of MUFA + PUFA and in the percentage of SFA were calculated according to Eq. (4). There was an increase of 31.4% in MUFA + PUFA content and a decrease of 75% in SFA content in the non-urea complexing fraction, and there was an increase of 85.35% in the EPA + DHA content. Such values are similar to the ones mentioned by Liu et al. [2]. They found for the urea complexation method with tuna oil a total increase of DHA and EPA of 85.02% with a urea-to-fatty acid ratio of 15 mol/mol, at −5 °C for 20 h. The non-urea complexing fraction is a rich source of MUFA + PUFA with a total amount of 88.9% of these acids (Table 4). This amount is similar to the one cited by

Table 3 Fatty acids profiles (%) of the crude and bleached oils and non-urea complexing fraction

Fatty acids	Crude oil (%)^A	Bleached oil (%)^A	Non-urea complexing fraction (%)^A
C12:0	0.16 ± 0.02^{a}	0.18 ± 0.01^{a}	0.19 ± 0.01^{ab}
C14:0	2.51 ± 0.01^{a}	2.51 ± 0.02^{a}	0.89 ± 0.01^{b}
C15:0	1.08 ± 0.02^{a}	1.08 ± 0.03^{a}	0.28 ± 0.01^{b}
C16:0	16.41 ± 0.02^{a}	16.43 ± 0.03^{a}	4.38 ± 0.02^{b}
C17:0	1.29 ± 0.01^{a}	1.30 ± 0.01^{a}	0.26 ± 0.01^{b}
C18:0	3.96 ± 0.01^{a}	3.95 ± 0.01^{a}	0.46 ± 0.01^{b}
C20:0	0.41 ± 0.02^{a}	0.42 ± 0.01^{a}	0.05 ± 0.01^{b}
C21:0	0.36 ± 0.01^{a}	0.37 ± 0.02^{a}	0.05 ± 0.01^{b}
C22:0	0.27 ± 0.02^{a}	0.28 ± 0.02^{a}	0.04 ± 0.02^{b}
C23:0	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	0.06 ± 0.01^{b}
C14:1ω5	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.10 ± 0.01^{ab}
C16:1ω7	5.47 ± 0.03^{a}	5.48 ± 0.04^{a}	7.02 ± 0.01^{b}
C18:1ω9	26.20 ± 0.02^{a}	26.21 ± 0.03^{a}	30.80 ± 0.01^{b}
C20:1ω9	1.31 ± 0.03^{a}	1.29 ± 0.05^{a}	0.66 ± 0.02^{b}
C22:1ω9	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.07 ± 0.01^{ab}
C24:1ω9	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}
C18:2ω6	18.50 ± 0.01^{a}	18.52 ± 0.01^{a}	25.90 ± 0.02^{b}
C18:3ω6	0.26 ± 0.01^{a}	0.25 ± 0.02^{a}	0.35 ± 0.01^{b}
C18:3ω3	7.20 ± 0.01^{a}	7.19 ± 0.01^{a}	9.71 ± 0.01^{b}
C20:2ω6	0.59 ± 0.02^{a}	0.60 ± 0.02^{a}	0.81 ± 0.01^{b}
C20:3ω6	0.47 ± 0.01^{a}	0.47 ± 0.02^{a}	0.70 ± 0.01^{b}
C20:3ω3	0.76 ± 0.01^{a}	0.76 ± 0.01^{a}	0.98 ± 0.01^{b}
C20:4ω6 (AA)	1.53 ± 0.01^{a}	1.52 ± 0.02^{a}	2.12 ± 0.01^{b}
C20:5ω3 (EPA)	2.62 ± 0.02^{a}	2.62 ± 0.01^{a}	4.59 ± 0.02^{b}
C22:2ω6	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.04 ± 0.01^{a}
C22:6ω3 (DHA)	2.51 ± 0.01^{a}	2.50 ± 0.02^{a}	4.90 ± 0.01^{b}
$\sum$ufa	5.75 ± 0.03^{a}	5.70 ± 0.02^{a}	4.42 ± 0.01^{b}

$\sum$ufa sum of unidentified fatty acids

AA arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

[A] Mean value ± standard error ($n = 3$)

Different superscript letters in the same line show that the values are significantly different ($p < 0.05$)

Table 4 Lipids classes (% total fatty acids) in bleached oil and non-urea complexing fraction

Fatty acids	Bleached oil (%)[A]	Non-urea complexing fraction (%)[A]
∑SFA	26.64 ± 0.02[a]	6.68 ± 0.01[b]
∑MUFA	33.21 ± 0.01[a]	38.75 ± 0.01[b]
∑PUFA	34.45 ± 0.01[a]	50.15 ± 0.01[b]
∑(MUFA + PUFA)	67.66 ± 0.01[a]	88.90 ± 0.01[b]
∑(EPA + DHA)	5.12 ± 0.01[a]	9.49 ± 0.01[b]

∑SFA sum of saturated, ∑MUFA sum of monounsaturated, ∑ PUFA sum of polyunsaturated, ∑(EPA+DHA) sum of eicosapentaenoic acid and docosahexaenoic acid

[A] Mean value ± standard error ($n = 3$)

Different superscript letters in the same line show that the values are significantly different ($p < 0.05$)

Wanasundara and Shahidi [4], who found a total of ω-3 fatty acids of 88.2% for urea-to-fatty acid ratio of 4.5 (w/w), at −10 °C for 24 h.

Conclusions

The recovery of crude oil obtained from carp heads through the fishmeal processes was approximately 83% in relation to the oil in the carp heads. PUFA found in higher amounts in the carp oil were C18:1ω-9 (oleic) and C18:2ω-6 (linoleic). The MUFA + PUFA content in the bleached oil of carp heads was approximately 67.7%.

In the hydrolysis study, the higher amount of alcohol (1:39 oil:ethanol molar ratio) resulted in a higher yield of FFA. These fatty acids were submitted to urea complexation (temperature of −10 °C, crystallization time of 20 h, and urea-to-fatty acid ratio of 4.5), and a percentage increase of MUFA + PUFA content, a decrease of SAF content, and an increase of 85.4% in the EPA + DHA content were found. The non-urea complexing fraction may be considered a rich source of polyunsaturated and monounsaturated fatty acids with a total of 88.9% of these fatty acids.

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