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Production and refinement of oil from carp (*Cyprinus carpio*) viscera

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ABSTRACT

Carp viscera oil can be obtained by both ensilage and fishmeal processes. This study examined the refinement of carp (*Cyprinus carpio*) oils obtained by both processes, and compared crude, neutralised, bleached, winterised and deodorised oils' characteristics and lipid profiles. Refined oils obtained by the two processes did not present significant difference ($p > 0.05$) for Lovibond colour, free fatty acids, and thiobarbituric acid values. The major fatty acids identified in the carp crude, bleached and refined oils were oleic, palmitic, palmitoleic, linoleic and linolenic, constituting approximately 69.6% of the total fatty acids of the oils. The $n-3/n-6$ ratio was approximately 1.05 for refined oil. Therefore, carp viscera refined oil can be considered a rich source of essential fatty acids of the $n-3$ and $n-6$ series.

1. Introduction

The common carp (*Cyprinus carpio*) is a major fish species in world aquaculture production. It is exclusively farmed in traditional extensive or semi-intensive pools (Vandeputte et al., 2008). Therefore, there is potential for producing oil from their by-products, including heads, skins, skeletons and viscera. There are reports on production of good quality fish oils from herring (Aidos et al., 2003) and catfish (Sathivel, Prinyawiwatkul, King, Grimm, & Lloyd, 2003) by-products. Fish oils are considered the major commercial source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and have received much attention in the scientific and industrial communities because of their positive role in human health (Fournier et al., 2007; Zhong, Madhujith, Mahfouz, & Shahidi, 2007).

Fish oil can be obtained by the ensilage acidification process (Reece, 1981; Zhou, Paulson, & Ackman, 1995) or by the fishmeal process (Zhong et al., 2007). However, crude oils require purification in order to meet standards for quality oil production and generation of a product acceptable for human consumption (Hafidi, Pioch, & Ajana, 2005). The refinement operations remove undesirable components, thus guaranteeing satisfactory purity and stability characteristics (acidity, colour, oxidative and sensorial).

The chemical or alkaline refinement includes the following steps: degumming, which removes gums; neutralisation with caustic soda for free fatty acids (FFA) removal; washing, drying and bleaching for removal of soap and trace metals; and deodorisation by vacuum distillation for removal of residual FFA, aldehydes, ketones, alcohols and other compounds (Antoniassi, Esteves, & Meirelles, 1998; Berdeaux et al., 2007; Hafidi et al., 2005). Winterisation is an additional step in oil refinement, and is used for concentrating PUFAs, especially eicosapentaenoic acid and docosahexaenoic acid (Ganga et al., 1998).

The $n-3$ polyunsaturated fatty acids (PUFA), especially (C20:5$n-3$) and (C22:6$n-3$), have biochemical effects on the prevention or treatment of several human diseases. EPA is the precursor of prostaglandins, thromboxanes and leukotrienes. DHA is a component of the phospholipid membrane of brain and retina cells; consequently, it is essential for human health (Fournier et al., 2007; Zhong et al., 2007).

The lipid classes and fatty acid profiles of major fish species and their muscle tissues have been determined; however, there are very few studies on lipid classes and fatty acid profiles of viscera and other by-products (Zhong et al., 2007). In view of these facts, it seems necessary to carry out a study on the characteristics and fatty acid profile of crude and refined oils extracted from carp viscera. The objectives of this work were to study the refinement of carp (*Cyprinus carpio*) viscera crude oils obtained by ensilage acidification and fishmeal processes, and to compare their characteristics and lipid profiles.

2. Materials and methods

Carp (*Cyprinus carpio*) viscera, from fish of approximately the same size (average weight 1.30 kg) and age (over 2 years old), from a commercial fish-processing plant, was utilised as raw material

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for oil extraction by ensilage and fishmeal processes; these residues were transported and immediately frozen in plastic containers at the Unit Operations Laboratory/FURG.

2.1. Ensilage acid process methodology

Initially, 35 kg of carp viscera were thawed overnight at ambient temperature and minced in a meat grinder, conditioned in 5 L plastic buckets and soon afterwards acidified with glacial acetic acid 10% (v/w); also, BHT antioxidant (250 ppm) was added to reduce lipid oxidation. During the initial liquefaction period, the silage was stirred occasionally. Temperature during liquefaction ranged from 25 to 35 °C. After a 15-day period the silage was sifted in a 14 tyler sieve for removal of spines and scales, for subsequent centrifugation.

Silage temperature was increased to 50 °C in a thermostatic bath afterwards, the material was separated in a centrifuge for 20 min at 7000*g*. The resulting product included three fractions: the bottom solid cake, an aqueous middle layer, and the upper oil layer. Oil fractions were separated from the other fractions and stored in an amber bottle at −20 °C for analysis.

2.2. Fishmeal process methodology

Processing of raw viscera was carried out for crude oil extraction on a laboratory scale, in conditions similar to commercial fish-processing plants. Processing included grinding, cooking, screening and centrifugation.

Prior to the fishmeal process, 35 kg of carp viscera were thawed overnight at ambient temperature and minced in a meat grinder. Afterwards, the raw material was cooked at 95–100 °C for 30 min. It was then sifted in a 14 tyler sieve for removal of spines and scales. Centrifugation of material for separation of fractions and storage of the oil fraction were carried out under conditions similar to the ensilage process.

2.3. Oil refinement methodology

The steps of the crude oil refinement process (degumming, neutralisation, washing, drying, bleaching, winterisation and deodorisation) were carried out employing the operating conditions described below.

The degumming step was carried out for 30 min at 80 °C and 500 rpm agitation, with addition of 1.0% of phosphoric acid (85% v/v) in relation to the oil mass. The neutralisation step occurred for 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 7000*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 a contact time of 20 min. Filtration was carried out in a Büchnner funnel with a pre-layer of diatomaceous earth. The refining steps (degumming, neutralisation, washing, drying and bleaching) were carried out at a manometric pressure in a vacuum of approximately 720 mm Hg.

The winterisation step was accomplished in three stages. First, the nucleation process occurred in a refrigerated bath with a water and alcohol mixture, from 30 °C to 5 °C, with a refrigeration rate of 0.62 °C/min and agitation of 500 rpm. In the second and third stages, the crystallisation processes were carried out without agitation, from 5 °C to −4 °C with a refrigeration rate of 2.7 °C/h, and from −4 °C to −5 °C with a refrigeration rate 0.25 °C/h, respectively. Crystallisation must be driven under slow refrigeration in order to supply large crystals and stable polymorphic forms. Small crystals, formed from fast refrigeration, cannot be easily filtered. Separation of the liquid olein and solid stearin fractions was carried out by centrifugation at 7000*g* for 20 min.

In the deodorisation step, oil was loaded in a vessel under vacuum (750 mm Hg) with one opening connected to a condenser, in order to remove the volatiles from the system. This vessel also possessed a steam inlet, provided by a steam boiler, with a valve controlling the outflow. Deodorisation of the winterised oil was carried out at 220 °C for 60 min with 5% steam (based on oil mass).

2.4. Analytical methodology

Free fatty acids (FFA, Ca 5a-40) and peroxide value (PV, Cd 8–53) were determined according to American Oil Chemists Society (AOCS) (1980) methodologies. The anisidine value (AV, Cd 18–90) and phosphorus content (PC, Ca 12–55) were determined according to American Oil Chemists Society (AOCS) (1997) methodologies.

FFA method was used, based on titration with a sodium hydroxide solution (phenolphthalein as an indicator) of the oil, suitably diluted with an ethyl alcohol-ethyl ether mixture. Results are expressed in % oleic acid.

The peroxide value method was used, based on titration with a sodium thiosulfate solution of the oil diluted with an acetic acid–chloroform mixture and then treated with potassium iodide. Results are expressed as milliequivalents/kg oil.

The anisidine value (AV) was determined in fish oil using a spectrophotometer (Quimis model Q-108DRM, Quimis, Diadema, Brazil), based on the reaction between α- and β-unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent. AV is expressed as 100 times the absorbance measured at 350 nm in a 1-cm path length cuvette.

Thiobarbituric acid value (TBA) was determined according to Vyncke (1970). TBA was determined using a spectrophotometric method, calculated from a standard curve obtained by reacting known amounts of 1,1,3,3 tetramethoxypropane with TBA. Results are expressed as mg malonaldehyde/kg oil.

Phosphorus content (PC) was determined in fish oil using a spectrophotometric method (Quimis model Q-108DRM).

Oil colour was determined, using the Lovibond method (Lovibond Colour Staler Tintometer, Model F, England), as described by Windsor and Barlow (1984), fixing the colour yellow at 30 units and varying the colour red.

The analyses of FFA, PV, AV, TBA, PC and colour were done in crude, degummed, neutralised, bleached, winterised and deodorised oils, in triplicate.

For fatty acid identification and quantification, chromatographic analysis was carried out for crude, bleached and refined carp oils. Fatty acid profiles were determined by preparation of methyl esters as described by Metcalfe and Schimitz (1966).

The fatty acid methyl esters (FAME) were identified by gas chromatography (Varian 3400 CX, Palo Alto, CA) equipped with an Agilent DB-23 (Santa Clara, CA) capillary column (60 m × 0.25 mm, 0.25 μm film thickness). FAME analysis was carried out, in duplicate, by injecting 1.0 μl; split ratio 1:50). GC conditions were as follows: injection temperature 215 °C, flame ionisation detector temperature 215 °C, flow rate of helium carrier gas 1.0 ml/min and linear speed 24 cm/s, oven temperature held at 110 °C for 5 min, then increased to 215 °C at 5 °C/min and held at 215 °C for 24 min. The FAME were identified by direct comparison of their retention times with standards (NU-CHEK GLC-87), and were quantified as the percentage area of each FAME mixture.

2.5. Statistical methodology

Characterisation values for the carp crude, neutralised, degummed, bleached, winterised and deodorised oils, as well as fatty acid profiles and lipid classes, were compared using the Tukey HSD test of differences of means (Box, Hunter, & Hunter, 1978), with Statistica 6.0 (Statsoft, Tulsa, OK). Values were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Fish oil characteristics

Table 1 presents free fatty acids percentage (FFA), peroxide value (PV), anisidine value (AV), thiobarbituric acid (TBA), phosphorus content (PC) and Lovibond colour (LC) values for carp crude, degummed, neutralised, bleached, winterised and deodorised oils obtained by fishmeal and ensilage processes. Also in this table are comparisons of oil characteristics by Tukey test of differences of means.

Crude oils obtained (Table 1) possessed significant differences ($p < 0.05$) for FFA, AV, TBA, PC and LC between processes. The PV values for both processes were not significantly difference ($p > 0.05$). For both processes FFA, PV, AV, TBA, PC and LC values were significantly affected ($p < 0.05$) by neutralisation oils.

Crude oil obtained by the ensilage possessed higher FFA values than crude oil produced by the fishmeal process (Table 1). FFA contents are usually associated with undesirable flavour and textural changes when they are present in fats and oils. In the oil processing industry, FFA are determined for providing an indication of the amount of alkali to be used for their removal as soaps during the refinement process (Sathivel et al., 2003).

The initial FFA content in ensilage crude oil is due to action of endogenous enzymes present in the viscera prior to acidification (Reece, 1981). Zhou et al. (1995) studied the FFA content of lipids during ensilage acidification of minced herring; in this study, the maximum FFA level was about 6%. FFA content of fishmeal crude oil presented in Table 1 is similar to the one found by Chantachum, Benjakul, and Sriwirat (2000). The authors studied the separation and quality of fish oil from precooked and non-precooked tuna heads, obtaining FFA of approximately 3% for oils separated from non-precooked heads heated at 95 °C for 30 min.

In Table 1 it is observed that there was a significant difference ($p < 0.05$) between the crude and degummed oils in the two processes. It can also be verified that there was an increase in FFA and LC values. Therefore, the degumming step effectively removed PC and decreases in PV, AV, TBA occurred after oil degumming. Traces of phosphoric acid remaining in the oil after acid degumming treatment are responsible for the increase of acidity observed in the degummed oil. The reduction in PV, AV, and TBA was probably due to absorption of primary and secondary oxidation compounds by the hydrated gum.

For neutralised, bleached, winterised and deodorised oils (Table 1) obtained by both processes, there was observed a decrease of FFA in relation to the crude oil; this is consistent with the function of the neutralisation step, which is to remove most of the FFA; in the bleaching step, removal of the soapstocks residues occurred (Rossi, Gianazza, Alamprese, & Stanga, 2003), and during the deodorisation step, vaporisation of odoriferous compounds and free fatty acids took place (Ceriane and Meirelless, 2007). Under appropriate processing conditions, FFA can be reduced by up to 50% during deodorisation. An acceptable level of FFA in refined fish oil is between 1.8% and 3.5% (Sathivel et al., 2003).

The winterised oils (Table 1) did not differ ($p > 0.05$) between both processes. The winterisation step is used to concentrate PUFAs (Ganga et al., 1998). In addition, the parameters analysed in Table 1 not are affected by winterisation.

The first stage of the oxidation process is characterised by the production of hydroperoxides, which is usually measured as the peroxide value (Aidos et al., 2003). After degumming and neutralisation steps, the bleached and winterised oils obtained through the fishmeal and ensilage processes possess PV in relation to the crude oil of 47% and 43%, respectively. This is consistent with the function of the bleaching earth to remove impurities, such as primary oxidation products. The removal of these impurities improves the sensory quality and oxidative stability of the refined oil (Rossi et al., 2003). The PV is below the demanded level for human consumption of 8 meq/kg oil (Boran, Karaçam, & Boran, 2006), and for both crude oils provides an index of oxidative quality.

In the present study, anisidine values (AV) and thiobarbituric acid (TBA) values of the fishmeal crude oil were higher than values of ensilage crude oil (Table 1); this was due to the use of high temperatures during the fishmeal manufacturing process, which potentially causes increased oxidation. After degumming and neutralisation steps, the bleached and winterised oils obtained by both processes were lower in AV in relation to the crude oil by approximately 65% and 72%, respectively. It was observed that TBA reduction in relation to the crude oil was approximately 79% and 71%, respectively.

AV is associated with the second stage of oxidation represented by further degradation of lipids through a radical oxidation process initiated by hydroperoxides. The resulting nonvolatile secondary end-products (high molecular weight saturated and unsaturated carbonyl compounds; Aidos et al., 2003). TBA value is widely used as an indicator of the degree of lipid oxidation, with the decomposition of hydroperoxides and formation of secondary products of oxidation such as aldehydes, ketones and alcohols (Boran et al., 2006). An acceptable AV for good-quality crude fish oils is lower than 20 (Hamilton, Kalu, McNeill, Padley, & Pierce, 1988). In Table 1, AV values for deodorised oils were similar to results cited by Aidos et al. (2003), of 8.9 for herring oil. The TBA value in Table 1 is in agreement with the demanded patterns for quality and acceptability of oils for human consumption, of 7–8 mg malonaldehyde/kg oil (Boran et al., 2006). In this manner, AV value and thiobarbituric acid value for both deodorised oils indicate good oxidative quality.

In the deodorised oils there is an increase in PV, AV and TBA, in relation to the bleached and winterised oils. The increase of values is due to the high temperatures used in the deodorisation step. Increased exposure of the oil to heat will increase the susceptibility to oxidation and peroxide formation, and decomposition of hydroperoxides, with the formation of secondary products of oxidation, such as aldehydes, ketones and alcohols (Ceriane & Meirelless, 2007; Manral, Pandey, Jayathilakan, Radhakrishna, & Bawa, 2008).

In the degumming step the phosphorus content (Table 1) in oils obtained by ensilage and fishmeal processes decrease by approximately 53% and 52%, respectively. After the neutralisation step, all phosphorus was removed. Values for crude oils were lower than results cited by Immanuel, Sathaivan, Shankar, Peter and Palavesam (2009), for *Sufflamen capistratus* oil (343 ppm). Sathivel et al. (2003) studied the oil production from catfish viscera and found phosphorus concentrations of 107.6 ppm and 99.2 ppm in crude oil and degummed oil (citric acid), respectively. After the neutralisation step the phosphorus was removed.

Lovibond colour (LC) was higher in ensilage crude oil than that obtained by fishmeal process (Table 1). Oil pigmentation during ensiling is shown to be caused by the release of the acid hydrolysis product of haemoglobin, haem (Reece, 1981). In ensilage oil an increase in free fatty acids (FFA) content occurs, resulting in formation of lipid–protein complexes, and consequently increasing colour.

Table 1
Characterisation of carp oils obtained by two processes.

Step process/indices	Fishmeal oil						Ensilage oil					
	Crude	Degummed	Neutralised	Bleached	winterised	Deodorised	Crude	Degummed	Neutralised	Bleached	Winterised	Deodorised
Free fatty acids (FFA)% oleic acid	3.35 ± 0.02^{e}	5.31 ± 0.02^{f}	0.56 ± 0.02^{d}	0.45 ± 0.02^{b}	0.47 ± 0.02^{b}	0.08 ± 0.01^{a}	6.63 ± 0.01^{g}	7.23 ± 0.01^{h}	0.52 ± 0.01^{c}	0.44 ± 0.01^{b}	0.46 ± 0.01^{b}	0.09 ± 0.02^{a}
Peroxide value (PV) meq/ kg oil	3.38 ± 0.01^{e}	2.50 ± 0.01^{c}	1.98 ± 0.03^{b}	1.79 ± 0.02^{a}	1.78 ± 0.01^{a}	4.18 ± 0.03^{f}	3.36 ± 0.02^{e}	2.80 ± 0.02^{d}	2.00 ± 0.02^{b}	1.80 ± 0.01^{a}	1.79 ± 0.01^{a}	4.20 ± 0.3^{f}
Anisidine value (AV)	13.4 ± 0.4^{i}	12.3 ± 0.3^{h}	8.2 ± 0.3 ^{d}	4.8 ± 0.2^{b}	4.7 ± 0.1^{b}	9.1 ± 0.3^{e}	10.3 ± 0.3^{g}	9.8 ± 0.2^{f}	7.4 ± 0.4^{c}	3.1 ± 0.3^{a}	2.9 ± 0.2^{a}	8.6 ± 0.3^{e}
Thiobarbituric acid (TBA) mg malondialdehyde/ kg oil	6.7 ± 0.1^{i}	5.6 ± 0.1^{g}	3.2 ± 0.1^{f}	1.5 ± 0.3^{e}	1.49 ± 0.1^{e}	6.1 ± 0.3^{h}	1.10 ± 0.1^{d}	0.77 ± 0.1^{c}	0.58 ± 0.1^{b}	0.40 ± 0.2^{a}	0.41 ± 0.2^{a}	6.0 ± 0.3^{h}
Phosphorus content (PC) ppm	201^{c}	94.2^{a}	–	–	–	–	251^{d}	12^{1b}	–	–	–	–
Lovibond colour (LC) Red colour (30 Yellow)	5.0 ± 0.1^{c}	7.3 ± 0.1^{d}	4.0 ± 0.2^{b}	2.4 ± 0.2^{a}	2.3 ± 0.2^{a}	4.0 ± 0.1^{b}	16 ± 0.2^{f}	18 ± 0.2^{g}	11.3 ± 0.2^{e}	2.0 ± 0.2^{a}	2.1 ± 0.2^{a}	4.2 ± 0.2^{b}

Mean value ± standard (in triplicate).
Different superscript letters in the same line are significantly different ($p < 0.05$).

In Table 1, a decrease in LC in relation to the crude oil can be observed for after neutralisation and bleaching. The decrease in LC in bleached oils is due to the fact that the adsorbents used for bleaching can remove pigments and other impurities, such as trace metals, phospholipids, and oxidation products (Rossi et al., 2003). However, the increase in LC in refined oils and the alteration of final colour (Table 1) is related to the oxidation of colourless components or the existing colour pigments becoming fixed by high temperatures (Antoniassi et al., 1998).

The characteristic yellow-orange colour of fish oil is the result of deposition of dietary carotenoids (Luterotti, Franko, & Bicanic, 1999). Carotenoids are lipid-soluble antioxidants and their biological properties are related to their structures. The single and double bonds repeated in the polyenic chain determine their antioxidant properties (Hidalgo, Brandolini, Pompei, & Piscozzi, 2006). Oil colour depends on the temperature to which it is submitted. Increase of colour intensity is due to accumulation of nonvolatile decomposition products such as oxidised triacylglycerols and FFA (Antoniassi et al., 1998).

Refined oils of both processes (Table 1) were similar ($p > 0.05$) for FFA, PV, AV, TBA and LC values. PV, AV, TBA and LC increased after the deodorisation step.

3.2. Fatty acids profiles

The fatty acids profiles of carp oil from the refinement step are shown in Table 2. It can be verified that the crude and bleached oils were not significantly different between both processes. However, significant difference ($p < 0.05$) was observed for refined oils in relation to crude and bleached oils. Also, it can be verified that the refined oils were not significantly different between both processes. The major identified fatty acids were C18:1$n-9$ (oleic), C16:0 (palmitic), C16:1 (palmitoleic), C18:2$n-6$ (linoleic), and C18:3$n-3$ (linolenic), constituting around 67% of the total fatty acids of fishmeal and silage oils.

Palmitic acid was the predominant saturated fatty acid (SFA), accounting for about 50% of all SFA (Table 2). Oleic acid was identified as the most abundant monounsaturated fatty acid (MUFA) for refined oils. Palmitoleic acid was the second most abundant MUFA for crude, bleached and refined oils for both processes. High levels of oleic, palmitoleic, and arachidonic acids have been reported as a characteristic property of freshwater fish oils (Guler, Kiztanir, Aktumsek, Citil, & Ozparlak, 2008). Linoleic and linolenic acids were the predominant polyunsaturated fatty acids (PUFA).

The increase of *trans* FA content for oleic and linoleic acids in refined oils is due to the high temperatures used in the deodorisation step. FA are labile molecules which, when exposed to heat treatment, can be lost through different chemical transformations (Berdeaux et al., 2007; Ceriane and Meirelless, 2007; Fournier et al., 2007). The initial content of *trans* PUFA in crude oils may increase in refined oils, due exclusively to deodorisation, since this reaction does not occur during preceding steps of the refinement process. In European countries, one quality parameter for refined edible oils is that the level of *trans* FA should be lower than 1.0% (Ceriane and Meirelless, 2007). In this manner, both refined oils obtained in this study were acceptable in relation to the *trans* level.

DHA and arachidonic (AA) contents in crude, bleached and refined oils were lower than those in carp oil cited by Rasoarahoma, Barnathan, Bianchini, and Gaydou (2004), of 6.7% and 5.9%, respectively, and higher than cited by Druzian, Marchesi, and Scamparini (2007), of 1.02% and 1.16% respectively. However, EPA content was higher than EPA contents quoted by these authors, of 3.4% and 2.4%, respectively. The amount of EPA and DHA suggested for daily ingestion is within the range 200–1000 mg (Inhamuns & Franco, 2008).

The reason for the variation in the results observed for EPA, DHA and AA is likely to be the feeding habits of the fish. The percentage of PUFA, such as EPA and DHA, in fish muscle, is dependent on their diets. Variations in fatty acid composition might be related to changes in the nutritional habits of the fish (Druzian et al., 2007; Inhamuns and Franco, 2008).

The lipid classes in carp oil from the refinement step are shown in Table 3. It can be verified that there was no significant difference for Σ SFA and Σ PUFA of crude and bleached oils for the fishmeal and ensilage processes. The refined oils in relation to crude and bleached oils showed significant differences ($p < 0.05$) for Σ SFA, Σ PUFA and $n-3/n-6$ ratio for both processes. In general, fish are relatively low in SFA (<30%), except for certain species; in Table 3 it can be verified that SFA content was around 27% for crude and bleached oils. MUFA contents of carp oil were higher than SFA in crude, bleached and refined fishmeal and silage oils (around 41.8%). The publications of Guler et al. (2008) and Druzian et al. (2007) reported similar results for carp oil in the winter, of 41.1% and 43.1%, respectively. PUFA contents in carp have been reported to vary over a very wide range, from 11.6–15.7% (Bieniarz, Koldras, Kaminski, & Mejza, 2000) to 32.3–34.5% (Geri, Poli, Gualtieri, Lupi, & Parisi, 1995) of total fatty acids. In this work, PUFA contents of crude, bleached and refined fishmeal and silage oils (Table 3) were superior to that of oil examined by Druzian et al. (2007), of 23%.

Refined oils presented a decrease in Σ SFA content and an increase in the Σ PUFA content in relation to the bleached oils due to the winterisation. This step is used to concentrate PUFAs; it is based on differences in melting points, using low-temperature crystallisation, which was originally developed to separate certain triacylglycerides (TAG), fatty acids, esters and other lipids which are highly soluble in organic solvents. Winterisation is a thermomechanical separation process where the high and low-melting TAG are separated by partial crystallisation, followed by filtration. Fat solubility in organic solvents decreases with increasing mean molecular weight and increases with increasing unsaturation (Ganga et al., 1998; Shahidi & Wanasundara, 1998). Thus, the concentration of unsaturated and polyunsaturated fatty acids in refined oil occurred, with consequent decrease in Σ SFA and increase in Σ PUFA.

Table 3 shows that the unsaturated and polyunsaturated fatty acids (MUFA + PUFA) contents in refined fishmeal and silage oils represent 69.6% of the total fatty acids. Therefore, they are a rich source of unsaturated and polyunsaturated fatty acids. In this table, the $n-3/n-6$ ratio in crude and bleached fishmeal and silage oils were 1.14 and 1.15, respectively; for refined oils of the two processes the $n-3/n-6$ ratio was 1.05. This ratio is similar to the one cited by Guler et al. (2008) for carp oils. The $n-3/n-6$ ratio in the total lipids of freshwater fish is usually between 0.5 and 3.8 (Henderson & Tocher 1987). The $n-3/n-6$ ratio has been suggested as a useful indicator for comparing relative nutritional values of fish oils. An increase in the human dietary $n-3/n-6$ fatty acid ratio is essential in the diet, to help prevent coronary heart disease by reducing plasma lipids, and to reduce cancer risk. An increase in the dietary $n-3/n-6$ ratio also seems to be effective in preventing toxic shock syndrome and cardiomyopathy (Guler et al., 2008).

Table 2
Fatty acid profiles (%) of carp crude, bleached and refined oils obtained by two processes.

Fatty acids	Fishmeal Oil			Ensilage Oil		
	Crude*	Bleached*	Deodorised*	Crude*	Bleached*	Deodorised*
11:0	0.08 ± 0.01^{b}	0.08 ± 0.01^{b}	0.06 ± 0.01^{ab}	0.08 ± 0.01^{b}	0.08 ± 0.01^{b}	0.05 ± 0.01^{a}
12:0	0.30 ± 0.01^{ab}	0.29 ± 0.01^{ab}	0.28 ± 0.01^{a}	0.31 ± 0.02^{ab}	0.32 ± 0.02^{b}	0.29 ± 0.01^{ab}
13:0	0.24 ± 0.01^{b}	0.23 ± 0.01^{b}	0.20 ± 0.01^{a}	0.25 ± 0.01^{b}	0.23 ± 0.02^{b}	0.20 ± 0.01^{a}
14:0	3.79 ± 0.02^{b}	3.80 ± 0.01^{b}	3.11 ± 0.02^{a}	3.82 ± 0.03^{b}	3.81 ± 0.01^{b}	3.10 ± 0.01^{a}
15:0	1.20 ± 0.03	1.22 ± 0.01	1.20 ± 0.01	1.20 ± 0.01	1.21 ± 0.01	1.20 ± 0.01
16:0	16.19 ± 0.03^{b}	16.20 ± 0.02^{b}	15.50 ± 0.01^{a}	16.14 ± 0.04^{b}	16.16 ± 0.01^{b}	15.50 ± 0.01^{a}
17:0	1.11 ± 0.01^{b}	1.10 ± 0.02^{b}	1.00 ± 0.01^{a}	1.10 ± 0.02^{b}	1.12 ± 0.01^{b}	1.00 ± 0.01^{a}
18:0	3.15 ± 0.01^{c}	3.13 ± 0.02^{abc}	3.10 ± 0.01^{a}	3.17 ± 0.02^{c}	3.15 ± 0.01^{c}	3.11 ± 0.01^{ab}
20:0	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
21:0	0.17 ± 0.02	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
22:0	0.18 ± 0.02	0.17 ± 0.01	0.18 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
23:0	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
14:1$n-5$	0.17 ± 0.01^{c}	0.16 ± 0.01^{bc}	0.14 ± 0.01^{a}	0.18 ± 0.01^{c}	0.15 ± 0.03^{abc}	0.14 ± 0.01^{ab}
15:1$n-5$	0.42 ± 0.01^{b}	0.43 ± 0.01^{b}	0.35 ± 0.01^{a}	0.43 ± 0.02^{b}	0.44 ± 0.01^{b}	0.36 ± 0.02^{a}
16:1$n-7$	8.08 ± 0.04^{b}	8.06 ± 0.02^{b}	7.64 ± 0.01^{a}	8.04 ± 0.03^{b}	8.09 ± 0.02^{b}	7.65 ± 0.01^{a}
17:1$n-7$	1.27 ± 0.03^{b}	1.28 ± 0.01^{b}	1.20 ± 0.01^{a}	1.30 ± 0.03^{b}	1.26 ± 0.04^{b}	1.43 ± 0.01^{c}
18:1$n-9$ *cis*	25.82 ± 0.03^{a}	25.84 ± 0.01^{a}	26.45 ± 0.01^{b}	25.80 ± 0.02^{a}	25.82 ± 0.01^{a}	26.47 ± 0.01^{b}
18:1$n-9$ *trans*	0.23 ± 0.02^{a}	0.25 ± 0.01^{a}	0.35 ± 0.01^{b}	0.21 ± 0.02^{a}	0.23 ± 0.02^{a}	0.33 ± 0.01^{b}
20:1$n-9$	1.83 ± 0.05^{a}	1.80 ± 0.02^{a}	1.92 ± 0.01^{b}	1.85 ± 0.03^{a}	1.83 ± 0.01^{a}	1.90 ± 0.01^{b}
22:1$n-9$	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.18 ± 0.02^{b}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}	0.18 ± 0.01^{b}
24:1 $n-9$	3.99 ± 0.02^{b}	4.00 ± 0.01^{b}	3.38 ± 0.01^{a}	3.99 ± 0.01^{b}	3.98 ± 0.01^{b}	3.39 ± 0.01^{a}
18:2$n-6$ *trans* 9,12	0.31 ± 0.01^{a}	0.31 ± 0.01^{a}	0.51 ± 0.01^{b}	0.30 ± 0.01^{a}	0.31 ± 0.01^{a}	0.52 ± 0.01^{b}
18:2 $n-6$ *cis*	9.17 ± 0.01^{a}	9.17 ± 0.01^{a}	9.68 ± 0.01^{b}	9.17 ± 0.01^{a}	9.16 ± 0.01^{a}	9.69 ± 0.02^{b}
18:3$n-6$	0.34 ± 0.02^{abc}	0.33 ± 0.01^{ab}	0.35 ± 0.02^{bc}	0.32 ± 0.01^{ab}	0.30 ± 0.02^{a}	0.37 ± 0.01^{c}
18:3 $n-3$	7.16 ± 0.01^{a}	7.17 ± 0.01^{a}	7.52 ± 0.01^{b}	7.17 ± 0.01^{a}	7.18 ± 0.01^{a}	7.50 ± 0.01^{b}
20:2$n-6$	0.43 ± 0.02^{a}	0.43 ± 0.01^{a}	0.61 ± 0.02^{b}	0.42 ± 0.01^{a}	0.41 ± 0.01^{a}	0.60 ± 0.01^{b}
20:3 $n-6$	0.42 ± 0.02^{a}	0.41 ± 0.01^{a}	0.59 ± 0.01^{b}	0.43 ± 0.02^{a}	0.42 ± 0.01^{a}	0.58 ± 0.01^{b}
20:3$n-3$	1.43 ± 0.01^{a}	1.41 ± 0.02^{a}	1.56 ± 0.01^{b}	1.44 ± 0.02^{a}	1.42 ± 0.01^{a}	1.57 ± 0.01^{b}
20:4$n-6$ (AA)	1.24 ± 0.02^{a}	1.23 ± 0.01^{a}	1.70 ± 0.01^{b}	1.22 ± 0.01^{a}	1.21 ± 0.01^{a}	1.68 ± 0.01^{b}
20:5$n-3$ (EPA)	3.82 ± 0.01^{a}	3.83 ± 0.01^{a}	3.90 ± 0.01^{b}	3.81 ± 0.2^{a}	3.83 ± 0.01^{a}	3.91 ± 0.01^{b}
22:2$n-6$	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.07 ± 0.02^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.07 ± 0.02^{b}
22:6$n-3$ (DHA)	1.20 ± 0.02^{a}	1.20 ± 0.01^{a}	1.30 ± 0.01^{b}	1.20 ± 0.01^{a}	1.22 ± 0.01^{a}	1.31 ± 0.01^{b}
Σnu**	5.73 ± 0.30^{b}	5.76 ± 0.38^{b}	5.11 ± 0.50^{a}	5.71 ± 0.20^{b}	5.79 ± 0.22^{b}	5.10 ± 0.26^{a}

AA: arachidonic acid; EPA: eicosapentanoic acid; DHA: docosahexanoic acid.
Different superscript letters in the same line are significantly different ($p < 0.05$).
* Mean value ± standard ($n = 3$).
** Σ sum of unidentified fatty acids.

Table 3
Lipids classes (% of total fatty acids) in carp crude, bleached and refined oils obtained by two processes.

	Fishmeal oil			Ensilage oil		
	Crude	Bleached	Deodorised	Crude	Bleached	Deodorised
Σ SFA*	26.86 ± 0.19[b]	26.85 ± 0.15[b]	25.27 ± 0.14[a]	26.87 ± 0.20[b]	26.86 ± 0.14[b]	25.25 ± 0.13[a]
Σ MUFA**	41.89 ± 0.20	41.90 ± 0.11	41.83 ± 0.22	41.88 ± 0.15	41.87 ± 0.16	41.85 ± 0.10
Σ PUFA***	25.54 ± 0.15[a]	25.51 ± 0.12[a]	27.79 ± 0.14[b]	25.50 ± 0.13[a]	25.48 ± 0.12[a]	27.80 ± 0.13[b]
$n-3$	13.61 ± 0.10[a]	13.61 ± 0.15[a]	14.28 ± 0.10[b]	13.62 ± 0.10[a]	13.65 ± 0.10[a]	14.29 ± 0.10[b]
$n-6$	11.93 ± 0.10[a]	11.90 ± 0.10[a]	13.51 ± 0.10[b]	11.88 ± 0.15[a]	11.83 ± 0.10[a]	13.51 ± 0.10[b]
$n-3/n-6$	1.14 ± 0.01[b]	1.14 ± 0.01[b]	1.05 ± 0.01[a]	1.15 ± 0.01[b]	1.15 ± 0.01[b]	1.05 ± 0.01[a]

Mean value ± standard error ($n = 3$).
Different superscript letters in the same line are significantly different ($p < 0.05$).
* Σ SFA: sum of unsaturated.
** Σ MUFA: sum of monounsaturated.
*** Σ PUFA: sum of polyunsaturated.

4. Conclusions

In this study, carp (*Cyprinus carpio*) crude oils obtained by the ensilage and fishmeal processes showed significant differences ($p < 0.05$) for FFA, TBA, AV, PC and LC values. Also, differences ($p < 0.05$) were observed between the crude and bleached oils. The FFA, TBA, AV, PV and LC values decreased after bleaching of the crude oils. The refined oils obtained by both processes did not differ. Carp oil refinement improved the fish oil characteristics, because it removed components that cause colour, free fatty acids and lipid oxidation products.

The fatty acids profiles of refined carp oils were significantly different ($p < 0.05$) in relation to crude oil and bleached oils for both processes. Refinement of the carp oils decreased the Σ SFA content and increased the Σ PUFA content; this occurred due to the winterisation step. The unsaturated and polyunsaturated fatty acids (MUFA + PUFA) contents in refined fishmeal and silage oils were around 69.6% of the total fatty acids. In this study, the $n-3/n-6$ ratio was 1.05 for refined oils, similar to values cited in the literature. Therefore, based on the results obtained in this work, carp viscera refined oil can be considered a rich source of essential fatty acids of the $n-3$ and $n-6$ series.

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