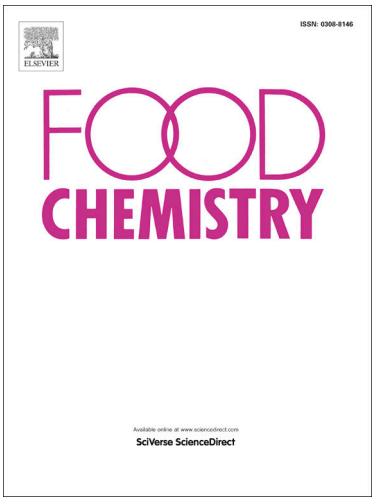
Journal Pre-proofs



Optimization of oil extraction from caiman fat. Characterization for use as food supplement

Luciana Vera-Candioti, Pamela M.L. Leiva, Florencia Valli, Claudio A. Bernal, Carlos I. Piña, Melina S. Simoncini, Marcela A. González

PII: S0308-8146(21)00761-5
DOI: https://doi.org/10.1016/j.foodchem.2021.129755
Reference: FOCH 129755

To appear in: *Food Chemistry*

Received Date: 29 October 2020
Revised Date: 29 March 2021
Accepted Date: 2 April 2021

Please cite this article as: Vera-Candioti, L., Leiva, P.M.L., Valli, F., Bernal, C.A., Piña, C.I., Simoncini, M.S., González, M.A., Optimization of oil extraction from caiman fat. Characterization for use as food supplement, *Food Chemistry* (2021), doi: https://doi.org/10.1016/j.foodchem.2021.129755

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Ltd.

Journal Pre-proofs

OPTIMIZATION OF OIL EXTRACTION FROM CAIMAN FAT. CHARACTERIZATION FOR USE AS FOOD SUPPLEMENT.

Luciana VERA-CANDIOTI[1,2], Pamela M.L. LEIVA[2,3,4], Florencia VALLI[3,4], Claudio A. BERNAL[1,2], Carlos I. PIÑA[3,4], Melina S. SIMONCINI[3,4] and Marcela A. GONZÁLEZ[2,*]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Avda. Rivadavia 1917, CP C1033AAJ, Buenos Aires, Argentina.

[2]Departamento de Ciencias Biológicas, Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Ciudad Universitaria, 3000, Santa Fe, Argentina.

[3]CICyTTP-CONICET/Prov. Entre Ríos/UADER, España 149, Diamante, Entre Ríos, Argentina.

[4]Proyecto Yacaré, Laboratorio de Zoología Aplicada: Anexo Vertebrados, FHUC/UNL, Aristóbulo del Valle 8700, Santa Fe, Santa Fe, Argentina.

[*] **Corresponding Author:** Prof. Marcela González. PhD, Cátedra de Bromatología y Nutrición. Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, C.C. 242. (3000) Santa Fe, Argentina. Telephone: 54-342-4575211 Fax: 54-342-4575221, e-mail: maidagon@fbcb.unl.edu.ar

Journal Pre-proofs

Abstract

The leather of *Caiman latirostris* is highly appreciated in the fashion industry and the meat is valued as an important food but its fat are usually discarded because it has no commercial value. However it is an alternative source of essential fatty acids and could be used for human consumption. The aim was to optimize the oil extraction from *Caiman latirostris* fat and to carry out the chemical and microbiological characterization for its use as food supplement. The oil obtained by fusion method contains fatty acids with high nutritional quality such as oleic acid (34%), linoleic acid (30%) and α-linolenic acid (2%). The atherogenicity index was 0.29 and the thrombogenicity index 0.47. The presence of mesophilic aerobic bacteria, coliform bacteria, *Escherichia coli* and *Salmonella* were not observed, and the oil is stable for 4 months at 25ºC and for at least 8 months in an inert atmosphere at 25ºC.

Keywords: BROAD-SNOUTED CAIMAN, OIL EXTRACTION, CAIMAN FAT, FOOD SUPPLEMENT, ESSENTIAL FATTY ACIDS.

1. Introduction

In Argentina, Proyecto Yacaré aims to conserve the Broad-snouted caiman (*Caiman latirostris*; Larriera and Imhof, 2006; Larriera, 2011) through sustainable use as a renewable resource. The leather of *Caiman latirostris*, like that of many other crocodilians, is highly appreciated in the fashion industry (Larriera, 2011). The meat is beginning to be valued as an important source of high-quality animal protein and essential fatty acids (FA) important for human nutrition, especially if it can be enriched by modification of the animals' diets (Piña et al., 2016; Simoncini et al., 2020). On the other hand, the fat is usually discarded, increasing environmental contamination and a potential source of valuable natural oils (Kluczkovski et al., 2016).

Journal Pre-proofs

In Argentina and other countries of the world, the commercialization of products and by-products of species such as *Caiman latirostris* born in captivity is allowed, as long as they do not damage their survival in their habitat, they comply with the established regulations and carry out their operations under certain guidelines and effective controls (Department of the Interior-Fish and Wildlife Service of United States-Federal Register Vol. 78, No. 122; Government Secretary for the Environment and Sustainable Development of Argentine-EX-2019-66176112-APN-DRIMAD#SGP Regulation of the live specimens, products and by-products of the species broad-snouted caiman (*Caiman latirostris*) and *Caiman yacare*.

Foods and fats rich in poly-unsaturated fatty acids (PUFA) are observed with great attention by consumers, due to their close relationship with health benefits. There are two essential FA, linoleic acid (LA: c9,c12-18:2, n-6) and α-linolenic acid (ALA: c9,c12,c15-18:3, n-3) that may modulate circulating lipids and reduce the risk of cardiovascular disease (CVD) (Simopoulos, 2016). These two essential FA, as well as, long-chain FA derived from them such as eicosapentanoic acid (EPA: c5,c8,c11,c14,c17-20:5), docosahexaenoic acid (DHA:c4,c7,c10,c13,c16,c19-22:6) and arachidonic acid (ARA:c5,c8,c11,c14-20:4), are also required for the regulation of many biological functions. In addition, a balanced relationship between dietary PUFA n-6 and n-3 (5:1-10:1) is recommended for the reduction of the incidence of CVD and inflammatory disorders (Simopoulos, 2016).

Based on the characteristics of crocodile meat, we hypothesized that the oil extracted from fat could be used for food supplement. However, doing so requires compliance with national legislation. **C**hapter VII of the Argentine Food Code (CAA, Código Alimentario Argentino) specifies the requirements for fats and oils in food in Argentina. According to the CAA, edible animal fats or animal dietary fats are defined as those separated from the fatty tissues and clean and unaltered fat parts of bovine animals, sheep, pigs or goats, slaughtered for consumption under sanitary conditions, under official health inspection. The CAA does not specify the extraction of oils from non-traditional animal, such as caimans. Furthermore, the American Oil Chemists' Society (AOCS) has published official methods to determine the oil content

in matrices such as meat, fruit, seeds, fish, etc., and many of them are based on solvent extraction but does not include any method for extracting oil from caiman fat.

The methodology to extract oil from food depends on the sample matrix; ranging from conventional technologies such as mechanical pressing (Kajal and Deepu, 2015; Bonilla-Mendez et al., 2018) and extraction with solvents such as Soxhlet, Bligh-Dyer and Folch (Folch et al., 1956; Buthtelezi et al., 2012; Vidal et al., 2012; Huang et al., 2015; Kajal and Deepu, 2015), to more recent technologies based in extraction methods such as supercritical fluids, microwave assisted extraction, ultrasound assisted extraction and enzymatic hydrolysis (Bucio et al., 2016; Kaspars, 2019). Most of the latest technologies require specific equipment and the addition of external agents to promote oil extraction.

Huang et al. (2015) used a mixture of dichloromethane and methanol to extract oil from crocodile fat, but they only evaluated oil extraction yield and its antioxidant capacity. Buthelezi et al. (2012), using an adaptation of the Folch method to extract oil from crocodile fat, only evaluated antimicrobial and anti-inflammatory activities of the oil obtained. Similarly, Kluczkovski et al. (2016) performed total lipid extraction using the Folch method and only evaluated the FA content of samples of fat of *Melanosuchus niger*, to understand their nutritional quality.

To the best of our knowledge, there are no published analytical methodologies for the extraction of oil from caiman fat for use as food supplement. Consequently, the aim of this work was to optimize the oil extraction from *Caiman latirostris* fat and to carry out the chemical and microbiological characterization for its use as food supplement. The optimization procedure included a quality control of the oil minimizing the content of extraction solvents and microbial load, as well as obtaining oil with low oxidability and high stability over time.

2. Materials and Methods

2.1 Animal breeding and sample collection

Journal Pre-proofs

The Broad-snouted caimans used for this study were provided by "Proyecto Yacaré" a sustainable management program developed in Santa Fe province, Argentina, where *Caiman latirostris* are utilised through "ranching", i.e., harvesting of wild eggs with the collaboration of local people, artificial incubation and raising of hatchlings on the farm (Larriera, 2011). Hatchlings are kept in captivity for approximately one year; then, about 10% are reintroduced to their nesting sites and 90% are used for the production of meat and skins (Larriera et al., 2008), which are marketed by *Yacarés Santafesinos* (SENASA- National Service of Health and Food Quality of Argentina – DAPFV – CAPA, Official authorization of the establishment n° 4081 Expte N°: 7005/02, for the commercialization of *Caiman latirostris* meat from breeding establishments in captivity through the ranching system in Santa Fe, Argentine.

Caimans were produced through artificial incubation and reared under controlled captive conditions: within heated greenhouses (30ºC) with access to wet (water) and dry areas. During the study, animals were fed *ad libitum* six days per week, with a base diet composed of ground chicken heads (70%) and dry feed formulated for reptiles (30%) (Simoncini et al., 2020). The caimans are raised on average for two years, when they reached the weight and length suitable for marketing and for this study (total length 89±4 cm, snout-vent length 44±2 cm, weight 3.7±0.4 kg, approximately). It is important to note that all animals used in the experiment appeared healthy, without any pathological signs during the feeding time.

The animals were handled following the protocol of the Proyecto Yacaré (Yacarés Santafesinos/MUPCN, approved slaughterhouse N° 4081) and according to the sanitary conditions established by SENASA for production for human consumption. The management guide of the Crocodile Specialists Group (CSG / IUCN) "Best management practices for crocodilian farming", available on the group's website (http://www.iucncsg.org/content_images/attachments/CSG-BMP.pdf), explicitly specifies the slaughter methods recommended by the IUCN-SSC Crocodile Specialist Group (Expert

Panel, 2013; Hutton, 1992). In the particular case of Yacarés Santafesinos / yacaré project, "Cervical Dislocation" is carried out.

Approximately, between 100 and 120 g of visceral adipose tissue from each of 8 animals used in this study were removed and stored at -20ºC in a freezer until the time of oil extraction. Visceral adipose tissue is all the fat that is in the abdominal cavity.

2.2 Chemicals and Reagents (see in supplementary material)

2.3 Optimization of oil extraction

Four methods of oil extraction based on solvent extraction and melting were evaluated. These methodologies were based on the specifications of the CAA to extract lard of the fat tissues of pigs (Chapter VII of the CAA Article 541) and on solvent extraction used to extract oil from crocodile (Buthtelezi et al., 2012; Huang et al., 2015, Kluczkovski et al., 2016).

Samples were quickly cut into small pieces or crushed, preventing defrosting to prevent softening. To perform extraction, four 10-g portions of finely divided fat were placed in four different glass beakers (S1, S2, S3, and S4). In two of them, 60 mL of solvent (n-hexane, S1), or a mixture of chloroform-methanol-water (30:20:10, v/v/v, S2) were added, and were stirred during 4 hours at a 60 °C. Then, the solvents along with the oil were separated from the solid residue through a filtering process and the solvent was evaporated using a rotary evaporator.

The third portion of these fat was placed in an oven at 60ºC for 5 hours (S3). After the extraction time, the mixture was placed on a porous fabric mesh and with the help of pressure, the oil was extracted.

The last portion was placed in a glass vessel (S4) and heated under constant stirring, controlling the temperature such that it did not exceed 80ºC. As the fat began to melt it was transferred to another container (C2) avoiding the passage of tissue without melting. For this operation, a reticulated mesh was used to retain the solid material, which was returned to S4 for later fusion. Once the melting process was

completed, the resulting solid tissue was placed on a porous fabric mesh and with the help of pressure, the oil was extracted, which was collected and unified with the other oil portions collected in C2.

To each extraction, the weight of the crude oil obtained (WO) was gravimetrically determined and expressed as % w/w of the wet edible tissue (WT). Total yield (%) was expressed as Equation 1.

$$yiel\ (\%) = \frac{WO\ (g)}{WT\ (g)} \times 100 \qquad \text{Equation 1}$$

2.4 Fatty acid composition and nutritional quality indices

The FA composition of the oil and fat of caiman were determined by the standard gas chromatography (Masson et al. 2015). Briefly, FA were converted to fatty acid methyl esters (FAME) and were quantified using a gas chromatograph (Shimatzu GC-2014, Shimadzu Corporation, Kyoto, Japan) fitted with a capillary column CP-Sil 88, 100 m, 0.25 mm id (Varian, Lake Forrest, CA) and flame ionization detector (*see separations conditions in supplementary material*).

The nutritional quality of oil extracted was evaluated by four indices from the ~~fatty acid~~ FA composition data: by calculating the ratio between PUFA and saturated fatty acids (SFA); the Atherogenicity Index (AI, Equation 2); the Thrombogenicity Index (TI, Equation 3) (Ulbricht and Southgate 1991); and, the ratio between Hypocholesterolemic and Hypercholesterolemic fatty acids (HH, Equation 4) (Santos-Silva et al. 2002). Indices based on the functional effects of different fatty acids allow a better assessment of the nutritional quality of lipids in food.

$$AI = \frac{12{:}0 + 4 \times 14{:}0 + 16{:}0}{\sum (MUFA + PUFA)} \qquad \text{Equation 2}$$

$$TI = \frac{12{:}0 + 14{:}0 + 16{:}0}{0.5 \times \sum MUFA + 0.5 \times \sum PUFA} \qquad \text{Equation 3}$$

$$HH = \frac{\text{Hypocholesterolemic fatty acids}}{\text{Hypercholesterolemic fatty acids}} \qquad \text{Equation 4}$$

Journal Pre-proofs

where PUFAs are poly-unsaturated fatty acids, MUFAs are mono-unsaturated fatty acids, hypocholesterolemic FA are c9-18:1+c9,c12-18:2+c5,c8,c11,c14-20:4+c9,c12,c15-18:3+c5,c8,c11,c14,c17-20:5+c7,c10,c13,c16,c19-22:5+c4,c7,c10,c13,c16,c19-22:6; and hypercholesterolemic FA are 14:0 and 16:0.

2.5 Oil physicochemical characterization and initial oxidative parameters

Different parameters related to the oxidative stability of extracted oils were measured using AOCS official methods (AOCS International, 2017) available for oils and fats: iodine value (Cd 1-25), peroxide value (Cd 8-53), saponification index (Cd 3-25), acid index (Cd 3d-63), and *p*-anisidine value (Cd 18-90).

Conjugated Dienes (CD, Equation 5) and Trienes (CT, Equation 6) content were determined by the IUPAC method (1987) measuring the absorbance at 234 and 270 nm of a solution of the oil dissolved in isopropanol. CD and CT were calculated using the following equations:

$$\mathrm{CD} = \frac{\mathrm{Abs}_{234}}{\mathrm{C\ x\ d}} \qquad \text{Equation 5}$$

$$\mathrm{CT} = \frac{\mathrm{Abs}_{270}}{\mathrm{C\ x\ d}} \qquad \text{Equation 6}$$

where: Abs_{234}= absorbance of the solution at 234 nm, Abs_{270}= absorbance of the solution at 270 nm, C= concentration of the oil solution (g/100 mL) and d= length of the cell in cm.

The presence of secondary oxidation compounds was determined initially by means of a qualitative test based on the colorimetric reaction between the phloroglucinol and the aldehyde epidirinic, in hydrochloric acid medium. This test is commonly called the Kreiss index. When the result of the Kreiss index was positive, the secondary oxidation compounds were quantified by the *p*-anisidine value.

Journal Pre-proofs

The antioxidant capacity was measured using DPPH method (free radical scavenging activity) measuring the absorbance at 517 nm of a solution of the oil dissolved in MeOH: ethyl ether (1:1). To quantify the antioxidant capacity, a calibration curve prepared with Trolox was used and the results were reported in µmol of Trolox per 100 g of sample (Brand-Williams, et al. 1995).

Total phenols content was determined by the Folin-Ciocalteau method described by Herchi et al. (2011), with some modifications. An aliquot of 3 g of extracted oil was mixed with 3 mL of chloroform and 3 mL of HCl 0.05 mol/L. This mixture was vortexed and centrifuged at 3500 rpm during 10 minutes. An aliquot of 2.5 mL of the aqueous phase extract was placed in a volumetric flask (10 mL) and 0.1 mL of Folin-Ciocalteau reagent added. After 3 min, saturated sodium carbonate (1 mL) was added. The flask was filled with water up to 10 mL. After 1 h, the absorbance at 765 nm was measured using a UV-Vis spectrophotometer (Shimadzu UV-1800) with a 1-cm cell. Total phenolic content was determined after the preparation of a standard curve performed with gallic acid. The results were reported in gallic acid equivalent (GAE) per g of sample.

2.6 Oil microbiological analysis

Microbiological studies were carried out on the extracted oil to determine the presence of pathogenic microorganisms for human health according to official methods (Microbiological Analysis of Foods Official Analytical Methodology, 2011). The presence/absence of total mesophilic aerobic bacteria (CFU/mL), total coliform bacteria (NMP/100 mL), *Escherichia coli* in 100 mL and *Salmonella* in 25 g of sample were determined.

2.7 Oil storage conditions for oxidation testing

The oil with the lowest microbial load, the lowest amount of organic solvents and primary and secondary oxidation compounds was used to determine the oxidative stability.

Journal Pre-proofs

This selected oil was placed in an amber bottle and stored at 25ºC to estimate the stability of oil to oxidation; the temperature was chosen in order to reproduce a moderate oxidation condition. The stability test was carried out for 4 months. The rate of oxidation was followed by periodically measuring peroxide value, Kreiss index, and *p*-anisidine value. The FA composition was determined at the beginning and the end of the oxidation test.

To assess the effect of vacuum packaging, another aliquot of extracted oil was placed in an amber bottle into which a stream of nitrogen was injected to remove oxygen and prevent further oxidation. This sample was stored at 25ºC for 8 months and after that period its stability was determined by the peroxide, *p*-anisidine, Kreiss index and conjugated dienes and trienes assessments. Likewise, FA composition was determined to evaluate any possible modification during the storage period.

2.8 Software and statically analysis

All analytical determinations were performed in triplicate (n= 3). Data analysis was performed by using *Statgraphics Plus 5.1* software. To compare the values of parameters related to FA composition, oil characterization and stability obtained with the four proposed extraction procedures, analysis of variance (ANOVA) was performed followed by a multiple comparisons test. The multiple comparisons test applies a multiple comparison procedure to determine the means that are significantly different from others. The method currently used to discern between the averages is the procedure of the smallest differences Fisher significant (LSD). With this method, there is 5.0% of the risk of considering each pair of means as significantly different when the real difference is equal to 0.

3. Results and Discussion

3.1. Fatty acid composition and nutritional quality

The FA composition of the four types of oil extracted and caiman fat before extraction are in Table 1 and the indices used to evaluate nutritional quality of oil extracted are in Table 2. The ANOVA

Journal Pre-proofs

analysis indicated that, although there are statistically significant differences between the content of some FA in the oils extracted with the four extraction methods and in the fat (14: 0 and c9, c12, c15- 18: 3), this difference did not modify the statistical significance between the FA relationships that allow us to evaluate the nutritional quality of the oil (SFA, MUFA, PUFA, Ratio n -6/n-3, PUFA/SFA ratio, AI, TI, HH index) (Table 2). These parameters are essential not only to select the extraction method but also to conclude on the nutritional quality of the oil.

Considering the animal source of the lipids, the nutritional value of the oil extracted is due to the content of PUFA, mainly oleic (c9-18:1 n-9; 34%), linoleic (30%) and α-linolenic (2%) acids. These results are comparable to those obtained for captive crocodilians (Gunstone and Russell 1954, Osthoff et al. 2010). In addition the total SFA content was 26%, with palmitic acid (16:0) being the most abundant (20%).

In recent years, consumers have been giving greater importance to nutritional contributions and their effect on health (Hocquette et al, 2012). Fat obtained from the caiman has favorable characteristics for human health, with a low content of SFA, high content of PUFA such as LA and ALA. This profile positions it as a better alternative compared to other products such as bovine and pork. Bovine fat contains a large percentage of SFA that are associated with the development of CVD and obesity, among other problems. On the other hand, if we compare the fat of caiman with that of fish, both have a low amount of SFA and a high content of PUFA such as FA n-3, with their content being higher in the fat of sea fish (Huang et al., 2020; Conchillo et al., 2006).

Foods with a ratio PUFA/SFA below 0.45, have been considered as undesirable to the diet (Department of Health and Social Security, Diet and cardiovascular disease, London, 1984), as they may result in increased blood cholesterol. In this study, this ratio was between 1.29 and 1.36, values that exceed the recommended minimum.

The Atherogenic index (AI) and Thrombogenicity index (TI), relating pro- and anti-atherogenic FA and pro- and anti-thrombogenic FA, respectively, are biomarkers related to the nutritional quality of

the diet. High levels of AI and TI (greater than 1.0) were shown to be positively associated with obesity and therefore with the incidence risk of CVD (Zhu et al., 2018). In oil, AI and TI are defined as the ratio between the content of FA (lauric-12:0, myristic-14:0 and palmitic acids-16:0) and the content of protective FA (MUFA and PUFA; Castro-Bolaños et al. 2005). Myristic acid and palmitic acid have more influence on the AI, because they are the main promoter of increases in serum cholesterol. The denominator includes all FA that have a protective action, that is, those that tend to reduce the risk of developing coronary heart disease. In caiman oil the AI was between 0.27 and 0.29 and the TI between 0.45 and 0.47, values less than the recommended maximum limit.

The relationship between hypocholesterolemic and hypercholesterolemic FA (HH index) is considered as the specific effect of FA on cholesterol metabolism. Thus, high values of this ratio are desirable, indicating that the amount of hypercholesterolemic FA is less than that of hypocholesterolemic FA. In the present study, we verified that the HH index values were between 3.27 and 3.50, a value considered as acceptable. Based on the PUFA/SFA ratio AI, TI, and HH indices obtained for extracted caiman oil, we could suppose that its consumption would not be risky in the development of chronic non-communicable diseases.

The recommended n-6/n-3 ratio for the organism must be between 5:1 and 10:1 (Yang et al. 2015). The problem is that this ratio is very far from the current diets of western countries, which contain a high amount of n-6 FA (Palou et al. 2008). The n-6/n-3 ratio of FA is fundamental in terms of their physiological effects, since a high ratio promotes diseases such as cancer, inflammatory and cardiovascular, in addition leading to a decrease in insulin sensitivity in the muscle and promotes the accumulation of lipids in adipose tissue (Simopoulos et al. 2008, Simopoulos 2016). Consuming different types of FA has different effects on metabolic pathways. The oils rich in FA of the n-3 family have anti-lipid and anti-atherogenic properties, as well as anti-inflammatory. They have the ability to regulate the expression of different genes associated with obesity-related energy metabolism.

Despite the fact that *Caiman latirostris* oil has a PUFA n-6/n-3 value of around 14:1 (Table 2)

and the recommended intake is between 5:1 and 10:1, this oil has a lower ratio than two more oils consumed by our population, such as sunflower (IA = 65:1) and corn (IA = 54:1), consequently crocodile oil could be used as a dietary supplement.

Because crocodiles are monogastric animals, their diet strongly influences the FA composition of fat. Therefore, it would be novel to modify the diet of these animals in order to increase the amount of PUFA n-3 in their fatty tissues. Caiman in captivity have a diet composed of ground chicken heads and dry feed formulated for reptiles, while crocodilians in their natural state eat mainly fish, insects or molluscs according to the availability of food from the environment where they live (Osthoff et al. 2009, Borteiro et al. 2009). Therefore, the composition of FA will differ substantially, since they have a different diet.

Although we show that the oil obtained from *Caiman latirostris* fat contains essential FA such as PUFA n-3 (1.89-2.35%) and PUFA n-6 (31.2-33.6%), it is possible to increase the amount of compounds of high nutritional value in the tissue by adding sources of n-3 FA such as flaxseed to the diet, an action that would even increase the commercial value of the oil because a healthier food could be obtained (Piña et al. 2016). From the above, it is shown that the oil obtained from fat of *Caiman latirostris* could be used for human nutrition due to its excellent FA composition.

3.2 Oil physicochemical characterization and initial oxidative parameters

The physicochemical parameters that characterize the oil produced with the four procedures are in Table 3. There were no statistically significant differences in the iodine index, saponification index, *p*-anisidine value, Kreiss index, and acid value ($p>0.05$), but differences were detected between recovery, peroxide index, conjugated dienes and trienes, antioxidant capacity and reducing substances ($p<0.05$). The multiple comparisons test was used to determine the means that are significantly different from others. Means and least significant difference (LSD) intervals are shown on Figure 1.

Journal Pre-proofs

Iodine index is a measure of the degree of unsaturation of the fat components, increasing with the number of double bonds per unit. It is used to verify the purity and identity of the oils and fats. Saponification index is a measure of the free and esterified ~~fatty acids (~~FA~~)~~ existing in the fat and is inversely proportional to the average molecular weight of the triglyceride FA that constitutes the fat. Caiman oil obtained by different procedures showed similar iodine and saponification index to those published by Gunstone and Russell (1954) (Table 3). Because there are no statistically significant differences between the iodine and saponification index values obtained with the four different extraction methods, we can conclude that there is no difference in the extracted FA. This is also evidenced when analyzing FA composition (Table 1).

The highest yield was obtained for sample S4 (89±1%) followed by sample S3 (51±6%), S1 (42±6%) and S2 (35±3%). The extraction efficiency obtained by Huang et al. (2015) to extract oil from crocodile fat using a mixture of dichloromethane and methanol was higher than that obtained in our work using hexane or the chloroform-methanol-water mixture (Bligh-Dyer method). This could be explained by the fact that the polarity of the solvent and the extraction time plays a fundamental role in the extraction of oil lipid. There is evidence that the Bligh-Dyer method underestimates the total lipid content in samples with high fat content (Vidal et al., 2012, Pérez-Palacios et al., 2018) or that it does not have the capacity to extract all the lipids present in the sample matrix under study.

On the other hand, the relationship between the sample quantity and the extracting solvent mixture used by Huang et al. (2015) was 1/20 (g/mL), while 1/6 (g/mL) was used in our work. This would be related to the fact that when a small amount of sample and a large amount of extraction solvent are used, the contact between the phases is facilitated, which increases the extraction efficiency. In any case, the use of organic solvents to obtain edible oil would not be a good alternative since it would require a phase of solvent removal and there could also be residues harmful to health.

Since the oil is intended to be used as food supplement, it is necessary to have an efficient extraction method able to produce high quality oil. Fusion extraction was the most efficient (89±1%),

but also the best in terms of economy and oil quality. Likewise, the oil obtained by this method does not contain toxic substances or contaminants because no external agent or organic solvents are added.

Peroxide and conjugated dienes values in oil were higher when extracted with S3 and S4 methods than with S1 and S2. This demonstrates that performing the extraction with heat generates a greater amount of primary oxidation compounds. The *p*-anisidine value method measured the content of aldehydes generated during the decomposition of hydroperoxides. Kreiss index is a semi-quantitative test that measures the presence of the epidrinic aldehyde produced by oxidation of linolenic acid. Many secondary oxidation compounds formed by degradation of peroxides have conjugated triennial structures that have a maximum absorbance at 270 nm. Because undetectable *p*-anisidine values were obtained, the Kreiss index were negative and the values obtained for the conjugated trienes were negligible (although there is a statistically difference between the different types of extractions), it can be affirmed that no secondary oxidation compounds were generated during the oil extraction with the four strategies used.

The acid value (AV) is a measure of the content of free fatty acids (FFA) present in fats and oils, derived from the hydrolysis of triglycerides. Its result allows conclusions to be drawn about the degree of alteration of the sample and determines whether a refinement step will be necessary. Based on the results obtained from AV we can conclude that there were no statistically significant differences between the AV of the oil produced with the four methods, with values lower than 2.0 mg KOH/g oil. Therefore we can conclude that the extraction processes did not increase the hydrolytic processes and it is not necessary to perform a refinement step.

The antioxidant capacity determined by the DPPH method was greater in methods S1, S3 and S4 with values between 25 and 28 μmol Trolox/100g, while method S2 had a value of 19 μmol Trolox/100g. Although statistically significant differences were detected between the antioxidant capacities of the oils extracted by the proposed methods, it is important to highlight that all the oils presented antioxidant activity, which leads us to highlight that the oil has free radical scavenging substances in its composition.

Journal Pre-proofs

The total phenols content was greater in methods S3 and S4 with values between 1.8 and 2.1 mg GA/100g, while in methods S1 and S2 values were 0.6 and 0.08 mg GA/100g respectively. Results indicate that heat extraction was more efficient recovering phenolic compounds than solvent extraction. Caimans were fed with ground chicken head (70%) and dry balanced feed for reptiles (30%). To preserve the food from deterioration, 0.05% (w/w) of a mixture of synthetic antioxidants (composed of butylated hydroxytoluene –BHT-, ethoxyquin,*tert*-butylhydroxiquinone –TBHQ-) is added to the food. These compounds are of the phenolic type, and have the ability to capture and neutralize free radicals. Therefore, the moderate antioxidant activity detected in caiman oils could be due to the presence of these antioxidants in the diet. We observed a relationship between the content of phenolic compounds and the antioxidant activity in methods S3 and S4. In general, the oils with a higher content of total phenolic compounds had higher antioxidant activity, however, it can be seen that the oils obtained from method S1 and S2 had a greater antioxidant activity than were expected. This is indicative that the antioxidant capacity is due to the combined effect of various factors, such as the presence of other types of antioxidant metabolites, or a pro-oxidative activity that contrasts with the potent antioxidant.

In most countries where crocodile meat is used for human consumption, and/or exported, a concern has usually been *Salmonella*. For this reason it is important to evaluate if the processing of the fat to obtain oil eliminates the natural microorganisms of crocodiles. In this study we consider evaluating the presence of any type of contaminating bacteria that cause pathologies in humans could be detrimental to consumer health (Soto Varela et al. 2016). The presence of mesophilic aerobic bacteria, coliform bacteria, *Escherichia coli* (in 100 mL of the sample) and *Salmonella* (in 25 g of sample) was not observed for the oils obtained with the four strategies evaluated.

Based on the results, the fusion method was selected to extract the oil from the *Caiman latirostris* fat because is the extraction methodology that has the highest performance, absence of solvents, and microbial load, can be scaled and has low oxidability.

Journal Pre-proofs

Considering the specifications of Article 540 and 543 of Chapter VII of the CAA (which specifies the requirements that edible animal fats must meet), we can conclude that caiman oil could be considered "virgin" because it was obtained by thermal procedures, purified by filtration and centrifugation and did not needed refinement processes. Also, the obtained oil meets the specified oxidative and hydrolytic stability parameters for other oils obtained from animal fat (acidity index<1.6 mg KOH/g oil and peroxide index<10.0meq O_2/kg oil).

Many oils, once extracted, require a purification process to achieve the quality characteristics that make it acceptable for consumption since they may contain insoluble impurities, phospholipids, FFA, moisture, oxidation products primary, pigments and even persistent organic pollutants (Bonilla-Méndez et al. 2018). The acidity index and the peroxide index are essential indicators to determine the completion of the refining process. According to the CAA, the refining of virgin fats that have excessive free acidity values is allowed, provided that their peroxide indexes do not exceed 20.0 meqO_2/kg, and must not exceed the limits specified in the corresponding articles in the refined product. Since the oil obtained from the fat of *Caiman latirostris* has a PV of 5.6 meq O_2/kg and the acid number is not excessive (1.4 mg KOH/g), it is not necessary to carry out a refining step.

3.3 Stability of oil

Oxidation rate was followed by periodically measuring peroxide value, Kreiss index, and *p*-anisidine value. The FA composition was determined on the same day (day 0) and at the end of the test and no difference in FA composition was detected in storage time (data not are shown). Therefore it is concluded that storage at 25ºC does not affect the composition of FA.

The peroxide value is a measure of the concentration of peroxide and hydroperoxide forms in the initial stage of lipid oxidation. The number of peroxides present in oils reflects its oxidative level and thus its tendency to become rancid. Results (Figure 2A) showed that although the PV increases gradually until 120 days of testing, the maximum value reached is less than 10.0 meq O_2/kg oil.

Journal Pre-proofs

In the second phase of oxidation, the primary product of oxidation, peroxides decompose and develop substances such as aldehydes, which are responsible for the rancid smell and taste. *p*-anisidine value test and Kreiss index measures this secondary oxidation (Figure 2B). Before 60 days of storage at 25ºC, the *p*-anisidine value (*p*-AnV) was undetectable and the Kreiss index was negative. This indicates that no secondary oxidation compounds were generated. After 60 days the *p*-AnV begins to increase until reaching a value of 116 at 8 months.

Due to the PV and *p*-AnV obtained and taking into account the specifications of Article 540 and 543 of Chapter VII of the CAA (IP less than 10.0 meq O_2/kg), and the maximum limit allowed of *p*-AnV is 10.0 (Rossell 1989) to infer rancidity in oils we can conclude that there is no significant amount of primary and secondary oxidation compounds, in the oil obtained by fusion of fat of caiman, to assume advanced oxidation state and rancidity if it is preserved at 25ºC for 4 months.

On the other hand, for an aliquot of extracted oil stored in the absence of oxygen and stored at 25°C for 8 months, stability was determined by the peroxide, *p*-anisidine, Kreiss index, conjugated dienes and trienes and FA composition. The values obtained were PV 4.68±0.8 meq O_2/kg, *p*-AnV 0.57±0.03, Kreiss index was negative, CD 3.00±0.06 Abs_{234nm}/(g/100 mL), and CT 0.35±0.05 (Abs_{270nm}/(g/100mL). These values did not differ significantly from those initially measured (see Table 3). Likewise, there was no statistically significant difference between the FA composition (data not shown) of the freshly obtained oil and that stored at 25°C for 8 months. We conclude that the oil obtained by fusion has excellent oxidative stability and can be stored in an inert atmosphere protected from light and at 25°C for at least 8 months, maintaining its initial physicochemical and organoleptic properties.

4. Conclusions

The fusion method used to extract oil from *Caiman latirostris* fat demonstrated an excellent performance (89% w/w). It was determined that the extraction method did not alter the FA composition in caiman fat. The oil obtained did not contain organic solvents and had a low microbial load. Also, due

to its excellent oxidative stability, it can be produced and stored at 25ºC during 4 months and in an inert atmosphere for at least 8 months, maintaining its physicochemical properties and nutritional quality. This may be due to the presence of reducing substances and free radical scavenging that increase their antioxidant capacity.

Due to its excellent nutritional quality the oil obtained from caiman fat could be used for human nutrition. Given that the majority of FA are anti-atherogenic, anti-trombogenic and hypocholesterolemic, we could suppose that the consumption of caiman oil would be a preventive factor for the development of chronic non-communicable diseases.

Obtaining oil from a waste implies a reduction in the environmental impact while economically benefiting the organization by adding value to by-products and waste. This work is part of the Save Food program issued by FAO in conjunction with other organizations, with actions aimed at preventing and reducing food waste.

Acknowledgements

We wish to thank for the appreciated comments and suggestions to the manuscript of Dr. Charlie Manolis. We also thanks the financial support received from Universidad Nacional del Litoral by CAI+D Orientado 2016 and by the Proyecto Yacaré/Yacarés Santafesinos, PICT 2014 Nº2212 (to Simoncini), PICT 2019 N°04300 (to Leiva), with the support of FUNDACIÓN NATURA, COMFAUNA and funding from GORDON AND BETTY MOORE FOUNDATION (GRANT 9258) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Author contributions

Luciana Vera Candioti and Marcela González were responsible for the overall conception and design of the study, data handling, writing and interpretation. Pamela Leiva and Florencia Valli participated in the collection and the analysis of the samples. Melina Simoncini, Carlos Piña and Claudio

Journal Pre-proofs

Bernal collaborated in the analysis, and interpretation of results, and in the revision of the article. All authors have read an approved the final version, and have agreed to submit the manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

Funding sources

This work was supported by "Programa de I+D Orientado a Problemas Sociales y Productivos". Convocatoria 2016.Resol CS Nº 632/17.-Secretaría de Ciencia y Técnica - Universidad Nacional del Litoral and and PICT2014-2212 (to M. Simoncini), PICT 2019 N°04300 (to Leiva), with the support of FUNDACIÓN NATURA, COMFAUNA and funding from GORDON AND BETTY MOORE FOUNDATION (GRANT 9258).

References

Argentine Food Code (2018). Chapter XII. Fatty Foods and Food Oils. Article 540 and 543.

AOCS (2017) *Official Methods and Recommended of Practice of the American Oil Chemists' Society*. 7th edn., American Oil Chemists' Society.https://www.aocs.org/attain-lab-services/methods/methods/search#section-c-commercial-fats-and-oils

Bonilla-Mendez, J. R. & Hoyos-Concha, J.L. (2018) Métodos de extracción, refinación y concentración de aceite de pescado como fuente de ácidos grasos omega-3. *Corpoica Ciencia Tecnología Agropecuaria*, Mosquera (Colombia), 19(3), 621–644.

Borteiro C., Gutiérrez F., Tedros M. & Kolenc F. (2009) Food habits of the Broad-snouted Caiman (*Caiman latirostris*: Crocodylia, Alligatoridae) in northwestern Uruguay, Studies on Neotropical *Fauna and Environment* 44 (1), 31–36.

Journal Pre-proofs

Bucio, S. L., Sanz, M. T., Beltrán, S., Melgosa, R., Solaesa, A. G., & Ruiz M. O. (2016) Study of the influence of process parameters on liquid and supercritical CO2 extraction of oil from rendered materials: Fish meal and oil characterization. *Journal of Supercritical Fluids* 107, 270–277.

Buthelezi, S., Southway, C., Goviden, U., Bodenstein, J. & du Toit, K. (2012) An investigation of the antimicrobial and anti-inflammatory activities of crocodile oil. *Journal of Ethnopharmacology,* 1243, 325–330.

Brand-Williams W., Cuvelier M. E. & Berset C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensm Wiss u Technology Journal*, 28, 25-30.

Castro-Bolaños, M., Herrera-Ramírez, C.H. & Lutz-Cruz, G. (2005) Composición, caracterización y potencial aterogénico de aceites, grasas y otros derivados producidos o comercializados en Costa Rica. *Acta médica Costarricence,* 47(1), 36–42. ISSN 0001-600.

Conchillo A., Valencia I., Puente A., Ansorena D. & Astiasarán I. (2006) Functional components in fish and algae oils. *Nutricion Hospitalaria* 21, 369-373

Expert Panel (2013). Analysis of Humane Killing Methods for Reptiles in the Skin Trade, ed. by Swiss Federal Veterinary Office. Federal Veterinary Office: Berne, Switzerland.

Folch, J., Lees, M. & Stanley, S. (1956). A simple method for the isolation and purification of total lipids from animal tissues. From the McLean Hospital Research Laboratories, Waverley, and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts.

Gunstone, F.D., & Russell, W.C. (1954). The component acids of crocodile fat. *Animal Fats,* 57, 462–465.

Herchi, W., Sakouhi, F., Arráez-Román, D., Segura-Carretero, A., Boukhchina, S., Kallel, H., & Fernández-Gutierrez, A., (2011). Changes in the content of phenolic compounds in flaxseed oil during development. Journal of the American Oil Chemists Society 88, 1135–1142.

Journal Pre-proofs

Hocquette, J. F., Botreau, R., Picard, B., Jacquet, A., Pethick, D. W., & Scollan, N. D. (2012). Opportunities for predicting and manipulating beef quality. *Meat science, 92*(3), 197-209.

Huang C., Chiba L.I., Magee W.E., Wang Y., Rodning S.P., Bratchera C.L., Bergen W.G. & Spangler E.A. (2020) Effect of flaxseed oil, poultry fat, and vitamin E supplementation on physical and organoleptic characteristics and fatty acid profile of pork, and expression of genes associated with lipid metabolism. *Livestock Science* 231, 103849.

Huang, Y., Dong, S., & Liu, Y. (2015) Effects of solvent polarity and acidity on the extraction efficiency and fatty acid composition of oil from Nile crocodile. *International Journal of engineering Advanced Research Technology (IJEART),*1(1), 1–6.

Hutton, J.M. (1992). Humane killing of crocodilians. Pp. 194-196 in Crocodiles. Proceedings of the 11th Working Meeting of the IUCN-SSC Crocodile Specialist Group. IUCN: Gland, Switzerland).

IUPAC, (1987) *Standard Methods for the Analysis of Oils, Fats and Derivatives* (pp. 210–211), 7thedn., edited by C. Paquot & A. Haufenne, IUPAC, Blackwell Scientific, Oxford.

Kajal, C., & Deepu, J. (2015). Cooking and pressing is an effective and eco-friendly technique for obtaining high quality oil from Sardinella longiceps. *European Journal of Lipid Science and Technology,* 117(6), 837–850.

Kaspars, I., & Dagnija, B. (2019) Extraction of fish oil using green extraction methods: a short review. *Energy Procedia* 128, 477–483.

Kluczkovski, A. J., De Francisco, A., Beirao, L., Kluczkovski, A., & Barbosa H. (2016). Lipids of Amazon Caimans: A source of fatty acids. *African Journal of Biotechnology*, 15(29), 1559–1565.

Larriera, A., & Imhof, A. (2006). Proyecto Yacaré. Cosecha de huevos para la cría en granjas del género *Caiman* en la Argentina. En M. L. Bolkovic, y D. Ramadori (Eds.), Manejo de Fauna Silvestre en la Argentina. Programas de uso sustentable (pp. 51–64). Dirección de Fauna Silvestre, Secretaría de Ambiente y Desarrollo Sustentable. Buenos Aires, Argentina.

Journal Pre-proofs

Larriera, A., Imhof, A., & Siroski, P. (2008). Estado actual de los programas de conservación y manejo del género Caiman en Argentina. *Contribución al Conocimiento del género Caiman de Sudamérica* (pp.141–180). Castroviejo J, Ayarzaguena J, Velasco A (eds). Public. Asoc. Amigos de Doña Ana. Madrid.

Larriera, A. (2011). Ranching the broad-snouted caiman (*Caiman latirostris*) in Argentina: an economic incentive for wetland conservation by local inhabitants. En M. Abensperg-Traun, D. Roe, & C. O Criodan (Eds.), Proceedings of an International Symposium on The relevance of Community-Based Natural Resources Management (CBNRM) to the Conservation and Sustainable Use of CITES-Listed Species in Exporting Countries" (pp 86-92). European Commission Directorate General Environment. Vienna. Austria.

Masson L., Alfaro T., Camilo C., Carvalho A., Illesca P.G., Torres R., Tavares do Carmo M., Mancini-Filho J., Bernal C.A. (2015) Fatty acid composition of soybean/sunflower mix oil, fish oil and butterfat applying the AOCS Ce 1j-07 method with a modified temperature program. *Grasas y Aceites* 66, e064. doi:10.3989/gya.0692141.

Microbiological Analysis of Foods Official Analytical Methodology. Pathogenic Microorganisms Volume 1. (2011). National Network of Official Laboratories of Food Analysis (RENALOA). ANMAT National Ministry of Health Presidency of the Nation.

Palou, A., Pico, C., Bonet, M.L., Serra, F., Oliver, P., Rodriguez, A.M. & Ribot, J. (2008). Lípidos dietéticos y salud. El libro Blanco de las Grasas en la Alimentación Funcional; pp. 41–61.

Osthoff, G., Hugo, A., Bouwman, H., Buss, P., Govender, D., Joubert, C.C. &Swarts, J.C. (2009). Comparison of the lipid properties of captive, healthy wild, and pansteatitis-affected wild Nile crocodile (*Crocodylus niloticus*). *Comparitve Biochemistry and Physiology, Part A* 155, 64–69.

Pérez-Palacios, T., Ruiz, J., Martín, D., Muriel, E. & Antequera, T. (2008) Comparison of different methods of total lipid quantification in meat and meat products. *Food Chemistry,* 110(4), 1025–1029.

Journal Pre-proofs

Piña, C.I., Lucero, L.E., Simoncini, M.S., Peterson, G.B. & Tavella, J.M (2016). Lipid profile of yacares overo meat fed with diets enriched with flax seeds. *Zootecnia Tropical*; 34(2), 25-33.

Rossell, J. B. (1989). Measurement in rancidity. In J. C. Allen, &R. J. Hamilton (Eds) *Rancidity in food* (pp 22–51) Elsevier, London.

Santos-Silva, J.; Bessa, R.J.B. & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science*, 77(2-3), 187–194.

Simoncini, M. S., Lábaque, M. C., Perlo, F., Fernandez, M. E., Leiva, P. M. L., Paez, A. R., & Piña, C. I. (2020). Caiman latirostris meat characterization: Evaluation of the nutritional, physical and chemical properties of meat from sustainable ranching program in Argentina. *Aquaculture* 515:734570.doi:10.1016/j.aquaculture.2019.734570.

Simopoulos, A.P. (2008).The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine*, 233(6), 674-688.

Simopoulos, A. (2016). An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*, 8(3), 128–144.

Soto Varela, Z., Pérez Lavalle, L., &Estrada Alvarado, D. (2016) Bacteria causing of foodborne diseases: an overview at Colombia. Salud Uninorte ISSN 2011-7531. *Barranquilla,* 32(1),105–122.

Ulbricht, T.L.V. & Southgate, D.A.T. (1991) Coronary heart disease: seven dietary factors. The Lancet, London, 338(773), 985–992.

Vidal, N.P., Yusta, A.M., Manzanos, M.J., Goicoechea, E. & Guillen, M.D. (2012) Métodos para la extracción de lípidos alimentarios. Ventajas y limitaciones. Actas del VII Congreso Español de Ingeniería de los Alimentos. Ed: USB-ISBN: 978-84-695-4196-8. Ciudad Real (Spain), 43–49.

Journal Pre-proofs

Yang, L.G., Song, Z.X., Yin,H., Wang, Y.Y., Shu, G.F., Lu, H.X. Sun, G.J. (2015). Low n-6/n-3 PUFA ratio improves lipid metabolism, inflammation, oxidative stress and endothelial function in rats using plant oils as n-3 fatty acid source. Lipids; 51(1):49-59.

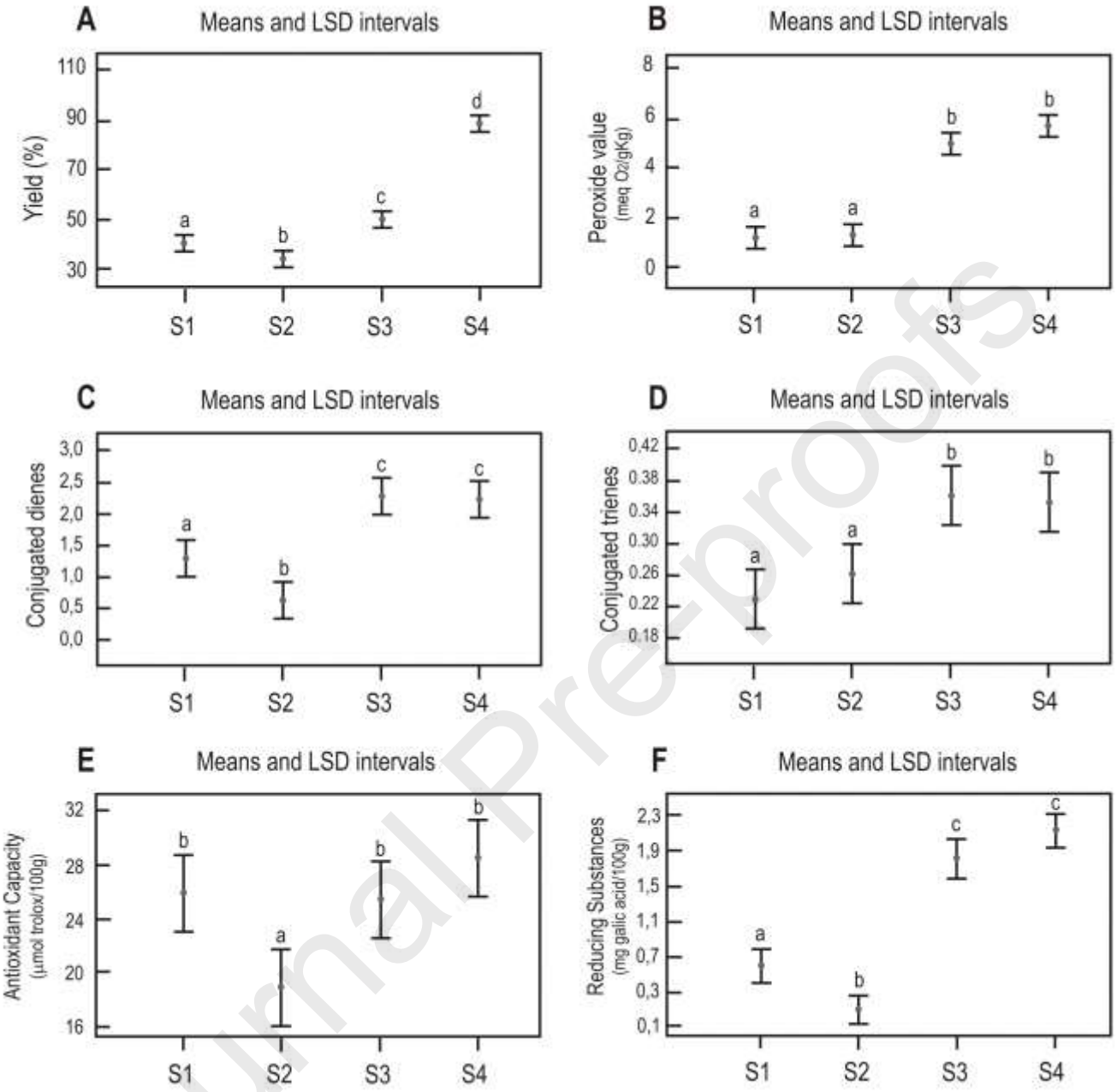
Zhu, X., Ting Lei, T., Hu, J., Xu, W., Yi, N. & Lei, S. (2018) Atherogenic index of plasma is a novel and better biomarker associated with obesity: a population-based cross-sectional study in China . *Lipids in Health and Disease* 17 (1):37-42.

Figure Captions

Figure 1, Luciana Vera-Candioti: Means and least significant difference (LSD) intervals of the extraction parameters that show statistically significant differences.

Figure 2, Luciana Vera-Candioti. Change in the content of peroxide value (PV) and *p*-anisidine value (*p*-AnV) during the oxidative stability test.

Journal Pre-proofs



Journal Pre-proofs

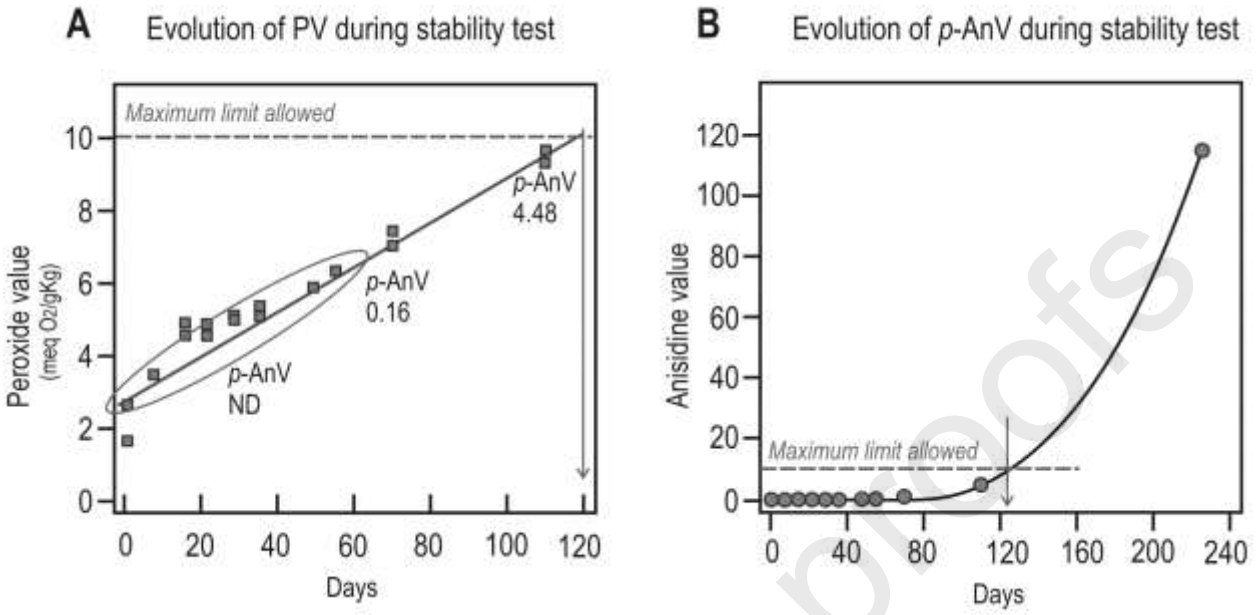


Table 1: Fatty acid composition of the four types of oils extracted and the fat of the caiman before its extraction (data presented as mean of fatty acid content expressed as percent of total fatty acid methyl esters ±standard deviation).

	Extraction methods					
al fatty acids	S1	S2	S3	S4	Fat	*p*-value
	0.383±0.001[a, b]	0.362±0.006[a]	0.403±0.024[b, c]	0.367±0.006[a]	0.411±0.001[c]	0.0024
	18.796±0.222	19.949±0.085	19.294±0.598	19.758±0.284	19.163±0.669	0.2488
	0.331±0.024	0.298±0.026	0.367±0.002	0.294±0.015	0.295±0.029	0.0542
	4.239±0.030	4.339±0.053	4.833±0.343	4.075±0.101	4.391±0.444	0.2870

Journal Pre-proofs

	0.125±0.015	0.118±0.024	0.148±0.037	0.124±0.019	0.127±0.023	0.7566
	5.592±0.054	5.151±0.300	5.533±0.124	5.624±0.058	5.486±0.411	0.5533
	34.190±0.480	32.942±0.037	33.376±0.067	33.490±0.380	33.383±0.719	0.0914
1	2.096±0.122	1.965±0.147	2.047±0.130	2.038±0.125	2.137±0.108	0.5872
18:2	29.736±0.250	30.062±0.393	29.263±0.692	29.624±0.041	29.693±0.921	0.9520
c15-18:3	0.126±0.108	0.115±0.034	0.167±0.071	0.116±0.007[a]	0.169±0.072	0.8080
c15-18:3*	1.910±0.109[a]	2.176±0.144[b]	2.028±0.041[a,b]	1.953±0.038[a]	1.907±0.032[a]	0.0290
1	0.318±0.049	0.256±0.008	0.322±0.059	0.174±0.131	0.357±0.005	0.1996
-20:2	0.348±0.056	0.268±0.011	0.295±0.005	0.281±0.009	0.300±0.011	0.1597
,c17-20:3	0.260±0.023	0.239±0.009	0.267±0.022	0.198±0.039	0.234±0.054	0.5080
1,c14-20:4	0.802±0.001	0.935±0.072	1.035±0.050	0.715±0.055	0.920±0.262	0.4580
	0.013±0.001	0.016±0.008	0.023±0.010	0.012±0.001	0.109±0.083	0.2020
	0.312±0.057	0.282±0.012	0.275±0.029	0.253±0.026	0.245±0.034	0.4310
c13,c16,c19-22:5	0.146±0.059	0.117±0.017	0.149±0.012	0.103±0.030	0.099±0.046	0.5370
0,c13,c16,c19-22:6	0.160±0.061	0.138±0.016	0.177±0.022	0.135±0.016	0.116±0.049	0.3520

Abbreviations: NI means unidentified, (S1) extraction with n-hexane , (S2) extraction with a mixture of chloroform-methanol-water (30:20:10, v, v, v) , (S3) extraction where fat was placed in an oven at 60^{0}C for 5 hours , (S4) extraction where fat was heated under constant stirring, controlling that the temperature did not exceed 80^{0}C. A *p*-value > 0.05 indicates that there is no statistically significant difference between groups, [a. b. c.] different superscripts letters in the same row indicate statistically significant difference between groups at p< 0.05, * fatty acids where statistically significant difference is observed between groups.

Journal Pre-proofs

Table 2: Nutritional quality indices of the four types of oils extracted and the fat of the caiman before its extraction (SFA, MUFA, PUFA, PUFA n-3 and PUFA n-6 are presented as mean of the sum of fatty acid content expressed as percent of total fatty acid methyl esters ±standard deviation).

	Extraction methods					
Index	S1	S2	S3	S4	Fat	*p*-value
SFA	24.90±0.23	25.58±0.31	25.4±0.61	25.87±0.29	25.19±0.79	0.7119
MUFA	40.86±0.56	39.52±0.36	40.6±0.84	40.79±0.62	40.38±0.91	0.1205
PUFA	33.80±0.66	34.33±0.43	33.7±0.70	33.38±0.09	33.68±0.92	0.4026
PUFA n-3	2.22±0.59	2.43±0.15	2.35±0.05	2.19±0.05	2.12±0.07	0.4697
PUFA n-6	31.58±0.29	31.90±0.40	31.3±0.70	31.19±0.74	31.56±0.93	0.7706
Ratio n-6/n-3	14.25±0.27	13.12±0.06	13.30±0.03	14.23±0.03	14.87±0.06	0.5997
Ratio PUFA/SFA	1.36±0.02	1.34±0.02	1.33±0.03	1.29±0.03	1.34±0.02	0.7332
AI	0.27±0.02	0.29±0.02	0.28±0.02	0.29±0.02	0.28±0.02	0.3379
TI	0.45±0.02	0.47±0.02	0.46±0.02	0.47±0.02	0.46±0.02	0.3827
HH index	3.49±0.03	3.27±0.03	3.35±0.03	3.33±0.03	3.38±0.03	0.5732

Abbreviations: Sum of saturated fatty acids (SFA) = (14:0; 16:0; 17:0; 18:0), Sum of mono-unsaturated (MUFA) = (c9-16:1; c9-18:1; c11-18:1; c11-20:1; 22:1), Sum of poly-unsaturated (PUFA) = (c9,c12-18:2; c6,c12,c15-18:3; c9,c12,c15-18:3; c11,c14-20:2; c11,c14,c17-20:3; c5,c8,c11,c14-20:4; 22:4; c7,c10,c13,c16,c19-22:5; c4,c7,c10,c13,c16,c19-22:6), Sum of PUFA n-3= (c9,c12,c15-18:3; c11,c14,c17-20:3; c7,c10,c13,c16,c19-22:5; c4,c7,c10,c13,c16,c19-22:6), Sum of PUFA n-6 = (c9,c12-18:2; c6,c12,c15-18:3; c11,c14-20:2; c5,c8,c11,c14-20:4), AI= atherogenic index, TI= thrombogenicity index, HH index= relation between hypocholesterolemic and hypercholesterolemic fatty acids. A *p*-value > 0.05 indicates that there is no statistically significant difference between groups.

Journal Pre-proofs

Table 3. Oil caiman chemical characterization (yield, saponification index, iodine index, peroxide value, conjugated dienes and trienes, *p*-anisidine value, kreiss index, acid value, antioxidant capacity and reducing substances) obtained by four methods of extraction.

	S1	S2	S3	S4	p-value
Yield (% w/w)	42±6[a]	35±3[b]	51±6[c]	89±1[d]	<0.0000
Saponification index (mg KOH/g oil)	199±4[a]	197±7[a]	194±7[a]	194±7[a]	0.7997
Iodine index (gI_2/100 g oil)	92±3[a]	99±5[a]	87±9[a]	88±2[a]	0.4923
Peroxide value (meq O_2/Kg)	0.9±0.4[a]	1.0±0.4[a]	4.8±0.2[b]	5.6±0.8[b]	<0.0000
Conjugated dienes (Abs_{234nm}/(g/100 mL)	1.3±0.2[a]	0.6±0.5[b]	2.3±0.3[c]	2.30±0.03[c]	0.0004
p-anisidine value	ND	ND	ND	ND	–

Kreiss index	negative	negative	negative	negative	–
Conjugated trienes (Abs_{270nm}/(g/100mL)	0.23±0.05[a]	0.26±0.01[a]	0.36±0.03[b]	0.35±0.06[b]	0.0075
Acid value (mg KOH/g oil)	1.3±0.4[a]	2.0±0.9[a]	1.04±0.07[a]	1.4±0.2[a]	0.2412
Antioxidant capacity (µmol trolox/100g)	26±3[a]	19±2[b]	25±3[a]	28±4[a]	0.0222
Reducing substances (mg GA/100g)	0.6±0.1[a]	0.08±0.04[b]	1.8±0.3[c]	2.1±0.4[c]	<0.0000

Abbreviations: ND means no detectable, (S1) extraction with n-hexane, (S2) extraction with a mixture of chloroform-methanol-water (30:20:10, v, v, v), (S3) extraction where fat was placed in an oven at 60^0C for 5 hours , (S4) extraction where fat was heated under constant stirring, controlling that the temperature did not exceed 80^0C, A *p*-value > 0.05 indicates that there is no statistically significant difference between groups, [a. b. c] Different superscripts letters indicate statistically significant difference between groups at $p < 0.05$.

Journal Pre-proofs

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proofs

Author contributions

Luciana Vera Candioti and Marcela González were responsible for the overall conception and design of the study, data handling, writing and interpretation. Pamela Leiva and Florencia Valli participated in the collection and the analysis of the samples. Melina Simoncini, Carlos Piña and Claudio Bernal collaborated in the analysis, and interpretation of results, and in the revision of the article. All authors have read an approved the final version, and have agreed to submit the manuscript.

Highlights

- The fusion method used to extract caiman oil is the most efficient of methods.
- The caiman oil obtained does not contain organic solvents or microbial load.
- The caiman oil is stable for at least 8 months in an inert atmosphere at 25°C.
- Caiman oil can be used for human nutrition due to its high nutritional quality.

Journal Pre-proofs