Artigo

Incorporation of Omega-3 Fatty Acids in Nile Tilapia (*Oreochromis niloticus*) By-Products Containing Sacha Inchi Oil

Silva, M.; Rydlewski, A. A.; Oliveira, M.; Biondo, P. B. C.; Maldaner, L.* Visentainer, J. V.

Rev. Virtual Quim., 2020, 12 (2), 414-423 Data de publicação na Web: 9 de Abril de 2020

<http://rvq.sbq.org.br>**Incorporação de Ácidos Graxos Ômega-3 em Subprodutos da Tilápia do Nilo (*Oreochromis niloticus*) Alimentados com Dieta Contendo Óleo de Sacha Inchi**

Resumo: Este estudo avaliou a incorporação de ácidos graxos (AGs) da série ômega-3 em subprodutos (cabeça, fígado e vísceras) de tilápia do Nilo (*Oreochromis niloticus*), a partir da suplementação nas rações dos peixes com óleo Sacha inchi (SIO), (tratamento II - TII), em substituição ao óleo de soja (SO), (tratamento I - TI). Houve um aumento nas concentrações de ácido alfa-linolênico (LNA, 18:3n-3) de 3.73 % nas amostras de cabeça, 4.67 % no fígado e 1.43 % nas vísceras. Nos teores do ácido eicosapentaenoico (EPA, 20:5n-3) a elevação foi de 1.47 % na cabeça e no ácido docosahexaenoico (DHA, 22:6n-3) foi de 1.6 % na cabeça e 2.56 % no fígado dos peixes tratados com SIO. A análise estatística realizada por PCA (análise dos componentes principais) separou os tratamentos em grupos distintos, de acordo com a dieta fornecida. A suplementação com SIO na dieta da tilápia do Nilo aumentou a qualidade lipídica dos seus subprodutos.

Palavras-chave: Tilápia do Nilo; subprodutos de peixe; suplementação; enriquecimento de ração; PCA.

Abstract

This study evaluated the incorporation of omega-3 fatty acids (FAs) in Nile tilapia (*Oreochromis niloticus*) by-products (head, liver and viscera) with addition of Sacha inchi oil (SIO) (Treatment II – (TII)) replacing soybean oil (SO) (Treatment I – (TI)). Regarding the alpha-linolenic acid (LNA, 18:3n-3) concentration, an increase of 3.73 % in the head, 4.67 % in the liver and 1.43 % in the viscera was observed. Whereas eicosapentaenoic acid (EPA, 20:5n-3) concentration, the increase observed was 1.6 % in the head and 2.56 % in the liver. The principal components analysis (PCA) statistical analysis separated the treatments into two groups according to the diet provided. And, as expected, the supplementation with SIO in the fish diet increased the lipid quality of Nile tilapia by-products.

Keywords: Nile tilapia; fish by-products; supplementation; feed enrichment; PCA.

* Universidade Estadual de Maringá, Departamento de Química, Av. Colombo, 5790, CEP 87020-900, Maringá-PR, Brazil.

jesuiv@gmail.com

DOI: [10.21577/1984-6835.20200033](https://doi.org/10.21577/1984-6835.20200033)

Incorporation of Omega-3 Fatty Acids in Nile Tilapia (*Oreochromis niloticus*) By-Products Containing Sacha Inchi Oil

Michele da Silva,^a Adriela A. Rydlewski,^b Marina Oliveira,^a Polyana B. F. Biondo,^a
Liane Maldaner,^{a,c,*} Jesuí V. Visentainer^{a,b,c}

^a Universidade Estadual de Maringá, Programa de Pós-Graduação em Química, Av. Colombo, 5790, CEP: 87020-900, Maringá-PR, Brasil.

^b Universidade Estadual de Maringá, Programa de Pós-Graduação em Ciência de Alimentos, Av. Colombo, 5790, CEP 87020-900, Maringá-PR, Brasil.

^c Universidade Estadual de Maringá, Departamento de Química, Av. Colombo, 5790, CEP 87020-900, Maringá-PR, Brasil.

*jesuiv@gmail.com

Recebido em 9 de Julho de 2019. Aceito para publicação em 15 de Março de 2020

1. Introduction

2. Material and Methods

- 2.1. Experimental diets
- 2.2. Ethics and sampling
- 2.3. Diets proximal composition
- 2.4. Fatty acids composition
- 2.5. Fatty acids quantification
- 2.6. Statistical analysis

3. Results and Discussion

- 3.1. Feed and oils
- 3.2. Tilapia by-products

4. Conclusion

1. Introduction

Fisheries and aquaculture provide are currently around 158 million tons of fish, being the proportion used for direct human consumption about 136 million tons, and the remaining fraction (21.7 million tons) considered by-product.^{1,2} About 50-70 % of underutilized fish parts, result in wastes, that are improperly discarded in nature, contributing to increased environmental pollution and to biological imbalance.^{3,4} This practice occurs,

mainly, due to the lack of efficient management and sustainable reuse of fish by-products, which are a potential economically viable source of oils, proteins, and other nutrients.⁵

Over the past two decades, there has been a global increase in awareness of the environmental and nutritional importance of underutilized fishery resources.⁶ In Europe, one of the main objectives of the Landing Obligation of the European Commission (EU) Common Fisheries Policy, from 2019, is for Landing Obligation to implement strategies, using alternative technologies of the recovery of this waste to develop products for

human consumption.² By-products such as heads, liver and viscera are valuable source of nutrients and may be used in the production of a extensive variety of products with nutraceutical and food applications, such as enriched animal feed, fish meal, dietetic products, cosmetics, chitin, chitosan, collagen, chondroitin, among others.^{1,5,7}

Nile tilapia (*Oreochromis niloticus*) is the most common category of aquaculture in the world.¹ (FAO, 2016). However, this freshwater fish has low levels of polyunsaturated fatty acids (PUFAs) of the omega-3 series (PUFA n-3), including alpha-linolenic acid (LNA, 18:3n-3) in comparison to saltwater fish, such as salmon and tuna.^{8,9} Therefore, researches has been carried out in order to increase the n-3 fatty acid (FA) content in cultured fish, from the diet provided.¹⁰

LNA is considered an essential FA, since it cannot be produced by the human body, and is a precursor of important long chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) which are of fundamental importance for the maintenance of health, as both FA are related to the prevention of cardiovascular diseases and cancer, in addition it contributes to the reduction of LDL cholesterol and blood triglycerides levels.^{11,12} DHA performs an important role in the formation and development of the brain and retina in the biogenesis and function of photoreceptors and neuroprotection.^{10,13} EPA is associated with protection of the hepatic profile, leading to the lower risk of developing metabolic syndrome, related to the lipid profile.¹⁴

Due to the innumerable benefits of n-3 FAs to human health, the investigation for new food sources has been increased. A potential source of omega-3 is Sacha inchi oil (SIO).¹⁵ Originally from Sacha inchi (*Plukenetia volubilis* L.) is an oleaginous plant of the family Euphorbiaceae, native to the tropical rainforest of the Andean region of South America, typical of the Peruvian Amazon region, also known as "Inca peanuts", "wild peanuts" and "mountain of peanuts". It produces star-shaped green fruits and dark-brown edible seeds, rich in oil (35-60 %) and proteins (27 %). Its oil also has several bioactive compounds with high antioxidant activity, such as tocopherols, polyphenolic compounds and phytosterols. Furthermore, it can also be used as lipid source to supplement fish food in order to increase the nutritional and lipid quality of its diets

and, consequently, of these fish by-products.^{10,15-17} In this context, the objective of this study was to evaluate the incorporation of omega-3 PUFAs in the head, liver and viscera of Nile tilapia fed with diet enriched with SIO, replacing soybean oil (SO).

2. Materials and methods

2.1. Experimental diets

The fish were submitted to two treatments: Treatment I (TI) and Treatment II (TII). TI was considered the control treatment, with addition of 4.2 % soybean oil (SO). Soybean oil was used as the lipid source, once it is generally used in foods of this species. Plant oils rich in 18:2n-6, such as soybean, corn, and sunflower oils, have been identified as lipid sources effective in promoting tilapia growth.¹⁰ TII was added 4.2 % Sacha inchi oil (SIO), replacing SO. SIO was obtained from the US herbal and spice industry. The diets were isonitrogenous (30.00 g 100 g⁻¹ of crude protein) and isoenergetic (3.20 kcal of digestible energy *per* kg) and formulated to contain 4.00 g 100 g⁻¹ lipid according to the nutritional requirements of the species.¹⁸ The ingredients used in the preparation of the treatments are listed in Table 1.

2.2. Ethics and sampling

In order to carry out this study, it was necessary to request authorization from the Committee of Ethics in Animal Use in Tests of the State University of Maringá, protocol n°. 012/2014, Opinion n°. 037/2014. The experiment was performed at the Aquaculture Experimental Station of the State University of Maringá, UEM/CODAPAR, located in the district of Floriano, Maringá, Paraná State, Brasil, 23°31'7.29"S and 52°2'20.81". A total of 48 specimens of tilapia were utilized, weighing on average 101.9 ± 0.1 g, with five months of age, distributed in 2 tanks, totalizing 24 animals in each of the tanks. Each tank has an individual capacity of 23 L of water and 20 % of daily renewal fee. Before the start of the experiment, the fish were acclimatized for fourteen days. The food was given 4 times a day (8h, 11h, 14h and 17h), manually, until apparent satiety. The fish were submitted to two treatments, with two replicates each, using a completely randomized design.

Table 1. Formulation of diets used in treatments (% by mass)

Ingredients (%)	Treatment	
	TI	TII
CornBran	21.18	21.18
Soybean Bran	53.65	53.65
Wheat Bran	8.62	8.62
Brewers Rice	7.66	7.66
Dicalcium phosphate	2.87	2.87
Premix*	0.48	0.48
NaCl (Salt)	0.48	0.48
L-Lysine	0.19	0.19
DL-Metionina	0.14	0.14
L- Threonine	0.14	0.14
Antifungal	0.10	0.10
Vitamin C (mono)	0.10	0.10
Antioxidant	0.04	0.04
L-Tryptophan	0.05	0.05
Cholinechloride	0.10	0.10
Soy oil	4.2	-
Sacha inchi oil	-	4.2

TI = treatment with 4.2 % of SO, TII = treatment with 4.2 % of SIO, SO = soybean oil, SIO = Sacha inchi oil, *Mineral and vitamin supplement.

Prior to the experiment, fish received control treatment (no addition of SIO) for diet adaptation during seven days. After this period, the treatment containing SIO was introduced for thirty days. On day 0 and day 30, four fish from each treatment were euthanized under ice, with longitudinal sectioning to the body. The heads, livers and viscera were carefully removed, separated, rinsed in tap water, vacuum packed in polyethylene bags in N₂ atmosphere and frozen stored at -18 °C. Samples were homogenized before the subsequent analysis.

2.3. Proximal composition of diets

The moisture, ash, and crude protein in the prepared diets were determined according to AOAC.¹⁹

2.4. Fatty acids composition

The total lipids (TL) were determined according to Bligh and Dyer.²⁰ Fatty acid methyl ester (FAME) was prepared by methylation of TL, as describe by Hartman and Lago.²¹ FAMES were separated by gas

chromatography (GC) using a Thermo 3300 gas chromatograph equipped with a flame ionization detector (FID) and a fused-silica CP-7420 (SELECT FAME; Agilent Technologies) capillary column (100 m x 0.25 mm i.d. x 0.25 µm of cyanopropil polysiloxane).²² The temperatures of injector and detector were 200 °C and 240 °C, respectively. The column temperature remained at 165 °C for 7 min, following a heating ramp for 4 °C min⁻¹ up to 185 °C, remaining for 4.67 min. Afterward, a new heating ramp raised the temperature to 235°C at a rate of 6 °C min⁻¹, maintained for 5 min, totalizing 30 min of analysis. Was injected 2 mL of sample with split injection at 1:80 ratio. For identification, the FA retention times were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Retention times and peaks area percentages were automatically computed by Chromquest software 5.0 (Thermo Scientific).

2.5. Quantification of fatty acids

Quantification (mg fatty acid/g of total lipids) was calculated using tricosanoic acid methyl ester as internal standard (IS) (23:0). Theoretical

FID correction factor values were used to obtain concentration values.²³ FA contents were calculated by using Eq. 1. The results were recalculated from mg g⁻¹ of TL to mg 100 g⁻¹ of sample.

$$\text{FA (mg/ g TL)} = \frac{A_X \cdot W_{IS} \cdot CF_X}{A_{IS} \cdot W_X \cdot CFAE} \times 100 \quad (1)$$

In Eq. 1, FA represents mg of FAs per g of total lipids, A_X is the FAs peak area, A_{IS} is the IS methyl ester peak area of tricosanoic acid (23:0), W_{IS} is the IS weight (mg) added to the sample, W_X is the sample weight (mg), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of FAs rather than as methyl esters.

2.6. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and the means were compared by Tukey's test ($p < 0.05$). The results were analyzed by principal component analysis (PCA) using Statistica 7.0 software. Data before treatment was not necessary.

3. Results and Discussion

3.1. Feed and oils

The proximate and the results of FAs compositions in the diets of TI and TII are displayed in Table 2.

The results presented in Table 2 did not expose significant difference in crude protein and total lipid contents between TI and TII ($p < 0.05$). The diets of both treatments were considered isoproteic (30.00 g 100 g⁻¹) reaching desirable parameters for the growth and development of this fish species.¹⁸ Both treatments TL values were around 10 %. According to Chou (2001), for good growth and development in tilapia, diets containing 5 % to 15 % of lipids are within a range adequate to meet the metabolic needs of this species.²⁴

The main saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FAs in TI and TII were palmitic (16:0), oleic (18:1n-9) and linoleic (18:2n-6) acids, respectively. Both SFA, 16:0 and 18:0 indicated no significant difference

Table 2. Principal fatty acids composition (mg 100 g⁻¹ fatty acid of sample), and proximate composition of feed TI and TII

Fatty Acids	Treatment	
	TI	TII
16:00	1020.43 ^a ± 36.21	892.80 ^a ± 66.44
18:00	234.06 ^{ab} ± 17.42	266.65 ^a ± 25.85
18:1n-9	1510.59 ^a ± 51.98	1216.97 ^b ± 85.20
18:1n-7	60.69 ^a ± 2.91	48.15 ^b ± 3.96
18:2n-6 (LA)	3381.10 ^a ± 83.37	3254.49 ^a ± 72.27
18:3n-3 (LNA)	343.61 ^a ± 30.32	2312.24 ^b ± 98.83
SFA	1254.48 ^a ± 28.53	1159.45 ^a ± 92.24
MUFA	1571.27 ^a ± 54.67	1265.11 ^b ± 87.48
PUFA	3724.70 ^b ± 112.66	5566.73 ^a ± 168.14
n-6/n-3	9.88 ^a ± 0.65	1.41 ^b ± 0.034
Proximate composition		
Ash (%)	6.79 ^a ± 0.18	7.07 ^a ± 0.21
Crude protein (%)	28.42 ^a ± 0.87	28.81 ^a ± 0.68
Moisture (%)	5.20 ^a ± 0.09	5.46 ^a ± 0.05
Total lipids (%)	10.36 ^a ± 0.93	10.42 ^a ± 0.85

Data provided by Aquaculture Laboratory, Zootechnics Department of the State University of Maringá. Results expressed as means of three replicates. Different letters in the same line are significantly different ($p < 0.05$) by Tukey test. TI = treatment with 4.2 % of SO; TII = treatment with 4.2 % of SIO; LA = linoleic acid, LNA = alpha-linolenic acid, SFA = total saturated fatty acid, MUFA = total monounsaturated fatty acid, PUFA = total polyunsaturated fatty acid, n-6 = total omega-6 fatty acid, n-3 = total omega-3 fatty acid.

between the treatments, what reflected in the SFA sum (Σ SFA), which remained statistically constant in both treatments. LA (18:2n-6) concentrations between both treatments also did not present significant difference. On the other hand, the MUFA 18:1n-9, 18:1n-7 and the MUFA sum (Σ MUFA) were different between TI and TII.

According to Anvisa,²⁵ foods with more than 0.6 g of LNA per 100 g of food are considered high in omega-3. Sacha inchi has approximately 50 % of oil, resulting in 2.31 g of LNA *per* 100 g of seed and can be considered a source of this FA. Supplementation with SIO promoted a 6-fold increase in LNA values, which was 343.61 mg from 100 g⁻¹ in TI to 2312.24 mg of 100 g⁻¹ in TII, exposing significant difference between treatments. A study carried out by Schneider *et al.*²⁶ where they used 4.2 % of chia oil as a source of n-3 to complement the Nile tilapia diet, obtained lower LNA values (1.598 mg of LNA *per* 100 g⁻¹) than the values found in this work. These results indicate that supplementation with SIO may be a great alternative for the incorporation of n-3 FAs, in substitution to sources commonly used as chia.

The content of LNA found in TII, reflected in the lower n-6/n-3 ratio (1.41) of this treatment, which was 7 times lower than the TI (9.88), besides being lower than 4.0, which is the value recommended by the University of Maryland Medical Center.²⁷

3.2. Tilapia by-products

The major FAs found in the by-products of TI and TII tilapia are exhibited in Table 3. The highest levels of palmitic, oleic and linoleic acid were found in tilapia viscera (TI). In TI, the MUFA, PUFA and SFA sum was 7290.09 mg 100 g⁻¹, 4154.82 mg 100 g⁻¹ and 6157.96 mg 100 g⁻¹ (of sample), respectively, differing significantly from samples supplemented with SIO (TII). These results confirm the efficiency of SIO supplementation in increasing the lipid quality of its by-products, and consequent reduction in the SFA sum. The dietary intake of foods with high levels of FAs of this class is related to the increase of several diseases, including coronary and cardiovascular diseases.²⁸

The concentration of the essential fatty acid LNA (18:3n-3) increased in the proportion of 3.73, 4.67 and 1.43 times in head, liver and viscera samples, respectively (TII), presenting significant difference between treatments. In a study by Oliveira *et al.*²⁹ where they supplemented feed

with 4.2 % of Japanese grape oil to evaluate the incorporation of n-3 FAs into Nile tilapia fillets, the results showed that the LNA values were 1.3 times higher than the group control, lower than those found in our by-products. These results indicate, therefore, that SIO can also be an efficient source of LNA in fish food supplementation.

The incorporation of LNA into tilapia heads resulted in 260 % increase in the sum of these FAs (Σ n-3), while liver and viscera samples had an increase of 228 and 110 %, respectively, in TII when compared to TI. The results of the incorporation of the LNA in the TII samples elevate of the nutritional value and the consequent quality of the products that can be generated from these by-products.⁹ Intake of omega-3 FAs is strongly associated with the prevention and treatment of cardiovascular diseases and several other health problems, such as systemic arterial hypertension (SAH) and dyslipidemia. However, there are few rich sources of these FAs available in nature. Furthermore, consumption of omega-3 FA sources is low in the occidental countries, due to cultural factors, which contributes even more to the low intake of LC-PUFAs and its precursor.^{12,30}

According to Aguiar *et al.*⁸ during the elongation and desaturation processes of omega-3 FAs, may occur storage of the precursor LNA in the liver of Nile tilapia rather than being fully converted to EPA and DHA. In addition, during the elongation and desaturation process, n-3 and n-6 FAs share the same enzymes responsible for the conversion of LNA and LA to their long chain fatty acids (LC-PUFAs). Therefore, the competition between FAs of the different series may occur, where the excess of one group may cause a decrease in the conversion yield of the other group.³¹ These metabolic processes may justify EPA (20:5n-3) values found in the liver and viscera, and also in the DHA (22:6n-3) values in the viscera's samples, which did not present significant difference between TI and TII.

On the other hand, the EPA stood out in the TII head samples (12.04 mg 100 g⁻¹), differing significantly from TI (8.19 mg 100 g⁻¹). DHA also showed higher values in the head samples (67.50 mg 100 g⁻¹ in TII and 41.29 mg 100 g⁻¹ in TI) and liver (209.32 mg 100 g⁻¹ in TII and 81.69 mg 100 g⁻¹ in TI). These LC-PUFAs are recognized to perform vital roles in human nutrition because it present protective effect against the development of various chronic degenerative

diseases. For these reasons, the increase in lipid quality of tilapia products must be encouraged.²⁷

It was possible to observe that the increase in n-3 FA concentration provided considerable differences in the n-6/n-3 ratio of TII. The samples of head, liver and TII viscera indicated reduction of 2.53, 2.21 and 1.65 times, respectively, in comparison to the TI samples. The lowest proportion n-6/n-3 was found in the liver and head samples of TII (1.33 and 2.91, respectively). These values are in accordance with the recommendations of several nutrition societies, which guide a ratio of approximately 5:1 from n-6 to n-3 for the prevention of diseases such as diabetes, cancer and obesity.^{30,32} The highest values of the n-6/n-3 ratio were observed in TI for viscera (9.48) sample and in the head (7.38) samples. It is important accentuate that SO is one of the most widely used lipid sources in the manufacture of diets for cultured fish, which may also have influenced the n-6/n-3 ratio values of these samples.³³

For establish correlations between the variables SFA, MUFA, PUFA, n-6 FAs sum, n-3 FAs sum, n-6/n-3 ratio, and the samples, statistical

analysis of principal component analysis (PCA) was used (Figure 1).

Figure 1 displays samples/score (H-TI, H-TII, L-TI, L-TII, V-TI and VTII) and variables/loadings (SFA, MUFA, PUFA, n-6 FA sum, n-3 FA sum and n-6/n-3 ratio). Two principal components (PC) were necessary to explain the total variance of the all data analyzed. The first principal component (PC1) explained 85.64 % of the variance, while the second component (PC2) explained 13.54 %. Thus, PC1 and PC2 accounted for 99.18 % of the total variance of the data.

The first group contributed positively in PC1 and was composed of V-TI, which stood out in the MUFA, SFA, n-6 content and n-6/n-3 ratio. In addition, the H-TII and V-TII samples were responsible for the high (variable) loads of n-3, and for this reason, it was positively clustered in PC1. These samples were also separated in the positive quadrant of PC2, as well as L-TII, because it is characterized by high concentrations of PUFAs and FA n-3 (SIO).

The second group formed by L-TI, H-TI and L-TII, were grouped in the negative quadrant of PC1, since these samples obtained the lowest

Table 3. Principal fatty acids composition (mg fatty acid 100 g⁻¹ of sample), summation and ratios of the heads, liver and viscera of tilapia of the treatments I and II

	Head		Liver		Viscera	
	TI	TII	TI	TII	TI	TII
	(4,2 % SO)	(4,2 % SIO)	(4,2 % SO)	(4,2 % SIO)	(4,2 % SO)	(4,2 % SIO)
Fatty acids						
Main SFA 16:0	1336.58a ± 58.17	1382.82a ± 67.91	384.34a ± 19.33a	427.32a ± 40.19	3760.10a ± 0.35	2742.00b ± 7.32
Main MUFA 18:1n-9	2030.31a ± 86.19	2197.38a ± 83.27	372.05a ± 13,64	413.79a ± 34.56	5857.83a ± 15.78	4300.20b ± 22.20
Main PUFA 18:2n-6	1394.67a ± 35.65	1443.27a ± 35.65	231.85a ± 19.97	281.28a ± 28.81	3174.89a ± 21.27	2135.24b ± 11.55
18:3n-3	104.08b ± 2.87	388.50a ± 19.30	12.32b ± 1.31	57.61a ± 4.13	147.27b ± 7.82	210.96a ± 0.87
20:4n-3	15.12b ± 1.14	47.69a ± 2.16	4.77b ± 0.27	20.23a ± 1.30	29.54a ± 1.57	33.89a ± 0.63
20:5n-3	8.19ab ± 0.16	12.04a ± 0.53	2.88a ± 0.12	5.63a ± 0.49	20.30a ± 0.84	24.63a ± 1.24
22:4n-3	49.65a ± 3.12	52.00a ± 3.16	20.50a ± 0.92	21.02a ± 1.48	109.15a ± 4.98	78.25b ± 5.80
22:6n-3	41.29b ± 2.21	67.50a ± 2.86	81.69b ± 5.49	209.32a ± 20.55	84.97a ± 4.54	85.84a ± 7.53
Σ SFA	2007.85a ± 94.15	2101.80a ± 89.61	803.23a ± 41,10	748.25a ± 53.55	6157.96a ± 7.28	4406.17b ± 5.98
Σ MUFA	2583.6a ± 71.47	2827.18a ± 61.33	550.35a ± 73.30	558.36a ± 49.17	7290.09a ± 6.83	5316.87b ± 17.84
Σ PUFA	1829.67a ± 64.77	2220.57a ± 94.62	539.53a ± 16.02	730.02a ± 58.83	4154.82a ± 60,51	2909.45b ± 7.98
Σ n-6	1611.45a ± 54.63	1652.84a ± 74.54	403.86a ± 29.35	416.21a ± 39.63	3758.36a ± 30.88	2477.87b ± 4.14
Σ n-3	218.34b ± 10.07	567.73a ± 30.87	137.35b ± 3.02	313.81a ± 19.80	396.46ab ± 21.45	431.58a ± 4.03
n-6/n-3	7.38a ± 0.21	2.91b ± 0.16	2.94a ± 0.13	1.33a ± 0.10	9.48a ± 0.03	5.74b ± 0.00

Results expressed as means of three replicates, Equivalent parts of the fish were compared between TI and TII, Different letters in the same line are significantly different (p<0.05) by Tukey test, ΣSFA = total saturated fatty acid, ΣMUFA = total monounsaturated fatty acid, ΣPUFA = total polyunsaturated fatty acid, n-6 = total n-6 FA, n-3 = total n-3 FA, SO = soybean oil, SIO = Sacha inchi oil

Figure 1. PC: principal component. Biplot of scores and loadings from the principal component analysis (PCA) of heads (H), liver (L) and viscera (V) of TI and TII

loads of n-3 in relation to the others. The negative portion of PC2 was composed of samples L-TI, H-TI and V-TI, which were supplemented with SO and presented high amounts of SFA, MUFAs, n-6 FAs and n-6/n-3 higher ratio, confirming the results found in the analysis of FAs.

4. Conclusion

All samples treated with SIO revealed significant increase in LNA concentration in comparison to samples of Nile Tilapia fed with SO. EPA excelled in the head samples and DHA in the liver and head samples, supplemented with SIO. PCA statistical analysis exposed the formation of two groups according to the diets supplied, confirming which samples treated with SIO presented higher levels of LNA and LC-PUFAs. Our results indicated that Nile Tilapia by-products submitted to treatment TII have become valuable sources of omega-3 PUFAs and can be considered as an alternative to supplement fish diet. Therefore, the recovery of this biomass can be potentially applied in the production of various products, for becoming of the elevate lipid value.

Acknowledgments

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação Araucária de Apoio ao

Desenvolvimento Científico e Tecnológico do Estado do Paraná (FAPPR) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support and fellowships.

References

- ¹ FAO, 2016. Available on: <<http://www.fao.org/3/a-i5555e.pdf>>. Accessed on: 30 june 2019.
- ² European Commission. Regulation (EU) N° 1380/2013 of the European Parliament and the Council of 11 December 2013 on the Common Fisheries Policy, Amending Council Regulations (EC) N° 1954/2003 and (EC) N° 1224/2009 and Repealing Council Regulations (EC) N° 2371/2002 and (EC) N° 639/2004 and Council Decision 2004/585/EC; European Commission: Brussels, Belgium, 2013.
- ³ Ezejiofor, T. I. N.; Enebak, U. E.; Ogueke, C. Waste to wealth-value recovery from agro-food processing wastes using biotechnology: a review. *British Biotechnology Journal* **2014**, *4*, 418. [CrossRef]
- ⁴ Olsen, R. L.; Toppe, J.; Karunasagar, I. Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. *Trends in Food Science & Technology* **2014**, *36*, 144. [CrossRef]
- ⁵ Vázquez, J. A.; Meduñña, A.; Durán, A. I.; Nogueira, M.; Fernández-Compás, A.; Pérez-Martín, R. I.; Rodríguez-Amado, I. Production of valuable compounds and bioactive metabolites from by-products of fish discards using chemical processing, enzymatic hydrolysis, and bacterial fermentation. *Marine Drugs* **2019**, *17*, 139. [CrossRef] [PubMed]

- ⁶Sheriff, S. A.; Sundaram, B.; Ramamoorthy, B.; Ponnusamy, P. Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of *Rastrelliger kanagurta* by proteolytic enzymes. *Saudi Journal of Biological Sciences* **2014**, *21*, 19. [CrossRef] [PubMed]
- ⁷Wisniewski, A.; Wiggers, V.R.; Simionatto, E.L.; Meier, H.F.; Barros, A. A. C.; Madureira, L. A. S. Biofuels from waste fish oil pyrolysis: Chemical composition. *Fuel* **2010**, *89*, 563. [CrossRef]
- ⁸Aguar, A. C.; Morais, D. R.; Santos, L. P.; Stevanato, F. B.; Visentainer, J. E. L.; De Souza, N. E.; Visentainer, J. V. Effect of flaxseed oil in diet on fatty acid composition in the liver of Nile Tilapia (*Oreochromis niloticus*). *Archivos Latinoamericanos de Nutricion* **2007**, *57*, 273. [PubMed]
- ⁹Chen, C.; Sun, B.; Li, X.; Li, P.; Guan, W.; Bi, Y.; Pan, Q. N-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: Quantification of optimum requirement of dietary linolenic acid in juvenile fish. *Aquaculture* **2013**, *416*, 99. [CrossRef]
- ¹⁰Ng, W. K.; Chong, C. Y.; Wang, Y.; Romano, N. Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil finishing diets. *Aquaculture* **2013**, *372*, 97. [CrossRef]
- ¹¹Mazza, M.; Pomponi, M.; Janiri, L.; Bria, P.; Mazza, S. Omega-3 fatty acids and antioxidants in neurological and psychiatric diseases: An overview. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **2007**, *31*, 12. [CrossRef]
- ¹²Eltweri, A. M.; Thomas, A. L.; Metcalfe, M.; Calder, P. C.; Dennison, A. R.; Bowrey, D. J. Potential applications of fish oils rich in omega-3 polyunsaturated fatty acids in the management of gastrointestinal cancer. *Clinical Nutrition* **2017**, *36*, 65. [CrossRef] [PubMed]
- ¹³Koletzko, B. Human Milk Lipids. *Annals Nutrition & Metabolism* **2016**, *69*, 28. [CrossRef] [PubMed]
- ¹⁴Machado, G.; Puton, B. F.; Bertol, C. Nutracêuticos: Aspectos Legais e Científicos. *Revista Eletrônica De Farmácia* **2019**, *16*. [CrossRef]
- ¹⁵Chirinos, R.; Zuloeta, G.; Pedreschi, R.; Mignolet, E.; Larondelle, Y.; Campos, D. Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity. *Food Chemistry* **2013**, *141*, 1732. [CrossRef] [PubMed]
- ¹⁶Fanali, C.; Dugo, L.; Cacciola, F.; Beccaria, M.; Grasso, S.; Dachà, M.; Mondello, L. Chemical characterization of Sacha inchi (*Plukenetia volubilis* L.) oil. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 13043. [CrossRef] [PubMed]
- ¹⁷Kumar, B.; Smita, K.; Cumbal, L.; Debut, A. Sacha inchi (*Plukenetia volubilis* L.) shell biomass for synthesis of silver nanocatalyst. *Journal of Saudi Chemical Society* **2017**, *21*, S293. [CrossRef]
- ¹⁸National Research Council, Nutrient Requirements of Fish, The National Academies Press: Washington, DC, 1993.
- ¹⁹Association of Official Analytical Chemists; Official Methods of Analysis of AOAC International; Cunniff, P. A., ed.; AOAC: Arlington, 1998.
- ²⁰Bligh, E. G.; Dyer, W. J. A. Rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **1959**, *37*, 911. [CrossRef]
- ²¹Hartman, L.; Lago, R. C. A. Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice* **1973**, *22*, 475. [PubMed]
- ²²Martin, C. A.; de Oliveira, C. C.; Visentainer, J. V.; Matsushita, M.; de Souza, N. E. Optimization of the selectivity of a cyanopropyl stationary phase for the gas chromatographic analysis of trans fatty acids. *Journal of Chromatography A* **2008**, *1194*, 111. [CrossRef]
- ²³Visentainer, J. V. Aspectos analíticos da resposta do detector de ionização em chama para ésteres de ácidos graxos em biodiesel e alimentos. *Química Nova* **2012**, *35*, 274. [CrossRef]
- ²⁴Chou, B. S.; Shiau, S. Y.; Hung, S. S. O. Effect of dietary cod liver oil on growth and fatty acids of juvenile hybrid tilapia. *North American Journal of Aquaculture* **2001**, *63*, 277. [CrossRef]
- ²⁵ANVISA, Regulamento Técnico Sobre Informação Nutricional Complementar; Accessed on: 01 July 2019. [Link]
- ²⁶Schneider, V. V. A.; Carbonera, F.; Montanher, P. F.; Lopes, A. P.; Matsushita, M.; Visentainer, J. V. Incorporation of alpha-linolenic acid and enhancement of n-3 fatty acids in Nile tilapia: A factorial design. *Journal of the American Oil Chemists' Society* **2015**, *92*, 693. [CrossRef]
- ²⁷Young, K. Omega-6 (n-6) and omega-3 (n-3) fatty acids in tilapia and human health: A review. *International Journal of Food Sciences and Nutrition* **2009**, *60*, 203. [CrossRef] [PubMed]
- ²⁸De Souza, R. J.; Mente, A.; Maroleanu, A.; Cozma, A. I.; Ha, V.; Kishibe, T.; Uleryk, E.; Budylowski, P.; Schünemann, H.; Beyene, J.; Anand, S. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* **2015**, *351*, h3978. [CrossRef]

- ²⁹ Oliveira, M.; Ribeiro, R. P.; Silva, M. C.; Montanher, P. F.; Carbonera, F. C.; Visentainer, J. V.; Maldaner, L. Effects of a diet supplemented with japanese grape (*hovenia dulcis*) seed oil on the omega-3 and nutritional lipid quality in Nile Tilapia (*Oreochromis niloticus*). *Journal of the Brazilian Chemical Society* **2018**, 29, 463. [\[CrossRef\]](#)
- ³⁰ Simopoulos A. P. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* **2016**, 8, 128. [\[CrossRef\]](#) [\[PubMed\]](#)
- ³¹ Dubois, V.; Breton, S.; Linder, M.; Fanni, J.; Parmentier, M. Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology* **2007**, 109, 710. [\[CrossRef\]](#)
- ³² De Caterina, R.; Madonna, R.; Zucchi, R.; La Rovere, M.T. Antiarrhythmic effects of omega-3 fatty acids: From epidemiology to bedside. *American Heart Journal* **2003**, 146, 420. [\[CrossRef\]](#) [\[PubMed\]](#)
- ³³ Hossain, M. A. Fish as source of n-3 polyunsaturated fatty acids (PUFAs), which one is better-farmed or wild? *Advance Journal of Food Science and Technology* **2011**, 3, 455. [\[Link\]](#)