

Whole Chia Flour as Yield Enhancer, Potential Antioxidant and Input of n-3 Fatty Acid in a Meat Product

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Abstract

In this paper, the effect of the addition of whole chia flour and water at cooked fish burgers on yield, water and fat retention, n-3 fatty acids content and oxidative stability were studied. The results showed that 94% of yield, 92% of water retention and 97% of fat retention were achieved with the addition of 5.92 g of whole chia flour and 15 g of water per 100 g of burger. The n-3 fatty content increased from 704.57 ± 21.66 mg to 1551.71 ± 47.71 mg/100g of cooked burger when the chia flour was added, enhancing nutritional quality indices of lipid. A good oxidative stability, with minimum formation of conjugated dienes, hydroperoxides and thiobarbituric acid reactive substances during 30 days frozen storage was obtained too, probably due to the presence of polyphenolic compounds with antiradical activity and reducing power in the fibrous fraction of the flour chia. The results from the present study highlight remarkable technological applications of whole chia flour as food ingredient in the design of healthier fish meat commodities.

Keywords

Freshwater Fish, Whole Chia Flour, Potential Antioxidant, Yield, Fortified Foods

1. Introduction

At present, food industries are investing in the development of new beneficial food products; in this sense,

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products have been marketed as “low fat” or “light” in saturated fatty acids (SFA), enriched with n-9, n-3 and/or n-6 fatty acids. To achieve this, they have resorted to the incorporation of a wide variety of ingredients into foods, whose consumption could prevent diseases, such as flax seed [1] and chia seed among others, as well as in oils rich in unsaturated fatty acids [2], including sunflower oil [3] and olive oil [4]. Other good sources of unsaturated fatty acids are products of ictio origin, fish meats and oils [5] mainly of marine origin.

The river fish have lipids with less n-3 fatty acids content than seafish [6], but they represent an attractive alternative to design and technological development of new fortified products especially when the access to seafood is limited due to the long distances from distribution centers, being for this reason more expensive. One of the most consumed water fish from Paraná River is the spotted catfish (*Pseudoplatystoma corruscans*). This fish, which belongs to the family Pimelodidae, is characterized by a high fat content (12.2 g/100g), being also an important source of protein. This fat contains mainly triglycerides of unsaturated fatty acids (56% of the total fat) among which are the monounsaturated fatty acids (MUFA) n-9 oleic acid, and the polyunsaturated fatty acids (PUFA) n-6 linoleic acid and n-3 docosahexaenoic acid (DHA) [7].

With respect to meat and processed derivatives, storage and cooking are necessary for safe and palatable product but these methods may also play a significant role in the development of oxidation processes, which affect the physico-chemical state of proteins and their amino acid bioavailability [8]. It may also affect the lipid composition of the meat, especially favors the loss of essential fatty acids and consequently, reducing the nutritional value [9]. Also, depending on the intensity and duration of heat treatment, as well as the intrinsic characteristics of the meat emulsion, many authors have reported significant weight loss, water and fat after the meat products have undergone cooking and storage [10]-[12]. So it is necessary to incorporate different additives to try to minimize this negative effect.

Chia seed (*Salvia hispanica* L.) contains between 30 - 33.5 g oil/100g with high concentrations of polyunsaturated fatty acids (PUFA), being α -linolenic acid (ALA), a n-3 fatty acid the main essential fatty acid present in it (57 - 65 g/100g) [13]. Another benefit of chia seed is its high fiber with excellent technological properties as a texturizing, stabilizing, which intervenes in the gel formation, improves the ability of water and fat retention, etc., among other properties [14] [15]. In addition, it is known that the chia seeds [16] contain a great amount of polyphenolic compounds with potent antioxidant activity, mainly flavonoids such as quercetin, kaempferol, myricetin and others. The insoluble fraction of the chia flour (dehydrated and degreased fraction) also showed antioxidant activity comparable to that of Trolox [17]. It is important to highlight the nutritional importance of chia seeds, which make them, an ideal ingredient to add a great number of culinary preparations.

Therefore, bring together the benefits of one freshwater fish with a rich source of n-3 fatty acids, such as whole chia flour, in only one product seems to be an interesting perspective.

The aims of this research were: 1) to optimize the effect of addition of different levels of whole chia flour and water on the technological parameters (yield, water and lipids retention) of precooked surubi meat burgers; 2) to evaluate the changes on nutritional values of the optimized burgers with the whole chia flour addition; 3) to characterize fibrous fraction of whole chia flour used in this research with respect to its antioxidant properties and 4) to analyze the oxidative stability of the precooked products during storage frozen.

2. Materials and Methods

2.1. Materials and Samples

Samples of stained surubi specimen (*Pseudoplatystoma corruscans*) were extracted from Parana River (27° 17'30"S latitude and 58° 59'50"W longitude) by fishermen through trawls. The specimens once sacrificed were eviscerated and their head and tail removed in the place of arrival of the boats; immediately the pieces of fish were transferred under refrigeration at Food Industries II laboratory.

Chia seeds were provided by Nutraceutica Sturla SRL (Argentina) from crops grown in Salta state (25° 04'02"S latitude and 65.5° 28'00"W longitude) and kept in plastic packages hermetically sealed to 5°C ± 1°C. Then, whole chia flour (moisture: 4.35 g/100g, protein: 17.90 g/100g, fat: 37.11 g/100g, carbohydrates: 35.76 g/100 and ash: 5.07 g/100g) was obtained by grinding through a concentric disc manual grinder (Toolcraft® tc 2541) and frozen at -18°C until its use.

2.2. Burger Manufacture

Fish meat was ground and emulsified with Philips® processor for 3 minutes. NaCl (2 g/100g and sodium poly-

phosphate (0.5 g/100g-Bernecol[®]) were mixed with emulsion. The amounts of whole chia flour and water were added according to a factorial multilevel design. The mixes were kneaded for 15 minutes and then were molded in samples of approximately 100 g each one (100 mm of diameter and 20 mm of height). Afterward, one batch of meat burger was cooked in a static oven (Tecnodalvo[®]) at 200°C for 15 minutes until the thermal center reached 73°C during 15 seconds to reach food safety; and another batch was left raw for control. The burgers were weighed before and after cooking to calculate cook losses. Once cooked and cooled, they were packed in polyethylene film with an oxygen permeability 2000 cm³/m² 24 h and frozen at -18°C.

2.3. Experimental Design

The factorial multilevel design adopted was 3² (two factors with three levels). The levels of the water factor were A: 15 g, B: 25 g and C: 35 g per 100 g, and the levels of whole chia flour factor, E: 0 g, F: 5 g and G: 10 g per 100 g. A response surface methodology was used to analyze the effect of the two independent variables (water and whole chia flour) on the responses (yield, water and lipid retention). The treatment structure was completely randomized with 2 replications. A multiresponse optimization was used to find the optimum formulation, using a desirability function approach which is based on the idea that the “quality” of a product or process has multiple quality characteristics, with none of them outside some “desired” limits. The method finds operating conditions that provide the “most desirable” response values [18]. The individual maximum response function to yield, lipid and water retention was combined and the maximum desirability was obtained.

2.4. Cooking Measurements

Cooking yield, and the lipids and water retention of burgers were calculated as follows:

$$\text{Yield (\%)} = (\text{Cooked Product Weight} / \text{Raw Product Weight})$$

$$\text{Water Retention (\%)} = (\text{Water Cooked Product Weight} / \text{Water Raw Product Weight}) * 100$$

$$\text{Lipid Retention (\%)} = (\text{Lipid Cooked Product Weight} / \text{Lipid Raw Product Weight}) * 100$$

2.5. Chemical Analysis

2.5.1. Proximate Analysis

Moisture was determined by weight loss after 24 h at 105°C in an assisted air circulation oven according to AOAC Method 950.46 [19]. Crude protein content was analyzed by the Kjeldahl procedure (AOAC Method 960.52) [19] and the fat content was determined in samples previously dried by the Soxhlet method, using petroleum ether (B_p: 30°C - 60°C) as extraction solvent. Proximate composition of burgers was done in triplicate. Total dietary fiber (TDF) was determined by an enzymatic-gravimetric method (AOAC Method 985.29) [19].

2.5.2. Fatty Acid Composition and Nutritional Quality of Lipids

Extraction and purification of fat were performed according to the method of Bligh & Dyer (1959) [20]. The fatty acid methyl esters were prepared according to the technique of the AOAC Method 969.33 [19], and quantified with Agilent Technologies gas chromatograph equipped with a 60 capillary column Supelco 2340[®] and a FID detector. The oven temperature was held at 140°C for 5 min and subsequently increased to 4°C/min to reach 240°C and maintained this temperature for 15 min. Identification of fatty acid methyl esters was based on retention time of standard methyl esters (Supelco[®] 37 Components FAME Mixture, Bellefonte, PA), plus Conjugated Linoleic Acids (Sigma-Aldrich) and Methyl cis-7, 10, 13, 16, 19-Docosapentaenoate (Supelco) eluting from the capillary column. Peak areas were integrated using chromatography data software, and concentrations of each ester were calculated as a percentage of the total area of the chromatogram. Data were expressed as mg fatty acid per 100 grams of product. The Index of Atherogenicity (IA) [21], Index of Thrombogenicity (IT) [21] and HH [22] were calculated as follows:

$$IA = [C12:0 + (4 \times C14:0) + C16:0] / [\Sigma MUFA + \Sigma n-6PUFA + \Sigma n-3PUFA]$$

$$IT = [C14:0 + C16:0 + C18:0] / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n-6PUFA) + (3 \times \Sigma n-3PUFA) + (\Sigma n-3PUFA / \Sigma n-6PUFA)]$$

$$HH = (C18:1cis9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0)$$

2.5.3. Antioxidant Properties of Chia Flour

1) Preparation of extracts

The whole chia flour was prewashed degreased and dried to obtain only the fibrous fraction. The extracts for analysis of antioxidant properties were obtained by dissolving the fibrous fraction in 70% ethanol overnight in a shaker at room temperature, and subsequent filtration through 0.45 μm .

2) Analysis of antioxidant properties

The Folin-Ciocalteu reagent assay was used to determine the total phenolic content [23] [24]. At 1 ml sample, distilled water was added to obtain 10 ml solution, followed by addition of 0.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 1 ml of saturated sodium carbonate (Na_2CO_3) was added and then kept in darkness for an hour. Subsequently, the absorbance at 735 nm was measured. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of dry matter.

The determination of the free radicals scavenging capacity of fibrous fraction of whole chia flour was determined on DPPH^{*} (1,1-diphenyl-2-picrylhydrazyl radical) and ABTS⁺, [2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation].

Both assays were carry out according to the method descript by Siddhuraju *et al.* (2002) [25]. Ascorbic acid (AA) was used as a reference and the spectrophotometric methods consisted in measuring the decrease in absorbance at 517 nm and 734 nm respectively, of 10 μl samples added to 3 ml of the DPPH^{*} solution (10^{-3} M) and ABTS⁺ radical cation solution (7×10^{-3} M) for 10 minutes at room temperature. The absorbance was read in a UV-Vis spectrophotometer (Thermo Scientific UV-Vis, Evolution 600). The results were expressed as mg of vitamin C equivalent (AAE) per gram of dry matter, using as reference a standard curve.

The reducing power was determined according to the method of potassium ferricyanide in presence of ferric chloride [26], by measuring absorbance at 700 nm. Ascorbic acid was used as standard, expressing the results in mg of vitamin C equivalents (AAE) per gram of dry matter.

2.5.4. Evaluation of Oxidative Stability of Optimized Burger

The cooked fish burgers stored at -18°C for 90 days were sampled at 0, 30, 60 and 90 days.

The evaluation of lipid oxidation was based on the determination of primary and secondary lipid oxidation products. The primary lipid oxidation products included conjugated dienes and lipid hydroperoxides, whereas the secondary lipid oxidation products were determined as malondialdehyde.

Conjugated dienes (CD) were quantified spectrophotometrically according to the AOAC Method 957.13 [19] of polyunsaturated fats and oils. 10 ± 2 mg of fat was weighed and dissolved in 4 ml of hexane. The absorbance was measured at 233 nm in a UV-Vis spectrophotometer (Thermo Scientific UV-Vis, Evolution 600). Data were expressed in mg of conjugated linoleic acid (CLA) equivalent per gram of fat, using a calibration curve of conjugated linoleic acid (Sigma Aldrich), according to Ponginebbi *et al.* (1999) [27].

Lipid hydroperoxides were determined according to the FIL-IDF 74A method (International Dairy Federation 1991) [28] slightly modified. Lipids (2 - 5 mg depending on the extent of peroxidation), were mixed with 9.9 ml chloroform-methanol (70:30 v/v) in a glass tube. Ammonium thiocyanate solution (0.05 ml) was added and the sample was mixed again. Then 0.05 ml iron II from a ferrous chloride solution was added to reaction mixture and after 5 min incubation at room temperature, the absorbance was measured at 500 nm. The results of peroxide value (PV) were expressed in milliequivalents of peroxides per kg fat.

The secondary oxidation products of lipid oxidation were measured as TBARS (thiobarbituric acid reactive substances) by spectrophotometric method described by Romero *et al.* (2005) [29]. TBARS were expressed as mg of malondialdehyde (MDA) per kg of fat in samples using 1, 1, 3, 3-tetramethoxypropane (TEP) as a standard.

2.5.5. Statistical Analysis

All results were expressed as mean \pm standard deviation ($M \pm SD$) and an analysis of one-way variance (ANOVA) was applied. To media comparisons the Tukey's test was used (p value < 0.05).

3. Results and Discussion

3.1. Optimization of the Formulation

Table 1 gives codification, Yield (% w/w) Water and Lipid Retention (% w/w) for all tested formulations of burgers. The factor 1 (Water) decreased significantly the yield, water and lipid retentions, while factor 2 (Whole chia flour) influenced positively on the response variables and showed a parabolic behavior.

The decreasing of cooking yield and water and the lipid retention obtained when the water addition was increased is according to Velioğlu *et al.* (2010) [11], who reported that the yield by cooking decreased when the amount of water in the formulation of beef burgers was increased. Valenzuela Melendres *et al.* (2014) [30] also found that the addition of tomato paste with high water content to beef burgers produced a decreased cooking yield and that could be due to an increase in the percentage of moisture in the product.

On the other hand, the flour chia addition showed a different behavior in cooking yield of beef burgers presenting higher percentages, except to 10% of chia flour and 35% of water. Valenzuela Melendres *et al.* (2014) [30] reported that flaxseed flour produced an increment of the dry matter of the beef burgers, and then a higher cooked yield.

The optimization criteria were to maximize yield and water and lipid retention. The mathematical model used to find a global desirability value is a geometric media of individual desirability values. **Figure 1** shows the desirability function to water and whole chia flour for Yield (%w/w) Water and Lipid Retention (% w/w) in all formulations tested burgers. The optimum combination of ingredients to add intosurubí burgers were 15 g of water (−1) and 5.92 of whole chia flour (0.18) per 100 g of product, with a global or overall desirability of 0.98. The optimal formulation gave a cooking yield of $93.94\% \pm 0.41\%$ w/w and moisture content of $70.36\% \pm 0.34\%$ w/w and fat content of $7.27\% \pm 0.10\%$ w/w.

As regards lipid and water retention, the optimal formulation retained $97.10\% \pm 0.31\%$ w/w and $91.87\% \pm 0.27\%$ w/w respectively. Higher fat and moisture retention of optimal burgers can be attributed to binding and stabilizing effect produced by the fibrous portion of whole flour chia [15]. This is according to Anderson & Berry (2001) [31], who hypothesized that the mechanism of water and fat retention could be predominantly physical. Then, the swelling of the starch and the fiber, in addition to some fat absorption by the fiber, could interact with the protein of the ground meat to form a matrix, which would prevent the coalescence and migration of fat from coming out of the product.

3.2. Nutritional Value and Lipid Profile

To evaluate changes in nutritional value, the control burger (formulation without adding chia flour) and the burger added with 5.92 g/100g the whole chia flour (Optimal), both cooked and stored at -18°C for 90 days were compared.

Table 1. Yield, water and lipid retention of burgers (% w/w).

Formulation	Water		Whole chia flour		Yield (% w/w)	Water retention (% w/w)	Lipid retention (% w/w)
	Codified	g/100g	Codified	g/100g			
AE	−1	15	−1	0	$92.52^a \pm 0.06$	$90.13^b \pm 0.31$	$80.65^b \pm 0.51$
BE	0	25	−1	0	$92.70^a \pm 0.24$	$90.82^b \pm 0.95$	$83.84^b \pm 0.76$
CE	1	35	−1	0	$92.78^a \pm 0.37$	$90.31^b \pm 0.22$	$92.97^b \pm 1.53$
AF	−1	15	0	5	$94.24^b \pm 0.41$	$92.19^a \pm 0.27$	$95.67^a \pm 0.41$
BF	0	25	0	5	$93.58^{ab} \pm 0.12$	$91.08^{ab} \pm 0.02$	$91.62^{ab} \pm 2.82$
CF	1	35	0	5	$93.08^{ab} \pm 0.54$	$91.06^{ab} \pm 2.06$	$96.71^{ab} \pm 8.61$
AG	−1	15	1	10	$93.42^{ab} \pm 0.34$	$90.61^{ab} \pm 0.11$	$96.11^{ab} \pm 0.24$
BG	0	25	1	10	$93.11^{ab} \pm 0.21$	$89.53^{ab} \pm 0.36$	$80.87^{ab} \pm 2.78$
CG	1	35	1	10	$92.86^a \pm 0.01$	$88.98^b \pm 1.48$	$74.69^b \pm 6.83$

Different letters in the same column indicate significant differences ($p < 0.05$).

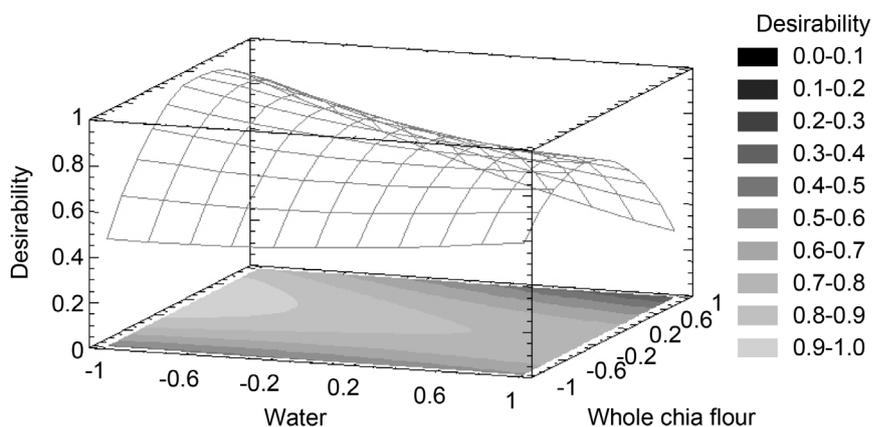


Figure 1. Response surface for overall desirability. It combined individual maximum desirability function to yield, lipid and water retention. Desirability was maximized with respect to water and whole chia flour contents expressed as codified values. The lighter shade indicates highest value.

The moisture content decreased slightly from 74.05 ± 0.01 to 70.36 ± 0.34 g/100g and protein content varied from 18.57 ± 0.13 to 17.47 ± 0.77 g/100g respectively. Optimization of the product showed a small loss of water and no change in protein content after cooking ($p < 0.5$). Gök *et al.* (2011) [32] observed a similar behavior in moisture content, a reduction in fat level and an increase in protein content for meatballs with fat replaced with 20 g of ground poppy seeds per each 100 g with respect to control. Moreover, the optimized hamburger provided dietary fiber (4.23 ± 0.36 g/100g of product) while the control hamburger did not contribute with fiber.

The addition of 5.92 g whole chia flour to the formulation increased the total fat content from 9.42 ± 0.03 to 10.19 ± 0.10 g/100g, and the fatty acid profile of the control and the optimal burger, cooked and stored at -18°C for 90 days are shown in the **Table 2**.

In the optimal burger the n-3 fatty acids content was 1551.71 ± 47.71 mg of n-3/100g of burgers, doubling the content of control (704.57 ± 21.66 mg of n-3/100 g of burgers), being 1237.13 ± 38.03 mg/100g provided by ALA, and 214.41 ± 6.59 mg/g by the docosahexaenoic acid (DHA) plus the eicosapentaenoic acid (EPA), thereby increasing the contribution of n-3 fatty acids of marked form.

This significant n-3 contribution is probable due to high amounts of fat present in chia flour (30% approx.), where the content of alpha linolenic acid represents the 60% of the total fatty acids approximately [13].

That is according to Delgado-Pando *et al.* (2010) [33], who reported that, in the frankfurters with 11.5 g fat/100g of product, 2.5 g/100g were n-3 PUFAs (2 g/100g of ALA and 500 mg/100g of EPA and DHA), when the pork backfat was replaced with healthier oils combination (olive, linseed and fish oils).

Considering that the dietary recommendation of health world organizations establish that it is necessary to consume between 1.4 and 3 g of n-3 PUFAs/day, and between 180 and 1000 mg/day for the long chain n-3 PUFAs, 100g this product would satisfy this recommendation.

In the same way, the human diets should have a PUFA/SFA ratio above 0.45 [34] and n-6/n-3 fatty acids ratio between 1:1 and 2:1 [35], because lower ratios of PUFA/SFA or higher n-6/n-3 fatty acids ratio in the diet increase the incidence of cardiovascular diseases [36].

In this study, the PUFA/SFA ratio in burgers increased from 1.02 (control burger) to 1.77 (optimal burger) and the n-6/n-3 ratio decreased from 1.25 in control to 0.76 in optimal formulation (**Table 2**). Although the optimal formulation became healthier, the n-6/n-3 ratio value is still above the recommendations for fat intake, however, it is very lower than 10, value informed for western diets the n-6/n-3 ratio.

On the other hand, in western diets the n-6/n-3 ratio was recently increased from 10:1 to 20:1 (due to a high n-6 proportion in the diet, largely made up by linoleic acid from vegetable oils) which is highly inappropriate for normal growth and development of human [35].

The indexes of atherogenicity (IA) and thrombogenicity (IT) were also lower when the chia flour was added (**Table 2**). These indexes indicate the potential to promote platelet aggregation. So, fats with low values of IA and IT can inhibit the aggregation of platelets and decrease the levels of esterified fatty acids, cholesterol and

Table 2. Fatty acid composition and nutritional quality indexes of lipids from cooked and stored at -18°C for 90 days burgers. Control (without chia flour added) and optimal (with the whole chia flour added).

Fatty Acids Indexes *	Burger	
	Control	Optimal
$\Sigma\text{n-3}$	$704.57 \pm 21.66^{\text{a}}$	$1551.71 \pm 47.71^{\text{b}}$
$\Sigma\text{n-6}$	$880.93 \pm 27.08^{\text{a}}$	$1185.39 \pm 36.44^{\text{b}}$
$\Sigma\text{n-9}$	$1666.24 \pm 51.23^{\text{a}}$	$1587.91 \pm 48.82^{\text{a}}$
ΣSFA	$1561.46 \pm 48.01^{\text{a}}$	$1550.71 \pm 47.67^{\text{a}}$
ΣMUFA	$2009.77 \pm 61.79^{\text{a}}$	$1933.46 \pm 59.44^{\text{a}}$
ΣPUFA	$1585.49 \pm 48.74^{\text{a}}$	$2737.10 \pm 84.15^{\text{b}}$
n-3 ALA	$349.52 \pm 10.75^{\text{a}}$	$1237.13 \pm 38.03^{\text{b}}$
n-3 EPA + DHA	$241.67 \pm 7.43^{\text{b}}$	$214.41 \pm 6.59^{\text{a}}$
n-6/n-3	$1.25 \pm 0.00^{\text{b}}$	$0.76 \pm 0.00^{\text{a}}$
P/S	$1.02 \pm 0.00^{\text{a}}$	$1.77 \pm 0.00^{\text{b}}$
IA	$0.38 \pm 0.00^{\text{b}}$	$0.28 \pm 0.00^{\text{a}}$
IT	$0.41 \pm 0.00^{\text{b}}$	$0.23 \pm 0.00^{\text{a}}$
HH	$2.44 \pm 0.00^{\text{a}}$	$3.47 \pm 0.00^{\text{b}}$

*Means \pm standard error. Different letters in the same row indicate significant differences ($p < 0.05$). Data were expressed as mg fatty acids/gram of fat.

phospholipids, thereby preventing the appearance of micro and macrocoronary diseases [21].

According to Tonial *et al.* (2011) [37], there are no recommended values for IA and IT, but it is understood that lower values of these indexes indicate a healthier ratio. In this study, the addition of whole chia flour reduced the IA and the IT significantly ($p < 0.05$). Similar IA and IT indexes values have been reported in enriched frankfurters with replacement of pork backfat with konjac gel and by the addition of healthier oils stabilized [38].

According to Santos-Silva *et al.* (2002) [22], a higher HH ratio indicates a more suitable nutritionally food. The HH index value of cooked optimal burger was higher than the control, reaching values lower than those reported by Fuchs *et al.* (2013) [39] when Nile tilapia croquettes were enriched with 12.5 g of flaxseed flour per 100 g (HH ratio of 7.90), however, it is appropriate to note that the burger with chia flour added showed an increase of 40% of HH index.

3.3. Antioxidant Properties of Fibrous Fraction of Chia Flour

The fibrous fraction hydroalcoholic extracts of whole chia flour had a polyphenol content of 5.28 ± 0.10 mg GAE per gram of dry matter. This result was higher than the informed in other foods such as sesame cakes (1.94 mg GAE per gram of dry matter) [40] but less than alcoholic extracts of *Salvia ringens* (208.27 mg GAE/g dry extract) [41].

Free radicals capture capacity equivalent were 5.10 ± 0.18 mg AAE/g of dry matter for DPPH[•] and 3.73 ± 0.05 mg AAE/g of dry matter for ABTS^{•+} per gram. These values were higher than those found by other authors. Chaalal M. *et al.* (2013) [42] reported that prickly pear seeds of red-yellow variety exhibited the strongest scavenging capacity on DPPH[•] (0.9081 mg AAE/g for whole seeds and 1.147 mg AAE/g for ground seeds) while that Alimpic *et al.* (2015) [41] informed that the scavenging activity on ABTS^{•+} radicals were 2.44 mg AAE/g dry extract to alcoholic extracts of *Salvia ringens*.

The fibrous fraction hydroalcoholic extracts of whole chia flour presented a reducing power of 0.50 ± 0.01 mg of AAE equivalent per gram of dry matter. Amarowicz *et al.* (2000) [43] expressed the reducing power is a function of the total concentration of polyphenols, so a high content of polyphenolic supposed a good reducing power. Duh *et al.* (1997) [44], Manian *et al.* (2008) [45] and Rumbaoa *et al.* (2009) [46] also reached to similar conclusions.

3.4. Evaluation of Lipid Oxidation in Burger

Table 3 shows the effect of whole chia flour on DC, VP and TBARS values of cooked fish burger during storage at -18°C for 90 days. As the unsaturated fatty acids from natural product presents double bonds interrupted by methylene groups, when their oxidation occurs, the double bonds shift to create conjugated systems, which can then be measured spectrophotometrically [47].

The burger without adding chia flour reached higher values than those with the whole chia flour added. This means that the incorporation of whole chia flour retarded the formation of CD in cooked fish burger until 30 days of storage, losing its effect at 60 days.

The hydroperoxides are primary oxidation products that determine the extent of lipid oxidation at initial stages of oxidation [47]. The results of this research with respect to the evolution of this parameter showed a similar behavior to CD formation, evidencing the effect antioxidant of chia flour during the 30 days of frozen storage. It should be noted further that, the peroxide value of optimal burgers remained below 2 milliequivalents of peroxides/kg fat during all 90 days test.

TBARS analysis measures the formation of secondary products of lipid oxidation, mainly malondialdehyde, which contribute to off-flavour of oxidized fat. TBARS values of optimized burger was considerably lower ($p < 0.05$) than the control for each sampling during storage, indicating high protection of whole chia flour against lipid oxidation in cooked fish burger.

This antioxidant effect of whole chia flour on the formation of DC, VP and TBARS can be due to the presence of polyphenolic compounds with antiradical activity and reducing power before determined.

4. Conclusions

The addition of whole chia flour and water significantly increased the yield, water and fat retention in freshwater fish burgers.

Small amounts of whole chia flour (5.92 g/100g of product) added to the formulation of freshwater fish burgers increased the content of n-3 fatty acids significantly, so that the intake of a burger (100 g approximately) would be enough to cover the daily intake recommendations of international health organizations.

Furthermore, that addition increased significantly the level of polyunsaturated fatty acids and the P/S ratio,

Table 3. Evaluation of lipid oxidation in control formulation (without adding chia flour) and the optimal formulation (added with the whole chia flour), cooked and stored at -18°C for 90 days.

Time (days)	Control formulation	Optimal formulation
	CD (CLA equivalent/g of fat)	
0	5.45 ± 0.06 ^b	4.70 ± 0.02 ^a
30	5.34 ± 0.01 ^b	4.79 ± 0.10 ^a
60	4.85 ± 0.12 ^a	4.87 ± 0.08 ^a
90	5.52 ± 0.07 ^b	4.76 ± 0.12 ^a
PV (milliequivalent of peroxides/kg fat)		
0	2.78 ± 0.12 ^b	1.68 ± 0.10 ^a
30	5.09 ± 0.38 ^b	1.64 ± 0.06 ^a
60	1.12 ± 0.05 ^a	1.57 ± 0.08 ^b
90	1.69 ± 0.11 ^b	1.12 ± 0.08 ^a
TBARS (mg MAD/kg fat)		
0	9.86 ± 0.69 ^a	10.24 ± 0.72 ^a
30	8.68 ± 0.22 ^b	4.39 ± 0.28 ^a
60	4.05 ± 0.22 ^a	4.48 ± 0.19 ^b
90	6.46 ± 0.32 ^b	4.39 ± 0.35 ^a

^aMeans ± standard error. Different letters in the same file indicate significant differences ($p < 0.05$). CD: Conjugated dienes. PV: peroxide value. TBARS: thiobarbituric acid reactive substances. CLA: conjugated linoleic acid MDA: malondialdehyde.

and decreased the n-6/n-3 ratio in values less than 1; also reduced the IA and the IT indexes and increased the HH index, which represents a positive effect on human health and indicates a more nutritionally suitable food. Furthermore, the chia flour provided the product with a high content of dietary fiber that improved its nutritional value and exerted an antioxidant effect on the lipids, evidencing oxidative stability on the formation of CD, PV and TBARS during 30 days of frozen storage.

This is important because river fish meat with the addition of chia flour can be used as a new product fortified with n-3 fatty acids in regions far from the marina zone. This information will enable the future development of other meat products (chicken, beef, etc.) healthier and with better benefits on human health.

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