academicJournals

Vol. 9(27), pp. 675-683, 22 July, 2015
DOI: 10.5897/AJPP2014. 4308
Article Number: 1B50D5A54534
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Influence of a homeopathic product on performance and on quality flour and cookie (Grissini) of Nile tilapia

Mariana Manfroi Fuzinatto[1]*, Denise Pastore De Lima[1,2], Ana Paula Andretto[1], Leidiane Accordi Menezes[3], Aloisio Henrique Pereira Souza[1], Maria Luiza De Souza Franco[4], Nádia Cristina Steinmacher[2], Saraspathy Naidoo Terroso Gama De Mendonça[2] and Lauro Vargas[4]

[1]Programa de Pós Graduação em Ciência de Alimentos - PPC, Universidade Estadual de Maringá Brazil.
[2]Centro de Tecnologia e Engenharia de Alimentos, Universidade Tecnológica Federal do Paraná, Campus Medianeira Brazil.
[3]Programa de Pós Graduação em Tecnologia de Alimentos – PPGTA, Universidade Tecnológica Federal do Paraná, Campus Medianeira Brazil.
[4]Departamento de Zootecnia, Universidade Estadual de Maringá Brazil.

Received 2 March, 2015; Accepted 1 July, 2015

Animals can also benefit from homeopathic remedies by having their immunological system and organic responses to stress reduction stimulated, as well as their balance reestablished. In this context, the objective of this study was to assess the performance of Nile tilapias (*Oreochromis niloticus*) treated with food containing a homeopathic product (Homeopatila 100®), as well as the physical, chemical, technological and sensory quality of flour and cookie (grissini) based on the fish co-product. An eighty-four days assessment was conducted on two types of diet for fishes with sexual inversion: control (CT), added with 40 ml of hydroalcoholic solution (alcohol 30° GL) per kilo of food; and homeopathic treatment (HT) added with 40 ml of homeopathic product Homeopatila 100® per kilo of food. The flours (NTF) were obtained using the head and neck-ends of Nile tilapias with subsequent physical, chemical and technological analyses. In order to assess the applicability of the flours from both treatments, we formulated the grissini (cookies) added with 10% NTF substituting wheat flour; physical, chemical, technological and sensory characteristics were assessed. Considering the studied period, the fish treated with food containing the homeopathic product Homeopatila 100® presented final total weight significantly higher ($p < 0.05$) than fish in the control group. The homeopathic product had no significant influence ($p < 0.05$) on the physical and chemical of the NTF, as well as the results of the assessments on cookies (grissini) produced with the addition of 10% NTF, including the sensory test.

Key words: Homeopathy, *Oreochromis niloticus,* fish, co-product, principal component analysis.

INTRODUCTION

Aquaculture provides about one-third of fish-based products worldwide with a major role of Nile tilapia (*Oreochromis niloticus*) production. The production of this type of fish has expanded since the last decade because

*Corresponding author. E-mail: lvargas@uem.br

Table 1. Composition of homeopathic product *Homeopatila 100®*.

Compound	/1000 ml
Iodum 12cH[1]	250 ml
Sulphur 30cH	250 ml
Natrum muriaticum 200 cH	250 ml
Streptococcinum 30cH	250 ml
Vehicle (Ethyl alcohol 30° GL)	Q.s.f.[2]

[1]Centesimal Hahnemannian dilution. [2]Quantity sufficient for. Source: REALH Homeopathy – Brazil.

of the technological advances associated with more intensive growth practices, especially in China, Philippines, Thailand, Indonesia and Egypt (Josupeit, 2010; Yarnpakdee et al., 2014).

Nile tilapias are the most cultivated fresh-water fish in Brazil; according to the Ministry of Fishing and Aquaculture, in 2011, the production exceeded 253 thousand tons (Brazil, 2011). The increase in the fish production also increases the quantity of generated sub-products that may be used to develop processed foods due to excellent sensory and nutritional characteristics. In this context, the development of new products, based on fish, residues represent a viable technological alternative since fish are historically associated with healthy food (Brazil, 2011).

Homeopathy is a form of complementary medicine that uses high dilutions of substances derived from plants, minerals or animals based on the principle of similarity (Khuda-Bukhsh and Pathak, 2008). Bell and Koithan (2012) proposed an innovative model for the action of homeopathic remedies on living organisms; the research indicates that homeopathic remedies are constituted by nanoparticles and this model provides a basis to guide researches on the function of nanomaterial in living systems, the action mechanism of homeopathic remedies and their use in nanomedicine. Animals can also benefit from homeopathic products by having their immunological system and organic responses to stress reduction stimulated, as well as their balance reestablished. In addition to contributing with prophylaxis by reducing stress, these products may also reduce the application of chemotherapy and antibiotics, preventing environmental risks for animals and consumers (Siena et al., 2010).

The use of homeopathic products in Nile tilapias diet (40 ml kg^{-1} of food) benefited fish' survival, muscle fiber hypertrophy, reduced the hepatic lipid inclusion, hepatosomatic index and total lipids content in the muscle tissue (Valentim-Zabott et al., 2008; Júnior et al., 2012; Andretto et al., 2014). However, there is only one study approaching the development and evaluation of food products (nuggets) based on fish treated with homeopathic medicines (Lima et al., 2015). In this study the aim was to evaluate the performance of Nile tilapia treated with foods containing a homeopathic product (Homeopatila 100®), as well as physical, chemical, technological and sensory quality of flour and biscuit (grissini) produced as a co-product of this fish.

MATERIALS AND METHODS

Experimental protocol and sampling

The experiment was carried out at the experimental station for fishery of the State University of Maringá (UEM) – CODAPAR, district of Floriano, city of Maringá, state of Paraná. A hundred and sixty Nile tilapia males with sexual inversion were weighed, measured and distributed in eight fiber glass tank with capacity of 600 L, maintained at the density of 20 fish per box. The design was completely randomized with two treatments – control (CT) added with 40 ml hydro-alcoholic solution (30° GL) (vehicle as the homeopathic product) per kilo of food; homeopathic (HT) added with 40 ml Homeopatila 100® per kilo of food; each with four replicates (C1, C2, C3 and C4; H1, H2, H3 and H4, respectively). Table 1 presents the composition of the studied homeopathic product. The eighty-four day experiment was approved by the Animal Experimentation Ethics Committee of UEM (protocol 019/2013). The fish were treated with food containing 32% crude protein; the quantity provided was *ad libitum* in two daily portions (9 am and 4 pm). On a weekly basis, the homeopathic product Homeopatila 100® was incorporated into the food sprinkled with a manual sprayer; subsequently, the food was homogenized and dried at room temperature with periodical removal for 24 h. The food was stored in a dry, well-ventilated location without the presence of sunlight, chemical products or equipment emitting a magnetic field until reaching no alcohol odor and clusters. Equal inclusion process was carried out for the control treatment using hydroalcoholic solution.

At the final stage of the experiment, all fish were captured by nets, stunned with water and ice at 0°C and sacrificed through section of the spinal cord and separated per treatment. The whole fish were weighed and measured; heads were removed, washed, weighed and vacuum-packed in polyethylene bags. The neck-ends were extracted from the carcasses using High Tech® deboner (model HT C100), weighed and vacuum-packed in polyethylene bags. The heads and the neck-ends were stored at -18°C. The apparent feed conversion (AFC) was determined by the following equation:

$$AFC = (\text{Weight of meal provided during the period}) \times (\text{Final weight} - \text{Initial weight})^{-1}$$

Obtaining the Nile tilapia flour (NTF)

Four flours were obtained per treatment (one per replicates). The preparation of NTF followed the methodology by Franco et al. (2013), with modifications. Initially, heads and neck-ends were subjected to one-hour cooking process in a pressure pot; the residues were pressed in manual press for foods and grinded in a stainless steel mill (worm thread) Skymsen® (model PSEE-98MHD). Subsequently, the mass obtained was Quimis® kiln (model Q317B), allowed to remain at 60°C for 24 h. The obtained product was then grinded in cutting mill SOLAB® (model CE-430/Mini) and sieved at 60 mesh. The flours were weighed, vacuum-packed in polyethylene bags and stored at -18°C until the analyses and grissini production.

Characterizing Nile tilapia flour (NTF)

Moisture content, crude protein, ashes and total lipids were determined according to the methodology proposed by the Association of Official Analytical Chemists (AOAC, 2005). Minerals, calcium, iron and phosphorus were determined according to the analytical methods of the National Laboratory of Animal Reference (LANARA, 1981) and Institute Adolfo Lutz (IAL, 2008). Water activity (Aw) was assessed at 25°C in Aqualab® (model 4TE) equipment for the determination of water activity, according to the manufacturer's instructions. For color measurement, we used Minolta® colorimeter (model CR 400) with illuminant D65 and angle of view of 10°. The values of L (luminosity), a* (intensity of red color) and b* (intensity of yellow color) were expressed according to the color system by the Commission Internationale de L'Eclairage (CIELAB) (Minolta, 1998). The determination of the NTF yield was carried out gravimetrically through the relationship between the weight of heads and neck-ends in nature, and the weight of the extracted flours expressed in percentage. All of the analyses were carried in 3 replicates.

Cookie (grissini) formulation

In order to assess the applicability of the flours from both treatments, we formulated grissini added with 10% NTF substituting wheat flour. For the grissini production, the NTF from the four repetitions of TC (C1, C2, C3, and C4) were mixed and homogenized; equal process was employed for the HT with repetitions H1, H2, H3, and H4. The following ingredients and proportions were applied to produce the grissini: fresh yeast 2.3%; refined sugar 3%; distilled water 25%; salt 2.1%, oil 6%, wheat flour 56% and NTF (CT or HT) 5.6%. The cookies were baked in a Fisher® conventional oven at 200°C for 10 min; immediately after being cooked, the cookies were cooled at room temperature (25°C) and stored in polyethylene bags of high density and hermetically sealed glasses identified per treatment until the analyses.

Cookie (grissini) characterization

The moisture content, crude protein, ashes, total lipids, Aw, color and minerals (calcium, iron, phosphorus) were determined according to the description for NTF. Total carbohydrates were calculated by difference (100 g – total grams of moisture, crude protein, total lipids, and ashes). The content of soluble and insoluble dietary fibers was determined through the enzymatic-gravimetric method 985.29 by the AOAC (Prosky et al., 1985); total dietary fiber was calculated using the sum of these two fractions. All of the analyses were carried out in triplicate. The characteristics of the cookies were assessed according to method 10 - 50D (AACC, 2000); the dough was determined using pre- and post-heat weight expressed in grams. The width, thickness and length of the cookies were determined using millimeter scale ruler with pre- and post-heat values expressed in centimeters. The specific volume was calculated from the relationship between the apparent volume (determined using millet displacement method) and the post-heat weight of the cookies (Pizzinatto et al., 1993). The yield was determined through the difference between pre- and post-heat weight of the cookies. The results represented an arithmetic mean of 10 determinations derived from samples of a single test. The tensile strength was determined using Stable Micro Systems Texture Analyser TA.XT2 texture meter, with probe 3-Point bending Rig (HDP/3PB) and platform HDP/90. Results were expressed in Newton (N) representing an arithmetic mean of 15 determinations of tensile strength for samples derived from a single test randomly selected. The following parameters were employed: pretest velocity = 1.0 mm s^{-1}; test velocity = 3.0 mm s^{-1}; post-test velocity = 10.0 mm s^{-1}; distance 5 mm, with measure of compression force (Clerici et al., 2013).

Sensory evaluation

The sensory evaluation was approved by the Human Research Ethics Committee of UEM (623.527/2014). For the grissini acceptance test for both treatments, we applied a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) assessing the attributes of color, aroma, texture, taste and overall evaluation with the collaboration of 120 non-trained tasters – 56 female, 64 male, ages ranging from 15 and 57 years old, all employees and students at the Federal Technological University of Paraná (Dutcosky, 2013). After the sensory evaluation, we calculated the Acceptance Index (AI) for the attribute of overall evaluation by using the following expression:

$$AI\ (\%) = A \times 100 / B$$

Where A = average score for the attribute, and B = maximum score for the attribute (Lawless and Heymann, 2010).

Statistical and multivariate analyses

Fish performance results, the physical, chemical and technological analyses of the NTFs and grissini as well as the sensory evaluation were submitted to the t-Student means difference test ($p<0.05$). The multivariate analysis indicated the individual values for each replicate in the four NTF repetitions (n=24) for both treatments – CT and HT; the grissini had one repetition (n=6) and analyses in triplicate for both cases divided into data matrices. The first matrix contained the data of the NTF characterizations, and two matrices for the results obtained for the grissini. The data were pre-processed through self-scaling; in this case, data are centered on the mean and each individual value is divided by the standard deviation. The procedure is to guarantee that all of the variables have equal importance, that is, equal weight. Posteriorly, we applied the principal component analysis (PCA) using NIPALS algorithm with the scores (samples) and loadings (variables) decomposed in a dimensional graph. Statistical software, version 8.0 (StatSoft, 2007) was applied using 5% significance level ($p<0.05$) for the rejection of the null hypothesis and selection of the principal component.

RESULTS AND DISCUSSION

Fish performance

Table 2 presents the mean values of fish performance during the experimental period. No significant difference was observed between the treatments in relation to initial total weight, initial total length, final total length, head and neck-end weight, and apparent feed conversion (AFC); however, significant difference between the treatments ($p < 0.05$) was observed in relation to final total weight. The weight gain after 84 days for the tilapias treated with homeopathy (HT) was 207.16 g, while those in the control treatment (CT) presented 192.05 g. According to Rocha et al. (2002), weight gain is a very important factor when growing fish and determinant for the economic exploration of the area.

Intensive growth systems impose animals with

Table 2. Mean values of Nile tilapia performance for the different treatments.

Parameter	Treatments	
	Control (CT)	Homeopathy (HT)
Initial total weight (g)	101.12±17.73[a]	99.73±19.85[a]
Initial total length (cm)	18.52±6.00[a]	18.05±1.11[a]
Final total weight (day 84) (g)	293.17±56.76[b]	306.89±58.30[a]
Final total length (day 84) (cm)	26.42±1.81[a]	26.22±1.79[a]
Head weight (day 84) (g)	99.41±18.52[a]	100.86±19.81[a]
Neck-end weight (day 84) (g)	5.28±0.78[a]	5.99±1.59[a]
AFC	1.33[a]	1.27[a]

Mean ± standard deviation. Means followed by different letters (a-b) on a single line indicate significant differences through t-Student test ($p < 0.05$). AFC = Apparent Feed Conversion.

conditions that are opposite to the one found in their natural environment, such as high density and constant handling, factors that damage their well-being and have a negative influence on production. Stressed fish generally have their development compromised and possible hematological alterations followed by hyperglycemia and hemoglobin increase caused by the higher release of cortisol, which induces hepatic glycogenesis boost (Mazeaud et al., 1977; Carneiro, 2001).

Siena et al. (2010) treated Nile tilapia (*O. niloticus*) with food containing four levels of the homeopathic product Homeopatila 100® in hydroalcoholic solution per kilo of food (0.20, 40 and 60 ml) concluding that the fish treated with 40 ml kg^{-1} of food presented higher survival rate and lower hepatosomatic index than the control group. Braccini et al. (2013) demonstrated adding Homeopatila 100® (40 ml kg^{-1} of food) to young Nile tilapias' diet reduced the hepatosomatic index, increased the amount of hepatocytes and the percentage of intracellular glycogen compared with fish in the control group.

The formulation of the studied homeopathic product has four compounds (iodum, sulphur, *Natrum muriaticum* and Streprococcinum); according to Vijnovsky (2003), they have different indications for the treatment of behavior disorders. Therefore, the product was developed in order to reduce production stress by acting on the vital energy and maintaining the organic homeostasis of the fish.

Characterizing Nile tilapia flour (NTF)

According to the t-Student test ($p<0.05$), no minimum significant difference was observed between the NTF treatments in relation to the physical, chemical analyses (Table 3). The results of ashes and crude protein indicate that the NTF present high content of minerals in addition to being excellent sources of animal protein. Franco et al. (2013) studied the flour of Nile tilapia (*O. niloticus*) carcass and found contents of moisture, ashes and crude protein similar to those indicated in this study – 2.15, 38.03, and 45.32%, respectively. The chemical composition of fish-based products may vary according to the source of acquisition of heads and neck-ends, the food provided to the fish and the applied filleting method. Another factor that may have influence on the proximate composition of fish-based flour is the methodology employed in the production. Monteiro et al. (2012) developed a flour based on the head and carcass of Nile tilapia and verified contents of 4.01% moisture, 56.15% crude protein, 30.14% total lipids, and 3.89% ashes.

The residues of Nile tilapia (*O. niloticus*), especially head and bones, present high levels of calcium, iron and phosphorus (Oetterer, 2002). Calcium was the most abundant mineral present in the flours developed in this study followed by phosphorus and iron (Table 3). Petenuci et al. (2008) also found higher amount of calcium followed by phosphorus and iron when developing a flour based on Nile tilapia neck-end –2715.9 mg 100 g^{-1}, 1132.7 mg 100 g^{-1} and 1.3 mg 100 g^{-1}.

The intensity of yellow color (b*) in the NTF for the different treatments in this study was higher than the Brazilian catfish flour (*Brachyplatystoma vaillantii*) studied by Oliveira et al. (2015), while the luminosity parameter (L) and the intensity of red color (a*) presented lower values. The color of a certain food is influenced mostly by the employed raw materials and possible chemical and biochemical alterations occurring during processing and storage (Ribeiro et al., 2007).

Principal component analysis

The principal component analysis (PCA) was carried out to enable a wider exploration of the results, considering the weight of all of the obtained experimental measures. The data obtained from the four repetitions of NTF of the CT (C1, C2, C3 and C4) and the HT (H1, H2, H3 and H4) enabled the achievement of the significant principal components ($p<0.05$): PC1 and PC2, explaining 20.65 and 17.10%, respectively, of the results variability. We observe the formation of two distinguished groups (Figure

Table 3. Physical and chemical composition and quality of Nile tilapia flour (NTF) and grissini cookie for the different treatments.

Analyses [1]	Treatments – Flour (FTN) [2]		Treatments – Grissini	
	Control (CT)	Homeopathy (HT)	Control (CT)	Homeopathy (HT)
Yeld (%)	12.97±0.03	12.98±0.01	1.43±0.20	1.46±0.06
Moisture (g $100g^{-1}$)	2.53±0.53	2.60±0.49	8.35±0.01	8.17±0.16
Ashes (g $100g^{-1}$)	33.00±2.39	31.23±2.12	6.03±0.6	5.90±0.10
Total lipids (g $100g^{-1}$)	15.05±1.79	16.76±2.76	9.61±0.35	9.62±0.44
Crude protein (g $100g^{-1}$)	48.70±1.70	47.68±0.74	11.81±0.19	11.83±0.25
Carboidrato (g $100g^{-1}$)	-	-	64.33±0.59	64.35±0.42
Calcium (mg $100g^{-1}$)	2427.22±30.82	2447.60±31.95	167.65±7.30	170.11±4.50
Iron (mg $100g^{-1}$)	225.12±4.52	222.34±4.10	8.28±0.07	8.08±0.85
Phosphorus (mg $100g^{-1}$)	842.46±6.69	838.11±5.28	264.75±3.85	260.48±3.12
Aw	0.09±0.02	0.11±0.03	0.57±0.01	0.57±0.002
L	42.79±1.72	41.73±1.71	65.66±0.31	66.08±0.50
a*	1.47±0.54	1.80±0.46	3.46±0.09	2.93±0.52
b*	19.42±0.58	20.11±1.06	31.23±0.39	31.00±0.20
Soluble dietary fiber (g $100g^{-1}$)	-	-	1.77±0.13	1.81±0.13
Insoluble dietary fiber (g $100g^{-1}$)	-	-	8.89±0.29	9.44±0.29
Total dietary fiber (g $100g^{-1}$)	-	-	10.66±0.30	11.26±0.42
PreHD (g)	-	-	5.23±0.45	5.29±0.32
Post-HD (g)	-	-	3.80±0.25	3.83±0.33
PreHW (cm)	-	-	1.34±0.16	1.35±0.12
Post-HW (cm)	-	-	1.60± 0.25	1.65±0.12
PreHT (cm)	-	-	0.81±0.10	0.78±0.08
Post-HT (cm)	-	-	1.50±0.15	1.47±0.08
PreHL (cm)	-	-	4.82±0.28	4.82±0.11
Post-HL (cm)	-	-	4.97±0.33	4.95±0.19
SV (ml g^{-1})	-	-	3.36±0.51	3.52±0.43
TS (N)	-	-	46.55±6.25	48.66±9.60

[1]Means ± standard deviation. [2] Means for the four repetitions C1, C2, C3 and C4 (CT); H1, H2, H3 and H4 (HT). L = luminosity. a* = intensity of red color. b* = intensity of yellow color. PreHD = preheat dough. Post-HD = post-heat dough. PreHW = preheat width. Post-HW = post-heat width. PreHT = preheat thickness. Post-HT = post-heat thickness. PreHL = preheat length. Post-HL = post-heat length. SV = specific volume. TS = tensile strength – absent analysis.

1): the group formed by the blue ellipse containing the CT repetitions and the one formed by the red ellipse containing the HT repetitions. The HT repetitions obtained higher influence of the following loadings: water activity (0.8001), total lipids (0.7521) and moisture (0.6186). The loadings of crude protein (0.8464) and phosphorus (0.6619) (PC2) presented higher weight when distinguishing the CT repetitions (PC1); a possible explanation would be higher values of water activity, total lipids and moisture for the HT, and crude protein and phosphorus for the CT (Table 3).

Characterizing the (cookie) grissini

The results of the physical, chemical and technological analyses of the grissini for the different treatments are presented in Table 4. When statistically analyzed (t-Student $p<0.05$), the results demonstrated no significant difference between the treatments. Grissini are classified as cookies prepared with wheat flour, fat, water, salt and other food substances in the shape of thin, short cylinders (Brazil, 1978). Following the pattern in the NTF, calcium was the most abundant mineral found in the grissini followed by phosphorus and iron. The NTF has a dark color, consequently, the cookies presented more intense color than cookies produced exclusively with wheat flour (Sharmas and Gujral, 2014). Preheat weight, width, thickness and length of the grissini for both treatments presented no statistical differences ($p<0.05$), which indicated a high level of homogeneity during the processing. This allows the assumption that any differences possible to be observed after the cooking would derive from the NTF behavior in the dough. We observed that the width, thickness and length of the grissini in both treatments increased after the heat, while weight decreased; similar results were found by Fasolin et al. (2007) in cookies produced with banana flour, and

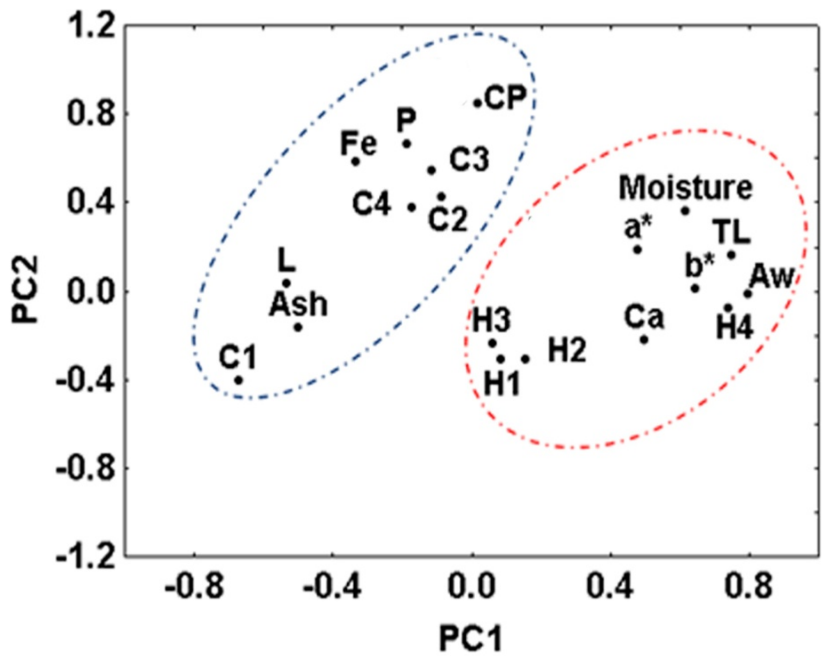

Figure 1. Principal component analysis for Nile tilapia flour (NTF) physical, chemical and technological analyses regarding control treatment (CT blue ellipse) and homeopathy (HT red ellipse). C1, C2, C3 and C4 = repetitions of CT. H1, H2, H3 and H4 = repetitions of HT. CP = Crude protein. P = Phosphorus. Fe = Iron. L = Luminosity. L = Total lipids. a* = intensity of red color . b* = intensity of yellow color. Aw = Water activity. Ca = Calcium.

Table 4. Mean values of the acceptance scores for grissini produced using Nile tilapia flour (NTF) for the different treatments.

Attributes	Treatments	
	Control (CT)	Homeopathy (HT)
Color	7.40±1.69	7.74±1.98
Aroma	7.06±1.65	7.17±1.78
Texture	6.74±1.90	7.15±1.07
Taste	7.02±1.83	7.30±1.90
Overall evaluation	6.96±1.90	7.00±1.97
AI (%)	77.33	77.78

Mean based on the 120 tasters. [1]Hedonic Scale: (9) like extremely, (8) like very much, (7) like moderately, (6) like slightly, (5) neither like nor dislike, (4) dislike slightly, (3) dislike moderately, (2) dislike very much, (1) dislike extremely. AI = Acceptance Index.

Moraes et al. (2010) in cookies with variations in the contents of sugar and lipids.

Sensory evaluation

No minimum significant difference (p<0.05) was observed among the means of the evaluation of attributes referent to the acceptance of the products (Table 4). All of the evaluations were within categories 'like slightly' and 'like regularly'. The attribute color received the most satisfactory score for both treatments; color is a major parameter to be considered when adding different types of flour into the cookies formulation since it may influence its appearance, and consequently its acceptance. Lima et al. (2015) carried out a sensory evaluation on nuggets containing fillet and mechanically separated meat of Nile tilapia treated with homeopathic product Homeopatila 100® and also found no significant differences (p<0.05) compared to the control treatment, for all attributes.

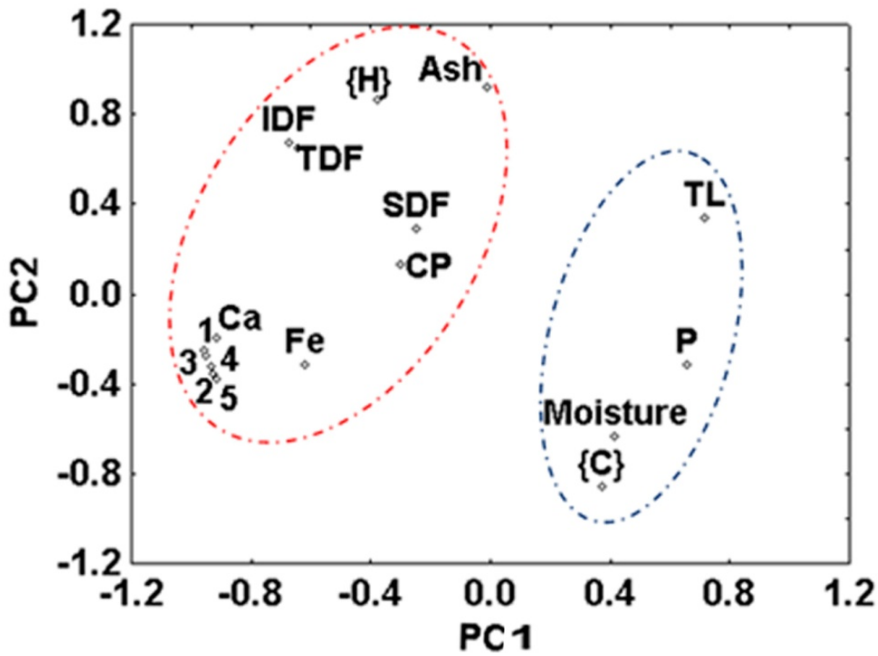

Figure 2. Principal component analysis of the proximate composition and sensory attributes of the control treatment grissini (CT blue ellipse) and homeopathic treatment (HT red ellipse). H = Homeopathic treatment. C = Control treatment. CP = Crude protein. IDF = Insoluble dietary fiber. TDF = Total dietary fiber. SFD = Soluble dietary fiber. Ca = Calcium. Fe = Iron. P = Phosphorus. Sensory attributes: 1 Color, 2 Aroma, 3 Texture, 4.Taste and 5 Overall evaluation.

Rebouças et al. (2012) produced salted cookie added with protein concentrate of Nile tilapia and obtained means for the sensory evaluation close to 5 ('indifferent') for all of the attributes. Rocha (2011) carried out a sensory evaluation on salted cookie containing Nile tilapia flour and obtained 56.66% acceptance with scores within 'like regularly' and 'like extremely'. Neiva et al. (2011) produced low commercial value cookies based on fish meat mechanically separated (*Menticirrhus americanus* and *Umbrina corides*), and verified that the samples presented good scores, varying from 6 ('like slightly') to 9 ('like extremely') for all of the evaluated attributes.

According to Dutcosky (2013), for a product to be considered well-received in consumers market in terms of sensory properties, it is required an Acceptance Index (AI) of at least 70%. As observed in Table 4, the grissini IA for both treatments was satisfactory.

Principal component analysis

The grissini developed with NTF from both the CT and the HT were grouped in two PCA dimensional graphs (Figures 2 and 3). Figure 2 presents the data on proximate composition (moisture, ashes, crude protein and total lipids), minerals as well as the sensory attributes, explaining a data variance of 73.78%. It was possible due to the selection of the PC1 with 47.12%, and PC2, responsible for 26.85% of the results variability presenting 5% significance level. According to Figure 2, the grissini containing NTF from the HT (red ellipse) obtained higher contribution in all of the evaluated organoleptic properties – color (-0.9534), odor (-0.9263), texture (-0.9512), taste (-0.9312) and overall impression (-0.9147) through PC1. The loadings ashes (0.9150), insoluble dietary fiber (IDF) (0.6681) and total dietary fiber (TDF) (0.6495) in PC2 also had an influence on the characterization of the HT grissini. The multivariate analysis enabled the identification of the differences between the treatments indicating better sensory preference for the grissini containing NTF from the HT (Figure 2), even though such results did not present minimum significant difference (p<0>05) through t-Student test (Table 4).

By conducting a multivariate analysis on the data of the technological characterization of the grissini, we observed a region with common loadings (Figure 3) for both treatments (CT and HT): pre- (0.9784) and post-heat thickness (-0.9852); pre- (0.9925) and post-heat width (0.9678); pre- (0.9733) and post-heat length (0.9949), and tensile strength (0.9005) in PC1 (48.54%). These parameters are used in the quality control during and after the processing of this type of product; it implies that,

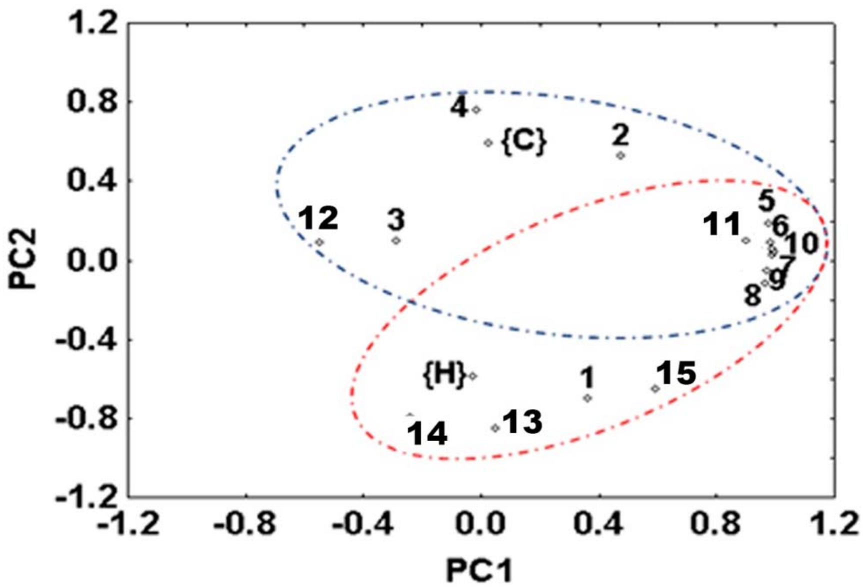

Figure 3. Principal component analysis of the control treatment grissini (CT: blue ellipse) and homeopathic treatment (HT: red ellipse) regarding the technological analyses results. C = Control treatment. H = Homeopathic treatment. 1 = L (luminosity). 2 = a* (intensity of red color). 3 = b* (intensity of yellow color). 4 = Aw. 5 = pre-heat thickness. 6 = post-heat thickness. 7 = pre-heat width. 8 = post-heat width. 9 = pre-heat length. 10 = post-heat length. 11 = Tensile strength. 12=Specific volume. 13 = pre-heat dough. 14 = post-heat dough. 15 = yield.

according to the statistical multivariate analysis, the use of NTF in both treatments had no influence on the grissini processing.

The HT grissini was characterized (red ellipse) by the following loadings: pre- (-0.8548) and post-heat dough (-0.7998); luminosity – L (-0.7010) and performance (-0.6555) in PC2 (21.91%, Figure 3). The CT grissini (blue ellipse) obtained better contribution of loadings a* (0.5292) and water activity (0.7624) in PC2 (Figure 3). According to studies carried out by Pagamunici et al. (2014) and Souza et al. (2014), the application of the PCA enables food products to be distinguished and characterized regarding their loading contributions.

Conclusion

During the studied period, the Nile tilapias treated with food containing the homeopathic product Homeopatila 100® presented final total weight significantly higher ($p<0.05$) than the fish in the control group. The homeopathic product had no significant influence ($p<0.05$) on the physical and chemical properties of the NTF, as well as the results of the assessment on the cookies (grissini) produced with addition of 10% NTF, including the sensory test. The multivariate analysis enabled an improved grouping and distinction of the samples through the respective physical, chemical compositions and technological and sensory characteristics. The obtained results suggest that the homeopathic product Homeopatila 100® used in Nile diet (40 ml kg^{-1} of food) is able to improve weight gain without compromising the physical, chemical, technological and sensory quality of the fish flour and cookie (grissini).

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel) for the financial support; the Federal Technological University of Paraná (UTFPR) – Medianeira Campus and the Federal University of Rio Grande (FURG) for having provided the proper technology for the development of this research.

REFERENCES

AACC (2000). American Association of Cereal Chemists. Approved methods of the American Association of Cereal Chemists. 10. ed. Saint Paul. v. 2.

Andretto AP, Fuzinatto MM, Bonafe EG, Braccini, GL, Mori RH, Pereira RR, Oliveira CAL, Visentainer JV, Vargas L (2014). Effect of a homeopathic complex on fatty acids in muscle and performance of the Nile tilapia (*Oreochromis niloticus*) Homeopathy 103:178-185.

AOAC (2005). Association Of Official Analytical Chemists. Official Methods of Analysis of the AOAC. 18th ed. Gaithersburg, M.D, USA.

Bell IR, Koithan M (2012). A model for homeopathic remedy effects: low dose nanoparticles, allostatic cross-adaptation, and time-dependent sensitization in a complex adaptive system. BMC Complementary Altern. Med. 12:191.

Braccini GL, Natali MRM, Ribeiro RP, Mori RH, Riggo R, Oliveira CA, Hildebrandt JF, Vargas L (2013). Morpho-functional response of Nile tilapia (*Oreochromis niloticus*) to a homeopathic complex. Homeopathy 102:233–241.

Brazil (1978). Agência Nacional de Vigilância Sanitária. Resolução – CNNPA (Comissão Nacional de Normas e Padrões para Alimentos) nº 12, de 1978. Padrões de identidade e qualidade para os alimentos e bebidas.

LANARA (1981). Ministério da Agricultura. Secretaria Nacional de defesa Agropecuária, Laboratório Nacional de Referencia Animal (LANARA). Métodos Analíticos Oficiais para Controle de Produtos de Origem Animal e seus ingredientes: Métodos Físico-Químicos.

Brazil (2011). Ministério da Pesca e Aquicultura. Boletim Estatístico da Pesca e Aquicultura.

Carneiro PCF, Urbinati EC (2001). Salt as a stress response mitigator of matrinxã, *Brycon cephalus* (Günther), during transport. Aquac. Res. 32:297–304.

Clerici MTPS, Oliveira ME, Nabeshima EH (2013). Qualidade física, química e sensorial de biscoitos tipo *cookies* elaborados com a substituição parcial da farinha de trigo por farinha desengordurada de gergelim. Braz. J. Food Technol. 16:139-146.

Dutcosky SD (2013). Análise sensorial de alimentos. Curitiba: Champagnat. 531 pp.

Franco MLRS, Abreu BB, Saccomani APO, El Vesco AP, Vieira V, Mikcha JMG, Gasparino E, Delbem ACB (2013). Elaboración de cookies y galletas con inclusión de harina de pescado. Infopesca Internacional. 53:30-33.

Fasolin LH, Almeida GC, Castanho PS, Netto-Oliveira ER (2007). Biscoitos produzidos com farinha de banana: avaliações química, física e sensorial. Ciênc. Tecnol. Aliment. Campinas 27:524-529.

IAL (2008). Instituto Adolfo Lutz (São Paulo). Métodos físico-químicos para análise de alimentos - 4ª Edição. 1ª Edição Digital.

Josupeit H (2010). World supply and demand of tilapia. Food and Agriculture Organization e FAO [Online]. Available:
<http://www.globefish.org/upl/Papers/World%20supply%20and%20demand%20of%20tilapia.pdf> .

Júnior RP, Vargas L, Valentim-Zabott M, Ribeiro RP, Silva AV, Otutumi LK (2012). Morphometry of white muscle fibers and performance of Nile tilapia (*Oreochromis niloticus)* fingerlings treated with methyltestosterone or a homeopathic complex. Homeopathy 101:154-158.

Khuda-Bukhsh AR, Pathak S (2008). Homeopathic drug discovery: theory update and methodological aspect. Expert Opin Drug Discov. 3:979-990.

Lawless HT, Heymann H (2010). Sensory Evaluation of Food: Principles and Practices, 2nd ed.; Springer: Berlin, Germany.

Lima DP, Fuzinatto MM, Andretto AP, Braccini GL, Mori RH, Canan C, Mendonça SNTG, Oliveira CAL, Pereira RR, Vargas L (2015). Mechanically separated fillet and meat nuggets of Nile tilapia treated with homeopathic product. Afr. J. Pharm. Pharmacol. 9:182-189.

Mazeaud MM, Mazeaud F, Donaldson EM (1977). Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 106:201-212.

Minolta (1998). Precise color communication - color control from perception to instrumentation. Japan: Minolta Co., Ltd. P. 59.

Monteiro MLG, Mársico ET, Viriato IM, Souza JML and Conte-Júnior CA (2012). Preparation of added value byproducts from the waste material of tilapia (Oreochromis niloticus) processing. J. Aquac. Res. Dev. 3:1-5.

Moraes KS, Zavareze ER, Miranda MZ, Salas-Mellado MM (2010). Avaliação tecnológica de biscoitos tipo *cookie* com variações nos teores de lipídio e de açúcar. Ciênc. Tecnol. Aliment., Campinas 30:233-242.

Neiva CRP, Machado TM, Tomita RY, Furlan EF, Lemos Neto MJ, Bastos DHM (2011). Fish crackers development from minced fish and starch: an innovative approach to a traditional product. Food Sci. Technol. (Campinas). 31:973-979.

Oetterer M (2002). Industrialização do pescado cultivado. Livraria e editora Agropecuária, Guaíba, RS. P. 200.

Oliveira IS, Lourenço LFH, Sousa CL, Joele MRSP, Ribeiro SCA (2015). Composition of MSM from Brazilian catfish and technological properties of fish flour. Food Control 50:38-44.

Pagamunici LM, Souza AHP, Gohara AK, Souza NE, Gomes STM, Matsushita M (2014). Development, characterization and chemometric analysis of a gluten-free food bar containing whole flour from a new cultivar of amaranth. Ciênc. Agrotec. Lavras 38:270-277.

Petenuci ME, Stevanato FB, Visentainer JEL, Matsushita M, Garcia EE, Souza NE de, Visentainer JV (2008). Fatty acid concentration, proximate composition, and mineral composition in fishbone flour of Nile Tilapia. Archivos Latinoamericanos de Nutricion. 58:87-90.

Pizzinatto A, Magno CPR, Campagnolli DMF, Vitti IP, Leito RFF (1993). Avaliação Tecnológica de Produtos Derivados de Farinhas de Trigo (pão, macarrão, biscoito). Campinas: ITAL. P. 54.

Prosky L, Asp N-G, Furda I, Devries JW, Schweizer TF, Harland BF (1985). Determination of total dietary fiber in foods and food products: collaborative study. J. Assoc. Official Analy. Chemists 68:677-679.

Rebouças MC, Rodrigues MCP, Castro RJS (2012). Biscoito salgado com adição de concentrado proteico de peixe: desenvolvimento e aspectos sensoriais. Alim. Nutr. Araraquara 23:45-50.

Ribeiro S CA, Ribeiro CFA, Park KJ, Araújo EAF, Tobinaga S (2007). Alteração da cor da carne de mapará (*Hypophthalmus edentatus*) desidratada osmoticamente e seca. Revista Brasileira de Produtos Agroindustriais 9:125-135.

Rocha JBS (2011). Utilização de tilápias de baixo valor comercial como fonte proteica na formulação de biscoito e sopas para a merenda escolar. Dissertação UFRB. P. 40.

Rocha MA, Ribeiro ELA, Mizubuti IY, Silva LDF (2002). Growth parameters and their correlations from ages 60 to 240 days in the carp (*Cyprinus carpio*). Semina: Ciências Agrárias, Londrina 23:29-34.

Sharmas P, Gujral HS (2014). Cookie making behavior of wheat-barley flour blends and effects on antioxidant properties. LWT - Food Sci. Technol. 55:301-307.

Siena CE, Natali MRM, Braccini GL, Oliveira AC, Ribeiro RP, Vargas L (2010) Efeito do núcleo homeopático *Homeopatila 100*® na eficiência produtiva em alevinos revertidos de tilápia do Nilo (*Oreochromis niloticus*). Semina: Ciências Agrárias. Londrina 31:985-94.

Souza NE, Rodrigues AC, Souza AHP, Matsushita M, Pedrão MR, Dias LF (2014). Quantification of minerals and tocopherols isomers in chestnuts approach chemometrics. Semina: Ciências Agrárias, Londrina. 35:2427-2436.

StatSoft (2007). Inc. Statistica: Data analysis software system, version 8.0.

Valentim-Zabott M, Vargas L, Ribeiro RP, Piau Júnior R, Torres MBA, Rönnau M, Souza JC (2008). Effects of a homeopathic complex in Nile tilapia (*Oreochromis niloticus* L.) on performance, sexual proportion and histology. Homeopathy 97:190–195.

Vijnovsky B (2003). Tratado de matéria médica homeopática. 1ª ed. Organon. São Paulo, SP, P. 2028.

Yarnpakdee S, Benjakul S, Penjamras P, Kristinsson HG (2014). Chemical compositions and muddy flavour/odour of protein hydrolysate from Nile tilapia and broadhead catfish mince and protein isolate. Food Chem. 142:210-216.