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Mechanically separated fillet and meat nuggets of Nile tilapia treated with homeopathic product

Denise P. Lima[1,3], Mariana M. Fuzinatto[3], Ana P. Andretto[3], Graciela L. Braccini[4], Ricardo H. Mori[2], Cristiane Canan[1], Saraspathy N. T. G. de Mendonça[1], Carlos A. L. de Oliveira[4], Ricardo R. Pereira[4] and Lauro Vargas[4]*

[1]Department in Food, Federal Technology University of Paraná, Medianeira - PR - Brazil.
[2]Aquiculture Center, Paulista State University, Jaboticabal – SP - Brazil.
[3]Department in Food, Maringá State University, Maringá – PR – Brazil
[4]Animal Science Department, Maringá State University, Av. Colombo, 5790, Bloco J 45 – Sala 115, CEP 87.020-900 - Maringá – PR – Brazil

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The homeopathic product Homeopatila 100® in the diet of Nile tilapia reduces stress during production and improves the well-being of fish. The objective of this study was to develop nuggets of tilapia fed with Homeopatila 100® and to assess their quality. Physical, chemical, microbiological and sensory analyses were performed on three formulations with 25, 50 and 75% mechanically separated meat (MSM) for each of the treatments. The nuggets with 75% MSM revealed a higher pH (5.89 ± 0.02), the tissue was softer (1.29 N ± 0.04), and they had a higher lipid value (15.96% ± 0.05). With 50 and 75%, the color (L^*) was darker (60.76% ± 0.91 and 60.03% ± 0.78), and there were lower protein amounts (15.54% ± 0.31 and 13.55% ± 0.35). Nuggets had an acceptable value of lipid oxidation (0.672 ± 0.007 mg MDA/kg). The microbiological analyses demonstrated that the product met the requirements of legislation. Nuggets with 25 and 50% MSM were deemed acceptable. There was no difference (p > 0.05) between the control treatment group and the Homeopatila 100® group for the analysis undertaken. The results indicated that the use of Homeopatila 100® in the diet of the Nile tilapia did not change the physical, chemical, microbiological and sensorial quality characteristics of the nuggets, ensuring consumer acceptability.

Key words: Co-product, aquaculture, homeopathy, fish, technology.

INTRODUCTION

Aquaculture provides one-third of the world's fishery products (Yarnpakdee et al., 2014). Tilapia is the second most popular fish cultivated globally (85 countries) and features low cost, firm white meat, mild flavor, high protein content and low lipid and energy content. It is a common item on the menu in Europe, Asia and the Americas. With the increase in the production of tilapia, it is desirable to develop products that enable the use of processing waste for human consumption (Zhang et al., 2011).

*Corresponding author. E-mail: lvargas@uem.br. Tel: +55 44 3011-8919

The filleting residue has 60 to 70% of the total weight of tilapia. Part of this residue is discarded, causing waste of natural resources (Taskaya and Jaczynski, 2009). The use of mechanically separated meat (MSM) enables the use of 14% of the total weight of the fish, reducing the cost, and presenting nutritional value equivalent to entire muscle. The MSM is little used as raw material for the production of fishburger, nuggets and sausages (Ninan et al., 2010; Gehring et al., 2011).

The nuggets are restructured, breaded and prepared from the disintegration of the flesh by mechanical methods (Marengoni et al., 2009). The manufacture of these products with MSM uses the processing residues, avoids health and environmental problems and adds commercial value to final product (Nunes et al., 2006). The products of homeopathy, a complementary and alternative medicine, are produced by dinamization, a process that involves sequentially stirred dilutions in small volumes applied to human and animal diets (Adler et al., 2011). The use of population homeopathy reduces stress to animals, especially in intensive systems that are very different from the natural environment, and increases the production potential and survival. It is a non-toxic product. The use of extremely diluted active ingredients ensures that there are no residues in meat and contamination in water and soil (Andretto et al., 2014).

Homeopatila 100® is a homeopathic complex designed to decrease stress during production and guarantee the well-being of Nile tilapia (*Oreochromis niloticus*) in the productive cycle of the commercial fish fry. Several studies have been undertaken with Nile tilapia and Homeopatila 100® (Siena et al., 2010; Braccini et al., 2013; Merlini et al., 2014). Because there are few reports on the products prepared from tilapia fillets and MSM treated with homeopathy, the current investigation provides the physical, chemical, microbiological and sensory evaluation of nuggets from MSM of Nile tilapia prepared with Homeopatila 100®.

MATERIALS AND METHODS

Prime matter and ingredients

The use of animals was approved by the Committee for Ethical Behavior in Animal Usage in Experiments of the State University of Maringá, Maringá PR Brazil (Protocol 019/2013). After sexual reversion, Nile tilapias (*O. niloticus*), variety Supreme, with a mean initial weight of 101.12 ± 17.73 g and mean initial length of 18.52 ± 6.00 cm for the control treatment and an initial mean weight of 99.73 ± 19.85 g and initial mean length of 18.05 ± 1.11 cm for the Homeopatila 100® treatment were randomly distributed in 16 fiber glass water boxes, with 20 animals per box. Homeopatila 100® and control treatments were analyzed with eight replications each by a totally randomized experimental design, with 160 fish per treatment. Fish were fed on commercial extruded meals with 32% crude protein and a diameter of 5 mm twice a day (10 and 16 h).

At the end of the experiment, when the fish weighed approximately 300 g, to make filleting easy, they were deprived of food for 24 h. All of the fish were captured by nets, desensitized with water and ice at 0°C (Scherer et al., 2006) and killed by breaking the spinal marrow. Two treatments were evaluated: a control treatment with a hydro-alcohol solution 30% v/v (addition of 40 ml/kg meal) and an experimental treatment with the homeopathic product Homeopatila 100® (addition of 40 ml/kg meal). We used this concentration because studies on Nile tilapia fed on the same product with the same concentration in the diet provided better results than those with other concentrations (Siena et al., 2010).

Homeopatila 100® was prepared by REALH, Campo Grande MS, Brazil, with registration of product for veterinary use number 024/05736-3 Ministry of Agriculture, Livestock and Food Supply, Brazil. The composition and respective Hahnemannian centesimal dilution of the homeopathic product Homeopatila 100®: Iodum (12 CH); Sulphur (30 CH); Natrum muriaticum (200 CH); Streptococcinum (30 CH) and vehicle - ethyl alcohol 30% v/v (sufficient amount). Fish were beheaded, eviscerated, filleted and skinned (Souza, 2002). The fillets were immediately vacuum-conditioned (Microvac CV 8, Selovac, BR) in smooth transparent nylon bags (thickness 0.280 mm) and frozen at -18°C until nugget preparation. MSM was obtained from the fillet wastes of the two treatments by the (HT C100, High Tech, BR), wrapped in polyethylene bags, frozen at -86°C in an ultrafreezer (IULT 90D, INDEL, BR) and stored at -18°C until the preparation of nuggets.

Preparation of nuggets

Three formulations were prepared for the Homeopatila 100® and control treatments with different concentrations as follows: 75% (F / 75), 50% (F / 50) and 25% (F / 25) with MSM, replacing the tilapia fillet. The F / 50 was chosen as the best formulation through the result of sensory analysis. Therefore, this formulation was prepared again, named F2 / 50, to verify the sensory acceptance and purchase intent among Homeopatila 100® and control. Further, 1200 g of emulsion was used for each assay, featuring the following formulation: 16% cold water; 10% hydrogenated fat; 4% soybean-concentrated protein; 2% corn starch; 2% salt; 0.15% dehydrated onion; 0.10% dehydrated garlic; 0.08% dehydrated parsley and chive; 0.07% dehydrated salvia; 0.03% dehydrated rosemary; 0.07% white pepper; 0.15% sodium tripolyphosphate; 0.05% sodium erythorbate; 0.25% citric acid solution 0.05%; and 65% meat portion (tilapia fillet and MSM).

Fillets and MSM were previously thawed at 4 ± 1°C for approximately 24 h, ground in an electric grinder (PCP-10 L, Poli, BR) and placed with MSM in a mini-cutter (Sire, Filizola, BR). Hydrated tripolyphosphate, the other ingredients and, finally, fat were added. After being homogenized for 2 min, the mass was placed on polyethylene and covered with a polyethylene film and stored for 24 h in a freezer at -18 ± 2°C. The frozen mass was cut in four parts (2.25 cm long and 2 cm wide), made breaded and then frozen at -18°C. For pre-frying, they were kept at 4 ± 1°C for 3 h and pre-fried in soybean oil for 1 min at 180 ± 1°C. For each formulation, 60 pieces were prepared with approximately 20 g not breaded, 24.5 g breaded and 23.5g breaded and pre-fried. The nuggets were prepared in triplicate, wrapped in polyethylene wrappers and frozen at -18 ± 2°C for 120 days.

Physical and chemical composition

The moisture, fixed mineral residue, lipid and crude protein amounts were determined on the 10th day after preparation, according to a protocol by the Association of Official Analytical Chemists (AOAC, 2006). Carbohydrate values were calculated by the difference: 100 - (% Moisture+ % fixed mineral residue + % crude protein + % total lipids). Further, pH analysis was performed at room temperature with a pH-meter (pH 21, Hanna®, Romania) on

10 g of the sample homogenized with 50 ml of distilled water. The evaluation of color is an important quality parameter used to observe natural changes of fresh food or changes during industrial processing (Sato and Cunha, 2005). The absorption was measured by a colorimeter (CR 400, Minolta, Japan) using D65 light and a vision angle of 10°. The absorption was measured at three different sites in the internal part of the product (Perlo et al., 2006), at approximately 45 ± 1°C. The luminosity (L*), red-green component (a*) and yellow-blue component (b*) values were presented according to the color system by the Commission Internationale de L'Eclairage (CIELAB) (Minolta, 1998).

Water activity (Aw) was evaluated at 25°C by a water activity apparatus (4TE, Aqualab, USA), a very important parameter to determine the preservation and shelf life of food. Microorganisms require different minimum Aw levels for growth. Usually, bacteria are more sensitive and almost all have their growth inhibited at Aw values between 0.90 to 0.91. Yeast and molds are more tolerant to lower Aw values, growing in bands ranging from 0.87 to 0.94 and 0.70 to 0.80, respectively (Abbas et al., 2009). To evaluate the shear force, the nuggets were cut into pieces measuring 1.5 cm height × 1.0 cm width × 2 cm length. Analyses were performed using a texture analyzer (TA.HD plus, Stable Micro Systems, UK) equipped with a Warner-Bratzler Blade and 5 g charge cell at a speed of 5.0 mm/s and a distance of 20 mm with a 0.001 mm resolution. The results of the minimum force needed for cutting are given in Newton (N). The samples of each formulation were used for the analyses. The chemical analyses were performed in triplicate. The color parameters, shear force, pH and Aw analyses were performed with 10 repetitions.

Microbiological evaluation

Twenty-four hours after preparation, the nuggets were evaluated for the presence of *Staphylococcus* coagulase and *Bacillus cereus*. The results were reported in colony forming units (CFU/g); coliforms were reported at 45°C and *Escherichia coli* in most probable number per gram (MPN/g). *Salmonella* sp. was determined by absence in 25 g and counting viable aerobic mesophilic and psychrotrophic expressed in Log_{10} CFU/g (ICMSF, 1982).

Lipid stability

After the preparation of the nuggets and pre-frying to an internal temperature of 75 ± 1°C, they were stored at -18°C. The products were then thawed at 4 ± 1°C for three hours prior to the lipid oxidation analysis at times 0 (24 h after preparation), 30, 60, 90 and 120 days by the thiobarbituric acid reactive substances (TBARS) method following Tarladgis et al. (1964), modified by Crackel et al. (1988) to evaluate the difference between means in storage days and between different treatments.

Sensory evaluation

The research was approved by the Research Ethics Committee and involvement of Human Beings of the State University of Maringá (297.336/2013). The 9-point hedonic acceptance test for nuggets were applied, ranging between 9 = I liked it very much and 1 = I definitely did not like it (Dutcosky, 2007). The attributes of color, aroma, tenderness, taste and overall impression were evaluated. It also approved the 5-point buying intention test ranging between 5 = I will surely buy it and 1 = I will surely not buy it (Ferreira et al., 2000). Sensory evaluations were undertaken in three stages. Each step used an untrained panel of 120 teachers and students, between 19 and 50 years of age, who represented consumers at a higher education level from the Federal Technology University of Paraná. In the first stage, the formulations F / 75, F / 50 and F / 25 of the control treatment were used; in the second stage, the same formulations with the Homeopatila 100® treatment were used. The formulation with the best overall evaluation and best buying intention was used in the third stage (50% MSM, F2 / 50) for the sensory analysis of nuggets made of fillet and MSM treated with Homeopatila 100®. The nuggets were thawed and baked until they reach a minimum temperature of 75 ± 1°C, cooled to approximately 45°C and served. The samples from both treatments were coded with random three digit numbers; therefore, the volunteer participant did not know which sample contained the homeopathic product. The equation IA (%) = (A × 100) / B, where A is the mean score for overall evaluation and B is the maximum score observed for overall evaluation, was employed to calculate the acceptability index of the formulations under analysis (Dutcosky, 2007; Monteiro, 1984).

Statistical analysis

Physical, chemical, microbiological and sensory evaluations for the formulations F / 75, F / 50 and F / 25 were evaluated by analysis of variance (ANOVA) and Tukey´s test ($p < 0.05$), and the results of the analysis for the F2 / 50 formulations underwent an analysis of variance at a 5% probability and Student´s t test using the statistical analysis system (SAS) 9.0 (SAS, 2009).

RESULTS AND DISCUSSION

Physical and chemical composition

Whereas the greatest difference ($p < 0.05$) in the instrumental analysis of color occurred in L* (luminosity) when a low value (25%) of MSM was added to the nuggets, no significant difference was reported in the a* (red/green) and b* (yellow/blue) of the other formulations and between the control and the Homeopatila 100® treatments (Table 1). A significant difference was reported in the L*, a* and b* values in chicken nuggets with MSM (Perlo et al., 2006). A 75% increase in MSM in the formulation of Nile tilapia nuggets significantly affects ($p < 0.05$) the shear force and pH. There was no significant difference ($p > 0.05$) in Aw in the various MSM additions (Table 1). Nuggets with 75% MSM had a more tender texture than nuggets with 25% MSM due to the MSM process that ruptures the muscle fiber. A similar effect has been reported in fish sausages, where MSM replacing the fillet, increased its softness (Oliveira Filho et al., 2010). The increase in pH due to MSM was probably due to a higher amount of phosphate caused by the fragmentation of the tilapias´ spines during processing (Gomide et al., 1997). The pH results meet Brazil's legislation for fresh fish (Brasil, 2001a). Higher values of pH were obtained by Oliveira Filho et al. (2010) and Dallabona et al. (2013) in sausages prepared with the addition of MSM at time 0 storage. The mean Aw value did not reveal any significant differences ($p > 0.05$) between the formulations and treatments. The value remained within the high Aw food range for microbial growth and was similar to the value for fresh fish (0.98). The cold storage was required (Oliveira Filho et al., 2010).

Table 1. Color (L*: luminosity a*: red/green; b*: yellow/blue), shear force, pH and Aw (water activity) of nuggets of Nile tilapia treated with Homeopatila 100®.

Parameter	L*	a*	b*	L*	a*	b*	Shear force (N)1		pH		Aw	
	Control			Homeopatila 100®			Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®
F / 75[2,3,5]	60.27±0.43^{bc}	0.85±0.04^{a}	31.56±0.14^{a}	60.03±0.78^{c}	0.90±0.03^{a}	32.05±0.24^{a}	1.45±0.08^{b}	1.29±0.04^{b}	5.88±0.03^{a}	5.89±0.02^{a}	0.975±0.002^{a}	0.972±0.003^{a}
F / 50[2,3,5]	60.34±0.28^{bc}	0.96±0.03^{a}	30.64±0.74^{a}	60.76±0.91^{bc}	0.89±0.04^{a}	32.02±3.14^{a}	2.57±0.10^{ab}	2.46±0.06^{ab}	5.86±0.02^{ab}	5.80±0.09^{ab}	0.962±0.007^{a}	0.963±0.001^{a}
F / 25[2,3,5]	64.42±0.21^{a}	0.99±0.06^{a}	28.58±0.41^{a}	62.66±0.14^{ab}	0.86±0.05^{a}	30.89±0.38^{a}	3.33±0.06^{a}	3.43±0.08^{a}	5.74±0.04^{b}	5.74±0.03^{b}	0.972±0.006^{a}	0.972±0.006^{a}
F2 / 50[2,4,5]	60.97±0.30^{a}	1.34±0.03^{a}	30.41±0.06^{a}	60.09±0.61^{a}	1.31±0.05^{a}	31.42±0.26^{a}	2.85±0.01^{a}	2.82±0.06^{a}	5.97±0.01^{a}	5.85±0.01^{a}	0.975±0.002^{a}	0.975±0.002^{a}

[1]N: Newton. [2]F / 75; F / 50; F / 25; F2 / 50 with 75%, 50%, 25% and 50% of MSM. [3]Value with different letters (a-b-c) on the same line and column differ significantly by Tukey´s test ($p<0.05$). [4]Line are not significantly different at 5% significance level by Student´s t test ($p<0.05$). [5]The results are given by the means ± standard error (n=10).

Table 2. Centesimal composition (%) of nugget from fillet and MSM of Nile tilapia treated with Homeopatila 100®.

Parameter	Moisture		Crude protein		Fixed mineral residue		Total lipids		Carbohydrate[4]	
	Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®
F / 75[1,2,5]	55.50±0.39^{a}	55.67±0.31^{a}	12.85±0.18^{b}	13.55±0.35^{b}	2.75±0.07^{a}	2.77±0.07^{a}	16.07±0.22^{a}	15.96±0.05^{a}	12.79±0.81^{a}	12.05±0.17^{a}
F / 50[1,2,5]	55.13±0.63^{a}	55.49±0.32^{a}	15.32±0.24^{b}	15.54±0.31^{b}	2.72±0.04^{a}	2.65±0.06^{a}	14.34±0.07^{b}	14.52±0.03^{b}	12.49±0.86^{a}	11.80±0.62^{a}
F / 25[1,2,5]	54.59±0.47^{a}	54.46±0.60^{a}	16.04±0.02^{a}	16.12±0.33^{a}	2.86±0.06^{a}	2.73±0.11^{a}	13.98±0.38^{b}	13.62±0.20^{b}	12.53±0.80^{a}	13.07±1.03^{a}
F2 / 50[1,3]	52.04±0.65^{a}	52.84±0.50^{a}	15.71±0.21^{a}	15.14±0.24^{a}	2.54±0.05^{a}	2.59±0.04^{a}	16.12±0.27^{a}	15.63±0.25^{a}	13.59±0.36^{a}	13.80± 0.77^{a}

[1]F / 75; F / 50; F / 25; F2 / 50 with 75%, 50%, 25% and 50% of MSM. [2]Value with different letters (a-b-c) on the same line and column differ significantly by Tukey´s test ($p<0.05$). [3]Line are not significantly different at 5% significance level by Student´s t test ($p<0.05$). [4]Total carbohydrates were calculated by difference: 100 - (% moisture + % fixed mineral residue + % crude protein + % total lipids). [5]The results are given by the means ± standard error (n=3).

2010).

There was no significant difference between treatments in the selected formulation (F2 / 50) in the pH measurement value, shear force, Aw and color (Table 1). The mean values for the moisture, fixed mineral residue and carbohydrates did not differ ($p > 0.05$) between the various MSM percentages between treatments (Table 2). The protein results were similar to those of the control group, but they were lower when the MSM was 50 and 75%. In the latter MSM proportion, the lipid values were higher in both treatment groups. There was no significant difference between the control and Homeopatila 100® in the 50% MSM (F2 / 50) formulation when the centesimal composition was taken into account. When the amount of MSM in sausages was increased, the protein value decreased, the fat increased and the moisture content and mineral residue were not changed (Oliveira Filho et al., 2010). The results were similar to those obtained for nuggets.

The high fat quantity in the nuggets when the MSM was increased may be related to its high lipid value because the ventral muscle parts in the carcass normally have a higher fat content (Oliveira Filho et al., 2010). The minimum protein content and the maximum level of total carbohydrates required by law in breaded products are 10 and 30%, respectively (Brasil, 2001b). The nuggets were developed following these requirements. Results similar to this research in lipids were obtained in croquettes of Nile tilapia MSM (Bordignon et al., 2010).

Microbiological quality

In the enumeration of coliforms at 45°C, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus* coagulase were not found and the presence of *Salmonella* sp. was not detected in the samples of nuggets for the two treatments. The counts of viable mesophilic and psychotrophic aerobic bacteria ranged from 1.54 ± 0.27 to 3.68 ± 0.11 and showed no significant difference between the products and treatments. The analyses were in agreement with those established by legislation (ICMSF, 1982). Results

Table 3. Sensorial evaluation of nugget from fillet and MSM of Nile tilapia treated with Homeopatila 100®.

Parameter	Stage 1			Stage 2			Stage 3	
	Control			Homeopatila 100®			Control	Homeopatila 100®
	F / 75[3,4,6]	F / 50[3,4,6]	F / 25[3,4,6]	F / 75[3,4,6]	F / 50[3,4,6]	F / 25[3,4,6]	F2 / 50[3,5,6]	F2 / 50[3,5,6]
Color[1,]	6.77±0.37^{b}	7.24±0.38^{a}	7.53±0.38^{a}	7.02±0.15^{b}	7.44±0.16^{a}	7.20±0.15^{ab}	6.96 ± 0.15^{a}	7.08 ± 0.16^{a}
Aroma[1,]	7.06±0.36^{b}	7.76±0.37^{a}	7.89±0.37^{a}	6.75±0.14^{a}	7.34±0.15^{a}	7.11±0.15^{a}	7.04 ± 0.16^{a}	7.24 ± 0.16^{a}
Tenderness[1,]	7.18±0.35^{b}	7.54±0.35^{ab}	7.66±0.35^{a}	6.68±0.13^{b}	7.70±0.15^{a}	7.69±0.15^{a}	7.53 ± 0.13^{a}	7.64 ± 0.14^{a}
Taste[1,]	6.49±0.47^{b}	7.39±0.48^{a}	7.94±0.49^{a}	6.64±0.16^{b}	7.21±0.18^{a}	6.99±0.17^{a}	6.72 ± 0.19^{a}	7.06 ± 0.20^{a}
Overall evaluation[1,]	5.75±0.33^{c}	6.47±0.33^{b}	7.10±0.33^{a}	6.41±0.13^{b}	7.28±0.15^{a}	7.31±0.15^{a}	6.94 ± 0.14^{a}	7.17 ± 0.15^{a}
Buying intention[2]	4.08±0.45^{b}	4.79±0.46^{a}	4.16±0.46^{a}	2.91±0.11^{b}	3.72±0.14^{a}	3.75±0.14^{a}	3.59 ± 0.12^{a}	3.77 ± 0.13^{a}

[1]Hedonic scale between 1 and 9 (1 I disliked it very much; 2 I disliked it; 3 I disliked it fairly; 4 I disliked it a little; 5 I didn´t like it nor disliked it/ I didn´t dislike it; 6 I liked it a little; 7 I liked it fairly; 8 I liked it; 9 I liked it very much). [2]Hedonic scale between 1 and 5 (1 I will certainly not buy it; 2 I would possibly not buy it; 3 Perhaps I will buy it, perhaps I will not; 4 I may buy it; 5 I will certainly buy it). [3]F / 75; F / 50; F / 25 and F2 / 50 with 75, 50, 25 and 50% of MSM. [4]Value with different letters (a-b-c) on the same line differ significantly by Tukey´s test (p<0.05). [5]Lines are not significantly different at 5% significance level by Student´s t test (p<0.05). [6]The results are given as the means ± standard error (n=120).

similar to this experiment were obtained in lyophilized mixtures of fish croquettes at time zero with the same main ingredient as the MSM of the Nile tilapia (Fuchs et al., 2013) and in croquettes of the MSM of tilapia after being pre-fried, (Bordignon et al., 2010) for *Salmonella* sp., Coliforms at 45°C, *B. cereus* and *S.* coagulase. However, higher values were found in 4 types of prepared fishburger with the MSM of the Nile tilapia for *E. coli* and coliforms at 45°C (Marengoni et al., 2009) and lower values for viable mesophilic and psycotrophic aerobic bacteria (Bordignon et al., 2010). The pre-frying process (180°C/1') helped in obtaining breaded nuggets with low microbial counts, agreeing with the results obtained in croquettes of MSM of Nile tilapia (Bordignon et al., 2010).

Stability of lipid oxidation

The TBARS values for the Homeopatila 100® treatment increased from 0.007 ± 0.003 mg MDA/kg to 0.672 ± 0.007 mg MDA/kg. In the case of the control, treatment increased the value of 0.004 ± 0.001 mg MDA/kg to 0.758 ± 0.007 mg MDA/kg. There was a gradual increase in lipid oxidation up to 120 days of storage (Figure 1), lthough no significant difference ($p > 0.05$) was found between 60 and 90 days. TBARS was not different ($p < 0.05$) for either treatment. Different values are cited as the mg MDA/kg limits in foods that might indicate rancidity by sensory evaluators. At 0.576, the oxidation value is low and there is no rancidity. Values greater than 1.51 are classified as unacceptable (Ke et al., 1984). In stored fishburger tilapia (-18°C/180 days), the values were lower than in nuggets (Tokur et al., 2004), and similar values were found in quenelles prepared with Nile tilapia (Angelini et al., 2013), ranging from 0.72 ± 0.50 to 0.88 ± 0.63 mg MDA/kg storage (-18°C/180 days). In the current assay, each formulation received an addition of the same amount of antioxidant (0.05% sodium erythorbate), which may have contributed to inhibiting oxidation. Therefore, the evaluated nuggets have an acceptable level of lipid oxidation.

Sensory evaluation

The first stage of sensory evaluation (Table 3) revealed a significant difference ($p < 0.05$) in formulation F / 75 for color, aroma, tenderness and taste. The three formulations showed differences ($p < 0.05$) in overall acceptability. Formulations with 50 and 25% MSM were preferred, whereas the sample with 75% MSM had the lowest acceptance. Sample F / 75 in the second stage of the sensorial test (Table 3) had a lower acceptability for color, tenderness, taste, overall evaluation and buying intention, but no significant difference for aroma. When the two sensorial evaluations were analyzed, acceptance tests showed results between 5.75 and 7.94 and 6.41 and 7.70, respectively, for the control and Homeopatila 100®. Buying intentions were close to 4 and 5 for the control and close to 3 and 4 for Homeopatila 100®. Five formulations provided acceptability values above 70% (Table 4). If these products were for sale, they would be accepted by consumers (Dutcosky, 2007). Formulation F / 50 for the control and Homeopatila 100® was within

Table 4. Acceptability index (%) by attribute and buying intention of fillet and MSM nugget.

Parameter	F / 75[1]	F / 75[1]	F / 50[1]	F / 50[1]	F / 25[1]	F / 25[1]	F2 / 50[1]	F2 / 50[1]
	Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®	Control	Homeopatila 100®
Color	75.22	78.00	80.44	82.67	83.67	80.00	77.33	78,67
Aroma	78.44	75.00	86.22	81.56	87.67	79.00	78.22	80.44
Tenderness	79.78	74.22	83.78	85.56	85.11	85.44	83.67	84.89
Taste	72.11	73.78	82.11	80.11	88.22	77.67	74.67	78.44
Total evaluation	63.89	71.22	71.89	80.89	78.89	81.22	77.11	79.67
Buying intention	81.56	58.22	95.78	74.44	83.22	75.00	71.80	75.40

[1]F / 75; F / 50; F / 25; F2 / 50 with 75, 50, 25 and 50% of MSM.

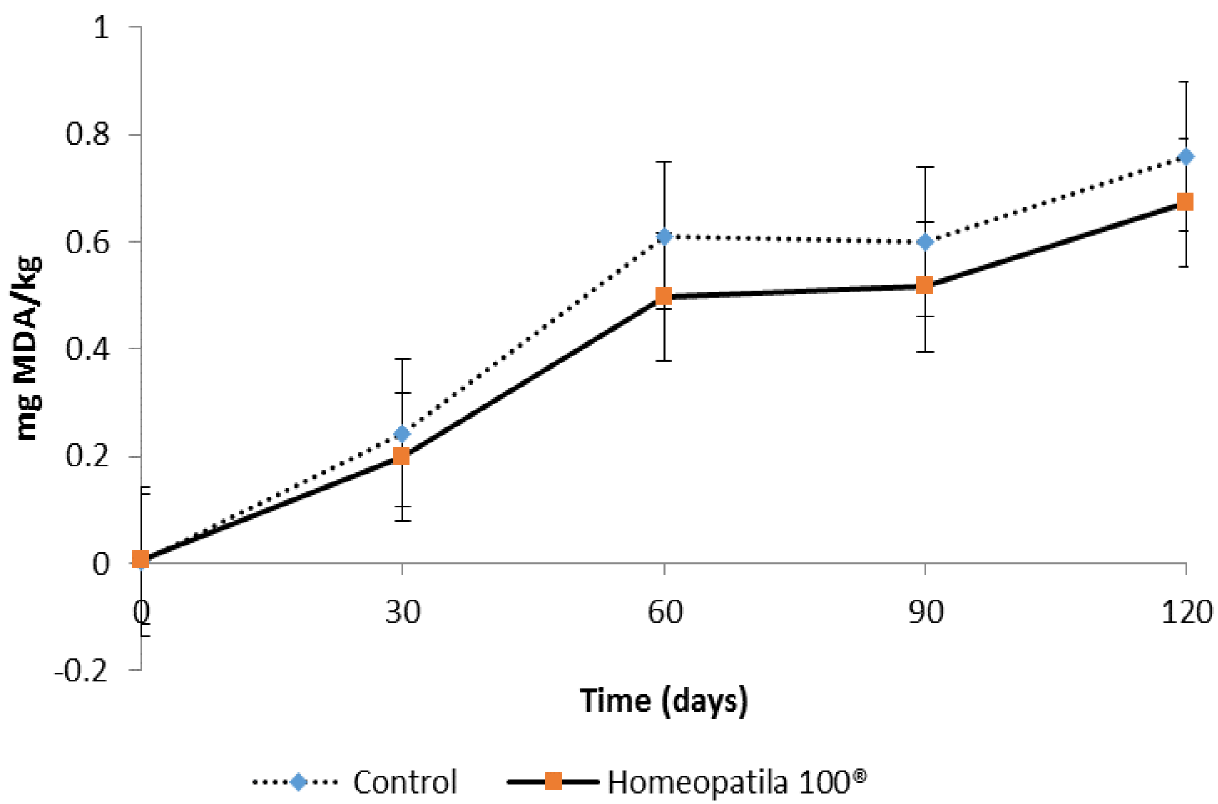

Figure 1. TBARS (thiobarbituric acid reactive substances) values of fillet and MSM 50% (F2 / 50) nuggets of Nile tilapia treated with *Homeopatila 100*® during storage of 120 days.

the range of “Surely I will buy it” and “Possibly I would buy it” because the acceptability indexes had values of 95.78 and 74.44%, respectively (Table 4), whereas the results were different only in the overall evaluation between F / 50 and F / 25. In Stage 1, the formulation with 50% MSM (F2 / 50) was chosen. The third stage of sensory analysis evaluated the consumer´s acceptability of nuggets with Homeopatila 100®. In the case of sausages prepared with fillet and MSM, the highest acceptance occurred between sausages prepared with 40 and 60% MSM, as in the current research (Oliveira Filho et al., 2010).

With regard to the sensory evaluation of F2 / 50 (Stage 3), the acceptability and buying intention did not have any significant difference between treatments. The acceptance test varied between 6.93 and 7.64, and tenderness had the highest score in each of the treatments (Table 3). Scores close to 4 for buying intention for the two treatments were reported, and the values for the acceptability index were 71.80 and 75.40% for the control and Homeopatila 100®, respectively (Table 4), with good acceptability (Dutcosky, 2007). In other research, tilapia MSM-based products were reasonably well accepted. In the case of tilapia breaded, scores were over 7.0 (Cortez Netto et al., 2010). Fishburgers with tilapia MSM had means between 7.14 and 7.46 for all attributes, and the mean scores for buying intention varied between 3.86 and 3.98 (Marengoni et al., 2009). Overall acceptance of sausages with 64% MSM of tilapia, smoked and pasteurized, ranged between 7.7 and 7.5, respectively (Dallabona et al., 2013).

In the instrumental evaluation of color (Table 1), nuggets with 25% MSM had a lighter color than those with 50% and 75%. In the qualitative analysis, the volunteer participants noted color differences with 75% MSM in both treatments. The shear force value was lower in formulation F / 75 (Table 1), which was also detected by the volunteers in the sensory analysis. In agreement with the results of this study, evaluators reported that the texture of sausages made with the highest percentage of MSM (80 and 100%) were softer than the other treatments (Oliveira Filho et al., 2010). The results (step 3) indicated no differences in color, aroma, tenderness, taste, overall evaluation and buying intention in the sensory evaluation with tilapia nuggets treated with Homeopatila 100®. F2 / 50 had the highest score with regard to tenderness. The same was reported in an assay with 40 and 60% MSM in sausages (Oliveira Filho et al., 2010). Buying intention varied for F2 / 50 between 71.80 and 75.40% for the control and Homeopatila 100® groups, respectively (Table 4), and 83.76% declared they had never eaten fish nuggets. With the results observed in the sensory analysis (steps 1 and 2), nuggets can be produced with 75% of filleting waste as a replacement for fillets of Nile tilapia without changing the physical, chemical, microbiological and sensory characteristics. However, to maintain a better acceptability and purchase intent for nuggets, the maximum amount of MSM as a substitute for the fillet can be 50%, reducing waste in fish processing and preventing negative environmental impact.

In the sensory evaluation of nuggets of Nile tilapia treated with Homeopatila 100® and control (stage 3), the results showed no significant differences in the color attributes, aroma, tenderness, taste, overall evaluation and purchase intent. The use of homeopathic product in the diet of tilapia did not affect the sensory quality of the nuggets. This effect is important because the homeopathic product has provided significant results in performance parameters in the cultivation of Nile tilapia.

Conclusion

The results provide no evidence that adjuvant treatment with Homeopatila 100® for fish growth improves the quality of meat and products made from Nile tilapia.

Conflict of interest

The authors declared no conflict of interest.

REFERENCES

Abbas KA, Saleh AM, Mohamed A, Lasekan O (2009). The relationship between water activity and fish spoilage during cold storage: A review. J. Food Agric. Environ. 7:86-90.

Adler UC, Paiva NMP, Cesar AT, Adler MS, Molina A, Padula AE, Calil HM (2011). Homeopathic Individualized Q-Potencies versus Fluoxetine for Moderate to Severe Depression: Double-Blind, Randomized Non-Inferiority Trial. Evid. Based Complement. Altern. Med. Article ID 520182, p.7.

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

Angelini MFC, Galvão JA, Vieira AF, Savay-da-Silva LK, Shirahigue LD, Cabral ISR, Modesta RCD, Gallo CR, Oetterer M (2013). Shelf life and sensory assessment of tilapia quenelle during frozen storage. Pesq. agropec. bras. Brasília 48(8):1080-1087.

AOAC (2006). Association Methods of Analysis of AOAC International, Volume I and II, 18th Edition, Rev. 1.

Bordignon AC, Souza BE, Bohnenberger L, Hilbig CC, Feiden A, Boscolo WR (2010). Elaboração de croquete de tilápia do Nilo (*Oreochromis niloticus*) a partir de CMS e aparas do corte em ‘V’ do filé e sua avaliação físico–química, microbiológica e sensorial. Acta Sci. Anim. Sci. 32:109-116.

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(4):233-241.

Brasil (2001a). Ministério da Agricultura, Pecuária e Abastecimento. Departamento Nacional de Inspeção de Produtos de Origem Animal (DIPOA). Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal – RIISPOA, Pescados e derivados, Capítulo VII. Brasília, DF.

Brasil (2001b). Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária. Instrução Normativa nº 6 de 15 de fevereiro de 2001. Regulamento técnico de identidade e qualidade de empanados. Diário Oficial da União, Brasília, DF, 19

fev, Seção 1, p 60.
Cortez Netto JP, Boscolo WR, Feiden A, Maluf MLF, Freitas JMA, Simões MR, (2010). Formulação, análises microbiológicas, composição centesimal e aceitabilidade de empanados de jundia (*Rhamdia quelen*), pacu (*Piaractus mesopotamicus*) e tilápia (*Oreochromis niloticus*). Rev. Inst. Adolfo Lutz. 69(2):181-187.
Crackel RL, Gray JI, Booren AM, Buckely DJ (1988). Effect of antioxidants on lipid stability in restructured beef steaks. J. Food Sci. 53:656-657.
Dallabona BR, Karam LB, Wagner R, Bartolomeu DAFS, Mikos JD, Francisco JGP, Macedo REF, Kirschnik PG (2013). Effect of heat treatment and packaging systems on the stability of fish Sausage. R. Bras. Zootec. 42:835-843.
Dutcosky SD (2007). Análise sensorial de alimentos. 2.ed., rev. e ampl. Curitiba: Champagnat p 239.
Ferreira VLP, Almeida TCA, Pettinelli MLC, Silva MAAP, Chaves JBP, Barbosa EMM (2000). Análise sensorial: testes discriminativos e afetivos. Campinas: SBCTA (Manual: Série Qualidade) p 127.
Fuchs RH, Ribeiro RP, Bona E, Matsushita MD (2013). Development of a freeze-dried mixture of Nile tilapia (*Oreochromis niloticus*) croquette using a GA-based multiobjective optimization J. Sci. Food Agric. 93(5):1042-1048.
Gehring CK, Gigliotti JC, Moritz JS, Tou JC, Jaczynski J (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review. Food Chem. 124:422-431.
Gomide LAM, Garcia AM, Pereira ASO, Mendonça RCS (1997). Avaliação físico-química e microbiológica da adição de carne de frango mecanicamente separada em embutido fermentado. Cienc. Tecnol. Aliment. 17:125-131.
ICMSF (1982). International Commission of Microbiological Specifications for Foods. Microorganimsm in foods. Their significance and methods of enumeration. 2nd ed. University of Toronto Press, Toronto p 436.
Ke PJ, Cervantes E, Roblemartinez C (1984). Determination of thiobarbituricacid reactive substances (TBARS) in fish tissue by an improved distillations pectrophotometric method. J. Sci. Food Agric. 35(440):1248-1254.
Marengoni NG, Pozza MSS, Braga GC, Lazzeri DB, Castilha LD, Bueno GW, Pasquetti TJ, Polese C (2009). Caracterização microbiológica, sensorial e centesimal de *fishburgers* de carne de tilápia mecanicamente separada. Rev. Bras. J. Saúde Prod. Anim. 10:168-176.
Merlini LS, Vargas L, Piau R, Ribeiro RP, Merlini NB (2014). Effects of a homeopathic complex on the performance and cortisol levels in Nile tilapia (*Oreochromis niloticus*). Homeopathy 103(2):139-142.
Minolta (1998). Precise color communication - color control from perception to instrumentation. Japan: Minolta Co., Ltd. p 59.
Monteiro CLB (1984). Técnicas de avaliação sensorial. 2.ed. Curitiba: CEPPA-UFPR p 101.
Ninan G, Bindu J, Joseph J (2010). Frozen storage studies of value-added mince-based products from tilapia (*Oreochromis mossambicus*, Peters 1852). J. Food Process. Preserv. 34: 255-271.
Nunes TP, Trindade MA, Ortega EMM, Castilho CJC (2006). Aceitação sensorial de reestruturados empanados elaborados com filé de peito de galinhas matrizes de corte e poedeiras comerciais. Cienc. Tecnol. Aliment. Campinas 26(4):841-846.
Oliveira Filho PRC, Netto FM, Ramos KK, Trindade MA, Viegas EMM (2010). Elaboration of sausage using minced fish of Nile tilapia filleting waste. Bras. Arch. Biol. Technol. 53:1383-1391.
Perlo F, Bonato P, Teira G, Fabre R, Kueider S (2006). Physicochemical and sensory properties of chicken nuggets with washed mechanically deboned chicken meat: Research note. Meat Sci. 72:785-788.
SAS (2009). Institute Inc. SAS/STAT 9.2 user's guide. 2nd ed. Cary, N.C.: SAS Institute Inc., c2009. The Genmod procedure p 37.
Sato ACK, Cunha RL (2005). Avaliação da cor, textura e transferência de massa durante o processamento de goiabas em calda. Bras. J. Food Technol. 8(2):149-156.
Scherer R, Augusti PR, Bochi VC, Steffens C, Fries LLM. Daniel AP, Kubota EH, Radünz Neto J, Emanuelli T (2006). Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods. Food Chem. 99(1):136-142.
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. Cien. Agrar. 31(4):985-994.
Souza MLR (2002). Comparação de Seis Métodos de Filetagem, em Relação ao Rendimento de Filé e de Subprodutos do Processamento da Tilápia-do-Nilo (*Oreochromis niloticus*). Rev. Bras. Zootec. 31(3):1076-1084.
Tarladgis BG, Pearson AM, Dugan LR (1964). Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods – II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15:602-604.
Taskaya L, Jaczynski J (2009). Flocculation-enhanced protein recovery from fish processing by-products by isoelectric solubilization/precipitation. LWT - Food Sci. Technol. 42:570-575.
Tokur B, Polat A, Beklevik G, Özkütük S (2004). Changes in the quality of fishburger produced from tilapia (*Oreochromis niloticus*) during frozen storage (18°C). Eur. Food Res. Technol. 218:420-423.
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.
Zhang X, Feng J, Xu M, Hu J (2011). Modeling traceability information and functionality requirement in export-oriented tilapia chain. J. Sci. Food Agric. 91:1316-1325.