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Physical, chemical and microbiological quality of fillets and mechanically separated meat, and sensory evaluation of fillets of Nile Tilapia treated with homeopathic product

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Current assay evaluates the physical, chemical and microbiological quality of fillets and mechanically separated meat (MSM) plus the sensory aspects of fillets of the Nile Tilapia (*Oreochromis niloticus*) treated with Homeopatila 100®. The moisture, fixed mineral residue, lipid, crude protein, pH, color, water activity (Aw), *Staphylococcus* coagulase, coliforms were reported at 45°C. *Escherichia coli*, *Salmonella* sp., counting viable aerobic mesophilic and psychrotrophic amounts of the fillets and MSM were determined. Acceptance, intention test and shear force of fillets also were performed. The fillets examined by softer tissue (6.08 N ± 0.04), lower Aw (0.990 ± 0.001), less total amounts of psychrotropic aerobic bacteria (2.12 ± 0.01 Log_{10} UFC/g) and by low component value of colour. L* (luminosity) (65.78 ± 0.37) and a* (red/green) (1.50 ± 0.01) for MSM and of high value of b* (yellow/blue) for MSM (13.54 ± 0.11) and fillets (4.93 ± 0.03) sensory acceptability was 80.11%. The colours of fillet and MSM were related to the results of fish stressed prior to slaughter. The results indicated that the use of Homeopatila 100® in the diet of the Nile Tilapia did not change the muscle quality and the sensory acceptability.

Key words: Aquaculture, tilapia, homeopathy, muscle.

INTRODUCTION

Homeopathy is a complementary and alternative medicine system and has user-friendly applications and extensive clinical literature (Bell and Koithan, 2012). Homeopathic products are manufactured by

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dynamization, which consists of dilutions by sequential stirrings in small volumes and has applications to both human and animal diets (Adler et al., 2011). Animal homeopathy is a science applied in several areas (Fisher, 2012), being ideal due to its low cost, efficiency and absence of toxicity. The use of extremely diluted active ingredients ensures no residues in meat and contamination in water and soil. Animals fed homeopathic product have better production potential, better quality and greater survival (Andretto et al*.,* 2014). Homeopatila 100® is a homeopathic complex that is designed to decrease stress in production and guarantee the well-being of Nile Tilapia *(Oreochromis niloticus)* in the productive cycle of the commercial fish fry.

Studies have been undertaken with this homeopathic product, revealing a high survival value and indicated a lower hepatosomatic index for Nile Tilapia (Siena et al., 2010). Research conducted by Braccini et al. (2013) in juvenile Tilapia provided satisfactory results for performance related to survival, prevalence and parasitic load. Study conducted by Merlini et al. (2013) with Nile Tilapia and Homeopatila 100® reported a low level of plasma cortisol level and high weight gain. Lima et al. (2015) investigated the effect of Homeopatila 100® in the diet of the Nile Tilapia on nuggets of fillet and MSM. The results indicated that it did not change the product's physical, chemical, microbiological and sensory characteristics, ensuring consumer acceptability. Nile Tilapia cultivated in high density that received the homeopathic product Homeopatila 100® incorporated into their feed presented better quality muscle tissues compared with the group control (Andretto et al., 2015).

Tilapia is the second most produced group of fish worldwide, and sales grow at a rate of 10% per year on average (Watterson et al., 2012). The world aquaculture production forecast for the year 2030 predicts between 79 and 110 million tons of fish (Watterson et al., 2012), including significant growth in the production of Nile Tilapia. Fish products provide important components of the human diet (Watterson et al., 2012), but are highly perishable products, as their quality and freshness deteriorate quickly after death (Medina et al., 2009). The results of physical, chemical, and microbiological analysis in addition to sensory evaluations provide the necessary information to measure the quality and freshness of fish muscle (Wills et al., 2004). The current investigation evaluated physical, chemical, and microbiological quality of fillets and mechanically separated meat (MSM) and rated sensory evaluation of fillets of Tilapia that consumed Homeopatila 100®.

MATERIALS AND METHODS

Fillet and MSM

Use of the animals was approved by the Ethic Committee for use of animals in experiments from the Maringa State University according to Protocol 019/2013. Males with sexual inversion from a homogenous Nile Tilapia population (*O. niloticus*; variety Supreme) used as a control had an initial mean weight of 101.12 ± 17.73 g and a length of 185.2 ± 60.0 mm. The Nile Tilapia treated with Homeopatila 100® had an initial mean weight of 99.73 ± 19.85 g and a length of 180.5 ± 11.1 mm. The fish were randomly distributed in 16 fiber glass water boxes with 600 L capacities each. Three hundred and twenty fish were distributed in the water boxes with 20 fish per box. The fish were conditioned 7 days prior to the start of the experiment, and the control and Homeopatila 100® treatments were evaluated in eight replications (eight boxes for each treatment) through a randomized experimental design with 160 fish per treatment.

The fish were fed on a commercial extruded diet with 32% crude protein of 5 mm diameter, which was provided twice per day (at 10:00 and 16:00 h). At the end of the experiment, the fish remained fasting for 24 h. All fish were captured by nets, de-sensitized with water and ice at 0°C (Scherer et al., 2006) and killed by spine medulla severance. The homeopathic product Homeopatila 100® was prepared by the firm REALH of Campo Grande MS and registered for veterinary use (No. 024/05736-3, Ministry of Agriculture, Livestock and Food Supply - Brazil). The composition and respective Hahnemannian centesimal dilution of the homeopathic product Homeopatila 100® were: Iodum (12 CH); Sulphur (30 CH); Natrum muriaticum (200 CH); Streptococcinum (30 CH) and vehicle - ethyl alcohol 30% v/v (sufficient amount). Two treatments were evaluated: a control with a hydro-alcohol solution of 30% v/v (addition of 40 mL/kg diet) and treatment with Homeopatila 100® (addition of 40 mL/kg diet).

Studies with Nile Tilapia fries that received the same product at the same concentration in the diet provided better results than those at other concentrations (Siena et al., 2010). 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 the time of analysis. 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 ultra freezer (IULT 90D, INDEL, BR) and stored at -18°C until the time of analysis.

Physical and chemical composition of fillets and MSM

Moisture, fixed mineral residue, lipids and crude protein value were determined according to procedures by the official Association of Analytical Chemists (AOAC, 2006). Further, pH was calculated at room temperature using a pH-meter (pH 21, Hanna®, Romania). 10 g of the sample was homogenized with 40 mL of distilled water. Colour was measured with a colorimeter (CR 400, Minolta, Japan), a D65 illuminant and 10° angle of vision. Colour measurements were performed at three different surface sites of the fillet and MSM. L* (luminosity), a* (red-green component), and b* (yellow-blue component) value were given according to the colour system of the Commission Internationale de L'Eclairage (CIELAB) (Minolta, 1998).

Water activity (Aw) was evaluated at 25°C in a water activity apparatus (4TE, Aqualab, USA). The shear force was evaluated by cutting the fillets in 1.5 cm high × 1.0 cm wide × 2.0 cm long pieces. Analysis 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) (Lima et al., 2015). The chemical analysis was performed in triplicate. The shear force, pH, Aw and colour analysis were performed with 10 repetitions, shortly after filleting and MSM extraction.

Microbiological evaluation of fillets and MSM

Fillets and MSM were microbiologically evaluated for *Staphylococcus* coagulase (CFU/g), *Salmonella* sp., *Escherichia coli* (MPN/g) and viable aerobic mesophilic microorganism and psychrotrophic counts, given in Log_{10} CFU/g, taking into consideration international legislation (ICMSF, 1982). Analysis was performed in triplicate, and the control and Homeopatila 100® treatments were compared.

Sensory evaluation of fillets

The committee for ethics in research involving human beings of the Maringa State University (Protocol 297.336/2013) authorized the Fillet Acceptance Test employing a 9 - point Hedonic Scale, where a score of 9 = "I like it very much," and a score of 1 = "I definitely do not like it" (Dutcosky, 2007). Colour, aroma, tenderness, taste and overall impression were evaluated. Further, a 5-point scale for the Buying Intention Test was also applied, with a score of 5 = "I will certainly buy it," and a score category of 1 = "I surely will not buy it" (Ferreira et al*.,* 2000). Each step used an untrained panel of 120 teachers and students, between 18 and 58 years of age, who represented consumers at a higher education level from the Federal Technology University of Parana.

Fillets were thawed (< 4°C), cut into 15 g - pieces measuring 4.5 × 4.5 × 2.0 cm seasoned with NaCl (2%) and grilled, till they reached an internal temperature between 72 and 75°C and were presented to the participants at approximately 40°C. The samples from both treatments were coded with three random digit numbers (Teixeira et al*.,* 1987); therefore, the volunteer participant did not know which sample contained the homeopathic product. The acceptability index of the formulations undergoing analysis was given by the equation:

$$IA\ (\%) = \frac{(A \times 100)}{B}$$

Where AI = acceptance index; A = the mean score obtained for total evaluation and B = the maximum score for overall evaluation (Dutcosky, 2007).

Statistical analysis

The results obtained, physical, chemical, microbiological and sensory analysis underwent analysis of variance (ANOVA) at 5% probability. A Student's t-test compared means using Statistical Analysis System (SAS) 9.0 (SAS, 2009).

RESULTS AND DISCUSSION

Physical and chemical composition of fillets and MSM

The mean centesimal composition value of the fillets, pH, L* and a* of the control and Homeopatila 100® groups failed to show any difference in significance. Shear force, b* and Aw were significantly higher ($p < 0.05$) in the control group (Table 1). The L*, a* and b* parameters of MSM revealed a significant difference ($p < 0.05$) between treatments, which did not occur for moisture, crude protein, fixed mineral residue, total lipids, pH and Aw (Table 1). Shear force value in the current experiment, 6.32 ± 0.39 and 6.08 ± 0.04 N, for the control and Homeopatila 100® treatment groups, respectively (Table 1), were similar to those obtained for *Sparus aurata* fish (*seabream*) (Mattos et al*.,* 2010), with values ranging between 5.0 ± 2.1 and 5.7 ± 1.8 N for raw fillets. The texture results disagree with research of raw fillets of fish exposed to stress before killing. Studies of different fish species under stress, such as turbot (*Psetta maxima*) (Morzelet al., 2003) and rainbow trout (*Oncorhynchus mykiss*) (Wills et al., 2004), provide a lower value of shear force and texture softening of the muscles. Other authors reported that there was no effect on the codfish (*Gadus morhua*) (Bjørnevik and Solbakken, 2010) and the gilt-head bream (Mattos et al., 2010), but increased hardness occurred with the Atlantic salmon (*Salmo salar*) (Skjervold et al*.,* 2001).

Quality characteristics of meat are affected by stress (Stien et al., 2005). Droval et al. (2012) and Santos et al. (2012) have used the classification by parameter L* to evaluate the quality of stressed animals muscles prior to killing. This type of classification has not been established for Tilapia. Research on rainbow trout revealed a paler color (> L*) in the meat of stressed fish (Poli et al., 2005). The lower the value of L*, the higher the percentage of myoglobin in meat with darker muscle (Ribeiro et al., 2007). Myoglobin is one of the main factors of colour formation in fish muscle, which is an important quality component for consumers in their decision to buy (Ocaño-Higuera et al*.,* 2009). Robb et al. (2000) showed that high levels of muscular activity had significant differences in L* and b* and low differences in a* in the colour of the rainbow trout muscle upon killing. They suggested that such a change may be caused by changes in the structure of the muscle tissue. Jittinandana et al. (2003) registered that stress immediately prior to euthanasia increased the L* value and decreased the a* value in the muscle of seasoned Arctic salmon (*Salvelinus alpinus*). The yellow component of the fillet in the experiment reported herein was significantly different ($p < 0.05$) from the control (Table 1) and had a more intense color. The results of the two treatments were similar to those obtained from juvenile Nile Tilapia (Girao et al*.,* 2012). In case of MSM, the L* and b* values were higher, although the a* value was lower in the control group (Table 1).

Moisture, fixed mineral residue, protein and lipid results in the fillets were close to those reported by Leonhardt et al. (2006). Similar results (Kirschniket al., 2013) were obtained from MSM Tilapia (Table 1). The higher fat content of MSM compared to that of fillets is due to the ventral muscular section in the carcass that normally contains more fat and is processed while obtaining MSM (Oliveira et al., 2010). The addition of 40 ml of Homeopatila 100® per kg of diet did not change the characteristics of the centesimal composition of the fillets and MSM. The pH of the fillets was similar to pH in Nile

Table 1. Physical and chemical evaluation of Nile Tilapia fillets and MSM.

Parameter	Fillet		MSM	
	Treatments		Treatments	
	Control[3,4]	Homeopatila 100®[3,4]	Control[3,4]	Homeopatila 100®[3,4]
Shear force (N)[1]	6.32 ± 0.39[a]	6.08 ± 0.04[b]	-	-
L^*	52.77 ± 0.40[a]	52.66 ± 0.40[a]	67.89 ± 0.37[a]	65.78 ± 0.37[b]
a^*	2.76 ± 0.03[a]	2.71 ± 0.03[a]	0.84 ± 0.01[b]	1.50 ± 0.01[a]
b^*	5.54 ± 0.03[a]	4.93 ± 0.03[b]	14.14 ± 0.11[a]	13.54 ± 0.11[b]
Moisture (%)	78.00 ± 0.11[a]	78.14 ± 0.11[a]	68.33 ± 0.43[a]	67.18 ± 0.43[a]
Crude protein (%)	18.64 ± 0.15[a]	17.45 ± 0.12[a]	15.62 ± 0.21[a]	16.06 ± 0.21[a]
Fixed mineral residue (%)	1.13 ± 0.01[a]	1.15 ± 0.01[a]	1.52 ± 0.04[a]	1.42 ± 0.04[a]
Total lipids (%)	1.93 ± 0.02[a]	1.95 ± 0.02[a]	14.05 ± 0.16[a]	14.71 ± 0.27[a]
pH	5.96 ± 0.02[a]	5.96 ± 0.02[a]	6.83 ± 0.04[a]	6.72 ± 0.04[a]
Aw[2]	0.995 ± 0.001[a]	0.990 ± 0.001[b]	0.989 ± 0.001[a]	0.988 ± 0.001[a]

[1]N: Newton; [2]Aw: water activity; [3]Value with different letters (a - b) on the same line differ significantly by the Student's t-test ($p < 0.05$); [4]The results are given as the mean ± standard error.

Table 2. Microbiological evaluation of Nile Tilapia fillets and MSM.

Parameters	Fillet		MSM	
	Treatments		Treatments	
	Control[3,4]	Homeopatila 100®[3,4]	Control[3,4]	Homeopatila 100®[3,4]
Escherichia coli counts (MPN/g)[1]	< 0.3	< 0.3	< 0.3	< 0.3
Research on *Salmonella* sp. 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g
Staphylococcus coagulase counts (CFU/g)[2]	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Viable aerobic mesophilic (Log_{10} CFU/g)	2.09 ± 0.14[a]	2.00 ± 0.14[a]	3.05 ± 0.17[a]	3.11 ± 0.68[a]
Viable aerobic psychrotrophic (Log_{10} CFU/g)	2.41 ± 0.01[a]	2.12 ± 0.01[b]	3.19 ± 0.20[a]	3.53 ± 0.20[a]

[1]MPN:most probable number; [2]CFU: colony forming unit; [3]Value with different letters (a - b) on the same line differ significantly by the Student's t-test ($p < 0.05$); [4]The results are given as the mean ± standard error.

Tilapia (Soares and Goncalves, 2012). The pH value in MSM was similar to pH in MSM (Kirschnik et al., 2013) of Tilapia at time 0 (Table 1). The Aw value for the two treatments complied with the classification for fish, as a food with high moisture content and were higher (Simões et al., 2007) and lower (Souza et al., 2005) than those in the fillets of the Nile Tilapia control and treatment group, respectively.

Pre-euthanasia stress decreases rigor mortis duration: stressed fish may develop rigidity faster than non-stressed animals, leading to effects on meat texture (Thiansilakul et al., 2011). The animals rigor mortis is characterized by a post mortem pH decrease due to an accelerated metabolic transformation of glycogen in to lactic acid, with paler meat due to liquid release (Nakayama et al., 1992). The results of this study relate with decreased stress in fish prior to slaughter. The color of the muscle treated with Homeopatila 100® proved less stressed fish and lower shear force value and free water in muscle compared to the control group.

Microbiological evaluation of fillets and MSM

Escherichia coli and *Staphylococcus* coagulase counts were nil, and *Salmonella* sp. did not occur in fillet and MSM samples in neither of the two treatments (Table 2). The viable aerobic mesophilic microorganisms did not reveal significant differences between treatments of fillet and MSM. It was only in the Tilapia fillets that viable aerobic psychrotrophic microorganisms were significantly higher ($p < 0.05$) for the control group (Table 2). Microbiological analysis of fillets and MSM in the control and Homeopatila 100® groups were complied with international legislation (ICMSF, 1982). The results similar to those in the current search were reported for fillets for *Salmonella* sp. and *Staphylococcus* coagulase (Simões et al., 2007), and higher levels were reported for viable aerobic psychrotrophic microorganisms, *Escherichiacoli* and *Salmonella* sp. (Bartolomeu et al., 2011).

There were similar counts (Rodrigues et al., 2008) for

Table 3. Sensory evaluation of Nile tilapia fillets.

Parameter	Treatments	
	Control[3,4]	Homeopatila 100®[3,4]
Color[1]	7.63 ± 0.33[a]	7.51 ± 0.32[a]
Aroma[1]	7.41 ± 0.32[a]	7.40 ± 0.32[a]
Tenderness[1]	7.95 ± 0.28[a]	7.82 ± 0.29[a]
Taste[1]	7.41 ± 0.49[a]	7.59 ± 0.49[a]
Total evaluation[1]	7.16 ± 0.32[a]	7.21 ± 0.33[a]
Buying intention[2]	4.18 ± 0.33[a]	3.97 ± 0.30[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 did not like it nor disliked it/ I did not 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]Value with different letters (a - b) on the same line differ significantly by the Student's t-test ($p < 0.05$); [4]The results are given as the mean ± standard error.

viable aerobic mesophilic microorganisms (2.8 Log_{10} CFU/g) and viable aerobic psychrotrophic microorganisms (2.0 Log_{10} CFU/g) and higher counts (Espirito et al., 2007) for viable aerobic mesophilic microorganisms (3.0 to 5.6 log_{10} CFU/g) compared to this study. Microbial counts of *Escherichia coli*, *Staphylococcus* coagulase and viable aerobic psychrotrophic microorganisms and *Salmonella* sp. in MSM were similar to those revealed by Poli et al. (2005) and viable aerobic mesophilic microorganisms demonstrated a higher value than those in the study of Mello et al. (2010). The use of Homeopatila 100® in fish diets may have decreased viable aerobic psychrotrophic microorganisms counts in the fillets due to stress reduction in the animals. Stress in fish favors the development of degrading microorganisms, which can lead to a shortening of fish products shelf life (Viegas et al., 2012).

Sensory analysis of fillets

The results of sensory analysis did not show any significant difference between the treatments (Table 3). The acceptance test yielded value between 7.16 (I liked it somewhat) and 7.95 (I liked it slightly), whereas tenderness had the best score in each treatment, with scores of 7.95 ± 0.29 and 7.82 ± 0.28 for the control and Homeopatila 100® groups, respectively. Scores close to 4 were attributed to the two treatments in the buying intention test, whereas values for the acceptability index were 79.60 and 80.11% for the control and Homeopatila 100® groups, respectively. The current research is the first investigation of sensory analysis of fillets of Nile Tilapia fed on a diet including a homeopathic product. Lima et al. (2015) investigated the first sensory analysis in nuggets.

Data on the sensorial evaluation of Tilapia fillets treated with Homeopatila 100® lay between the categories "I liked it somewhat" and "I like it very much". The above results demonstrated overall acceptance of the product, with tenderness being the attribute with the highest score in the two treatments (Table 3). The two treatments provided over a 70% acceptability value for all attributes. The above results suggest that fillets on sale will be accepted by consumers and provide the fishing industry with insight regarding potential strategies for development and marketing (Dutcoski, 2007).

When other Tilapia-based products are compared, the acceptance results for the two treatments prove to be similar to pates (6.5 to 7.4) (Minozzo et al., 2008) and fish burgers (7.1 to 7.5) (Marengoni et al., 2009). These results were similar to nuggets of file and MSM (7.28 and 7.31) treated with Homeopatila 100® (Lima et al., 2015).

The results also revealed that the panelists did detect differences in colour, aroma, tenderness and taste among the fillets that were subjected to different treatments. Several studies reported that pre-euthanasia stress has a slight effect on the smell but no effect on the taste of Salmonidae meat (Van et al., 2003). The instrumental evaluation of texture and colour (Table 1) showed that fish fillets treated with Homeopatila 100® were 3.8% more tender with an 11% increase in intense yellow colour, both of which were unperceived by the non-trained judging panel, the difference is very small. The above results are due to slight differences and the instrumental evaluation of grilled fillets was in contrast to raw fillets.

Conclusions

Nile Tilapia fed on a diet including Homeopatila 100® produced more tender fillets, less Aw, lower total psychrotrophic aerobic bacteria counts and good sensory acceptability. The end color of fillet and MSM relates to values for fish stressed prior to slaughter. The results indicate that the use of Homeopatila 100® in the diet of

Nile Tilapia did not change the fillet and MSM quality.

Conflict of Interest

The authors have not declared any conflict of interest.

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