



**UNIVERSIDADE ESTADUAL DE MARINGÁ**  
**CENTRO DE CIÊNCIAS AGRÁRIAS**  
Programa de Pós-Graduação em Ciência de Alimentos

**DESENVOLVIMENTO TECNOLÓGICO DE *NUGGETS***  
**DE FILÉ DE TILÁPIA DO NILO TRATADAS COM**  
***HOMEOPATILA 100*<sup>®</sup>**

**DENISE PASTORE DE LIMA**

Maringá  
2015

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DE FILÉ DE TILÁPIA DO NILO TRATADAS COM  
*HOMEOPATILA 100*<sup>®</sup>**

Tese apresentada ao programa de Pós  
Graduação em Ciência de Alimentos da  
Universidade Estadual de Maringá,  
como parte dos requisitos para  
obtenção do título de doutor em  
Ciência de Alimentos.

MARINGÁ  
2015

**Orientador**

Prof. Dr. Lauro Vargas

**Co-Orientador**

Prof. Dr. Ricardo Pereira Ribeiro

## **BIOGRAFIA**

DENISE PASTORE DE LIMA, nasceu em 13 de outubro de 1964, na cidade de Antônio Prado, Rio Grande do Sul. No ano 2000, concluiu a graduação em Tecnologia em Alimentos pela Universidade Tecnológica Federal do Paraná (UTFPR), Câmpus Medianeira. Em 2005 concluiu o mestrado em Desenvolvimento Regional e Agronegócio pela Universidade Estadual do Oeste do Paraná (UNIOESTE). É professora da Universidade Tecnológica Federal do Paraná, Câmpus Medianeira. Tem experiência nas áreas de Ciência e Tecnologia de Alimentos, atuando principalmente nos seguintes temas: segurança do alimento e tecnologia de carnes.

***Dedico***

*Ao meu marido, Ezequiel, pelo amor e carinho de todos os dias.*

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minha continuidade.*

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*As minhas irmãs pelo carinho e amizade.*

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Esta tese de doutorado está apresentada na forma dois artigos científicos, descritos a seguir:

Lima, D.P., Fuzinato, M.M., Andretto, A.P., Braccini, G.L., Mori, R.H., Canan, C., Mendonça, S.N.T.G., Oliveira, C.A.L., Ribeiro, R. Vargas, L. Physical, chemical, and microbiological quality of fillets and mechanically separated meat (MSM) and sensory evaluation fillets of Nile tilapia treated with homeopathic product. Artigo enviado à revista African Journal of Pharmacy and Pharmacology em 11 de março de 2015, atualmente sob o status revisão.

Histórico de submissões:

- 26.03.2014 - Evidence-Based Complementary and Alternative Medicine;
- 13.05.2014 - Homeopathy;
- 29.08.2014 - Journal of Food and Quality;
- 11.03.2015 - African Journal of Pharmacy and Pharmacology.

1. Lima, D.P., Fuzinato, M.M., Andretto, A.P., Braccini, G.L., Mori, R.H., Canan, C., Mendonça, S.N.T.G., Oliveira, C.A.L., Ribeiro, P.R. Vargas, L. Mechanically separated fillet and meat nuggets of Nile tilapia treated with homeopathic product. African Journal of Pharmacy and Pharmacology. Vol 9 (6), pp.182-189, 15 feb, 2015. DOI 10.5897/AJPP2014. 4173.

Histórico de submissões:

- 12.08.2014 - Journal the Science of Food and Agriculture.
- 12.09.2014 - African Journal of Pharmacy and Pharmacology (Publicado - Anexo II).

## GENERAL ABSTRACT

Homeopathy is a complementary and alternative medicine system and has user-friendly applications and extensive clinical literature. Homeopathic products are manufactured by dynamization, which consists of sequential dilutions by stirrings in small volumes and has applications to both human and animal diets. *Homeopatila 100*<sup>®</sup> is a homeopathic complex designed to decrease stress in production and guarantee the well-being of Nile tilapia (*Oreochromis niloticus*). Tilapia is the second group of fish most produced worldwide and marketing grows an average grow 10% per year. The world aquaculture production forecast for 2030 ranges among 79 and 110 million tons of fish, including significant growth in the production of Nile tilapia. Hence, it is desirable to develop products that enable the use of processing waste for human consumption. The filleting residue has 60-70% of the total weight of tilapia. The use of mechanically separated meat (MSM) enables the use of 14% of the fish total weight. The nuggets are restructured, breaded and prepared from the flesh disintegration by mechanical methods. The manufacture of these products with MSM uses the processing residues, avoids health and environmental problems and adds commercial value to the final product. This study aimed to evaluate the physical and chemical characteristics and microbiological quality of fillets, nuggets and MSM and to analyse sensory the fillets and nuggets of tilapia that consumed *Homeopatila 100*<sup>®</sup>. With the Nile tilapia we evaluated two diets: one control with 40 mL of a hydro-alcohol solution 30% v/v per kg of diet and the other *Homeopatila 100*<sup>®</sup> with 40 mL / kg of the homeopathic product. The tilapia had average weight of 101.12 g ( $\pm 17.73$ ) and 99.73 g ( $\pm 19.85$ ) and total average length of 18.52 cm ( $\pm 6.00$ ) and 18.05 cm ( $\pm 1.11$ ), respectively, 160 fish were distributed in 8 water tanks, totaling 20 animals in each of the boxes with individual capacity for 600L of water. Physical and chemical parameters of water and the performance of the fish were evaluated (they were killed after 84 days of treatment). At the end of the experiment, all fish were caught by nets, sensitized and killed by breaking the spine and obtained the fillets. The fillets were immediately vacuum-conditioned in smooth transparent nylon bags and frozen at -18 °C until nugget preparation. The MSM was retrieved and stored in polyethylene bags, separately each of the treatments, frozen at -86 °C and stored at -18 °C. During the experiment the physical and chemical parameters of the water and the fish performance were evaluated. Were realized physical, chemical and microbiological analyses of fillet and MSM and sensory analyses of fillet were performed. The nuggets were performed on three formulations with 25, 50 and 75% of MSM for each of the treatments. The stability of formulations were evaluated through analysis of Thiobarbituric Acid Reactive Substances (TBARS) (on days 0, 30, 60, 90 and 120). Microbiological analyzes were conducted to coliforms were evaluated at 45 °C, *Escherichia coli*, *Staphylococcus coagulase*, *Bacillus cereus*, counting viable aerobic mesophilic and psychrotrophic and *Salmonella* sp., and acceptance of attributes color, aroma, tenderness, taste and overall impression were evaluated, using 9-point hedonic scale and also Buying Intention, test the 5-point ranging. There was no significant difference ( $p > 0,05$ ) between the physical and chemical characteristics of water. Fish fed diet containing the homeopathic product enhanced tilapia production Greater



weight gain (207.16 g), fillets with softer tissue ( $6.08 \text{ N} \pm 0.04$ ), higher  $A_w$  ( $0.990 \pm 0.001$ ), less total amounts of psychotropic aerobic bacteria ( $2.12 \pm 0.01 \text{ Log}_{10} \text{ UFC/g}$ ) and low component value of color  $L^*$  (luminosity) ( $65.78 \pm 0.37$ ) and  $b^*$  (yellow/blue) ( $1.50 \pm 0.01$ ) for MSM and of high value of  $a^*$  (red/green) for MSM ( $13.54 \pm 0.11$ ) and fillets ( $4.93 \pm 0.03$ ). The sensory acceptability of the fillet was 80.11% indicating that it exposed for sale have good acceptance by the market. The nuggets with 75% MSM revealed a higher pH ( $5.89 \pm 0.02$ ), the tissue was softer ( $1.29\text{N} \pm 0.04$ ), and they had a higher lipid value ( $15.96\% \pm 0.05$ ). With 50% and 75%, the color ( $L^*$ ) was darker ( $60.76\% \pm 0.91$  and  $60.03\% \pm 0.78$ ), and there were lower protein amounts ( $15.54\% \pm 0.31$  and  $13.55\% \pm 0.35$ ). Nuggets had an acceptable value of lipid oxidation ( $0.672 \pm 0.007 \text{ mg MDA/kg}$ ). The results of fillet, MSN and nugget for the microbiological analyses demonstrated that the product met the requirements of legislation. The results of the colors of fillet and MSM these relate to the results fish stressed prior to slaughter. The results indicated that the use of *Homeopatila 100*<sup>®</sup> in the diet of the Nile tilapia did not change the muscle quality, ensuring consumer acceptability. 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*<sup>®</sup> group for the analysis undertaken with the Nugget. Therefore, the results indicated that the use of *Homeopatila 100*<sup>®</sup> in the diet of the Nile tilapia did not change the physical, chemical, microbiological and sensory quality characteristics of the nuggets.

**Keywords:** tilapia, co-product, aquaculture, homeopathy, technology, quality.

## RESUMO GERAL

Homeopatia é um sistema de medicina complementar e alternativa que tem aplicações de fácil utilização e extensa literatura clínica. Os produtos homeopáticos são fabricados por dinamização, que consiste em diluições sequenciais por agitação em pequenos volumes e tem aplicações nas dietas humanas e animais. A *Homeopatila 100*<sup>®</sup> é um produto homeopático elaborado com o propósito de diminuir o estresse na produção e garantir o bem estar de tilápias do Nilo (*Oreochromis niloticus*). A tilápia é o segundo grupo de peixes mais produzido em todo o mundo e sua comercialização cresce, em média, 10% ao ano. A previsão de produção na aquicultura mundial para 2030 situa-se entre 79 e 110 milhões de toneladas de peixe, incluindo um crescimento significativo na produção de tilápia do Nilo. Com isso, é desejável desenvolver produtos que permitam a utilização de resíduos de processamento para consumo humano. O resíduo da filetagem tem 60-70% do peso total de tilápia. A utilização de carne mecanicamente separada (CMS) permite o aproveitamento de 14% do peso total dos peixes. Os *nuggets* são reestruturados, preparados à milanesa a partir da desintegração da carne por métodos mecânicos. A fabricação destes produtos com CMS utiliza os resíduos de processamento, evita problemas ambientais e agrega valor comercial ao produto final. O objetivo deste estudo foi avaliar as características físicas, químicas e qualidade microbiológica dos filés, *nuggets* e CMS e analisar sensorialmente os filés e *nuggets* produzidos a partir de tilápia tratadas com *Homeopatila 100*<sup>®</sup>. Com as tilápias do Nilo foram avaliadas duas dietas: uma controle com 40 mL de uma solução hidro-álcool (álcool 30% v/v) por kg de ração e outra *Homeopatila 100*<sup>®</sup> com 40 mL/kg do produto homeopático. As tilápias tinham peso médio inicial de 101,12 g ( $\pm 17,73$ ) e 99,73 g ( $\pm 19,85$ ) e comprimento total médio de 18,52 centímetros ( $\pm 6,00$ ) e 18,05 centímetros ( $\pm 1,11$ ), respectivamente. Foram distribuídos 160 peixes em oito tanques de água, totalizando 20 animais em cada uma das caixas com capacidade individual de 600L de água. Avaliou-se os parâmetros físicos e químicos da água e do desempenho dos peixes (eutanasiados após 84 dias de tratamento). No final do experimento, todos os peixes foram capturados por redes, sensibilizados e mortos por rompimento da coluna vertebral e filetados. Os filés foram imediatamente acondicionados em sacos de nylon, lisos, transparentes e congelados a -18 °C, até a preparação do *nugget*. A CMS de cada um dos tratamentos foi elaborada e preparada separadamente, acondicionada em sacos de polietileno, congelada a -86 °C e armazenada a -18 °C. Durante o experimento, foram avaliados os parâmetros físicos e químicos da água e do desempenho dos peixes. Foram realizadas análises física, química, e microbiológicas de filé e CMS e análises sensoriais de filé. Foram preparadas três formulações com 25, 50 e 75% de CMS para cada um dos tratamentos. As formulações foram avaliadas quanto à estabilidade através da análise de *Thiobarbituric Acid Reactive Substances* (TBARS) (nos 0, 30, 60, 90 e 120 dias). Foram conduzidas análises microbiológicas para coliformes a 45 °C, *Escherichia coli*, *Staphylococcus* coagulase positiva, *Bacillus cereus*, contagem de aeróbios mesófilos viáveis e psicrotróficos e *Salmonella* sp. A aceitação dos atributos cor, aroma, maciez, sabor e impressão global, foi avaliada utilizando-se a escala hedônica de 9 pontos e a intenção de compra, em escala de 5

pontos. Não houve diferença significativa ( $p > 0,05$ ) entre as características físicas e químicas da água. Os peixes alimentados com dietas contendo o produto homeopático apresentaram maior ganho de peso (207,16 g), filés mais macios ( $6,08 \text{ N} \pm 0,04$ ), menor  $A_w$  ( $0,990 \pm 0,001$ ), menor contagem de bactérias aeróbias psicotróficas ( $2,12 \pm 0,01 \text{ Log}_{10} \text{ UFC/g}$ ) e menor componente de luminosidade  $L^*$  ( $65,78 \pm 0,37$ ) e  $b^*$  (amarelo / azul) ( $1,50 \pm 0,01$ ) para CMS e maior valor de  $a^*$  (vermelho/verde) para CMS ( $13,54 \pm 0,11$ ) e filés ( $4,93 \pm 0,03$ ). A aceitabilidade sensorial dos filés foi de 80,11% indicando que se expostos à venda teriam boa aceitabilidade pelo mercado consumidor. Os *nuggets* com 75% CMS apresentaram um pH mais elevado ( $5,89 \pm 0,02$ ), menor textura ( $1,29 \text{ N} \pm 0,04$ ), e valor mais elevado de lipídios ( $15,96\% \pm 0,05$ ). Com 50% e 75%, observou-se a coloração ( $L^*$ ) mais escura ( $60,76\% \pm 0,91$  e  $60,03 \pm 0,78\%$ ), e quantidade de proteína mais baixa ( $15,54\% \pm 0,31$  e  $13,55 \pm 0,35\%$ ). Os *nuggets* apresentaram um valor aceitável de oxidação lipídica ( $0,672 \pm 0,007 \text{ mg MDA/kg}$ ). Os resultados das análises microbiológicas do filé, CMS e *nuggets* demonstraram que o produto atendeu às exigências da legislação. Os resultados das cores de filé e CMS relacionam-se com os resultados de peixes estressados antes do abate. Os resultados indicaram que a utilização de *Homeopatila 100*<sup>®</sup> na dieta da tilápia não alterou a qualidade muscular, garantindo a aceitabilidade do consumidor. *Nuggets* com 25% e 50% de CMS foram satisfatoriamente aceitos. Não houve diferença ( $p > 0,05$ ) entre o controle e o tratamento *Homeopatila 100*<sup>®</sup> para as análises realizadas com o *nugget*. Portanto, indicaram que o uso de *Homeopatila 100*<sup>®</sup> na dieta da tilápia do Nilo não alterou as características de qualidade físicas, químicas, microbiológicas e sensoriais dos filés e CMS.

**Palavras chave:** tilápia, co-produto, aquicultura, homeopatia, tecnologia, qualidade.

## ARTIGO 1

### TITLE

Quality characteristics e acceptability of fillet and MSM of Nile tilapia treated with homeopathic product

### AUTHORS' NAME

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### ABSTRACT

Current assay evaluates the physical, chemical and microbiological quality of fillets and MSM plus the sensory aspects of fillets of the Nile tilapia (*Oreochromis niloticus*) treated with Homeopatila 100<sup>®</sup>. Physical and chemical parameters of water and performance of the fish were evaluated. The moisture, fixed mineral residue, lipid, crude protein, pH, color, Aw, *Staphylococcus* coagulase, *Bacillus cereus*, coliforms were reported at 45°C, *Escherichia coli*, *Salmonella* sp., counting viable aerobic mesophilic and psychrotrophic amounts were determined of fillet and MSM. It also acceptance, intention test and shear force of fillet were performed. There was no significant difference between the physical and chemical characteristics of water. Diet containing the homeopathic product enhanced tilapia production by more weight gain (207.16 g), the fillets by softer tissue (6.08 N ± 0.04), lower Aw (0.990 ± 0.001), less total amounts of psychrotrophic aerobic bacteria (2.12 ± 0.01 Log<sub>10</sub> UFC/g) and by low component value of color L\* (luminosity) (65.78 ± 0.37) and b\* (yellow/blue) (1.50 ± 0.01) for MSM and of high value of a\* (red/green) for MSM (13.54 ± 0.11) and fillets (4.93 ± 0.03). Sensory acceptability was 80.11%. The results indicated that the use of Homeopatila 100<sup>®</sup> in the diet of the Nile tilapia did not change the muscle quality, ensuring consumer acceptability.

**Keywords:** aquaculture, tilapia, homeopathy, fillet, MSM, acceptability.

## 1 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 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). The population homeopathy is the ideal for herds due to its low cost, efficiency and absence of toxicity. The use of extremely diluted active ingredients ensures that no residues in meat and contamination in water and soil. Animals fed homeopathic product has better potential production, better quality and greater survival (Andretto *et al.*, 2014).

Homeopatia 100<sup>®</sup> 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 the homeopathic product, the data provided by Siena *et al.* (2010) revealed a high survival rate and indicated a lower hepatosomatic index for Nile tilapia. Research conducted Braccini *et al.* (2013) in juvenile tilapia provided satisfactory results for performance related to survival, prevalence and parasite load. It also, study conducted Merlini *et al.* (2013) with Nile tilapia and Homeopatia 100<sup>®</sup> reported a low level of plasma cortisol level and high weight gain. 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 2030 lies 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 analyses in addition to sensory evaluations provide the necessary information to measure the quality and freshness of fish muscle (Wills, 2004).

The current search evaluates the physical, chemical, and microbiological quality of fillets and mechanically separated meat (MSM) and sensory evaluation fillets of tilapia that consumed Homeopatia 100<sup>®</sup>.

## 2. MATERIALS AND METHODS

### 2.1 PLACE AND PERIOD

The current search was performed on the Experimental Fish Farm of the Maringá State University – CODAPAR (Paraná Agricultural Development Company) in the district of Floriano in the municipality of Maringá PR - Brazil (23° 25' 30" S; 51° 56' 20" W) between April and June 2013 for a period of 84 days until the fish weighed approximately 300 grams and were ready for filleting.

Use of the animals was approved by the Ethic Committee for Use of Animals in Experiments from the Maringá State University de according to Protocol 019/2013.

### 2.2 ANIMALS, INSTALLATIONS AND FEED

Males with sexual inversion from a homogenous tilapia the Nilo population (*O. niloticus*; variety *Supreme*) used as a control had an initial mean weight of 101.12 g ( $\pm$  17.73) and a length of 18.52 cm ( $\pm$  6.00). The Nile tilapia treated with Homeopata 100<sup>®</sup> had an initial mean weight of 99.73 g ( $\pm$  19.85) and a length of 18.05 cm ( $\pm$  1.11). The fish were randomly distributed in 16 fiber glass boxes with 600 L capacities each and a 30% daily renewal rate. Finally, they were placed in a 120 m<sup>2</sup> hothouse covered with a polyethylene canvas. Three hundred and twenty fish were distributed in the water boxes with 20 animals per box. The fish were conditioned 7 days prior to the start of the experiment, and the control and Homeopata 100<sup>®</sup> 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 (CP) of 5-mm diameter, which was provided twice per day (at 10:00 h and 16:00 h) manually until apparent satiety. 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. Table 1 shows the percentage composition of diet given throughout the experimental period.

### 2.3 HOMEOPATHIC PRODUCT

The homeopathic product Homeopatila 100<sup>®</sup> was prepared by the firm REALH of Campo Grande MS, with registration of product for veterinary use number 024/05736-3 Ministry of Agriculture, Livestock and Food Supply - Brazil. Table 2 shows the composition and respective dilutions of the homeopathic product Homeopatila 100<sup>®</sup>.

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<sup>®</sup> (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).

The hydro-alcohol solution and the homeopathic product were sprinkled weekly in the diet for their incorporation. The diet was later homogenized and left to dry in the open air for 24 hours until it became loose without any trace of alcohol.

The diet was conditioned in an airy place without any sunlight, chemical products, or equipment involving magnetic fields.

### 2.4 PHYSICAL AND CHEMICAL PARAMETERS OF WATER

The values of the water's physical and chemical parameters such as temperature, pH, dissolved oxygen and electric conductivity were registered three times per week (Monday, Wednesday and Friday at 9:00 h and 16:00 h). Temperature and oxygen levels were monitored with an oxymeter (YSI-55/12 FT, Aquatic Eco-Systems, EUA), pH was monitored with a electronic pH meter (PH-1900, Instrutherm, BR), and electric conductivity was monitored with a portable conductimeter (CD-860, Instrutherm, BR).

### 2.5 NILE TILAPIA FILLET AND MSM YIELD

Fish from each treatment were measured at the start and end of the assay.

The fillet yield was determined according to the total weight of the fish. The MSM yield was calculated according to the total weight of fish and waste from filleting (carcass).

Filleting is performed by the same person. The fish were beheaded in a V-shape and eviscerated; the fins were removed, and fillets with the skin intact were retrieved. The skin was

then removed from the fillets with a knife. The fillets were obtained longitudinally from the dorsal muscle of the two sides of the fish throughout the whole extent of the spine and ribs (Souza, 2002). The fillets were washed in chlorine water at 5 ppm, weighed on a 0.01 g precision scale, and immediately vacuum conditioned in transparent nylon bags (0.280 mm thick) sealed (Microvac CV 8, Selovac, BR) and frozen at -18 °C until the time of analysis. The MSM was retrieved by the (HT C100, High Tech, BR), and prepared separately for treatment (control and Homeopatila 100<sup>®</sup>) with replications. After being weighed on a 0.01 g precision scale, the MSM was packed in polyethylene bags, frozen at -86 °C in an ultrafreezer (IULT 90D, INDEL, BR), and stored at -18 °C until analysis.

## 2.6 EVALUATION OF THE FILLETS AND MSM OF NILE TILAPIA

Three samples were randomly collected from each repetition of the two treatments after filleting and extraction of MSM for chemical and microbiological analysis of both the fillets and MSM. Analysis was performed in triplicate, and the control and Homeopatila 100<sup>®</sup> treatments were compared. Texture, pH, Aw and color analysis were conducted shortly after filleting and MSM extraction.

### 2.6.1 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<sup>®</sup>, Romania), 10 g of the sample was homogenized with 40 mL of distilled water.

Color was measured with a colorimeter (CR 400, Minolta, Japan) a D65 illuminant and 10° angle of vision. Color 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 color 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).

Texture (softness) for shear force was evaluated by cutting the fillets in 1.5 cm high x 1.0 cm wide x 2 cm long pieces. 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).

### 2.6.2 Microbiological evaluation of fillets and MSM

Fillets and MSM were microbiologically evaluated by *Staphylococcus* coagulase counts (CFU/g), research on *Salmonella* sp., *Escherichia coli* counts (MPN/g) and counting viable aerobic mesophilic microorganisms and psychrotrophic, given in Log<sub>10</sub> CFU/g, were determined, taking into consideration international legislation (ICMSF, 1982).

### 2.6.3 Sensory evaluation of fillets

The Committee for Ethics in Research Involving Human Beings of the Maringá 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 didn’t like it” (Dutcosky, 2007). Color, aroma, tenderness, taste and overall impression were evaluated. Further, a 5-point scale for the Buying Intention Test was also applied, where a score category of 5 = “I will certainly buy it,” and a score of 1 = “I surely won’t buy it” (Ferreira *et al.*, 2000). Prior to sensory analysis the 120 judges, all members of the staff and students of the Federal Technological University of Paraná at Medianeira PR - Brazil signed a term of free consent.

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 °C and 75 °C. Samples were coded with randomized three digits numbers (Teixeira *et al.*, 1987) and presented at approximately 40 °C to the judges.

The acceptability index of the formulations undergoing analysis was given by the equation  $IA (\%) = (A \times 100) / B$ , where A = the mean score obtained for total evaluation and B = the maximum score for overall evaluation (Dutcosky, 2007).

## 2.7 STATISTICAL ANALYSIS

The Mann-Whitney U test ( $p < 0.05$ ) was employed to determine the significance of the water's physical and chemical parameters (Zar, 1996).

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

## 3 RESULTS

### 3.1 PHYSICAL AND CHEMICAL PARAMETERS OF WATER

The parameters of water quality were evaluated to warrant fish development and health.

There was no significant difference ( $p > 0.05$ ) in the treatments in all parameters evaluated by U test (Table 3).

### 3.2 NILE TILAPIA AND FILLET AND MSM YIELD

Table 4 shows the performance and yield value of the control and Homeopatila 100<sup>®</sup> treatments. The total weight attained by the fish treated with Homeopatila 100<sup>®</sup> was significantly higher ( $p < 0.05$ ). There was no significant difference in the total weight of the fillets between treatments, and yield was significantly higher ( $p < 0.05$ ) in control group. The total weight of MSM was significantly higher ( $p < 0.05$ ) for fish treated with the homeopathic product, though no significant difference occurred between treatments with regard to MSM yield compared to the entire fish and carcass.

### 3.3 EVALUATION OF FILLETS AND MSM OF NILE TILAPIA

Table 5 demonstrates the mean value of the physical, chemical, microbiological and sensory analyses of the control and Homeopatila 100<sup>®</sup> treatment samples.

Table 6 shows the mean value of physical, chemical, and microbiological analyses of MSM samples of Nile tilapia for control and Homeopatila 100<sup>®</sup> treatment samples.

### 3.3.1 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<sup>®</sup> groups failed to show any difference in significance. Aw and shearing force were significantly higher (p<0.05) in the control group (Table 5). 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 6).

### 3.3.2 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 (Tables 5 and 6). The viable aerobic mesophilic microorganisms did not reveal significant differences between treatments of fillet and MSM. It was only in the tilapia fillets that of viable aerobic psychrotrophic microorganisms were significantly higher (p<0.05) for the control group (Table 5).

### 3.3.3 Sensory analysis of fillets

The results of sensory analysis did not show any significant difference between the treatments (Table 5). 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  $7.95 \pm 0.29$  and  $7.82 \pm 0.28$  for the control and Homeopatila 100<sup>®</sup> groups, respectively. Scores close to 4 were attributed to the two treatments in the buying intention test, whereas value for the acceptability index were 79.60% and 80.11% for the control and Homeopatila 100<sup>®</sup> groups, respectively (Fig.1).

## 4 DISCUSSION

### 4.1 PHYSICAL AND CHEMICAL PARAMETERS OF WATER

Throughout the experiment, the mean values of the water's physical and chemical parameters (Table 3) remained adequate for the reproduction of tilapia (Ribeiro, 2001) and were similar to those reported by Siena *et al.* (2010) and Braccini *et al.* (2013), with no significant difference between treatments.

### 4.2 PERFORMANCE OF NILE TILAPIA AND FILLET AND MSM YIELD

The filleting method led to a low fillet yield. Souza (2002) compared six filleting methods of Nile tilapia and reported a low yield when the skin was removed from the fillet with a knife after beheading the fish and eviscerating it. However, the literature reveals fillet yields tilapia of Nile, ranging between 25.4% and 42.0% of the fish gross weight (Souza, 2002).

### 4.3 PHYSICAL AND CHEMICAL COMPOSITION OF FILLETS AND MSM

Shear force value in the current experiment,  $6.32 \pm 0.39$  N and  $6.08 \pm 0.04$  N, for the control and Homeopatia 100<sup>®</sup> treatment groups, respectively (Table 5), were similar to those obtained (Mattos *et al.*, 2010) in *Sparus aurata*, with value ranging between  $5.0 \pm 2.1$  and  $5.7 \pm 1.8$  N for raw fillets. The texture results disagree with research of raw fillets of fish exposed to stress before killing. Searches with different fish species under stress, such as the turbot (*Psetta maxima*) (Morzel *et al.*, 2003) and the rainbow trout (*Oncorhynchus mykiss Walbaum*) (Wills *et al.*, 2004), provide a lower rate of shearing force and texture softening of the muscles. Other authors reported that there was no effect on the codfish (*Gadus morhua L.*) (Bjørnevik and Solbakken, 2010) and the gilt-head bream (*Sparus aurata*) (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). Researchers (Droval *et al.*, 2012; Santos *et al.*, 2012) have used the classification by parameter L\*

(luminosity) to evaluate the quality of stressed animals' muscles prior to killing. This type of classification has not been established for tilapia.

Myoglobin is one of the main factors of color formation in fish muscle, which is an important quality component for consumers in their decision to buy (Ocaño-higuera *et al.*, 2009).

Research on rainbow trout (*Oncorhynchus mykiss*) revealed a paler color ( $> L^*$ ) in the meat of stressed fish (Poli *et al.*, 2005). The lower the rate of  $L^*$ , the higher the percentage of myoglobin in meat with darker muscle (Ribeiro *et al.*, 2007). Robb *et al.* (2000) showed that high levels of muscular activity had significant differences in  $L^*$  and  $b^*$  (yellow component) and low differences in  $a^*$  (red component) in the color 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  $L^*$  rate and decreased the  $a^*$  value in the muscle of seasoned salmon from the Arctic (*Salvelinus alpinus*). The yellow component ( $b^*$ ) of the fillet in the experiment reported here in was significantly different ( $p < 0.05$ ) from the control (Table 5) and had a more intense color. The results of the two treatments were similar to those obtained in juvenile Nile tilapia (Girao *et al.*, 2012). In the case of MSM, the  $L^*$  and  $b^*$  value were higher, although the  $a^*$  rate was less in the control group (Table 6). The above results regarding the fillet and MSM color of Nile tilapia treated with Homeopatila 100<sup>®</sup> provided better quality characteristics and indicated that the homeopathic product was effective in reducing stress in tilapia by decreasing protein denaturation and releasing myoglobin in the muscle of the meat.

Moisture, fixed mineral residue, protein and lipid results in the fillets (Table 5) were close to those reported by Leonhardt *et al.* (2006). Similar results (Kirschnik *et al.*, 2013) were obtained from MSM tilapia (Table 6).

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 employment of 40 mL of Homeopatila 100<sup>®</sup> per kg of diet did not change the characteristics of the centesimal composition of the fillets and MSM.

The pH of the fillets the results were similar to those in Nile tilapia (Soares and Gonçalves, 2012). The pH value in MSM were similar to those in MSM (Kirschnik *et al.*, 2013) of tilapia at time 0 (Table 6).

Aw valor for the two treatments complied with the classification for fish, as a food with high moisture content (Simões *et al.*, 2007) 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 decrease *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). This study results relate with decreased stress in fish prior to slaughter the results showed a delay in onset of *rigor mortis*, a smaller shear force rate and free water in muscle compared to the control group.

#### 4.4 MICROBIOLOGICAL EVALUATION OF FILLETS AND MSM

Microbiological analysis of fillets and MSM in the control and Homeopatila 100<sup>®</sup> groups 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, *Escherichia Coli* and *Salmonella* sp. (Bartolomeu *et al.*, 2011). There were similar counts (Rodrigues *et al.*, 2008) for viable aerobic mesophilic microorganisms (2.8 Log<sub>10</sub> CFU/g) and viable aerobic psychrotrophic microorganisms (2.0 Log<sub>10</sub> CFU/g) and higher counts (Espírito Santo *et al.*, 2007) for viable aerobic mesophilic microorganisms (3.0 to 5.6 log CFU/g) compared to this study. Microbial counts of *Escherichia coli*, *Staphylococcus* coagulase and viable aerobic psychrotrophic microorganisms and research for *Salmonella* sp. in MSM were similar to those in (Poli *et al.*, 2005) and viable aerobic mesophilic microorganisms with higher value than those in the this study (Melo *et al.*, 2010).

The use of Homeopatila 100<sup>®</sup> in fish diets may have decreased viable aerobic mesophilic 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). Research on other animals treated with homeopathic products indicated a efficiency in the reduction of bacteria causing mastitis in animals with low counts of somatic cells (Klocke *et al.*, 2010).

#### 4.5 SENSORY ANALYSIS OF FILLETS

The current research described is the first search regarding the sensory analysis of fillets of Nile tilapia fed on a diet including a homeopathic product.

Data on the sensorial evaluation of tilapia fillets treated with Homeopatila 100<sup>®</sup> 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 5). The two treatments provided over a 70% acceptability rate for all attributes (Fig. 1). 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 fishburgers (7.1 to 7.5) (Marengoni *et al.*, 2009).

The results also reveal that the judges didn't detect differences in color, aroma, tenderness and taste among the fillets that were subjected to different treatments. Several studies report that pre-euthanasia stress has a slight effect on the smell but no effect on the taste of Salmonidae meat (Van de Vis *et al.*, 2003).

The instrumental evaluation of texture and color (Table 5) showed that fish fillets treated with Homeopatila 100<sup>®</sup> were 3.8% more tender with an 11% increase in intense yellow color, both of which were unperceived by the non-trained judging panel, the difference is very small. The above results are due to existing slight differences and the instrumental evaluation of grilled fillets provided in contrast to raw fillets.

#### 5 CONCLUSION

Nile tilapia fed on a diet including Homeopatila 100<sup>®</sup> had increased weight gain and muscle mass; they produced more tender fillets, less Aw, lower total psychrotrophic aerobic bacteria counts and good sensory acceptability. The results of the colors of fillet and MSM these relate to the results fish stressed prior to slaughter. The results indicated that the use of Homeopatila 100<sup>®</sup> in the diet of the Nile tilapia did not change the muscle quality.

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**Table 1.** Percentage of composition of commercial diet used in the experiment (diameter 5 mm).

Nutrient	Level of commercial guarantee (%) <sup>1</sup>	Result <sup>2</sup>
Moisture	Maximum 12	11.97 ( $\pm$ 0.05)
Crude protein	Minimum 32	40.61 ( $\pm$ 0.39)
Crude fiber	Maximum 9	3.35 ( $\pm$ 0.21)
Ether extract	Minimum 7	1.46 ( $\pm$ 0.01)
Mineral matter	Maximum 12	8.51 ( $\pm$ 0.08)

<sup>1</sup>Guaranteed level provided by supplier; <sup>2</sup>Chemistry Laboratory of the Federal Technology University of Paraná, Medianeira – PR; <sup>3</sup>Mean followed by standard deviation (n=3).

**Table 2.** Composition of Homeopatia 100<sup>®</sup>.

Compound	Dilution
<i>Iodum</i>	12 CH
<i>Sulphur</i>	30 CH
<i>Natrum muriaticum</i>	200 CH
<i>Streptococcinum</i>	30 CH
Vehicle (ethyl alcohol 30% v/v)	Q.s.p. <sup>2</sup>

<sup>1</sup> Hahnemannian centesimal dilution; <sup>2</sup> Sufficient amount.

Source: Real (2009).

**Table 3.** Mean value of physical and chemical parameters of water during the experimental phase.

Parameters	Treatments	
	Control <sup>1,2</sup>	Homeopatila 100 <sup>®1,2</sup>
Temperature (°C)	26.24 ± 2.32	25.78 ± 2.36
pH	7.47 ± 0.22	7.45 ± 0.12
Dissolved oxygen (mg/L)	6.65 ± 1.94	6.36 ± 1.80
Electric conductivity (µS/cm)	66.07 ± 28.60	69.03 ± 27.50

<sup>1</sup>Value are not significantly different at 5% significance level by Mann-Whitney's U test (p<0.05); <sup>2</sup>Mean followed by ± standard deviation.

**Table 4.** Performance and yield of fillets and MSM of Nile tilapia.

Parameter	Treatments	
	Control <sup>1,2</sup>	Homeopatia 100 <sup>®1,2</sup>
Initial total weight (g)	101.12 ± 17.73	99.73 ± 19.85
Total weight (84 days) (g)	293.17 ± 56.76 <sup>b</sup>	306.89 ± 58.30 <sup>a</sup>
Total weight of fillet (g)	84.33 ± 20.20	84.21 ± 19.81
Fillet yield (%)	27.80 ± 3.32 <sup>a</sup>	27.19 ± 2.64 <sup>b</sup>
Total weight of MSM (g)	25.04 ± 6.35 <sup>b</sup>	29.69 ± 3.63 <sup>a</sup>
MSM yield on weight of fish (%)	8.15 ± 3.68	9.12 ± 0.88
MSM yield on carcass weight (%)	60.80 ± 13.23	63.79 ± 4.70

Value with different letters (a-b) on the same line differ significantly by the Student's t-test ( $p < 0.05$ ). The mean is followed by  $\pm$  standard deviation.



**Table 5.** Physical, chemical, microbiological and sensory evaluation of Nile tilapia fillets.

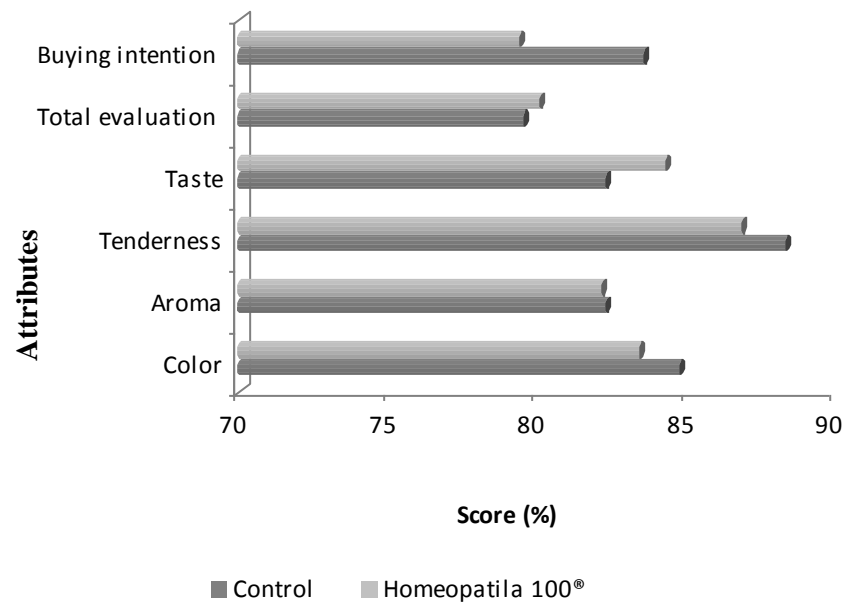
Parameters	Treatments	
	Control <sup>7,8</sup>	Homeopatila 100 <sup>®7,8</sup>
<b>Physical (n=10) and chemical (n=3) analyses</b>		
Shear force (N) <sup>1</sup>	6.32 ± 0.39 <sup>a</sup>	6.08 ± 0.04 <sup>b</sup>
L <sup>*</sup>	52.77 ± 0.40	52.66 ± 0.40
a <sup>*</sup>	2.76 ± 0.03	2.71 ± 0.03
b <sup>*</sup>	5.54 ± 0.03 <sup>a</sup>	4.93 ± 0.03 <sup>b</sup>
Moisture (%)	78.00 ± 0.11	78.14 ± 0.11
Crude protein (%)	18.64 ± 0.15	17.45 ± 0.12
Fixed mineral residue (%)	1.13 ± 0.01	1.15 ± 0.01
Total lipids (%)	1.93 ± 0.02	1.95 ± 0.02
pH	5.96 ± 0.02	5.96 ± 0.02
Aw <sup>2</sup>	0.995 ± 0.001 <sup>a</sup>	0.990 ± 0.001 <sup>b</sup>
<b>Microbiological analyses (n=3)</b>		
<i>Escherichia coli</i> counts (MPN/g) <sup>3</sup>	< 0.3	< 0.3
Research on <i>Salmonella</i> sp. 25g	Absence in 25g	Absence in 25g
<i>Staphylococcus</i> coagulase counts (CFU/g) <sup>4</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Viable aerobic mesophilic (Log <sub>10</sub> CFU/g)	2.09 ± 0.14	2.00 ± 0.14
Viable aerobic psychrotrophic (Log <sub>10</sub> CFU/g)	2.41 ± 0.01 <sup>a</sup>	2.12 ± 0.01 <sup>b</sup>
<b>Acceptability and buying intention (n=120)</b>		
Color <sup>5</sup>	7.63 ± 0.33	7.51 ± 0.32
Aroma <sup>5</sup>	7.41 ± 0.32	7.40 ± 0.32
Tenderness <sup>5</sup>	7.95 ± 0.28	7.82 ± 0.29
Taste <sup>5</sup>	7.41 ± 0.49	7.59 ± 0.49
Total evaluation <sup>5</sup>	7.16 ± 0.32	7.21 ± 0.33
Buying intention <sup>6</sup>	4.18 ± 0.33	3.97 ± 0.30

<sup>1</sup>N: Newton; <sup>2</sup>Aw: water activity; <sup>3</sup>MPN: most probable number; <sup>4</sup>CFU: colony forming units; <sup>5</sup>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); <sup>6</sup>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); <sup>7</sup>Value with different letters (a-b) on the same line differ significantly by the Student's t-test (p<0.05); <sup>8</sup>The results are given as the mean ± standard error.

**Table 6.** Physical, chemical and microbiological evaluation of MSM of Nile tilapia.

Parameters	Treatments	
	Control <sup>4,5</sup>	Homeopatia 100 <sup>®4,5</sup>
<b>Physical (n=10) and chemical (n=3) analyses</b>		
L*	67.89 ± 0.37 <sup>a</sup>	65.78 ± 0.37 <sup>b</sup>
a*	0.84 ± 0.01 <sup>b</sup>	1.50 ± 0.01 <sup>a</sup>
b*	14.14 ± 0.11 <sup>a</sup>	13.54 ± 0.11 <sup>b</sup>
Moisture (%)	68.33 ± 0.43	67.18 ± 0.43
Crude protein (%)	15.62 ± 0.21	16.06 ± 0.21
Fixed mineral residue (%)	1.52 ± 0.04	1.42 ± 0.04
Total lipids (%)	14.05 ± 0.16	14.71 ± 0.27
pH	6.83 ± 0.04	6.72 ± 0.04
Aw <sup>1</sup>	0.989 ± 0.001	0.988 ± 0.001
<b>Microbiological analyses (n=3)</b>		
<i>Escherichia coli</i> counts (MPN/g) <sup>2</sup>	< 0.3	< 0.3
Research on <i>Salmonella</i> sp. 25g	Absence in 25g	Absence in 25g
<i>Staphylococcus coagulase</i> counts (CFU.g <sup>-1</sup> ) <sup>3</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
Viable aerobic mesophilic (Log <sub>10</sub> CFU/g)	3.05 ± 0.17	3.11 ± 0.68
Viable aerobic psychrotrophic (Log <sub>10</sub> CFU/g)	3.19 ± 0.20	3.53 ± 0.20

<sup>1</sup>Aw: water activity; <sup>2</sup>MPN: most probable number; <sup>3</sup>CFU: colony forming unit; <sup>4</sup>Value with different letters (a-b) on the same line differ significantly by the Student's t-test (p<0.05); <sup>5</sup>The results are given as the mean ± standard error.



**Fig.1.** Acceptability index per attribute and buying intention of Nile tilapia fillets

## ARTIGO 2

### TITLE

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

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### ABSTRACT

The homeopathic product Homeopatila 100<sup>®</sup> 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<sup>®</sup> 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 ( $5.89 \pm 0.02$ ), the tissue was softer ( $1.29N \pm 0.04$ ), and they had a higher lipid value ( $15.96\% \pm 0.05$ ). With 50% and 75%, the color ( $L^*$ ) was darker ( $60.76\% \pm 0.91$  and  $60.03\% \pm 0.78$ ), and there were lower protein amounts ( $15.54\% \pm 0.31$  and  $13.55\% \pm 0.35$ ). Nuggets had an acceptable value of lipid oxidation ( $0.672 \pm 0.007$  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<sup>®</sup> group for the analysis undertaken. The results indicated that the use of Homeopatila 100<sup>®</sup> in the diet of the Nile tilapia did not change the physical, chemical, microbiological and sensory quality characteristics of the nuggets, ensuring consumer acceptability.

**Keywords:** 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).

The filleting residue has 60-70% 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*<sup>®</sup> 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*<sup>®</sup> (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*<sup>®</sup>.

## 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 g ( $\pm$  17.73) and mean initial length of 18.52 cm ( $\pm$  6.00) for the control treatment and an initial mean weight of 99.73 g ( $\pm$  19.85) and initial mean length of 18.05 cm ( $\pm$  1.11) for the Homeopatila 100<sup>®</sup> treatment were randomly distributed in 16 fiber glass water boxes, with 20 animals per box. Homeopatila 100<sup>®</sup> 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 h 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<sup>®</sup> (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<sup>®</sup> 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<sup>®</sup>: *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  $-86\text{ }^{\circ}\text{C}$  in an ultrafreezer (IULT 90D, INDEL, BR) and stored at  $-18\text{ }^{\circ}\text{C}$  until the preparation of nuggets.

### **Preparation of nuggets**

Three formulations were prepared for the Homeopatila 100<sup>®</sup> 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<sup>®</sup> 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  $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  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  $-18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ . The frozen mass was cut in four parts (2.25 cm long and 2 cm wide), made breaded and then frozen at  $-18\text{ }^{\circ}\text{C}$ . For pre-frying, they were kept at  $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for 3 h and pre-fried in soybean oil for 1 min at  $180\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .

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  $-18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  for 120 days.

### **Physical and chemical composition**

The moisture, fixed mineral residue, lipid and crude protein amounts were determined on the 10<sup>th</sup> day after preparation, according to a protocol by the Association of Official Analytical Chemists (AOAC, 2006). Carbohydrate values were calculated by the difference:  $100 - (\% \text{ Moisture} + \% \text{ fixed mineral residue} + \% \text{ crude protein} + \% \text{ total lipids})$ . 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  $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ . The  $L^*$  (luminosity),  $a^*$  (red-green component) and  $b^*$  (yellow-blue component) values were presented according to the color system by the Commission Internationale de L'Eclairage (CIELAB) (Minolta, 1998).

Water activity ( $A_w$ ) was evaluated at  $25\text{ }^{\circ}\text{C}$  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  $A_w$  levels for growth. Usually, bacteria are more sensitive and almost all have their growth inhibited at  $A_w$  values between 0.90 to 0.91. Yeast and molds are more tolerant to lower  $A_w$  values, growing in bands ranging from 0.87 to 0.94 and 0.70-0.80, respectively (Abbas et al., 2009).

To evaluate the shear force, the nuggets were cut into pieces measuring 1.5 cm height x 1.0 cm width x 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  $A_w$  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  $45\text{ }^{\circ}\text{C}$  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  $\text{Log}_{10}$  CFU/g (ICMSF, 1982).



## Lipid stability

After the preparation of the nuggets and pre-frying to an internal temperature of 75 °C ± 1 °C, they were stored at -18 °C. The products were then thawed at 4 °C ± 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, Pearson and Dugan (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, it were used the formulations F / 75, F / 50 and F / 25 of the control treatment; in the second stage, it were used the same formulations with the Homeopatila 100<sup>®</sup> treatment. 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<sup>®</sup>.

The nuggets were thawed and baked until they reach a minimum temperature of 75 °C ± 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  $IA (\%) = (A \times 100) / B$ , 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 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 Homeopatia 100<sup>®</sup> 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  $A_w$  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  $A_w$  value did not reveal any significant differences ( $p > 0.05$ ) between the formulations and treatments. The value remained within the high  $A_w$  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).

There was no significant difference between treatments in the selected formulation (F2 / 50) in the pH measurement value, shear force,  $A_w$  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 Homeopatia 100<sup>®</sup> 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  $1.54 \pm 0.27$  to  $3.68 \pm 0.11$  and showed no significant difference between the products and treatments. The analyses were in agreement with those established by legislation (ICMSF, 1982).

Results 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, *Bacillus cereus* and *Staphylococcus* coagulase. However, higher values were found in 4 types of prepared fishburger with the MSM of the Nile tilapia for *Escherichia coli* and coliforms at 45 °C (Marengoni et al., 2009) and lower values for viable mesophilic and psychotrophic 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<sup>®</sup> treatment increased from  $0.007 \pm 0.003$  mg MDA/kg to  $0.672 \pm 0.007$  mg MDA/kg. In the case of the control, treatment increased the value of  $0.004 \pm 0.001$  mg MDA/kg to  $0.758 \pm 0.007$  mg MDA/kg. There was a gradual increase in lipid oxidation up to 120 days of storage (Figure 1), although 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  $0.72 \pm 0.50$  to  $0.88 \pm 0.63$  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 sensory 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 sensory 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<sup>®</sup>.

Buying intentions were close to 4 and 5 for the control and close to 3 and 4 for Homeopatila 100<sup>®</sup>. 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<sup>®</sup> was within 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<sup>®</sup>. 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<sup>®</sup>, 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<sup>®</sup>. 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<sup>®</sup> groups, respectively (Table 4), and 83.76% declared they had never eaten fish nuggets.

With the results observed in the sensory analysis (Step 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<sup>®</sup> 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.

## CONCLUSIONS

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

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**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*<sup>®</sup>.

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

<sup>1</sup>N: Newton. <sup>2</sup>F / 75; F / 50; F / 25; F2 / 50 with 75%, 50%, 25% and 50% of MSM. <sup>3</sup>Value with different letters (a-b-c) on the same line and column differ significantly by Tukey's test (p<0.05).

<sup>4</sup>Line are not significantly different at 5% significance level by Student's t test (p<0.05). <sup>5</sup>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*<sup>®</sup>.

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

<sup>1</sup>F / 75; F / 50; F / 25; F2 / 50 with 75%, 50%, 25% and 50% of MSM. <sup>2</sup>Value with different letters (a-b-c) on the same line and column differ significantly by Tukey's test (p<0.05).

<sup>3</sup>Line are not significantly different at 5% significance level by Student's t test (p<0.05). <sup>4</sup>Total carbohydrates were calculated by difference: 100 - (% moisture + % fixed mineral residue + % crude protein + % total lipids). <sup>5</sup>The results are given by the means ± standard error (n=3).

**Table 3** Sensory evaluation of nugget from fillet and MSM of Nile tilapia treated with *Homeopatila 100*<sup>®</sup>.

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

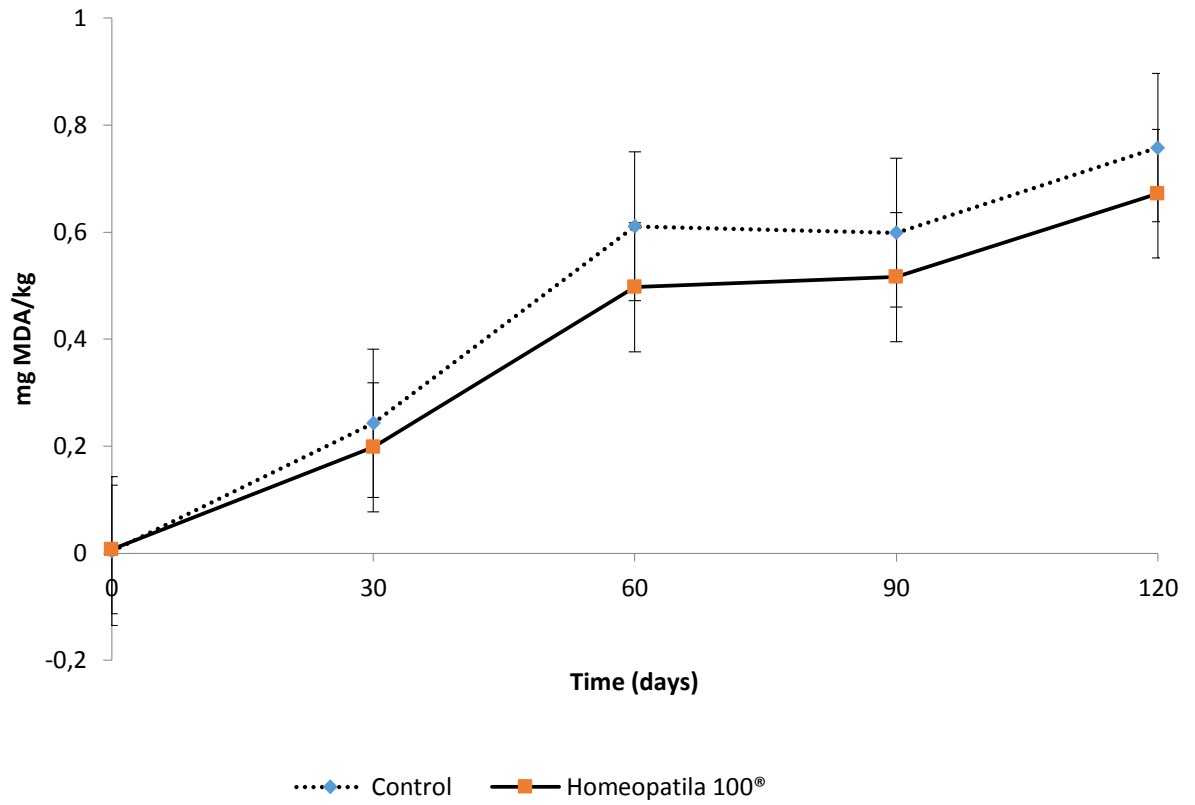
<sup>1</sup>Hedonic scale between 1 and 9 (1 I disliked it very much; 2 I disliked it; 3 I disliked it fairly; 4 I disliked it a little; 5 I didn't like it nor disliked it/ I didn't dislike it; 6 I liked it a little; 7 I liked it fairly; 8 I liked it; 9 I liked it very much). <sup>2</sup>Hedonic scale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

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

F / 75 <sup>1</sup>	F / 75 <sup>1</sup>	F / 50 <sup>1</sup>	F / 50 <sup>1</sup>	F / 25 <sup>1</sup>	F / 25 <sup>1</sup>	F2 / 50 <sup>1</sup>	F2 / 50 <sup>1</sup>
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	Control	<i>Homeopatila</i> <i>100</i> <sup>®</sup>	Control	<i>Homeopatila</i> <i>100</i> <sup>®</sup>	Control	<i>Homeopatila</i> <i>100</i> <sup>®</sup>	Control	<i>Homeopatila</i> <i>100</i> <sup>®</sup>
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

<sup>1</sup>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*<sup>®</sup> during storage of 120 days.

## **ANEXOS**

## Anexo I

### Submitted Manuscripts

Manuscript ID	Manuscript Title	Date Created	Date Submitted	Status
JFQ-2014-327	THE QUALITY CHARACTERISTICS OF THE MUSCLES OF THE NILE TILAPIA TREATED WITH HOMEOPATHIC PRODUCT <a href="#">[View Submission]</a> <a href="#">(Cover Letter)</a>	29-Aug-2014	29-Aug-2014	ADM: <a href="#">Boylston, Terri</a> • Awaiting AE Recommendation
				 <a href="#">top</a>



## Anexo II

academicJournals

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African Journal of Pharmacy and  
 Pharmacology

Full Length Research Paper

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

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The homeopathic product Homeopatila 100<sup>®</sup> 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<sup>®</sup> 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 ( $5.89 \pm 0.02$ ), the tissue was softer ( $1.29 \text{ N} \pm 0.04$ ), and they had a higher lipid value ( $15.96\% \pm 0.05$ ). With 50 and 75%, the color (L) was darker ( $60.76\% \pm 0.91$  and  $60.03\% \pm 0.76$ ), and there were lower protein amounts ( $15.54\% \pm 0.31$  and  $13.55\% \pm 0.35$ ). Nuggets had an acceptable value of lipid oxidation ( $0.672 \pm 0.007 \text{ mg MDA/kg}$ ). 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<sup>®</sup> group for the analysis undertaken. The results indicated that the use of Homeopatila 100<sup>®</sup> 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.

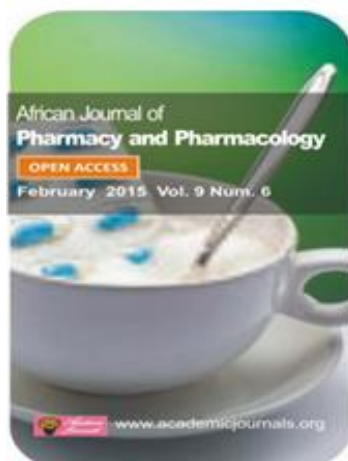
#### INTRODUCTION

Aquaculture provides one-third of the world's fishery products (Yampaldee 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).

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