# Elaboration of Sausage Using Minced Fish of Nile Tilapia Filleting Waste

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## ABSTRACT

*The objective of this study was to evaluate the inclusion of minced fish (MF) (0, 20, 40, 60, 80 and 100%), obtained from Nile tilapia filleting waste, in sausage and determine their physicochemical, nutritional and sensory properties. The sausages showed a decrease in protein and increase in fat content with increasing inclusion of MF. The nutritional quality of the products was high, with digestibility over 85%. The parameters of texture instrumental and yellow color (b\*) decreased with the increasing inclusion of MF. The sensory evaluation of the color showed that the maximum level of inclusion of MF was not well accepted by the panelists. The sausage with the best acceptance for the flavor attribute was those with 60% of MF. The results showed good nutritional quality of sausages utilizing MF of Nile tilapia filleting waste and according to the sensory evaluation, the maximum level of inclusion should not exceed 60%.*

**Key words:** *Oreochromis niloticus*, sausage, nutritional quality, minced fish, sensory acceptance, texture

## INTRODUCTION

The Nile tilapia, *Oreochromis niloticus,* is a fish species of tropical climate with a delicate flavored white meat and free from Y-shaped bones, which makes its culture interesting (Medri et al., 2009). In Brazil, it is the most produced species in aquaculture, reaching 69,078 tons in 2005 (FAO, 2006). The nutritional value of its meat is proven by its chemical composition, with protein between 15.0 and 20.0% and low fat amounts (1.0 to 4.0%) (Garduño-Lugo et al., 2003; Gryschek et al.,

2003). The industrial processing of tilapia in Brazil started in the early 1990s, with the priorization of the frozen fillets, which remains the same so far. However, the fillet yields (30.0 to 35.0%) are considered low, when compared to other freshwater fish raised in Brazil, such as the pacu, *Piaractus mesopotamicus*, (52.7%), rainbow trout, *Oncorhynchus mykiss*, (41.2%) and piracanjuba, *Brycon orbignyanus* (40.5%) (Viegas and Souza, 2004), generating a great amount of residues that are normally discarded (Ferraz de Arruda et al., 2007). The products derived from

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the minced fish (MF) obtained from the filleting waste process may increase the production of the edible portion for further utilization in other food products, which is a common activity, particularly in the processing of salmonids and catfish (Setiady et al., 2007).

The MF is obtained by passing the eviscerated and beheaded fish or fish waste through a machine which separates the meat from bones. This process allows additional recovery of the meat, in a range of 10.0 to 20.0%, of the whole eviscerated fish (Rasekh, 1987), although it may show a darker coloration due to the presence of hemopigments incorporated during the processing. This characteristic makes the MF more susceptible to lipid oxidation, since hemoglobin is known as an activator of lipid oxidation (Sánchez-Alonso and Borderias, 2008), thus a high proportion of MF in meat products may cause some problems, mainly of sensorial nature, due to the development of undesirable odor (rancidity) and texture defects (Trindade et al., 2005). The development of new products elaborated with MF, which yet retains all the nutritional advantages of the fish, might be a way of increasing the intake of this animal protein, besides adding value to the products generated by the Brazilian aquaculture.

Some studies have already been carried out utilizing the whole eviscerated tilapia for obtaining the MF (Gryschek et al., 2003) and surimi (Park et al., 1990). Other fish species have also been used for MF and elaboration of products, such as the silver carp, *Hypophthalmichthys molitrix* (Hu et al., 2008), trout, *Oncorhynchus mykiss* (Setiady et al., 2007) and catfish, *Clarias anguillaris* (Negbenebor et al., 1999). However, no reports are available on the utilization of MF from Nile tilapia filleting waste in the elaboration of sausages. Thus, the objective of this work was to elaborate the sausages with different inclusions (0, 20, 40, 60, 80 and 100%) of MF from Nile tilapia filleting waste in substitution to the fillet and evaluate its physical, chemical, nutritional and sensory characteristics.

## MATERIAL AND METHODS

### Raw material

Filleting wastes from Nile tilapia (*Oreochromis niloticus*) utilized in this study included the bones from the headless spine, skin, was supplied by a tilapia processing plant. These raw materials (150 kg) were transported frozen to the laboratory, defrosted for about 24 h and processed in an industrial fish deboning machine (HT 250, High Tech, Chapecó, Brazil). The MF, with an output of 53%, was packaged in 500 g-mass plastic bags and, immediately frozen (-40°C) in an ultra fast plate freezer (UCE - 20, Eco, São Paulo, Brazil). The frozen tilapia fillets (40 kg) were purchased from other fish processing plant. The chemical composition of the raw material is shown in Table 1.

**Table 1 -** Chemical composition[1] (mean ± standard deviation) of the raw material utilized in the formulation of the sausages.

| Raw-materials | Moisture (%) | Protein (%) | Fat (%) | Ash (%) |
|---|---|---|---|---|
| Fillet | 78.85 ± 2.69 | 18.74 ± 1.09 | 1.28 ± 0.89 | 1.05 ± 0.13 |
| MF | 75.47 ± 0.18 | 12.76 ± 0.61 | 10.54 ± 0.66 | 1.14 ± 0.17 |

[1]Values expressed in the wet base.

### Preparation of the sausages

The formulations were calculated in order to obtain 12 kg of sausages, substituting in different percentages of inclusion (0, 20, 40, 60, 80 and 100%), the tilapia fillet for the MF. The remaining ingredients utilized in the elaboration of sausages were added at the same proportions in all the treatments: soy protein 4% (Nutrisoy, Colombo, Brazil), tapioca starch 2%, salt 1%, curing salt 0.25%, antioxidant 0.5%, stabilizer 0.25% (Ibrac, Rio Claro, Brazil), sausage seasoning 1% (Kerry, Rio Claro, Brazil) and onion 2%.

The raw materials (MF and fillets) were defrosted for 24 h, weighed and ground in a cutter (V80, Tecmafrig, São Paulo, Brazil) along with the other ingredients for five minutes. The temperature of the mix when coming out of the cutter was 1°C. Afterwards, the emulsions were stuffed into 24 mm diameter cellulose casings (Viscofan, São Paulo, Brazil) and cooked in oven (SL 218,

Arprotec, Valinhos, Brazil) until the internal temperature reached 72ºC (approximately 80 min). After cooking, the sausages were cooled through water spraying until the internal temperature reached 40°C, vacuum packed (MI 60, Selovac, São Paulo, Brazil) after manual removal of the casings and stored at $0 \pm 1$ºC until the analyses.

## Chemical composition

The chemical composition of the raw material (fillet and MF) and the sausages were determined in triplicate according to the AOAC's methodology (1999). The protein content was determined by the micro-Kjeldahl method (N x 6.25), the fat was extracted in a Soxhlet extractor, the moisture was determined by the gravimetry and the ash content was determined by incineration in a muffle furnace.

## Amino acids analysis, chemical score of amino acids, in vitro digestibility and protein digestibility-corrected amino acid score (PDCAAS)

The amino acid composition of the lyophilized samples was determined after acid hydrolysis with HCl (6 M), at 110ºC for 22 h, according to the method described by Spackman et al. (1958). The chemical score of amino acids was determined by the division of the tested protein amino acid by the standard protein amino acid (FAO/WHO/UNU, 1985). The *in vitro* digestibility was determined by the pepsin–pancreatin system, according to the method described by Akeson and Stahmann (1964). The PDCASS index was determined by multiplying the *in vitro* digestibility by the chemical score of the product's most limiting essential amino acid and divided by 100, according to El and Kavas (1996).

## Water activity

The water activity was determined in triplicate at 25ºC in Aqualab CX-2 equipment (Decagon Devices, Pullman, USA).

## Instrumental texture

The instrumental Texture Profile Analysis (TPA) was performed in a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming, UK) previously calibrated with a 5 g-standard weight.

The sausages, 10 per treatment, were previously cut in slices of 23 mm long and compressed in up to 70%, utilizing an aluminum probe (SMS P/55) with pre-test, test and post-test speeds of 2.0 mm/s and distance from the platform of 16 mm at 25ºC. The studied parameters were hardness (g), cohesiveness (nondimensional) and chewiness (g x mm), calculated according to Bourne (1982).

## Instrumental color

The instrumental color was determined in triplicate using a portable colorimeter (Hunterlab, Miniscan XE, Reston, USA), previously calibrated with a black and white standard before each analysis, operating under D65 light source, observation angle of 10º and opening of measuring cell of 30 mm. The color was expressed utilizing the CIELab color system standards – "Comission Internationate de L'Eclairage": $L*$ (lightness), $a*$ (green-red color intensity) and $b*$ (blue-yellow color intensity).

## Sensory evaluation

For sensory evaluation, affective and acceptance tests were performed using the methodology described by Meilgaard et al. (1999). The sausages were heated in water for 2 minutes, cut in 2 cm long pieces and kept in an oven at 40ºC. A piece of each sample of sausages was served monadically in random order, along with water and cream crackers for the cleaning of the palate. The test was accomplished by 60 non-trained panelists for the attributes of flavor, texture, color, odor and overall acceptance, using a 9-point hedonic scale (1 – disliked very much to 9 – liked very much). In attendance to Resolution 196/96 of Brazilian Health Ministry, this study was approved by the Committee of Ethics in Research (FMRP-USP, under protocol number 0190/2006).

## Experimental design and statistic analysis

The experimental design was entirely randomized with six treatments (0, 20, 40, 60, 80 and 100% MF) and two repetitions using the variance analysis (ANOVA) and, in case of significant difference ($P<0.05$), the regression analysis was applied by using the SAS statistic software (Version 8, Cary, USA). The simple correlations were determined between the selected response variables.

## RESULTS AND DISCUSSION

### Chemical composition

The composition of the sausages in terms of proteins and fats varied linearly (*P<0.05*) with the addition of 0 to 100% of MF (Fig. 1). The protein content was highly negative correlated with the fat content (r = -0.86, *P<0.001*). These results reflected the differences in the raw material, i.e., the fillets had a higher protein percentage and lower fat percentage than MF (Table 1). The protein content in the sausages decreased (*P<0.05*) from 20.86 to 15.26% while the fat increased from 0 to 8.18% as long as MF content changed from 0 to 100%. The moisture (70.75 ± 0.59%) and ash content (3.40 ± 0.09%) showed no significant differences (*P>0.05)* in relation to the inclusion of

MF in the sausages. The Brazilian Legislation states that the sausages, elaborated with bovine, suine or poultry meat, must have the following composition: maximum moisture of 65%, minimum protein of 12% and maximum lipids of 30% (Brasil, 2000). Thus, according to the legislation, all the formulations studied met the requirements for the protein and lipids; however, they showed moisture values higher than that recommended by the legislation. These higher values were the consequence of the inherent composition of fish meat used in the present work, with high levels of moisture (over 75%) and also because of the non-addition of fats from other sources, which resulted in the products with high moisture and lipid levels much lower than the values established by Brazilian the legislation.
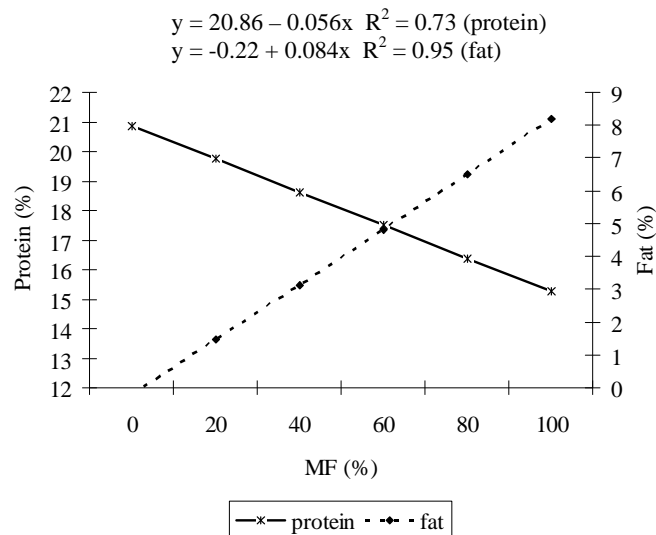
$$y = 20.86 - 0.056x \quad R^2 = 0.73 \text{ (protein)}$$
$$y = -0.22 + 0.084x \quad R^2 = 0.95 \text{ (fat)}$$



**Figure 1 -** Protein and fat content of sausages as function of inclusion of MF from Nile tilapia filleting waste.

### Amino acids profile (AA), chemical score of amino acids (CS), in vitro digestibility and protein digestibility-corrected amino acid score (PDCAAS)

The sausages formulated with 100% of MF showed a lower amount of essential amino acids than those formulated with 0% of MF, except for the histidine, which increased from 3.08 to 3.38 g/100 g of protein in the sausages formulated with 0 to 100% of MF (Table 2). Among the essential amino acids, the lysine was in higher amounts, varying from 8.07 g/100 g (100% of MF) to 8.57 g/100 g (0% of MF). These values were in accordance with the ones reported for the muscles

of fish which ranged from 7.9 to 9.8 g/100 g (Sikorski et al., 1990) and were higher than the ones found in the tilapia fillet (6.7 g/100 g) (Fernandes, 2000). Glycine and proline increased from 3.42 to 3.52 g/100 g and from 3.95 to 4.36 g/100 g, respectively in the sausages formulated with 0 to 100% of MF (Table 2), suggesting that collagen was incorporated in the material. The digestibility of the sausages was high, showing a variation of 85.58 to 91.11%. The values of chemical score of amino acids for all the treatments were equal or above 0.99 (Table 2), indicating a good protein quality, reaching the FAO/WHO/UNU standard (1985) for children

from 2 to 5 years of age. The essential amino acids that showed the lowest values of chemical score were leucine for the treatments with 0% (1.14), 40% (1.15), 60% (1.05), 80% (1.08) and 100% (1.06) of MF and the methyonine + cystine (0.99)

for the treatment with 20% of MF. For the PDCAAS index, the values varied from 0.90 to 1.00, being close to the one found in trout meat, *Salmo irideus*, (0.99) (El and Kavas, 1996).

**Table 2 -** Profile of amino acids, *in vitro* digestibility, chemical score of amino acids (CS) and protein digestibility-corrected amino acid score (PDCAAS) of sausages formulated with MF of Nile tilapia filleting waste.

| Amino acids (g/100 g) | Treatments (% MF) | | | | | | FAO/WHO/UNU[1] |
|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | 100 | |
| Aspartic Acid | 10.20 | 9.88 | 10.19 | 10.08 | 10.00 | 10.02 | - |
| Glutamic acid | 15.93 | 15.45 | 16.01 | 15.86 | 15.71 | 15.82 | - |
| Serine | 4.24 | 4.15 | 4.27 | 4.25 | 4.28 | 4.30 | - |
| Glycine | 3.42 | 3.44 | 3.63 | 3.51 | 3.48 | 3.52 | - |
| *Histidine | 3.08 | 3.09 | 3.15 | 3.23 | 3.29 | 3.38 | 1.9 |
| Arginine | 6.74 | 6.53 | 6.78 | 6.80 | 6.77 | 6.97 | - |
| *Isoleucine | 4.67 | 4.51 | 4.70 | 4.47 | 4.41 | 4.36 | 2.8 |
| Alanine | 5.73 | 5.47 | 5.66 | 5.61 | 5.65 | 5.60 | - |
| Proline | 3.95 | 3.90 | 4.22 | 4.30 | 4.28 | 4.36 | - |
| *Leucine | 7.56 | 7.24 | 7.58 | 6.95 | 7.14 | 6.97 | 6.6 |
| *Lysine | 8.57 | 8.36 | 8.59 | 8.27 | 8.13 | 8.07 | 5.8 |
| Methionine | 2.31 | 2.18 | 2.40 | 2.21 | 2.24 | 2.21 | - |
| Cystine | 0.67 | 0.30 | 0.53 | 0.51 | 0.56 | 0.59 | - |
| *Met + cyst. | 2.98 | 2.48 | 2.93 | 2.72 | 2.79 | 2.80 | 2.5 |
| Phenilalanine | 4.38 | 4.25 | 4.43 | 4.25 | 4.28 | 4.23 | - |
| Tyrosine | 3.71 | 3.39 | 3.47 | 3.34 | 3.23 | 3.52 | - |
| *Phe +Tyr. | 8.09 | 7.65 | 7.90 | 7.59 | 7.51 | 7.75 | 6.3 |
| *Threonine | 4.33 | 4.36 | 4.32 | 4.19 | 4.16 | 4.23 | 3.4 |
| *Valine | 4.81 | 4.61 | 4.80 | 4.64 | 4.53 | 4.49 | 3.5 |
| *Tryptophan | nd[2] | nd | nd | nd | nd | nd | 1.1 |
| Digestibility (%) | 85.58 | 91.11 | 89.38 | 90.46 | 89.96 | 89.96 | |
| CS | 1.14 | 0.99 | 1.15 | 1.05 | 1.08 | 1.06 | |
| PDCAAS | 0.97 | 0.90 | 1.0 | 0.95 | 0.97 | 0.95 | |

*Essential amino acids to man.
[1]FAO/WHO/UNU, 1985 (standard for kids from 2 to 5 years old)
[2]nd – no determined

**Water activity**
The average value for the water activity was 0.98 with nonsignificant differences (*P>0.05)* among the treatments. This value was within the range of foods with a great water activity for microbial growth, since it was slightly below 1.0 and was similar to the values reported for fresh fish (0.98) (Franco, 2004), thus requiring refrigerated storage.

**Instrumental texture**
The inclusion of the MF in substitution to the fillet caused an increase in the softness of the sausages, represented by the decrease (*P<0.05)* in the values of hardness, cohesiveness and chewiness (Fig. 2). The hardness decreased (*P<0.05)* from 13,196 g (0% of MF) to 881 g (100% of MF) and was represented by a quadratic regression. Meat

proteins are mainly composed of myofibrillar proteins (actin and myosin) that are responsible for the hardness of the sausages (Hedrick et al., 1994). In a study with the addition of mechanically separated poultry meat (0 to 100%) in sausages, a decrease in the hardness from 195.7 to 67.0 kPa was observed (Daros et al., 2005). The cohesiveness of the sausages showed a linear decrease (*P < 0.05)* from 0.63 (0% of MF) to 0.13 (100% of MF). The inclusion of MF caused a quadratic decrease (*P<0.05)* in the chewiness (7,347.3 to 163.3 g x mm) of the sausages formulated with 0 to 100% of MF. Desmond and Kenny (1998) also found decrease in the chewiness with the inclusion of 0% (345.4 N x mm) to 15% of surimi-like extract form beef hearts (225.2 N x mm) in frankfurters. The

parameters of hardness, cohesiveness and chewiness were highly positive correlated with protein content (r=0.87, 0.82 and 0.86, *P<0.001*, respectively) and negative correlated with fat content (r=-0.90, -0.92 and -0.86, *P<0.001*, respectively).
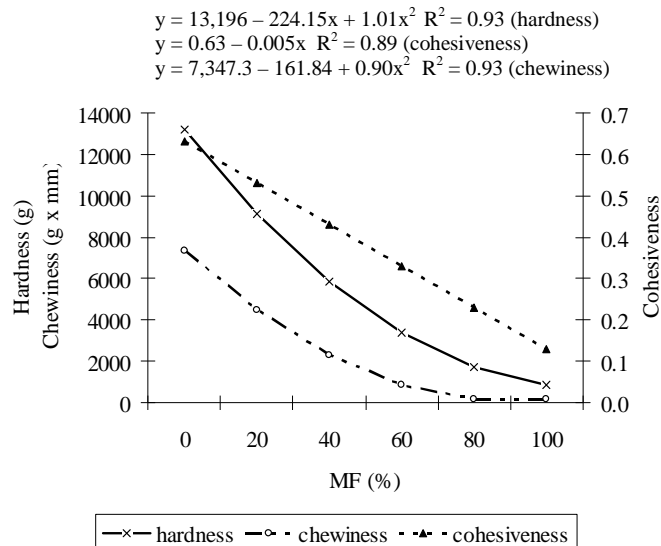
$$y = 13,196 - 224.15x + 1.01x^2 \quad R^2 = 0.93 \text{ (hardness)}$$
$$y = 0.63 - 0.005x \quad R^2 = 0.89 \text{ (cohesiveness)}$$
$$y = 7,347.3 - 161.84 + 0.90x^2 \quad R^2 = 0.93 \text{ (chewiness)}$$

**Figure 2 -** Values of instrumental texture for hardness, cohesiveness and chewiness of sausages formulated with different inclusions of MF from Nile tilapia filleting waste.

**Instrumental color**

The value of the instrumental color parameter $L*$ (lightness) did not show significant difference (*P>0.05*) with the inclusion of MF in the sausages (67.07 ± 1.25). The instrumental color parameter $a*$ (redness) did not show significant difference either (*P>0.05*) among the treatments (2.09 ± 0.22). Trindade et al. (2005) found a decrease in $a*$ with the inclusion from 0% (13.4) to 100% of the mechanically separated layer hen meat (8.4) in mortadella. The instrumental color $b*$ (yellowness) showed quadratic variation (*P<0.05*) with the increase in the inclusion of MF in the sausages (Fig. 3). The value of $b*$ of the sausages was decreased with the inclusion of 0% (16.18) to 100% of MF (11.98). Desmond and Kenny (1998) also noticed a decrease of $b*$ with the inclusion of 0% (11.4) to 15% (10.4) of surimi-like extract form the beef hearts in frankfurters.
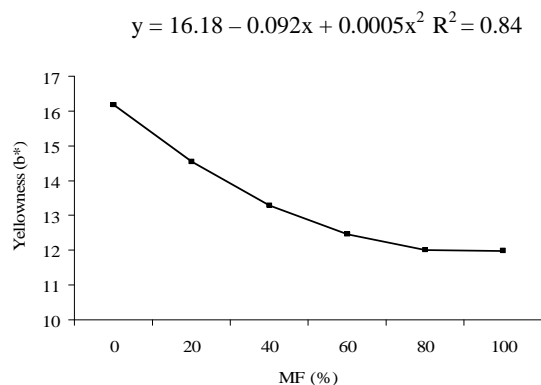
$$y = 16.18 - 0.092x + 0.0005x^2 \quad R^2 = 0.84$$

**Figure 3 -** Instrumental color yellowness (*b*)* of sausages formulated with MF of Nile tilapia filleting waste.

**Sensory evaluation**

The color attribute showed a linear decrease (*P<0.05)* in the scores with the increase of the MF inclusion (Fig. 4). The preferred treatment was the one without MF (I slightly liked it – 6.1). The least accepted treatment was the one which contained 100% of MF (I slightly disliked it – 4.1). The color attribute was highly positive correlated with the value $b*$ (r = 0.86, *P<0.001*), thus, the better color sensory results were obtained on yellow sausages. This could be explained by the darker coloration of the sausages formulated with higher inclusions of MF, which caused a decrease in the scores by the panelists. This attribute could be improved if the MF were submitted to washing processes, where the pigments were removed and the meat became lighter, or else with the addition of colorants into the sausages (Uyhara et al., 2008). However, in the studies carried out in India, the panelists gave the sausages with Japanese threadfin bream*, Nemipterus japonicus,* MF, a score of 9 (I thoroughly liked it) (Raju et al., 2003), showing that the acceptance of fish products could be related to the cultural factors. Thus, the maximum addition of MF in sausages to get the best acceptance could vary in different countries and even in different regions of the country.

The flavor of the sausages varied in a quadratic way (*P<0.05)*, inasmuch as the highest scores were obtained in the sausages with 53.8% of MF, i.e., among the treatments with 40 and 60% of MF (6.5 – I slightly liked it) (Fig. 4). The sausages which the panelists classified as less tasteful (5.4 – neither liked it/ nor disliked it) were those without the inclusion of MF; because according to the comments in the evaluation form, to their weak fish flavor and those with 100% of MF, that were considered with a strong fish flavor, possibly related to their high fat content (Fig. 1).

The texture of the sausages varied in a quadratic way (*P<0.05)* with the increasing inclusion of MF (Fig. 4). The highest scoring of this attribute was observed with the inclusion of 54.4% of MF, i.e., between the sausages formulated with 40 and 60% of MF (6.2 and 6.3 – I slightly liked it). The

sausages with 0 and 20% of MF were considered 'rubbery' by many panelists and consequently got lower scores (3.7 – I mildly disliked it and 5.3 – neither liked it/nor disliked it, respectively). In the case of the sausages formulated with 80 and 100% of MF, many panelists reported that the sausages were excessive soft, and rated them 5.8 (neither liked it/nor disliked it) and 4.5 (slightly disliked it), respectively. Daros et al. (2005) and Trindade et al. (2005) also observed that the texture of sausage and mortadella started getting softer with the increase in the percentage of mechanically separated poultry meat and mechanically separated layer hen meat. Beraquet et al. (1992) also suggested a decrease for this attribute with the increase from 40 to 100% of mechanically separated chicken meat in a Bologna-type sausage. As for the odor, the sausages showed a positive linear regression (*P<0.05)* with a slight increase of the scores with the inclusion of 0% of MF (5.6 – neither liked it/nor disliked it) to 100% of MF (6.1 – slightly liked it) (Fig. 4). This improvement could be attributed to the fact that the panelists preferred the strongest fish odor in the sausages that contained more MF as was observed for the flavor attribute.

The overall acceptance of the sausages varied in a quadratic way (*P<0.05)* with the inclusion of MF (Fig. 4). The highest acceptance was between the sausages elaborated with 40 and 60% of MF (6.1 and 6.2 – slightly liked it), followed by the sausages with 20 and 80% of MF (5.7 and 5.8 – neither liked it/nor disliked it) and, at last, those formulated with 0 and 100% of MF (4.9 – slightly disliked it and 5.1 – neither liked it/nor disliked it, respectively). The overall acceptance of the sausages was highly positive correlated with the texture and flavor (r = 0.95, 0.85, *P<0.001*, respectively), suggesting that texture and flavor were important attributes for the overall acceptance of the sausages. This limit of 60% of MF Nile tilapia filleting waste in sausages was in accordance with the Brazilian legislation (Brasil, 2000), in which the maximum allowed rate of mechanically separated meat in sausages could be 60%.
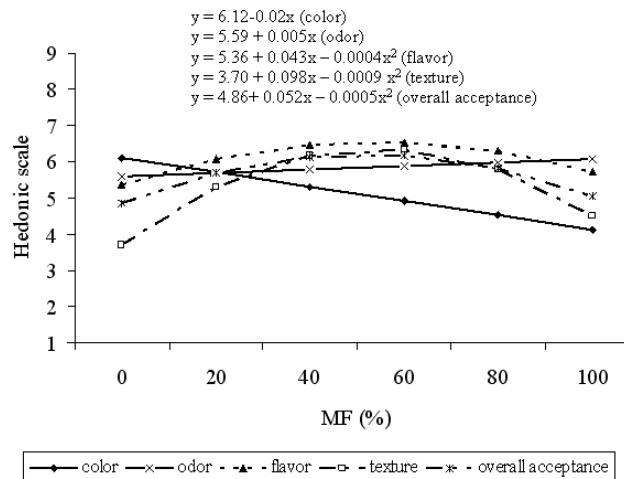
**Figure 4 -** Sensory evaluation of color, flavor, texture, odor and overall acceptance of sausages Bformulated with MF of Nile tilapia filleting waste.

## CONCLUSIONS

The sausages could be prepared with MF from Nile tilapia filleting waste, without hindering their nutritional and technological qualities, thereby showing the viability of their production. However, to keep a good overall sensory acceptance, the maximum rate of MF as a substitute for the fillet in sausages should be 60%.

## ACKNOWLEDGEMENTS

## RESUMO

O objetivo deste estudo foi avaliar a inclusão de carne mecanicamente separada (CMS) (0, 20, 40, 60, 80 e 100%), obtido de resíduos de filetagem de tilápias do Nilo, em salsichas e determinar suas propriedades físico-químicas, nutricionais e sensoriais. As salsichas apresentaram diminuição de proteína e aumento de lipídeos com a inclusão de CMS. A qualidade nutricional dos produtos foi alta, com digestibilidade acima de 85%. Os parâmetros de textura e cor instrumental amarela (b*) diminuíram com inclusão crescente de CMS. A avaliação sensorial da cor mostrou que o nível máximo de inclusão de CMS não foi bem aceito pelos provadores. As salsichas com melhor aceitação do atributo sabor foram aquelas com 60% de CMS. Os resultados demonstraram a boa qualidade nutricional das salsichas utilizando CMS de resíduos de filetagem de tilápias do Nilo e de acordo com avaliação sensorial, a porcentagem máxima de inclusão é de 60%.

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