Development and characterisation of cooked inlaid sausages with fillet and mechanically separated meat of Nile tilapia (*Oreochromis niloticus*)

Ângela Dulce Cavenaghi-Altemio[1] • João Luiz Zitkoski[1] • Gustavo Graciano Fonseca[2]

[1] Faculty of Engineering, Federal University of Grande Dourados, Dourados, Brazil
[2] Faculty of Natural Resource Sciences, School of Business and Science, University of Akureyri, Akureyri, Iceland

Correspondence

Gustavo Graciano Fonseca; University of Akureyri, Faculty of Natural Resource Sciences, Borgir v. Nordurslod, 600 Akureyri, Iceland
✉ gustavo@unak.is

Abstract

The limited consumption of fish is due to a limited availability of products based on this type of meat. Therefore, innovation and development of products that meet consumers' preferences are essential. The aim of this work was to develop and characterize cooked inlaid sausages prepared with fillet and mechanically separated Nile tilapia (*Oreochromis niloticus*) meat (MSM). Three sausages types were considered, F1 (100% MSM), F2 (50% MSM, 50% fillet) and F3 (25% MSM, 75% fillet). Microbiological quality of the raw materials and products obtained were within the standard limits. F1 had the lowest protein (34.68%) and the highest lipid (2.88%) contents. F1 and F2 had the highest luminosity L*, which was related to the MSM composition, as the L* was higher as the percentage of MSM increased in the formulation. The shear strength was higher for F2 (18.55 N) and lower for F1 (6.43 N) and differed among sausages types. The F2 sausages presented a better acceptability regarding the sensory attributes evaluated, with acceptation indexes of 88.00, 85.55, and 84.66% for flavour, texture, and odour respectively. This finding indicates that this formulation would be very well accepted in the market if available for commercialisation.

Keywords: fish composition; fish processing; fish products; fish proteins; fish sausages

1 | INTRODUCTION

Fish is essential in the population's diet, not only because it is an excellent source of nutrients such as proteins and minerals, but because it has a significant reserve of polyunsaturated fatty acids, especially of the omega series, which are considered essential for human development (Soccol and Oetterer 2003). Nile tilapia (*Oreochromis niloticus*) is a species of tropical fish with a delicate flavoured meat which makes its culture interesting. It is considered of great importance in world aquaculture (Medri *et al.* 2009; Fonseca *et al.* 2013). The fillet is the main form of commercialisation of Nile tilapia. It presents a

yield of around 30 – 33% of the fish processing. The remaining are residues usually utilised as raw material for animal feed or deposited in the environment, increasing environmental pollution (Martins *et al.* 2016).

The mechanically separated meat (MSM) is the fish flesh characterised by particles of the skeleton meat that have been mechanically separated by using a meat deboner that is essentially free of bones, viscera and skin, with suitable quality for human consumption (Cavenaghi-Altemio *et al.* 2018). In addition to providing a good use of these residues, it becomes a raw material of an appreciable nutritional quality that can be used for the prepara-

tion of different food products (Dallabona *et al.* 2013; Palmeira *et al.* 2016; Husein *et al.* 2020).

Fish emulsified products obtained from MSM offer great perspectives due to the ease of performing blends with different ingredients in order to improve and modify the sensory characteristics such as taste, odour, colour, and texture. The inlaid is a product made with meat or other edible animal tissue, which may be dyed, skinned, cured, seasoned, cooked, dried, and/or smoked (Cavenaghi-Altemio *et al.* 2020). It is obtained from an emulsion formed after the mixing of meat, additives, and condiments, promoting an intimate mixture of fat globules with a protein and water array (Lourenço *et al.* 2012). Thus, the aim of this work was to develop and characterize in terms of chemical, physical, microbiological analyses, and consumer acceptance, cooked inlaid sausages with fillet and mechanically separated meat of Nile tilapia (*Oreochromis niloticus*) as an alternative to fish consumption.

2 | METHODOLOGY

2.1 Fillets and mechanically separated meat (MSM) of Nile tilapia

Nile tilapia (*Oreochromis niloticus*) fishes were obtained from a local fish farm. They were transported live to the Laboratory of Food Technology from the Federal University of Grande Dourados, Dourados, MS, Brazil, where they were stunned by thermonarcosis in a mixture of ice and water (1:1), slaughtered, gutted, washed with 5 ppm chlorinated water spray, and filleted, under refrigerated conditions (12°C) (Fonseca *et al.* 2013). The MSM was produced from the carcasses in 3 mm particle size using a meat-bone separator (HT 250, High Tech, Brazil), operating at inlet 6°C and outlet 10°C (Cavenaghi-Altemio *et al.* 2018). Fillets and MSM were stored up to one week under freezing until processing.

2.2 Microbiological analysis

To assess microbiological analysis of the raw materials, duplicate 25 g samples were aseptically transferred into a stomacher bag containing 100 ml of sterile distilled water containing 0.1% peptone (1% for *Salmonella* sp. determination). Samples were homogenised for 1 min. Ten-fold serial dilution were prepared using sterile 0.1 peptone solution (9 ml) and spread plated (0.1 ml) in duplicate onto broths and/or agars for detection of typical colonies, biochemical confirmation, and identification, and plate counting for thermo-tolerant coliforms at 45°C, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella* sp., in accordance with the methodology described elsewhere (USDA/FSIS 1998).

2.3 Chemical analyses

Moisture, crude protein and crude ash contents of the raw materials and cooked inlaid sausages were determined in triplicate according to the methods described by AOAC (2012). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46), protein by the Kjeldhal method (method 928.08), crude fat by the ether extraction technique using a Soxhlet apparatus (method 991.36), and ash by using the muffle oven technique (method 920.153).

2.4 Cooked inlaid sausages obtained from fillet and MSM of Nile tilapia

For the preparation of the inlaid sausages, the fillets and MSM were both milled in a grinder with a 5 mm disc (Weg, Jaraguá do Sul, Brazil) at 1.5°C. Then, the ingredients of the formulations were added, according to the three treatments listed in Table 1, and the obtained masses were homogenised in a cutter (Model Sire, Filizola, São Paulo, Brazil) for 10 min at 4°C. Subsequently, the masses were embedded in a natural bovine casing in horseshoe format. After the mooring, the sausages were submerged in liquid smoke for 1 min. Then they were cooked until the internal temperature reached 72°C, when they received the thermal shock with cold water below 5°C for 20 min. After cooling, they passed through a varnish bath and an overnight drying at 4°C. The cooked sausages were identified as F1, F2 and F3, according to their formulations, vacuum packed, and utilised for further analysis. Additives and condiments were supplied by Conatril Industrial de Alimentos Ltda (Rio Claro, Brazil).

TABLE 1 Ingredients used in the preparation of three formulations of cooked inlaid sausages prepared with mechanically separated meat (MSM) and fillets of Nile tilapia (*Oreochromis niloticus*).

Ingredient	F1 (%)	F2 (%)	F3 (%)
MSM of Nile tilapia	89.63 (100)	44.82 (50)	22.41 (25)
Nile tilapia fillet	- (0)	44.82 (50)	67.22 (75)
Cold water	5.00	5.00	5.00
Textured soy protein	2.50	2.50	2.50
Refined sodium chloride	1.30	1.30	1.30
Spices	1.10	1.10	1.10
Sugar	0.40	0.40	0.40
Ascorbic acid	0.05	0.05	0.05
Sodium nitrite	0.015	0.015	0.015

Values in parenthesis are referred to the meat percentage, without considering the other ingredients of the formulation.

2.5 Physical analysis
2.5.1 Instrumental colour
The colour [CIE L*(lightness), a* (redness), b* (yellowness)] of the fish sausages elaborated with the inlaid sausages was evaluated using a colorimeter (Model CR 410, Konica Minolta, Ramsey, USA), with measurements standardised with respect to the white calibration plate (Jiménez and Gutiérrez 2001). Five readings were made

from the samples, after removing the external involucre.

2.5.2 Shear strength

Texture analysis of the cooked inlaid sausages obtained was carried out using a texture analyser (Model TAXTplus, Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Products kept at 2°C were equilibrated at room temperature (28 – 30°C) before analysis. Slices of 2 cm were cut, placed in the texture analyser and submitted to a cutting/shearing test (speed of 1.0 mm s^{-1}, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the shear strength (N), which indicated the firmness of the sample. A minimum of 10 replicates of each treatment were analysed (Kang and Chen 2015).

2.6 Sensory analysis

Sensory analyses of the ready-made dishes were conducted at the same day by 50 semi-trained panellists (52% female and 48% male). Their ages ranged from 18 to 44 years: 33% from 18 to 24 years old, 61% from 24 to 35 years old, and 5% over 35 years old. All judges were consumers of Nile tilapia and sausages. A nine-point hedonic scale (9 = like extremely; 1 = dislike extremely) was used for evaluation of the attributes colour, odour, texture and taste. The inlaid sausages were heated in a microwave oven for 5 sec, were cut transversely 2 mm thick, and served in disposable containers, coded with three-digit random numbers and presented in a monadic form (Cavenaghi-Altemio *et al.* 2018). The acceptation index (AI) was calculated according to the following equation: AI = (average of the attributed grades / maximum attributed grade) × 100. The sample was considered accepted if the value was greater than 70% (Stone and Sidel 2004).

2.7 Data analysis

Statistical results were evaluated through analysis of variance (ANOVA) and the Tukey test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 7.0. The sensory attributes results were presented in percentage (means ± standard deviations).

3 | RESULTS AND DISCUSSION
3.1 Microbiological analysis

Microbiological analyses of coliforms at 45°C, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella* sp. were carried out for the raw MSM and the fish fillets, and the three fish-based cooked inlaid sausage's formulations, in order to guarantee the food security of judgers priori the conduction of the sensory analysis (Table 2). Results obtained were within the limits established by Brazilian legislation (Table 2), which recommends a maximum of 1.0×10^2 CFU g^{-1} for thermotolerant coliforms at 45°C, a maximum of 5.0×10^2 CFU g^{-1} for positive coagulase staphylococci, and the absence of *Salmonella* sp. in 25 g of sample (ANVISA 2001). Although there is no specific legislation for MSM of fish, the results of microbiological analysis meet the criteria established for MSM of poultry, cattle and pork (MAPA 2000). According to international standards, these products should be free of Salmonella sp. and the levels of positive coagulase staphylococci and *C. perfringens* < 1.0×10^2 CFU g^{-1}. If exceed 1.0×10^3 CFU g^{-1}, it indicates poor handling practices, but if ≥ 1.0×10^4 CFU g^{-1}, they are considered potentially hazardous, may resulting in food borne illness if consumed (ICMSF 2011).

TABLE 2 Microbiological evaluation of raw material and cooked inlaid sausages prepared with mechanically separated meat (MSM) and/or fillets of Nile tilapia (*Oreochromis niloticus*) according to formulations F1 (100% MSM), F2 (50% MSM, 50% fillet) and F3 (25% MSM, 75% fillet).

Microbiological analysis	Nile tilapia		Formulations		
	MSM	Fillet	F1	F2	F3
Thermotolerant coliforms at 45°C (CFU g^{-1})	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1
Staphylococcus aureus (CFU g^{-1})	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1
Clostridium perfringens (CFU g^{-1})	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1	<1.0×10^1
Salmonella sp. (in 25 g)	Absent	Absent	Absent	Absent	Absent

3.2 | Proximate composition

The proximate composition of the MSM, fillets and cooked inlaid sausages are shown in Table 3. The lipid and ash contents of the MSM and the fillets were found close. However, higher contents of moisture and protein (75.53 and 19.36% respectively) were obtained for the Nile tilapia fillets (Table 3). It is well known that the biochemical composition of the fish meat can vary depending on the composition of the diet, the feed management, the species, sex, age, size of the fish and the type of body muscle or cut (Soccol and Oetterer 2003). Thus, variations in the composition are also expected depending on the nature of the raw material, e.g., the MSM from the entire fish or the fillet. These differences in protein and lipids were very evident when comparing the MSM and the fillets of Nile tilapia (Table 3).

The literature reports variables compositions for MSM of Nile tilapia, e.g., 74.7% moisture, 10.75% protein, 12.99% lipids, 1% ash (Sary *et al.* 2009); 79.83% moisture, 75.01% protein, 14.43% lipids and 6.69% ash (Kirschnik and Macedo-Viegas 2009); 72.75% moisture, 13.02% protein, 11.03% lipids, 1.08% ash (Mélo *et al.* 2011); 71%

moisture, 11.96% protein, 15.37% lipids, 1.22% ash (Dallabona *et al.* 2013), 73.87% moisture, 60.73% protein, 29.09% lipids (Fogaça *et al.* 2013), and 78.31% moisture, 64.96% protein, 28.91% lipids, 5.12% ash (Kirschnik *et al.* 2013), and Nile tilapia fillets with 77.91% moisture, 25.65% protein, 2.55% lipids, 1.04% ash (Souza *et al.* 2004), 78.80% moisture, 16.30% protein, 3.26% lipids,

0.96% ash (Dallabona *et al.* 2013). Differences are also observed for Nile tilapia filleting residues, with 77.24% moisture, 76.8% protein, 19.6% lipid, 4.48% ash (Rebouças *et al.* 2012), and minced Nile tilapia, with 75.47% moisture, 52.02% protein, 42.97% lipids, 2.69% ash (de Oliveira Filho *et al.* 2010).

TABLE 3 Proximate composition of raw material and cooked inlaid sausages prepared with mechanically separated meat (MSM) and/or fillets of Nile tilapia (*Oreochromis niloticus*) according to formulations F1 (100% MSM), F2 (50% MSM, 50% fillet) and F3 (25% MSM, 75% fillet).

Composition	Nile tilapia		Formulations		
	MSM	Fillet	F1	F2	F3
Moisture (%)	66.84 ± 0.04	75.53 ± 0.22	58.64 ± 1.56[a]	58.02 ± 2.71[a]	58.52 ± 0.53[a]
Protein (%)	14.73 ± 0.20	19.36 ± 1.85	34.68 ± 2.47[b]	38.19 ± 1.70[a]	37.15 ± 0.16[a]
	(70.68 ± 0.96)	(76.82 ± 0.79)	(84.54 ± 0.49)	(88.31 ± 0.46)	(87.53 ± 0.47)
Lipids (%)	4.00 ± 0.28	4.84 ± 0.02	2.88 ± 0.02[a]	2.83 ± 0.05[a]	2.78 ± 0.01[b]
	(19.19 ± 1.34)	(19.20 ± 1.11)	(7.02 ± 0.05)	(6.90 ± 0.11)	(6.77 ± 0.02)
Ash (%)	1.50 ± 0.07	1.25 ± 0.02	3.23 ± 0.08[a]	3.19 ± 0.05[a]	3.22 ± 0.00[a]
	(7.20 ± 0.34)	(4.96 ± 0.28)	(7.87 ± 0.17)	(7.38 ± 0.16)	(7.59 ± 0.17)
Calcium (mg/100 g)	120 ± 10	80 ± 10	150 ± 10[a]	130 ± 0[a,b]	110[b] ± 0
Sodium (mg/100 g)	nd	nd	685.18	651.18	716.5

Values in parenthesis are in dry basis. Values (means ± standard deviations) with the same superscript letter in the same row do not differ statistically at $p > 0.05$. nd, not detected.

It was observed that the three formulations had no significant difference ($p > 0.05$) for moisture and ash contents. However, the difference was significant for protein and lipids ($p < 0.05$). F1 had the lowest protein content, while F2 and F3 showed the highest values. F1 and F3 had the highest lipid content while F2 presented the lowest lipid content (Table 3). For the protein values, it was observed that in F1 (100% MSM) had a lower value (34.68%) due to the lower protein concentration contained in the MSM. Regarding lipids, this same formulation (100% MSM) had a higher value (2.88%) due to the higher lipid concentration contained in the MSM (Table 3). Although the ash content did not vary between formulations ($p > 0.05$), the calcium presented a significant difference ($p < 0.05$) for the three formulations. For fish-based products, the composition is naturally dependent of the composition of all ingredients. For example, the literature reports 64.98% moisture, 13.73% protein, 14.87% lipids, and 2.91% ashes in raw sausages made with MSM of Nile tilapia filleting residue (Dallabona *et al.* 2013).

3.3 Instrumental colour and shear strength
The instrumental colour parameters luminosity (L*), redness (a*), and yellowness (b*) of the fish-based cooked inlaid sausages developed for three formulations were determined (Table 4). The F1 and F2 had the highest L*, while F2 and F3 showed the highest a* ($p < 0.05$). The b* did not shown any variation ($p > 0.05$) between formulations. The highest values did not differ ($p > 0.05$) between them (Table 4). In these cases, differences may be related

to the MSM composition. The L* was higher as the percentage of MSM increased in the formulation. This relationship can be verified in the inlaid composition (Table 1) and the lipid content (Table 2). This increased fat content in the inlaid composition increases the reflection of light when incurred on the product. In agreement, it was reported elsewhere that due to the different compositions of hybrid sorubim fillets and MSM, there was a variation in the composition of the inlaids, mainly for lipids (Cavenaghi-Altemio *et al.* 2021).

The shear strength was also determined for all formulations, showing difference ($p < 0.05$) between them (Table 4). It was higher for F2 (18.55 N) and lower for F1 (6.43 N). In accordance, the lower the protein content, the lower the shear strength. The hardness is highly correlated with the high levels of proteins and low levels of lipids (Cortez-Vega *et al.* 2013).

3.4 Sensory analysis
Table 4 presents the scores of the sensory attributes of odour, colour, taste and texture of the fish-based cooked inlaid sausages by the acceptance test and index. All attributes presented a significant difference between the formulations ($p < 0.05$). F2 obtained the highest scores for the evaluated attributes. In addition, it also obtained the best scores for the acceptance index (Table 4).

The average scores of the sensory attributes varied from 6 (I liked it slightly) to 8 (I liked it a lot) on the hedonic scale, in an average range of 6.2 to 7.9, except for the texture of F1, which average score (5.0) was below

this range (Table 4). It is in agreement with both lower protein content (Table 3) and shear strength obtained for this formulation, which permits to conclude that the utilization of 100% of MSM of Nile tilapia (Table 1) negatively influenced ($p < 0.05$) the texture of the cooked inlaid.

The F2 presented the highest average scores for flavour (7.9), followed by texture (7.7) and odour (7.6), with acceptation indexes of 88.00, 85.55 and 84.66% respec-

tively (Table 4), being, therefore, the formulation most appreciated by the judgers. The attribute was accepted sensorially when the acceptance index was $\geq 70\%$ (Stone and Sidel 2004). In accordance, it was reported elsewhere that smoked sausage prepared with MSM of Nile tilapia showed good sensory acceptance (Dallabona *et al.* 2013).

TABLE 4 Instrumental color, shear strength, and sensory analysis of the cooked inlaid sausages prepared with mechanically separated meat (MSM) and/or fillets of Nile tilapia (*Oreochromis niloticus*) according to formulations F1 (100% MSM), F2 (50% MSM, 50% fillet) and F3 (25% MSM, 75% fillet).

Formu-lation	Instrumental colour			Shear strength (N)	Sensory analysis			
	L*	a*	b*		Odour	Colour	Taste	Texture
F1	50.1±0.5[a]	16.6±0.3[a]	10.4±0.5[a]	6.4±1.2[a]	7.0±1.4[a] (77.3±15.6)	6.5±1.6[a] (72.7±17.8)	6.3±1.8[a] (62.8±18.1)	5.0 ± 1.7[a] (63.0 ± 21.8)
F2	49.7±0.5[a]	19.5±0.2[b]	10.1±0.4[a]	18.6±1.2[b]	7.6±1.1[b] (84.7±12.0)	7.4±1.3[b] (82.7±14.1)	7.9±0.9[b] (88.0±10.3)	7.7 ± 1.0[b] (85.6 ± 11.4)
F3	46.9±0.5[b]	20.1±0.6[b]	10.6±0.5[a]	11.0±0.5[c]	7.0±1.5[a,b] (78.2±17.3)	7.2±1.3[a,b] (79.77±14.1)	7.1 ± 1.5[c] (78.4 ± 16.5)	6.2 ± 1.7[c] (69.1 ± 18.5)

L*: lightness); a*: redness, b*: yellowness. Values (means ± standard deviations) with the same superscript letter in the same column do not differ statistically at $p > 0.05$. Values in parenthesis are referred to the acceptation indexes ± standard deviations (%).

4 | CONCLUSIONS

In terms of microbiological quality, the raw materials and products obtained were within the standard limits established by Brazilian legislation. The proximate composition obtained was very close to that reported in the literature. The MSM and the fillets of Nile tilapia presented protein and lipid contents considered ideal for use in new products, with low calcium content. The cooked inlaid sausages prepared with a mixture (1:1) of fillet and MSM of Nile tilapia (F2) presented a better acceptability regarding the sensory attributes evaluated. This finding indicates that F2 would be very well accepted in the market if available for commercialization.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

ADCA research design; JLZ primary data collection; JLZ, ADCA & GGF data analysis; GGF manuscript preparation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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ORCID

ÂD Cavenaghi-Altemio https://orcid.org/0000-0002-3000-8869
JL Zitkoski https://orcid.org/0000-0003-1615-8497
GG Fonseca https://orcid.org/0000-0002-8784-661X