Original scientific paper

Evaluation of sausages obtained from mechanically separated Nile tilapia (*Oreochromis niloticus*) meat and prepared using different homogenizing and refining processes

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A b s t r a c t: *After filleting Nile tilapia (Oreochromis niloticus), unused parts can be processed to obtain the co-product, mechanically separated meat (MSM). The aims of this study were to use different processes for homogenizing and refining of Nile tilapia MSM sausages and to evaluate cooked sausages in terms of their microbiological, physical, chemical and sensory characteristics. Ingredients were processed according to three treatments: (T1) using a grinder and cutter, (T2) using a grinder and mixer, and (T3) using only the grinder. The protein content ranged from 15.08% (T3) to 15.91% (T1), lipids from 9.61% (T3) to 12.29% (T1), and ash from 1.83% (T1) to 2.73% (T3). The highest color lightness score was 57.28, for the sausage elaborated by the conventional method (T1). The obtained shear forces were 2.04 N (T1), 2.71 N (T2) and 1.77 N (T3). Only T2 sausages received an acceptability index higher than 70%. T2 sausages also were rated by 34% of panelists as "certainly would purchase" or "probably would purchase". In conclusion, it is feasible to produce sausages from Nile tilapia MSM by using a grinder and mixer for homogenizing and refining, which would be a good alternative method mainly for small producers.*
    Keyword: fish, fish flesh, quality, sensory analysis, process methods.

Introduction

The limited consumption of fish meat in some countries is not only due to economic and cultural factors, but it is also the consequence of a limited availability and diversity of species and products based on this type of meat (*Marques et al.*, 2020). Brazil has extremely favorable conditions for fish farming. In addition to the great market potential, the country has a favorable climate, good availability of land areas, extensive grain cropping to produce raw materials for animal feed, and good water potential (*Merengoni*, 2006). Nile tilapia (*Oreochromis niloticus*) is farmed worldwide due to its fast growth, easy handling, high yield and excellent quality meat (*Fonseca et al.*, 2013).

After filleting Nile tilapia, unused parts can be processed to obtain a co-product similar to mechanically separated red meat (MSM). A mechanized process separates the edible parts of the fish, generating skeletal meat particles free of viscera, bones and skin (*Cavenaghi-Altemio et al.*, 2018). Fish MSM is an intermediate product that serves as a raw material to produce surimi, fish burgers, fish fingers, nuggets, croquettes, pates, mortadella, sausages etc. (*Dallabona et al.*, 2013; *Palmeira et al.*, 2016; *Husein et al.*, 2020), so incorporating high-quality fish nutrients into these fish products (*Verdi et al.* 2020).

Sausages are the main products obtained from fish MSM. The sausages are made from an emulsion obtained by mixing water-soluble and fat-soluble ingredients in a cutter, preferably under vacuum and at low temperature. The resulting mixture, due to the extraction of soluble proteins, becomes viscous and the pieces of meat become adherent. The meat mixture is then filled into natural casings, bladders or other animal membranes or appropriate plastic casings. The sausages are made with meat or other edible animal parts, and can be dyed, skinned, cured, seasoned, cooked, smoked, and dried (*MAPA*, 2000).

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The aim of this research was to evaluate different processes for homogenizing and refining sausage obtained from Nile tilapia (*Oreochromis niloticus*) MSM in terms of their microbiological, physical, chemical and sensory characteristics.

Materials and Methods

Mechanically separated Nile tilapia meat (MSM)

About 100 Nile tilapia (*Oreochromis niloticus*) carcasses were utilized in the research. The fishes were produced in a fish farming system and weighed, on average, 0.700 ± 0.025 kg. After filleting by a local fish processing plant, about 67% of the total weight remained in the carcasses, totaling approximately 47 Kg. These carcasses were transported for 40 min under refrigerated conditions to the Laboratory of Bioengineering at the Federal University of Grande Dourados, Dourados, MS, Brazil, and immediately utilized to produce MSM. The MSM was produced in 3 mm particle size using a meat-bone separator (HT 250, High Tech, Brazil), operating at an inlet temperature of 6°C and outlet temperature of 10°C (*Cavenaghi-Altemio et al.*, 2018). Approximately 35 Kg of MSM obtained was immediately utilized to produce sausages.

Sausages produced from Nile tilapia MSM using different homogenizing and refining processes

To prepare the Nile tilapia sausages, the same formulation for all treatments was used (%): MSM, 89.14; soybean protein, 4.00; cassava starch, 2.00; refined salt, 1.80; spices, 1.30; sodium polyphosphate, 0.50; sugar, 0.40; liquid smoke, 0.40, cochineal carmine, 0.40; ascorbic acid, 0.05; and sodium nitrite, 0.015. The additives and the condiments were supplied by Cavenaghi Eireli (Dourados, MS).

The treatments differed according to the type of process by which the sausage was elaborated: treatment 1 (T1) using the grinder and cutter (conventional production); treatment 2 (T2) using the grinder and the mixer; and treatment 3 (T3) using only the grinder. For T1, the MSM was thawed and weighed, milled using a 5 mm disc grinder (Weg, Jaraguá do Sul) and emulsified in a cutter (*Sire Filizola, São Paulo*) along with the other formulation ingredients, previously weighed. For T2, the MSM was thawed and weighed, milled using a 3 mm disc grinder, and mixed in a mixer (CAF Máquinas, São Paulo) together with the other formulation ingredients, previously weighed. For T3, the MSM was thawed and weighed, milled using a 5 mm disc grinder and mixed manually together with the other formulation ingredients, previously weighed. Then, this mass was milled a second time using a 3 mm disc grinder.

After that, the respective sausage stuffings were filled manually into cellulosic casing, caliber 26 (*Picelli, Rio Claro*). All sausages were cooked by maintaining them at 55°C for 15 min, at 75°C, for 15 min, and at 85°C until the internal temperature of the product reached 74°C, before thermally shocking them at 0°C. The casings were removed, and the cooked sausages were left in a 5% solution of urucum dye for 20 min. They were then transferred to a phosphoric acid solution, pH 2.0 to 3.0, for 5 min. The sausages were refrigerated for 12 h, packed under vacuum, and kept under refrigeration prior the analyses. Each treatment was divided into three lots in order to evaluate the results of triplicates.

Chemical analysis

Moisture, crude protein, and crude ash contents of the 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 Kjeldahl method (method 928.08) and ash by the muffle oven technique (method 920.153). The lipid content was obtained in triplicate by the extraction method with cold organic solvent (*Bligh & Dyer*, 1959). The carbohydrate content was estimated by difference.

Physical analyses

Instrumental color

The color indices [CIE L*(lightness), a* (redness), b* (yellowness)] of the sausages elaborated from Nile tilapia MSM were determined using a colorimeter (Minolta Chroma Meter CR 410), with measurements standardized with respect to the white calibration plate (*Jiménez & Gutiérrez*, 2001). Six readings were made from the internal part of the sausages.

Shear force

Texture analysis of the sausages was carried out using a texture analyzer Model TAXTplus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Sausages were equilibrated at room temperature (28–30°C) before analysis. Samples of 15×15×20 cm were cut, placed in the texture analyzer and submitted to a cutting/shearing test (speed of 1.0 mm/s, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the

shear force (N), which indicated the firmness of the sample. A minimum of 10 replicates of each treatment were analyzed (*Kang & Chen*, 2014).

Microbiological analysis

Microbiological analyses of the sausages elaborated from Nile tilapia MSM were performed in triplicate for presence/absence of *Salmonella* spp., and counts of coagulase-positive staphylococci and thermo-tolerant coliforms at 45ºC, in accordance with the methodology described elsewhere (*USDA/FSIS*, 1998).

Sensory analysis

Sensory analyses of the sausages elaborated from Nile tilapia MSM were conducted by 50 non-trained panelists. A vertically structured nine-point hedonic scale of mixed category (9 = like extremely; 1 = dislike extremely) was used for evaluation of the attributes of color, taste, texture, odor, and overall acceptability. Samples (2 cm-long pieces) were prepared by steeping the sausages in boiling water for 3 min, draining the liquid, and holding the warmed sausage on a warming tray in covered plates for no longer than 30 min. Then, three slices of each treatment were presented to the panelists in monadic form, randomly coded with three digits. In the same sheet, the panelists recorded their purchase intention using a 5-point scale, where 5 = certainly would purchase, 4 = probably would purchase, 3 = perhaps would purchase / perhaps would not purchase, 2 = probably would not purchase and 1 = certainly would not purchase, which was expressed as the percentage of total score (*Cavenaghi-Altemio et al.*, 2018). The acceptability 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 AI was greater than 70% (*Stone and Sidel*, 2004).

Statistical analysis

Results were statistically evaluated using analysis of variance (ANOVA) and Tukey's test for comparison of means, at a level of 5% significance, using the software Statistica 7.0. The sensory attributes and the purchase intention were analyzed as percentages.

Results and Discussion

Microbiological analyses

Microbiological evaluations were conducted to ensure the safety of the raw materials and the efficiency of the sausage preparation processes. The microbiological results were within the limits established by Brazilian legislation (Table 1), which require the absence of *Salmonella* spp. in 25 g, and maximum counts of 3×10^3 CFU g^{-1} for *Staphylococcus aureus* and 5×10^3 CFU g^{-1} for coliforms at 45°C (*ANVISA*, 2001). These criteria are in accordance with the U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, which established a zero tolerance for *Salmonella* spp. and a limit of 10^4 CFU/g for *Staphylococcus aureus* in fish and fishery products (*FDA*, 2020). There was no clear relationship among the microbial microbiological results and the different treatments.

Chemical analyses

Proximate compositions obtained for sausages obtained from Nile tilapia MSM, elaborated using different homogenizing and refining processes are presented in Table 1. These values are very close

Table 1. Proximate composition and microbiological status of sausages prepared with mechanically separated Nile tilapia (*Oreochromis niloticus*) meat according to treatments T1, T2, and T3.

Treatment	Processing	Proximate composition (%)					Microbiological analyses (CFU/g)		
		Moisture	Protein	Lipids	Ash	Carbohydrates	TTC	CPS	*Salmonella* spp.
T1	grinder/cutter	60.66^a ± 2.37	15.91^a ± 1.70	12.29^a ± 1.23	1.83^a ± 0.17	9.31	<1×10^2	<1×10^3	Absent in 25g
T2	grinder/mixer	68.73^a ± 5.09	15.67^a ± 0.09	11.35^a ± 0.53	2.48^b ± 0.49	1.77	<1×10^2	<1×10^3	Absent in 25g
T3	Grinder	60.64^a ± 11.75	15.08^a ± 0.22	9.61^a ± 2.47	2.73^b ± 0.13	11.94	4,5×10^2	<1×10^3	Absent in 25g

Legend: Means with the same letter in the same column do not differ statistically at 5% (P>0.05). TTC: thermotolerant coliforms; PCS: coagulase-positive staphylococci bacteria; CFU: counting forming units

to those already reported for sausages from Nile tilapia MSM (*Uyhara et al.*, 2008; *Oliveira Filho et al.*, 2010; *Mélo et al.*, 2011).

Moisture, protein, and lipids did not differ between the three treatments (P<0.05). Ash was the unique parameter that differed significantly between the treatments (P>0.05).

The moisture content of the sausages (Table 1), on average, met the required level for identity and quality of sausages (maximum 65% moisture) (*MAPA*, 2000). Previously, an average moisture content of 70.75 % and range from 69.21 to 70.35% were reported in the literature for Nile tilapia MSM sausages, without significant differences in relation to the amount of MSM in the sausages (*Oliveira Filho et al.*, 2010), or to the nature of added colorant (*Uyhara et al.*, 2008), respectively.

The protein content ranged from 15.08% (T3) to 15.91% (T1) (Table 1). According to the Brazilian legislation, the minimum protein content in commercial meat products containing MSM is 12% (*MAPA*, 2000), and products containing lower protein levels can be considered as out of specification or even fraudulent. The prepared sausages contained superior protein levels and were considered satisfactory. Literature reported protein content in similar sausages ranged from 18.40 to 19.84% (*Uyhara et al.*, 2008), with average protein content of 13.02% (*Mélo et al.*, 2011), and 15.26% and 20.86% in Nile tilapia sausages containing 0% MSM and 100% MSM, respectively (*Oliveira Filho et al.*, 2010).

The average content of lipids found in the current study ranged from 9.61% (T3) to 12.29% (T1) (Table 1), which were also within the limits determined by Brazilian legislation (*MAPA*, 2000) that requires a maximum of 30% lipids. Lipid contents of 3.45% (*Uyhara et al.*, 2008) and 11.03% (*Mélo*

et al., 2011) were measured in sausage formulations containing Nile tilapia MSM, and 0% and 8.18% in Nile tilapia sausages containing 0% MSM and 100% MSM, respectively (*Oliveira Filho et al.*, 2010).

It was reported elsewhere that differences in composition could be related to differences in the raw materials and/or in the ingredients (*Cortez-Vega et al.*, 2013; *Cavenaghi-Altemio et al.*, 2018). This could explain the differences observed in relation to the results obtained by other authors. *Bordignon et al.* (2010) stated that MSM extracted from the abdominal muscle that is close to the cartilage of Nile tilapia has a high fat content. *Rebouças et al.* (2012) reported that the lipid content in the fish is very variable, depending on the species, age, body region, sexual cycle, and diet.

However, our present study utilized the same additives and condiments, and the Nile tilapia MSM was from the same batch; moreover, all ingredients were used at the same concentrations for the three treatments. On the other hand, the processing conditions might also affect the results (*Cortez-Vega et al.*, 2013), but statistical differences were not observed between the treatments in our study. This could affirm that utilization of grinder and cutter, grinder and mixer, or only grinder did not affect the sausage composition, so our particular types of sausage processing did not influence the characteristics of the MSM (*Mielnik et al.*, 2002), although our procedures could have altered the structure of biomolecules.

The average ash contents of the sausages were 1.83%, 2.48% and 2.73% for treatments T1, T2 and T3, respectively (Table 1). The ash content of T1 sausages differed from that of T2 and T3 sausages ($P > 0.05$). It is hard to explain how ash content could differ in the three treatments. However, the ash content found was more or less in accordance

Table 2. Instrumental color, shear force, and sensory analysis of sausages prepared with mechanically separated Nile tilapia (*Oreochromis niloticus*) meat according to treatments T1, T2, and T3.

Treatment	Instrumental color			Shear force (N)	Sensory analysis				
	L*	a*	b*		Color	Taste	Texture	Odor	OA
T1	57.28[a] ± 0.98	8.75[b] ± 0.22	8.56[a] ± 0.26	2.04[b] ± 0.36	5.47[a] ± 0.37 (60.8)	5.56[a] ± 1.02 (61.8)	6.36[a] ± 0.45 (70.7)	6.06[ab] ± 0.75 (67.3)	5.60[a] ± 0.28 (62.2)
T2	45.54[b] ± 2.62	14.31[a] ± 1.43	5.30[c] ± 0.69	2.71[a] ± 0.47	5.84[a] ± 0.11 (73)	5.84[a] ± 1.30 (73.0)	5.82[a] ± 1.28 (73.2)	6.24[a] ± 1.58 (78.0)	5.84[a] ± 1.30 (73.0)
T3	46.28[b] ± 0.78	14.94[a] ± 1.00	6.26[b] ± 0.76	1.77[b] ± 0.17	5.30[a] ± 0.91 (66.2)	4.74[b] ± 1.23 (59.2)	4.80[b] ± 0.14 (60.0)	5.50[b] ± 1.06 (68.7)	4.80[b] ± 1.27 (60.0)

Legend: T1, T2, and T3 treatments (see footnote to Table 1). L*: Lightness; a*: Redness, b*: Yellowness, OA: Overall acceptability. Means with the same letter in the same column do not differ statistically at 5% (P>0.05). Values in parenthesis are the acceptability index (%).

with the refined sodium mineral added in the form of NaCl (*Nowsad et al.*, 2000). Average ash contents of 3.40% and ranging from 3.2% to 3.7% were measured for Nile tilapia MSM sausages, without significant differences in relation to the inclusion of MSM (*Oliveira Filho et al.*, 2010) or the nature of added colorant (*Uyhara et al.*, 2008), respectively, and 1.08% in a Nile tilapia MSM emulsified-type sausage (*Mélo et al.*, 2011). These high ash levels in Nile tilapia sausages were due to the added curing salts that raised the mineral content (*Cavenaghi-Altemio et al.*, 2018; *Nascimento et al.*, 2017).

Physical analyses

Table 2 shows the results of instrumental color and shear force tests obtained for the Nile tilapia MSM sausages prepared using different homogenizing and refining processes.

Instrumental color

The values of L* (lightness), a* (redness), b* (yellowness) and W (whiteness) were significantly different (P>0.05) in all three treatments. When both grinder and cutter were utilized, sausages were lighter (P>0.05) but less red (P>0.05). Yellowness differed significantly (P>0.05) between the different treatments (Table 2).

The highest lightness value found was 57.28, for sausage elaborated by the conventional method (grinder and cutter). This value is comparable to that reported elsewhere (67.12) for sausage prepared from Nile tilapia MSM (*Lago et al.*, 2018). These differences in lightness could be related to different levels of incorporation of pigment from the remaining fins and skins present on the carcass frames during the passage of the residues by the meat and bone separator (*Uyhara et al.*, 2008). However, the significant difference (P>0.05) observed in the present study for all color parameters of T1 in relation to treatments T2 and T3 could be the result of some synergistic effect on meat rebinding during gelling, improving lightness and yellowness and reducing redness.

Shear force

The shear force differed among treatments T1, T2, and T3 (P>0.05) (Table 2). The obtained average shear forces were 2.04 N (T1), 2.71 N (T2) and 1.77 N (T3)). Similar results were obtained for Nile tilapia sausages without (2.02 N) and with 0.6% (2.95 N) of added transglutaminase (*Cavenaghi-Altemio et al.*, 2018).

Rupture force was reported to significantly correlate with the protein composition in cooked sausages, which suggests the increased gel rupture force is most likely due to the functional performance of the protein type rather than the protein content (*Wang and Xiong*, 1999). Despite the prepared sausages having the same composition, significant differences were obtained among the treatments (P>0.05), which could result from the different processes to which the fish meat (the main component) and the other ingredients were subjected. Therefore, we suggest the processing with different equipment could have differently altered the protein structure of the fish meat, by rupturing it at different levels. Moreover, the remaining protein structures have to be gelled to develop a proper sausage texture (*Jaczynski & Park*, 2003). The results indicate the combination of processing methods could have favored the disruption of the structures, and consequently the gelling during pasteurization, which could be beneficial to the firmness of the product. For example, despite the sausages having the same protein content, a greater exposure of functional groups in myofibrillar proteins would favor cross-linking interactions between –SH groups and the formation of S-S bonds (*Moosavi–Nasab et al.*, 2019).

Sensory analysis

The results of sensory analysis of the Nile tilapia MSM sausages are shown in Table 2. There was no significant difference in color scores between the three treatments (P<0.05). The mean color scores ranged from "like moderately" to "like very much".

The odor scores did not differ between treatments T1 and T2, or between T1 and T3, but the odor score of T3 differed significantly (P>0.05) from that of T2.

The texture score did not differ between treatments T1 and T2, but that of T3 differed significantly (P>0.05) from those of T1 and T2. This indicates the grinder itself does not favor good homogenization, and consequently, a more uniform texture was achieved when the grinder was combined with the cutter (T1) or the mixer (T2). With the continuous mechanical action, the released compounds can react with each other, forming new structures, so the meat and fat particles, or their mixtures, adhere to each other due to the force of mixture.

The taste scores for T3 sausages differed significantly (P>0.05) from those for T1 and T2 sausages (Table 2). This could also be explained by the sole utilization of the grinder to prepare T3 sausages.

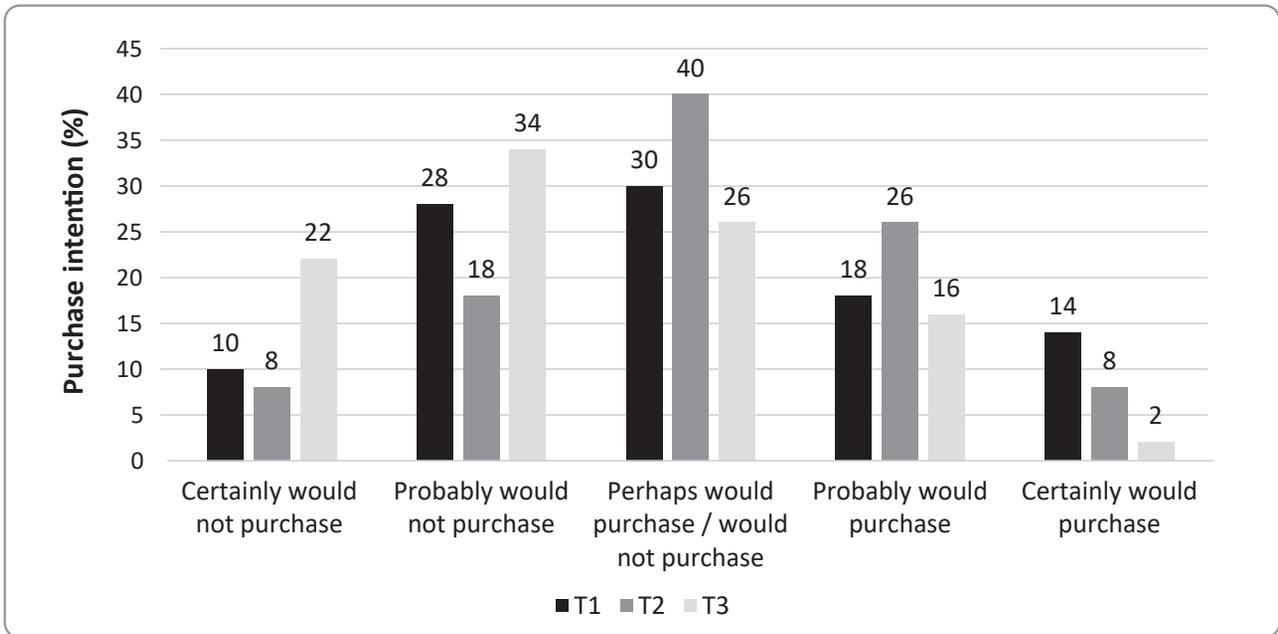**Figure 1.** Panelists' purchase intentions for Nile tilapia MSM sausages prepared using different treatments (T1: grinder and cutter; T2: grinder and mixer; T3: grinder).

Grinding uses mechanical energy to disorganize the tissue structure, leading to the formation of grains composed of more or less intact cells. However, the amounts of lipid and proteinaceous compounds obtained are scarce at this milling stage (*Cenci et al.*, 2018).

According to the hedonic sensory analysis test, T1 and T2 sausages received grades close to 6 (like slightly). The highest average AI was 72.2% for the T2 sausages. According to *Stone and Sidel* (2004), when the AI is equal to or greater than 70%, the product is considered accepted. Thus, only our T2 sausages were acceptable to the panelists.

When the panelists were asked about their intention to purchase sausages, for T2 sausages, 26.0% "probably would purchase", while 40.0% "perhaps would purchase, perhaps would not purchase", which were higher percentages than for T1 and T3. However, the highest score for "certainly would purchase" was obtained for T1 sausages (14.0%) (Figure 1). These results could be related to the greater consumption of red meat than fish derivative

products in the region where the research was conducted.

Rejection rates ("certainly would not purchase") around 10% and 8% were obtained for T1 and T2, respectively. T3 sausages received a much higher rejection rate of 22% (Figure 1), which is consequence of this sausage receiving the lowest scores for the texture and taste (Table 2), as already discussed.

Conclusion

In conclusion, it is feasible to produce sausages from Nile tilapia MSM using the grinder and mixer for homogenizing and refining (treatment 2), which would be suitable production means for small manufacturers. Considering that this product would be accepted on the market if made commercially available, sausage production using this basic equipment is an opportunity that could be exploited by the fish industry to augment the consumption of lower-cost Nile tilapia meat products.

Ocena kobasica dobijenih od mehanički odvojenog mesa nilske tilapije (*Oreochromis niloticus*) i pripremljenih različitim postupcima homogenizacije i prerade

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A p s t r a k t : *Posle filetiranja nilske tilapije (Oreochromis niloticus), neiskorišćeni delovi se mogu preraditi kako bi se dobio ko-produkt, mehanički odvojeno meso (MSM). Ciljevi ovog istraživanja su bili korišćenje različitih postupaka za homogenizaciju i preradu kobasica od mehanički odvojenog mesa nilske tilapije i ocena kuvanih kobasica u pogledu njihovih mikrobioloških, fizičkih, hemijskih i senzornih karakteristika. Sastojci su obrađeni prema tri tretmana: (T1) korišćenjem mašine za mlevenje mesa i rezača/kutera, (T2) korišćenjem mašine za mlevenje mesa i mešalice/miksera i (T3) samo korišćenjem mašine za mlevenje mesa. Sadržaj proteina kretao se od 15,08% (T3) do 15,91% (T1), lipida od 9,61% (T3) do 12,29% (T1), a pepela od 1,83% (T1) do 2,73% (T3). Najviša ocena za boju bila je 57,28, za kobasicu izrađenu konvencionalnom metodom (T1). Dobijene sile presecanja bile su 2,04 N (T1), 2,71 N (T2) i 1,77 N (T3). Samo kobasice T2 su dobile indeks prihvatljivosti veći od 70%. Kobasice T2 su bile ocenjene od strane 34% učesnika u panel diskusiji kao proizvod koji bi „sigurno kupili" ili „verovatno bi kupili". Zaključno, moguće je proizvesti kobasice od mehanički odvojenog mesa nilske tilapije upotrebom mašine za mlevenje mesa i mešalice/miksera za homogenizaciju i preradu, što bi bila dobra alternativna metoda uglavnom za male proizvođače.*

Ključne reči: *riba, meso ribe, kvalitet, senzorna analiza, metode prerade.*

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