Revista Ciência Agronômica, v. 51, n. 1, e20165496, 2020
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Stability of snacks made with minced Nile tilapia stored at room temperature[1]

Estabilidade de *snacks* elaborados com minced de tilapia do Nilo armazenados a temperatura ambiente

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ABSTRACT - Minced fish can be used in the preparation of fish products, despite being very susceptible to lipid oxidation and microbial growth. Fish snacks are widely appreciated in Asian countries, but still little studied in Brazil. Thus, the aim of this study was to produce snacks containing 20, 30, and 40% minced fish of Nile tilapia filleting waste and evaluate their stability at time zero and throughout 15, 30, and 45 days of storage at room temperature (25 °C) by physicochemical, microbiological, and sensory characterization. The inclusion of minced fish decreased ($P < 0.05$) the volatile nitrogenous base levels (0.71 to 0.56 mg $N.100g^{-1}$) and increased pH (5.45 to 5.90) of the snacks. An increase ($P < 0.05$) in lipid oxidation (1.10 to 2.03 mg. malonaldehyde. kg^{-1}) and pH (5.57 to 5.73) was observed during storage. Lower microbial counts were observed, which were within the limit allowed by the Brazilian legislation. Sensory acceptance of the attributes flavor, color, texture, global acceptance, and purchase intent of the snacks decreased with increasing the inclusion of minced fish, but remained with scores above 5, which is equivalent to "*not liked nor disliked*". During 45 days of storage, the sensory properties of the snacks remained stable with good acceptance scores. Therefore, fish snacks can be made with the addition of 20 to 40% of minced fish of Nile tilapia filleting waste without compromising their sensory acceptance and physicochemical and microbiological stability during 45 days of storage at 25 °C.

Key words: Shelf life. Fish snack. Preservation. Lipid oxidation.

RESUMO - O *minced fish* pode ser utilizado na elaboração de produtos de pescado, apesar de ser muito susceptível a oxidação lipídica e desenvolvimento microbiano. Os *snacks* de pescado são alimentos muito apreciados em países asiáticos, porém ainda pouco estudados no Brasil. Portanto, o objetivo do presente estudo foi elaborar *snacks* com 20, 30 e 40% de *minced* de resíduos de filetagem de tilápia do Nilo e avaliar a estabilidade aos zero, 15, 30 e 45 dias de armazenagem a temperatura ambiente (25 ºC), por meio de análises físico-químicas, microbiológicas e sensorial. A inclusão de *minced fish* diminuiu ($P<0,05$) os valores de bases nitrogenadas voláteis (0,71 a 0,56 mg $N.100g^{-1}$) e aumentou o pH (5,45 a 5,90) dos *snacks*. Durante a armazenagem, houve aumento ($P<0,05$) nos valores de oxidação lipídica (1,10 a 2,03 mg. malonaldeído.kg^{-1}) e pH (5,57 a 5,73) dos *snacks*. A contagem microbiana dos *snacks* foi baixa e estiveram dentro do limite permitido pela legislação Brasileira. A aceitação sensorial de sabor, cor, textura, aceitação global e intenção de compra dos *snacks* foi diminuindo com o aumento da inclusão de *minced fish*, porém permaneceram com notas acima de 5, que equivale a "não gostei nem desgostei". Durante a armazenagem de até 45 dias, os atributos sensoriais dos *snacks* permaneceram praticamente estáveis e com boa aceitação. Portanto, os *snacks* podem ser elaborados com inclusão de 20 a 40% de *minced* de resíduos de filetagem de tilápia do Nilo sem comprometer sua aceitação sensorial e estabilidade físico-química e microbiológica durante 45 dias de armazenagem a 25 °C.

Palavra-chave: Vida útil. Biscoito de pescado. Conservação. Oxidação lipídica.

DOI: 10.5935/1806-6690.20200002
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Received for publication 19/12/2016; approved on 05/08/2019
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INTRODUCTION

Snacks made with fish meat are known internationally as fish crackers, fish galletas, keropok, kerupuk, kaew krab pla, and banh tom phong and widespread in Asian countries like Malaysia, Indonesia, Thailand and Vietnam (HUDA; BONI; NORYATI, 2009). Fish snack production is performed by a homogeneous mixture of the ingredients, followed by molding and baking in water or steam (NURUL; BONI; NORYATI, 2009; TONGDANG; MEENUM; CHAINUI, 2008). In the cooking step, starch is gelatinized, causing swelling of the granules and leaching of polysaccharides, leading to higher solubility (NEIVA *et al*., 2011; WANG; ZHANG; MUJUMDAR, 2012).

For being a healthy food (rich in protein, fatty acids, minerals) fish can be used in the enrichment of high-energy products such as snacks. However, the inclusion of large amounts of fish meat can affect the product quality. During storage, the quality of snacks can also decrease due to several factors, including fat content, moisture, temperature and light, and lipid oxidation, while high moisture contents can negatively affect flavor, odor, and texture (SHAVIKLO *et al*., 2011).

Minced fish (MF) is a product obtained from a single fish species or mixture of fish species with similar sensory characteristics using a mechanized process to separate the edible parts, producing skeletal muscle particles absent of viscera, scales, bones, and skin (DURÃES *et al*., 2012; KIRSCHNIK; MACEDO-VIEGAS, 2009). This raw material is used in the preparation of fish products, although it is susceptible to lipid oxidation and microbial growth. In Brazil, the Ministry of Agriculture and Supply (BRASIL, 2000) states that mechanically separated meat (MSM) is obtained by a mechanical separation and grinding of animal bones from the butcher for the preparation of specific meat products. However, there are few studies evaluating the shelf life of snacks made with minced fish. Snacks made with corn starch and *Oncorhynchus mykiss*, *Hypophthalmichthys molitrix, Pollachius virens* meat showed good microbial stability, despite the changes in flavor and odor observed during storage for up to 6 months at 27 ± 2 °C, probably due to increased lipid oxidation (SHAVIKLO *et al*., 2011).

A previous study evaluated the physicochemical aspects and the sensory acceptance of snacks containing different levels (20 to 40%) of minced fish of Nile tilapia filleting waste (CORTEZ NETTO *et al*., 2014). It was observed that the addition of up to 40% of minced fish improved crude protein and minerals levels while maintaining the sensory acceptance above the threshold sensation (score 5 - neither liked /nor disliked). However, no study has assessed the microbiological, physicochemical, and sensory changes of snacks made with minced Nile tilapia during storage at room temperature. Therefore, the objective of this study was to evaluate the stability of snacks made with different percentages (20, 30, and 40%) of minced fish of Nile tilapia filleting waste, and to assess the physicochemical and microbiological changes, and the sensory acceptance throughout 45 days of storage at 25 ± 0.3 °C.

MATERIAL AND METHODS

Approximately 150 kg of Nile tilapia (*Oreochromis niloticus*) filleting waste were used, composed of the backbone, with no head, guts, and fins, obtained in fish market Royal Fish, Sumaré - SP. The cooled waste was transported to the Laboratory of Aquatic Products Processing, Campus Fernando Costa-USP in Pirassununga-SP. Fish filleting waste was washed with chlorinated water (5 ppm), headed and gutted, excess fat was removed, and the minced fish was again subjected to washing step. After cleaning, it was processed in a meat/bone separator with an endless worm gear (HT 250, High-Tech®). The minced fish with approximately 59% yield was mixed manually with 0.5% antioxidant sodium erythorbate (Doremus Alimentos Ltda, Guarulhos/SP, Brazil). Then, it was packaged in plastic bags (500g) and frozen in a ultra-fast plate freezer (UCE - 20, Eco®) until the internal temperature reaches approximately -40 °C and stored at -18 °C until the snack preparation (approximately one month).

The formulations were calculated by varying the minced fish/ starch ratios (60% cassava starch and 40% corn flour), as follows: Treatment 1 - 20% minced fish and 80% starch; Treatment 2 - 30% minced fish and 70% starch; and Treatment 3 - 40% minced fish and 60% starch. All treatments contained the same salt concentration, 2%. The water content was standardized as 60% in the formulation with the lowest level of minced fish inclusion (20% minced fish and 80% starch). For the other formulations, water content was adjusted according to the moisture content of the ingredients (53.79% and 47.58% for the formulations with 30 and 40% minced fish and 70 and 60% starch, respectively). Raw corn flour (Yoki Alimentos, Cambará/PR, Brazil) was mixed with total water of the formulation and cooked to obtain a firm and homogeneous dough. After cooling, the cooked corn flour was manually mixed with the other ingredients: minced fish, salt, and sour starch (Kisabor, Certa Ind Com de Alimentos Ltda, Guarulhos/SP, Brazil). After mixing, a cylindrical dough of approximately 45 g and 10 cm long without casing was obtained using a meat grinder (CAF® Standard) coupled with a sausage stuffer equipment.

The dough was cooked in boiling water (100 °C) for 10 minutes, cooled in ice-water, dried with the aid of cotton cloth, and then stored at 7 °C until achieving uniform drying and allow slicing. The formulations containing 20% and 30% minced fish remained approximately 36h in the refrigerator, while the treatment with 40% minced fish remained under refrigerated storage for 60h. After this step, the dough was sliced (1.5mm thick) using a meat slicer (Gural®), and the slices were dried in an oven (Fanem®) at 55 °C for 2 hours for treatments with 20% and 30% minced fish, and 2.5h for the treatment with 40% minced fish, aimed to achieve similar visual drying when compared to the other treatments. Then, the dough was cooled at room temperature, wrapped in plastic bags (300g) and stored at 25 ± 0.3 °C until analysis.

The total volatile nitrogenous bases (TVB-N) were determined in triplicate in non-fried snacks for each storage time (0, 15, 30, 45 days) according to Howgate (1976). The results were expressed as mg N.100 g^{-1}.

Lipid oxidation was determined in non-fried snacks for each storage time (0, 15, 30, 45 days) by thiobarbituric acid reactive substances (TBARS) assay, in triplicate according to Vyncke (1970). The results were calculated according to a standard curve and expressed as mg malondialdehyde kg^{-1} sample.

The pH was determined in triplicate in the non-fried snack at each storage time (0, 15, 30, 45 days) using an immersion electrode (HI 221, Hanna Instruments®). For that, 10g sample was ground in a food processor and homogenized (grinder, Nova Tecnica®) with 40 mL distilled water.

Salmonella spp counts, coagulase positive *Staphylococcus*, and total and fecal coliform bacteria were enumerated in 25g of non-fried snack for each treatment at 0, 15, 30, and 45 days of storage at 25 ± 0.3 ºC according to American Public Health Association (1992). For coagulase positive *Staphylococcus*, 0.1 mL aliquots of the dilutions were transferred to Petri dishes containing Baird-Parker agar supplemented with egg yolk emulsion. The plates were incubated in an inverted position in the oven for 48 hours at 35 ºC. *Salmonella* sp counts were determined using a kit (VIP Salmonella, Biocontrol®) with 0.1 mL sample incubated at room temperature for 10 min. The formation of a line in the control window (small) corresponded to a valid test, the line in the result window (large) indicated presumptive positive sample, and the absence line indicated negative sample.

Sensory acceptance test was performed in the sensory analysis laboratory, equipped with individual cabins with white fluorescent light. Affective acceptance testing was used in a balanced complete block with 60 untrained panelists of both sexes, each one representing a block in each storage time (0, 15, 30, and 45 days) according to Meilgaard, Civille and Carr (1999). Each assessor received 5 samples of each treatment, coded with a three-digit random number in balanced order. The attributes flavor, texture, color, odor, and overall acceptance were assessed using a 9-point hedonic scale (1 - very much disliked; 9 - liked very much). For sensory evaluation, the snacks from each sampling time (0, 15, 30, and 45 days were deep fried in oil at 200 °C for 4 seconds and served with water at room temperature. The study was previously approved by the Research Ethics Committee of the University of São Paulo Nursing School (CEP / EEUSP), Process n. 937/2010.

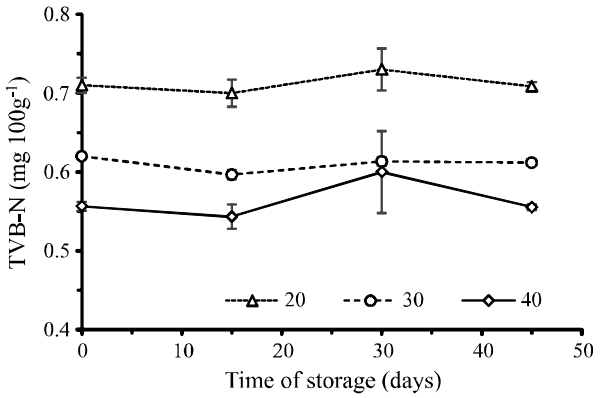
The experimental design was completely randomized with three treatments (20/80, 30/70, and 40/60 of minced fish/starch ratio) at time intervals (0, 15, 30, and 45 days). First, the sphericity on the variance-covariance matrix between times was performed. In the case of rejection of the sphericity assumption, the covariance structure that best fit the data was considered. Data were analyzed by ANOVA and significant difference ($P<0.05$) was analyzed by Tukey's test to verify the sources of variation *time and minced fish/starch*. All analyses were performed with the help of SAS statistical software version 9.1.3.

RESULTS AND DISCUSSION

The TVB-N values of the snacks decreased ($P<0.01$) with the increase in minced fish percentages, with mean values ranging from 0.71 to 0.56 mg N.100 g^{-1}, corresponding to the formulations containing 20% and 40% minced fish, respectively (Figure 1). No significant difference ($P>0.05$) was observed for the TVB-N values within 45 days of storage at 25 °C, with mean values ranging from 0.61 to 0.65 mg N.100 g^{-1} after 15 and 30 days of storage, respectively.

The decrease in TVB-N values of the snacks with increased minced fish can be due to cooking and drying processes, leading to different levels of TVB-N volatilization. The stability of the TVB-N values during storage may be due to little moisture and adequate storage conditions. In a previous study, a decrease from 9.71 to 7.46% moisture was observed in snacks with 20 to 40% minced fish, respectively (CORTEZ NETTO *et al*., 2014). Snacks with minced *Menticirrhus americanus* and *Umbrina coroides* also showed low moisture (3.12%) (NEIVA *et al*., 2011). The TVB-N values in snacks containing minced fish were lower than those found in other fish, including fish balls (8.13 - 32.21 mg N.100 g^{-1}) made with minced *Merlangius merlangus euxinus* (BORAN; KÖSE, 2007), *keropok lekor* (4.68 - 7.29 mg N.100 g^{-1}) (NOR-

Figure 1 - Mean TVB-N values of snacks containing 20 to 40% minced fish of Nile tilapia filleting waste stored for 45 days at 25 ± 0.3 °C. Vertical bars represent the standard deviation of average

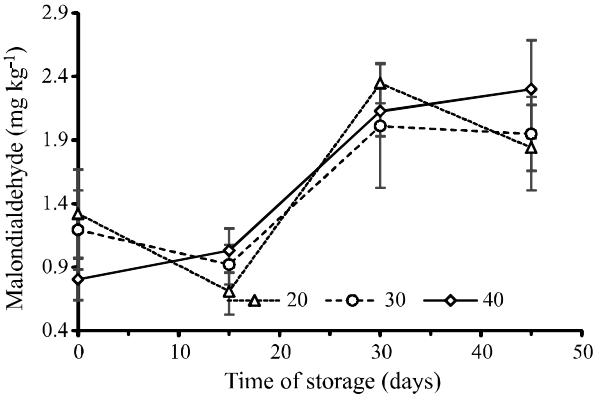


KHAIZURA *et al*., 2009) and snacks made with minced *Menticirrhus americanus* and *Umbrina coroides* (NEIVA *et al*., 2011) (1.54 to 18.16 mg N.100 g^{-1}).

An increase (P <0.01) in lipid oxidation was observed in the snacks during storage, from 1.10 (average value for day 0) to 2.03 mg malondialdehyde. kg^{-1} (average value for day 45) (Figure 2), with no significant difference (P>0.05) among the different minced fish levels. Similar values (1.19 to 1.41 mg malondialdehyde kg^{-1}) were observed in snacks made with minced *Menticirrhus americanus* and *Umbrina coroides* throughout 180 days of storage at 25 °C (NEIVA *et al*., 2011).

The process of obtaining minced fish can cause disruption of muscle membranes, allowing the interaction of oxidizing agents with polyunsaturated fatty acids resulting in the propagation of oxidative reactions

Figure 2 - Mean values of lipid oxidation - TBARS in snacks formulated with 20 to 40% minced fish of Nile tilapia filleting waste stored for 45 days at 25 ± 0.3 °C. Vertical bars represent the standard deviation of average

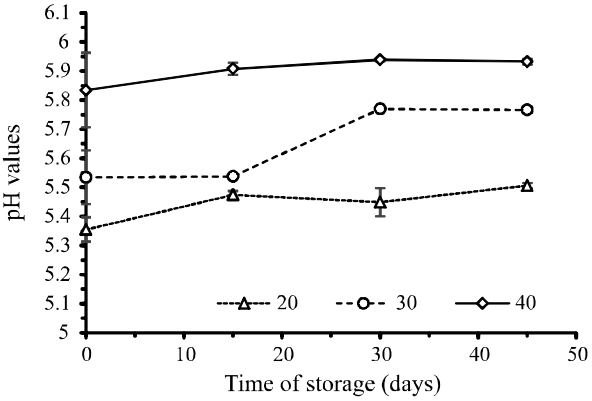


(ESTÉVEZ; VENTANAS; CAVA, 2007; OLIVEIRA FILHO *et al*., 2010), which may explain the increase in TBARS during 45 days of storage at 25 ± 0.3 °C. In addition, minced fish used in the preparation of snacks showed about 10% ether extract (CORTEZ NETTO *et al*., 2014), explaining the high susceptibility of snacks to lipid oxidation. The high concentration of polyunsaturated fatty acids in fish meat makes the product susceptible to lipid oxidation, which may lead to the formation of off-flavors during storage (JENSEN; RISBO, 2007).

Sausages made with minced fish of Nile tilapia filleting waste also exhibited an increase in TBARS values during storage (OLIVEIRA FILHO *et al*., 2010). Snacks made with corn starch and minced *Oncorhynchus mykiss*, *Hypophthalmichthys molitrix*, *Pollachius virens* fish species, stored at 27 °C for 6 months showed higher lipid oxidation (SHAVIKLO *et al*., 2011). However, the Brazilian legislation has not established maximum lipid oxidation values in fish and fish products.

The pH of the snacks increased (P<0.01) with the increase in minced fish concentration, with mean values ranging from 5.45 and 5.90 for the formulations with the addition of 20% and 40% minced fish, respectively. The storage time also affected the pH of the snacks, with mean values ranging from 5.57 to 5.73, corresponding to day 0 and after 45 days of storage, respectively (Figure 3).

Figure 3 - Average pH values of snacks containing 20 to 40% inclusion of minced fish of Nile tilapia filleting waste and stored for 45 days at 25 ± 0.3 °C. Vertical bars represent the standard deviation of average

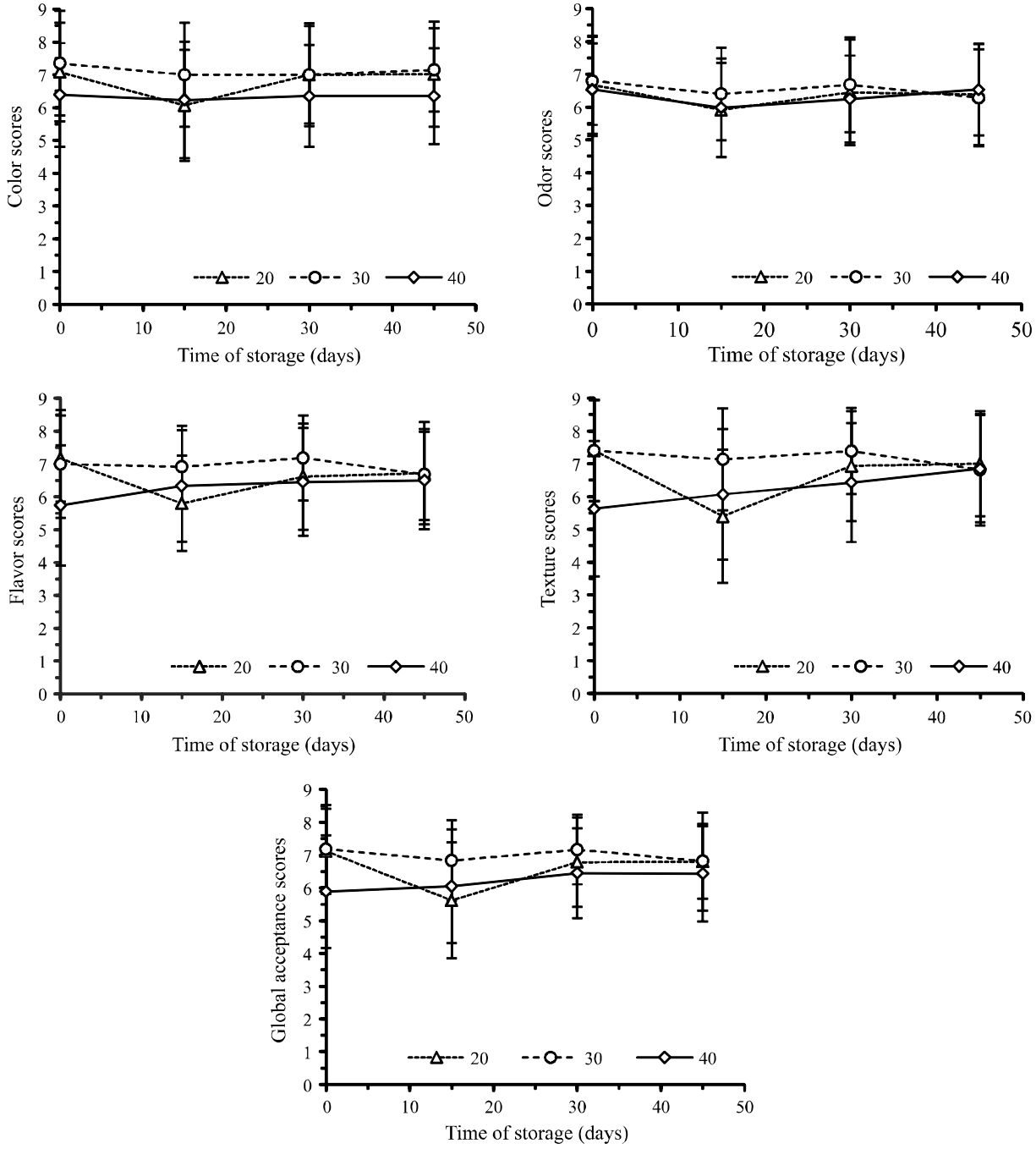


The pH behavior of minced fish during storage depends on the temperature, salt composition, buffering capacity of proteins, and enzymatic activity (KIRSCHNIK; MACEDO-VIEGAS, 2009). The pH values of fish snacks are below those found in Keropok lekor (6.61 to 6.87) (NOR-KHAIZURA *et al*., 2009), sardine patties

(6.20 to 6.48) (KILINC; CAKLI; TOLASA, 2008) and *Menticirrhus americanus* and *Umbrina coroides* snacks (6.88 to 6.90) (NEIVA *et al*., 2011).

During 45 days of storage, the presence of *Salmonella* sp. was not detected, and total coliform bacteria counts were lower than 3.0 CFU.g^{-1} sample, without the presence of fecal coliform bacteria. The coagulase positive *Staphylococcus* counts were less than 10 CFU.g^{-1} sample in all treatments. In general, the absence of pathogenic bacteria confirms good manufacturing practices and adequate storage. Similar results were observed by Shaviklo *et al*. (2011) for *Salmonella* in fish snacks made with *Pollachius virens*, confirming the safety of this type of product for consumers.

Figure 4 - Sensory scores for color, odor, flavor texture and global acceptance of snacks made with 20 to 40% minced fish of Nile tilapia filleting waste stored for 45 days at 25 ± 0.3 °C. Vertical bars represent the standard deviation of average



According to Brazilian law, *Salmonella* must be absent in a portion of 25g food made with fish meat, with a maximum limit of 2×10^2 g^{-1} coliform bacteria at 45 °C, and 5×10^2 coagulase positive *Staphylococcus* (BRASIL, 2001). Therefore, the present results have proven that the minced fish snacks were fit to the consumption during 45 days of storage at 25 ± 0.3 °C.

Differences ($P<0.01$) were observed for the sensory attributes flavor, color, texture, and overall acceptance of the samples containing 20 to 40% of minced tilapia filleting waste (Figure 4), with a better acceptance for the treatment containing 30% minced fish. However, all formulations presented a fairly narrow range of the score scale, ranging from 6.3-7.1; 6.3-6.9; 6.2-7.2; and 6.2-7.0, for the attributes color, flavor, texture, and overall acceptance, respectively. For the attribute odor, no significant differences were observed in acceptance ($P>0.05$) of snacks with different minced fish inclusion, which values ranged between 6.4 to 6.5.

Differences were also observed for color, odor, texture, and overall acceptance within 45 days of storage at 25 ± 0.3 °C. The higher differences ($P<0.01$) were found for flavor, texture and global acceptance among snacks made with 40% minced fish and the other formulations at time 0. However, again, the score range was very narrow, indicating that the acceptance remained stable during the 45 days of storage. Stability of scores was observed for the attribute flavor, which was not significantly different ($P>0.05$) throughout the 45-day storage time, with values between 6.3 to 6.7.

In general, for all attributes evaluated, the treatments with 20 to 40% inclusion of minced fish exhibited sensory scores above 5 ("did not like - or dislike"), showing it is possible to use this percentage inclusion in the formulations, without compromising the sensory acceptance. The maximum inclusion of minced fish in snacks in this study is higher than snacks containing 9% *Pollachius virens* flour (SHAVIKLO *et al*., 2011).

Darkening of snacks was observed during oven drying and after frying with increasing the inclusion of minced fish. This result was probably due to the Maillard reaction, leading to a lower acceptance of some attributes, as observed in color scores for snacks with 40% minced fish (Figure 4). Regarding the attribute odor, snacks with 40% minced fish were well accepted (scores above 5). Snacks made with *Oncorhynchus mykiss*, *Hypophthalmichthys molitrix*, and *Pollachius virens* meat showed changes in odor during 6 months of storage at 27 °C (SHAVIKLO *et al*., 2011).

Although lipid oxidation has been developed in the snacks, the panel were not able to identify the sensory degradation of the attributes color, odor, texture, and overall acceptance, which were stable in the consumers' opinion.

CONCLUSION

Snacks can be made with the inclusion of 20 to 40% of minced fish of Nile tilapia filleting waste without compromising the sensory acceptance and chemical and microbiological stability of the product during 45 days of storage at 25 ± 0.3 °C.

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