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Storage of vacuum-packaged smoked bologna sausage prepared from Nile tilapia

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ABSTRACT. Changes in smoked bologna sausage manufactured with Nile tilapia (*Oreochromis niloticus*), and wheat fibers in refrigerated storage (6 ± 2°C) during 30 days were evaluated. Minced fish (MF) obtained from waste (parings from the filleting fish operations) of Nile tilapia (*Oreochromis niloticus*) was used as raw material. In this experiment, were determined *Staphylococcus* count, the detection of *Salmonella* sp. (day 0), psychrotrophic count, total and fecal coliforms at 45°C, the acceptance sensory test (days 0 and 30), water activity, instrumental color, pH and lipid oxidation analysis (Thiobarbituric acid reactive substances - TBARS) days 0, 5, 10, 15, 20, 25 and 30 of storage. Lipid oxidation (TBARS) and water activity provided significant differences among storage days. However it was concluded that the bologna sausage remained stable and with acceptable quality for 30 days of storage may be a new alternative to adding value to fish products, because the researched microorganisms had a low count, changes in pH were not significant and sensory test indicated good acceptance of the product during the 30 days of storage.

Keywords: fish products, lipid oxidation, sensory test, refrigerated temperature.

Alteração durante o armazenamento de mortadela defumada embalada à vácuo elaborada com tilapia do Nilo

RESUMO. O objetivo foi avaliar as alterações na qualidade de mortadela defumada de peixe, adicionada de fibra de trigo durante 30 dias sob estocagem refrigerada a 6 ± 2°C. Utilizou-se como matéria-prima Carne Mecanicamente Separada (CMS) de recortes do filé de tilápia do Nilo (*Oreochromis niloticus*). Foram determinadas, contagem de *Staphylococcus*, detecção de *Salmonella* sp. (dia 0), contagem de psicrotróficos, coliformes totais e termotolerantes a 45°C, teste sensorial de aceitação (dias 0 e 30), atividade de água (a_w), cor instrumental, pH e análise da oxidação lipídica pelo método de substâncias reativas ao ácido tiobarbitúrico (TBARS), dias 0, 5, 10, 15, 20, 25 e 30 de estocagem. A oxidação lipídica e a_w apresentaram diferença significativa entre os dias de estocagem. Concluiu-se que a mortadela apresentou-se estável e com qualidade aceitável, durante 30 dias de estocagem refrigerada, podendo ser uma nova alternativa para agregar valor aos produtos à base de peixe, pois os microrganismos pesquisados apresentaram baixa contagem, as mudanças do pH não foram significativas e o teste sensorial indicou boa aceitação do produto durante os 30 dias de estocagem.

Palavras-chave: produtos à base de peixe, oxidação lipídica, teste sensorial, temperatura de refrigeração.

Introduction

Current world trend focuses on the intake of true healthy food. White meat, such as fish, underpins new trends since it is highly recommended due to its excellent and easily-digestible protein sources and to its provision of polysaturated fatty acids, vitamins and minerals for human nutrition (VENUGOPAL; SHAHIDI, 1995; SHAHIDI, 1998). Nevertheless, such a good food would be more complete if it contained alimentary fibre (SÁNCHEZ-ALONSO et al., 2007).

Although milk, meat, bread and other products are widely used, reference to fish and its derivates with insoluble dietary fibers is lacking in the literature (ANG; MILLER, 1991; YOON; LEE, 1990; BOLLINGER, 2000). However, the addition of fibers to fish products is highly important to improve the products' functionality, such as water bonds, gelification and others (SÁNCHEZ-ALONSO et al., 2007; ALISHAHI; AÏDER, 2012), but also as a means to develop a functional food with health benefits (BORDERÍAS et al., 2005).

There are two ways of introducing these fibers into fishery products. One is by injecting dispersions of fiber into fillets and another more effective way is by introducing fiber into restructured products. Restructured fishery products are products made from

minced and/or chopped muscle and which, with or without other ingredients, are used to make other products with a new appearance and texture (SÁNCHEZ-ALONSO et al., 2004).

Minced fish (MF) is obtained by passing the eviscerated and beheaded fish or waste, parings from the filleting fish operations, through a machine which separates the meat from the bones and this process allows additional recovery of the meat, in a range of 10.0 to 20.0% (RASEKH, 1987). 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 fishing and aquiculture (OLIVEIRA FILHO et al., 2010).

Besides the preservation of food by antimicrobial and antioxidant compounds, such as aldehydes, carboxylic and phenol acids (RIHA; WENDORFF, 1993), the smoking process may foment fish consumption and aggregate value to products through an increase in the fish sensorial features (SIGURGISLADOTTIR et al., 2000; LEROI; JOFFRAUD, 2000; EMERENCIANO et al., 2007).

The advantages of adding value to fish products and provide protein-rich foods and convenience (ease in preparation and use), have been investigated, for example, products made from minced fish, such as smoked fish pâté (ÜNLÜSAYÝN et al., 2007), tuna pâté (SÁNCHEZ-ZAPATA et al., 2011), fish burgers (MAHMOUDZADEH et al., 2010), cakes (KAMRUZZAMAN et al., 2006), fish sausages (LÓPEZ-CABALLERO et al., 2005; RAHMAN et al., 2007; OKSUZ et al., 2008; XU et al., 2010), fermented products such as Som-fug (RIEBROY et al., 2007).

Despite this trend, there are no studies regarding the development of smoked bologna sausage manufactured from MF with dietary fiber and on its stability during refrigerated storage. However, sausages with Japanese Threadfin Bream (*Nemipterus japonicus*) minced (RAJU et al., 2003) scored high in general acceptance, total counting and counting of aerobic spores submitted to storage temperatures and nisin concentrations. Other studies showed that samples of MF with caffeic acid and wheat fiber had their lipid oxidation totally inhibited after 10-day storage refrigeration (SÁNCHEZ-ALONSO et al., 2011). Results show that important quality loss may occur in products manufactured from this prime matter owing to the substitution of ingredients and storage conditions. Thus, is indispensable study the shelf life of these products.

The aim of this study was to evaluate the stability of physico-chemical, microbiological and sensory properties of smoked bologna sausage vacuum-packaged manufactured with MF of the Nile tilapia (*Oreochromis niloticus*), and wheat fiber in thirty days of storage under refrigeration temperature of 6 ± 2°C.

Material and methods

Smoked bologna sausage production

Raw materials consisted of Minced fish (MF) obtained from waste (parings from the filleting fish operations) of Nile tilapia (*Oreochromis niloticus*) provided by Cooperativa Agroindustrial Consolata (COPACOL).

Table 1 shows the other ingredients of the smoked bologna sausage's basic formulation.

Table 1. Formulation for smoked bologna sausage manufactured from MF.

Ingredients	Quantity (%)
Minced fish (MF)	62.95
Wheat fiber (Vitacel® WF200)	2.45
NaCl	0.8
Seasoning salt (Duas Rodas Industrial®)	0.15
Antioxidant (Duas Rodas Industrial®)	0.2
White pepper condiment (Duas Rodas Industrial®)	0.08
Polyphosphate (Duas Rodas Industrial®)	0.5
Bologna sausage condiment (Kraki®)	0.5
Cochineal crimson dye 3.0% (Saporiti do Brasil®)	0.07
Manioc starch	3.0
Isolated soybean protein	4.0
Ice	25.3

Smoked bologna sausage emulsion was manufactured according to technique by Moreira et al. (2008). However, hot smoking was employed so that the bologna sausage would reach internal temperature of 72°C (NUNES, 1999).

MF was thawed at 10 ± 1ºC in a refrigerator during 24 hours and then homogenized in a cutter (METVISA® CUT-3). The other ingredients mentioned above were added at this stage.

The emulsion was then placed in mechanical filler (JAMAR®) and filled into a natural bovine casing (Lopesco®) previously cured in acetic acid 4% solution. Casing was then sealed by a cotton string at every 20 cm, forming 500-gram pieces. The product was then pre-dried in an air-circulation buffer (Nova Ética®) at 50°C during 40 minutes and hot smoked in a smoker (Poly-térmica®) at a one-meter distance from a fire made from eucalyptus and *Mimosa scabrella* shavings, in a burning chamber, at approximately 100°C, during 4h.

When the smoking process was completed, the smoked bologna sausage were quickly cooled in a water and ice bath, vacuum packed with sealer Selovac 200B and stored in a refrigerator at 6 ± 2°C during 30 days.

Water activity

Water activity was determined at 0, 5, 10, 15, 20, 25 and 30 days of storage in triplicate and with two repetitions, by AquaLab CX-2, from Decagon Devices Inc. with temperature of sample at 25.0 ± 1ºC (IAL, 2005).

pH

Electrometric determination of pH was carried out by pHmeter HOMIS® at 0, 5, 10, 15, 20, 25 and 30 days of storage in triplicate and with two repetitions, following IAL (2005) methodology, with an electrode (SC18) inserted in the interior of the bologna sausage sample.

Analysis of lipid oxidation (TBARS)

Analysis of lipid oxidation was determined at 0, 5, 10, 15, 20, 25 and 30 days of storage in triplicate and with two repetitions, by Thiobarbituric Acid Reactive Substances (TBARS), following Vyncke (1970).

Instrumental color

Color was measured by system L*, a*, b* with reflectance spectrophotometer (Miniscan XE Plus 45/0-L, from Hunter Associates Laboratory Inc., Reston, VA, USA) provided with CIELab system and calibrated with black and white porcelain plates (HUNTERLAB, 1996). Each bologna sausage sample was previously cut in 2 cm (20 mm) slices and reading was carried out directly by the apparatus, in triplicate and with two repetitions.

Sensorial analysis

Acceptance test was undertaken employing the 9-scale hedonic test, according to NBR 14141 (ABNT, 1998). Seventy-one testers participated (MEILGAARD et al., 1999) and sensorial features, such as appearance, color, smell, taste, texture and total acceptance were evaluated at 0 and 30 days of storage

Microbiological analysis

Microbiological analyses for positive *Staphylococcus coagulase* and detection of *Salmonella* sp. were carried out according to official methodology of the American Public Health Association (APHA, 2001) at 0 day of storage in triplicate and with two repetitions. Total counting of psicrotrophic aerobic microorganisms, counting of total coliforms and thermotolerant coliforms at 45°C by Petrifilm® test were undertaken at 0, 5, 10, 15, 20, 25 and 30 days of storage, in triplicate and with two repetitions.

Statistical analysis

Data collected by physical and chemical analyses were analyzed by ANOVA and means were compared by Tukey's test at 5% probability, throughout the whole storage period. Means of results for sensorial tests were compared by test *t* at 5% probability, between days 0 and 30 of storage. Statistical analyses were carried out by software STATISTICA version 7.0 StatSoft, Inc., programmed in Microsoft Windows PC system.

Results and discussion

Water activity, pH and TBARS

Rates of water activity (a_w), humidity and pH should be known since they affect physical and chemical changes and are correlated to the development of microorganisms and to metabolic activities with results on food quality and stability (CHIRIFE; BUERA, 1996). Table 2 shows a_w, pH and TBARS rates for 0, 5, 10, 15, 20, 25 and 30 days of storage for smoked bologna sausage under refrigeration (6 ± 2°C).

Table 2. a_w, pH and TBARS (mg malondialdehyde kg^{-1}) of bologna sausage during of storage under refrigeration (6 ± 2°C) [(1)]

Analyses	Storage period (days)						
	0	5	10	15	20	25	30
a_w	0.982 ^{ab} ± 0.00	0.979 ^{b} ± 0.00	0.980 ^{ab} ± 0.00	0.982 ^{a} ± 0.00	0.981 ^{ab} ± 0.00	0.980 ^{ab} ± 0.00	0.981 ^{ab} ± 0.00
pH	6.42 ^{a} ± 0.08	6.42 ^{a} ± 0.03	6.31^{a} ± 0.03	6.27 ^{a} ± 0.05	6.36 ^{a} ± 0.02	6.27 ^{a} ± 0.02	6.27 ^{a} ± 0.12
TBARS	0.99^{a} ± 0.27	0.38^{c} ± 0.06	0.76^{ab} ± 0.15	0.69^{abc} ± 0.21	0.48^{bc} ± 0.04	0.51^{bc} ± 0.04	0.47^{bc} ± 0.05

[(1)]Means followed by different lowercase letters in the lines differ by Tukey's test at 5% probability.

Significant difference for a_w occurred only between days 5 and 15. Cáceres et al. (2008) registered rates between 0.96 and 0.98 for bologna sausage with fish oil. Same results were obtained in this research during storage. Changes in pH during storage were not significant. However, results show that smoked bologna sausage is a highly perishable product since its pH and a_w are respectively > 5.2 and > 0.95, with recommended storage temperature at ≤ 5°C (SABATAKOU, 2001).

TBARS index was widely employed to indicate degree of lipid oxidation. Lipid oxidation caused a decrease in nutritional quality and taste changes that might jeopardize acceptance of the product (BILGIN et al., 2008). TBARS rates (mg malondialdehyde kg^{-1}) of fish smoked bologna sausage provided significant differences for storing days (0 and 30). Rates oscillated throughout the storage period (Table 2) with a decrease between 0.99 mg kg^{-1} (day 0) and 0.47 mg kg^{-1} (day 30).

The addition of anti-oxidant in the product's formula and smoking components with anti-oxidant activity, such as phenolic compounds, may have

contributed towards rate decrease. TBARS rates of vacuum-packed sausages prepared from North African catfish (*Clarias gariepinus*) and Indian carps (*Labeo rohita*) strengthened with several levels of refined tuna oil (PANPIPAT; YONGSAWATDIGUL, 2008) gradually increased in proportion to storage time at 4°C. The authors reported moderate oxidation, probably due to the anti-oxidant activity of nitrates added to the sausages.

Silva et al. (2008) also registered a wide oscillation for TBARS rates during six-week storage of smoked fillets of the blue catfish (*Ictalurus furcatus*) treated with several anti-oxidants. Supplementary methods should also be taken into account so that lipid oxidation (rate of peroxide, specific aldehyde and ketone analysis by chromatography) could be better monitored (CARDOSO et al., 2008).

Brazilian legislation on the subject does not give a maximum TBARS rate for fish products. However, rates for smoked bologna fish sausage during 30 days of storage comply with those recommended for the good preservation from oxidation alterations in meat products, or rather, less than 3 mg kg^{-1} (AL-KAHTANI et al., 1996). Rates lower than 3 mg kg^{-1} were also reported in sausages with Nile tilapia (*Oreochromis niloticus*) minced, stored at 0 ± 0.3ºC (OLIVEIRA FILHO et al., 2010) and in bologna sausage with fish oil during storage at 4ºC (CÁCERES et al., 2008).

Instrumental color

It is a known fact that the consumer chooses food by its physical outward aspects, which comprises color, shape and size. Color is the most influential among the above characteristics, directly affects decisions on the choice of food to be consumed and is directly related to food acceptability (BLOUKAS et al., 1999). Although there was a trend towards an increase in luminosity (★L), red (★a) and yellow (★b) color during the storing period, significant differences were not detected (Table 3). Such instrumental results conformed to the human perception of color (sensorial data) which similarly did not identify significant differences in color during storage.

Table 3. Instrumental color of smoked bologna sausage during storage in refrigeration[(1)].

Parameters	Storage period (days)						
	0	5	10	15	20	25	30
L★	69.08 [a] ± 0.89	69.65 [a] ± 0.70	69.22 [a] ± 1.26	69.78 [a] ± 0.66	70.91 [a] ± 0.99	69.39 [a] ± 1.26	70.20 [a] ± 2.02
a★	11.94 [a] ± 0.29	11.99 [a] ± 0.38	11.16 [a] ± 0.69	11.47 [a] ± 0.30	10.89 [a] ± 0.57	11.54 [a] ± 0.51	11.22 [a] ± 0.65
b★	11.89 [a] ± 0.50	11.95 [a] ± 0.42	12.39 [a] ± 0.27	11.62 [a] ± 0.40	11.81 [a] ± 0.32	12.12 [a] ± 0.38	12.54 [a] ± 0.59

[(1)]Means followed by different lowercase letters in the lines differ by Tukey's test at 5% probability.

Smoke compounds may have contributed towards color stability during the storage period. Kristinsson et al. (2003) reported that CO-enriched filtered smoke had been employed to stabilize the color of fish muscles. They also reported that fish treated with filtered smoke significantly improved color stability during storing. Stabilization is due to a strong CO (carbon monoxide) bond with the heme of hemoglobin and myoglobin (Mb / Hb-Fe2 + ≡ CO) which become highly resistant to auto-oxidation and thus to de-coloration (SORHEIM et al., 1997).

Cod sausages (LÓPEZ-CABALLERO et al., 2005) provided similar results to those of the smoked bologna sausage under analysis. In fact, no significant changes occurred in L★ and b★ rates after 25 days of storage at 2°C. Al-Bulushi et al. (2005) also provided similar rates for L★ parameters during the storage period of minced underutilized fish burger in the Middle East. The same authors stated that L★ rates did not have any significant changes ($p > 0.05$) and maintained the product shiny throughout the storage period.

Sensorial analysis

Figure 1 shows means of scores in the sensorial acceptability test on days 0 and 30 of smoked bologna sausage storage.

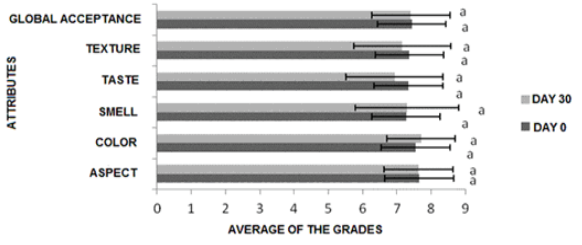

Figure 1. Graph representing means of sensorial analysis of smoked bologna sausage at 0 and 30 storage days under refrigeration (6 ± 2°C). NOTE: Means followed by same letters in the columns do not differ by t test at 5% probability among the storage days.

Means for general acceptance and all features evaluated in the smoked bologna sausage ranked between 7 ('I liked it somehow') and 8 ('I like it very much') of the hedonic scale employed, with no significant changes. These rates indicated a good acceptance of the product during the storage period. The evaluation of the sensorial quality of sausages manufactured with Japanese Threadfin Bream (*Nemipterus japonicus*) (RAJU et al., 2003) up to 15 days storage under refrigeration (6 ± 2°C) was similar to that of smoked bologna sausage in current study. Sensorial rates of dry sausage of the North African catfish (*Clarias gariepinus*) (OKSUZ et al., 2008) were higher at the start of storing at 4°C.

There was a significant rate decrease after 14 days of storage. Kumolu-Johnson et al. (2010) obtained similar results when they investigated such sensorial parameters as taste, texture, physical aspects and smell of the smoked North African catfish (*Clarias gariepinus*) during storage at 4°C. Since they reported that all sensorial results were above average, smoked African catfish may be acceptable up to 28 days after smoking.

Microbiological analysis

Besides the mandatory analysis (BRASIL, 2001) for fish products, coliforms at 45°C g^{-1}, *Staphylococcus coagulase* positive/g and *Salmonella* sp. / 25 g, were undertaken total counting of psicrotophic aerobic microorganisms and total coliforms. Degradation at low temperatures is caused by psicrophile bacteria which develop at 0°C. Brazilian legislation on the matter fails to state the limits for such microorganisms since they are not dangerous for public health. However, psicrotrophic microorganisms are the main degradation cause in chilled fish since they decrease the product's shelf life (OGAWA; MAIA, 1999; BORDIGNON et al., 2010). Table 4 shows the results of microbiological analysis of smoked bologna sausage for storage days under refrigeration.

Table 4. Microbiological analysis of smoked bologna sausage for storage days under refrigeration.

Analysis	Limits Brazilian legislation (BRASIL, 2001)	Storage periods (days)						
		0	5	10	15	20	25	30
Staphylococcus positive coagulase g^{-1}	10^3	$<10^{*(1)}$	(2)-	-	-	-	-	-
Salmonella sp 25 g^{-1}	Absent 25 g^{-1}	Absent 25 g^{-1}	-	-	-	-	-	-
coliforms at 45°C g^{-1}	10^3	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$
Total coliforms	-	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$	$<10^*$
Psicrotrophic aerobic	-	$<10^*$	$<10^*$	$<10^*2x\ 10^2$	$<10^*2x\ 10^2$			$<10^*$

(1)Estimated rate: no typical colonies have been found in the three dilutions (10^{-1}, 10^{-2} and 10^{-3}). (2) Analysis were not performed on the appointed days.

The simultaneous application of heat treatment (hot smoking), vacuum packing, storage under refrigeration (6 ± 2°C) and addition of salt allowed a good microbiological quality of the smoked bologna sausage during storage. Therefore, in accordance with Brazilian law (BRASIL, 2001), the smoked bologna sausage remained microbiologically stable during the 30 days of storage under refrigeration.

Result is in agreement with the findings of Cardoso et al. (2008) that evaluated the stability of sausages manufactured with MF (*Merluccius capensis*) added fiber and also claim that a higher aerobic microorganism growth might have been impaired because of packing conditions (vacuum) and storing temperature (2 ± 1°C). Sausages manufactured with Japanese Threadfin Bream (*Nemipterus japonicus*) minced (RAJU et al., 2003) presented psicrotrophic aerobic bacteria during 30 storage days at 6 ± 2°C. Similarly to what occurred with the smoked bologna sausage, the pathogenic microorganisms (*Staphylococcus aureus* and *Salmonella* sp.) were not detected in sausages manufactured with Nile tilapia (*Oreochromis niloticus*) minced and stored at 0 ± 0.3°C during 40 days (OLIVEIRA FILHO et al., 2010).

Conclusion

Most of the initial properties were retained during the storage period, but the water activity and lipid oxidation (TBARS) showed significant differences between days of storage. However, it was found that the sausage and bologna remained stable with acceptable quality during the 30 days of storage, because was not detected the pathogenic microorganisms (Staphylococcus aureus and Salmonella sp), changes in pH were not significant and sensory test indicate good acceptance of product during the 30 days of storage. So this product may be a new alternative to adding value to fishery products.

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