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The effects of vacuum and modified atmosphere packaging on quality changes in seasoned cobia (*Rachycentron canadum*) sticks stored under refrigeration

Efeitos das embalagens a vácuo e em atmosfera modificada sobre as alterações da qualidade da barrinha temperada de beijupirá (Rachycentron canadum) armazenada sob refrigeração

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Abstract

The objective of this research was to compare the quality attributes of seasoned cobia sticks packed under vacuum and using modified atmosphere packaging (MAP) and stored under refrigeration, as well as to establish the most suitable gas mixtures to maintain product freshness and provide an extended shelf life. The seasoned cobia sticks were breaded with a mixture of dehydrated spices (parsley, onion, garlic, tomato and bacon), packaged under vacuum; 100% aerobic package (control, with the presence of atmosphere air); MAP1 (15% N_2 + 80% CO_2 + 5% O_2), and MAP2 (20% N_2 + 70% CO_2 + 10% O_2), and stored at 5°C for 28 days. Samples were taken every 72 hours for the microbiological analyses (total mesophilic count, total psychrotrophic count, coagulase positive Staphylococcus, Salmonella sp., and thermotolerant Coliforms at 45°C) and physicochemical analyses (pH, the nitrogen from the total volatile bases, and trimethylamine), all carried out in triplicate. The results showed that the seasoned cobia sticks remained safe and with good microbiological, physical and chemical quality for a longer period when packed in a modified atmosphere. MAP1 (15% N_2 + 80% CO_2 + 5% O_2) was the system showing the best performance in the maintenance of quality and freshness, demonstrating the feasibility of ensuring quality and a longer shelf life (28 days) at 5°C.

Keywords: *Fish stick; MAP; Quality; Microbial stability; Physicochemical stability; Shelf life.*

Resumo

O objetivo desta pesquisa foi comparar os atributos de qualidade de barrinhas temperadas de beijupirá embaladas a vácuo e em atmosfera modificada (MAP), sob temperatura de refrigeração, bem como estabelecer a mistura de gás mais adequada para manter o frescor do produto e a vida de prateleira mais extensa. As barrinhas temperadas de beijupirá foram empanadas com uma mistura de especiarias desidratadas (salsa, cebola, alho, tomate e bacon), embaladas sob vácuo; 100% aeróbico (controle, na presença do ar atmosférico); MAP1 (15% N_2 + 80% CO_2 + 5% O_2); MAP2 (20% N_2 + 70% CO_2 + 10% O_2), e armazenada a 5°C durante 28 dias. A cada 72 horas, amostras foram retiradas, em triplicata, para as análises microbiológicas (contagem total de bactérias mesófilas, contagem total de bactérias psicrotróficas, *Staphylococcus* coagulase positiva, *Salmonella* sp., e coliformes termotolerantes a 45°C) e análises físico-químicas (pH, nitrogênio de bases voláteis totais e trimetilamina). Os resultados mostraram que as barrinhas temperadas de beijupirá permaneceram seguras e com qualidade microbiológica e físico-química por um longo período de tempo quando embalados em atmosfera modificada. O grupo MAP1 (15% N_2 + 80% CO_2 + 5% O_2) foi o que apresentou melhor desempenho na manutenção da qualidade e frescor, sendo o mais adequado demonstrando sua viabilidade em garantir a qualidade e maior prazo de validade (28 dias) a 5°C.

Palavras-chave: *Barrinha de peixe; ATM; Qualidade; Estabilidade microbiana; Estabilidade físico-química; Vida de prateleira.*

1 Introduction

The global expansion of aquaculture, which involves different fish and shellfish species, has changed the balance between supply and demand for these products. This factor, combined with a poor marketing structure, usually results in a loss in their commercial value (FARIA et al., 2011). In addition, the quality, shelf life and seafood safety are determined mainly by the presence and growth of pathogens and deteriorative microorganisms (SILBANDE et al., 2016). The feasibility of developing safe food is based on a proper understanding of microbial behaviour, besides intrinsic (pH, water activity, etc.) and extrinsic (temperature, atmosphere, etc.) factors which are essential to determine the shelf life (MASNIYOM, 2011; GONÇALVES, 2012; OKPALA et al., 2016).

Considering these characteristics, and to avoid undesirable deterioration, new processes and techniques for food packaging have been adopted in the fishery industry (FARIA et al., 2011; BONO; BADALUCCO, 2012, 2014; GONÇALVES, 2012). Modified atmosphere packaging (MAP) is a packaging system that influences the behaviour of the meat (according to changes in the internal environment), also known as a microenvironment. The system reduces meat contamination and provides an effective inbuilt water vapor barrier, preventing loss by evaporation. The atmospheres used in MAP combine different concentrations of oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) to maintain the appearance of the meat, either from a microbial or sensorial standpoint, and each one has a specific participation in extending the shelf life (MASNIYOM, 2011; GONÇALVES, 2012; KONTOMINAS, 2014; SAMPELS, 2015; PERNA, 2016). CO_2 is the main gas used, due to its bacteriostatic effect on fish microflora, and high CO_2 levels inhibit microbial growth, extend the shelf-life and maintain the quality of the fish products during the shelf-life (RODRIGUES et al., 2016). Kirtil et al. (2016) also mentioned that MAP prolongs the shelf life (in general, shelf life increases of 30–60% could be achieved) and maintains the general characteristics of fish that make it appear more “natural”.

Thus the aim of this research was to compare the quality attributes of seasoned cobia sticks packed under vacuum and using modified atmosphere packaging, and stored under refrigeration, as well as establishing the most suitable gas mixtures to maintain product freshness and extend the shelf life.

2 Material and methods

2.1 Raw material and sampling

Fresh specimens of cobia (*Rachycentron canadum*) were acquired directly from the company Camanor Produtos Marinhos Ltda. (Barra do Cunhaú, RN, Brazil), kept in insulated boxes with flaked ice (1:1), transported to the Seafood Technology and Quality Control Laboratory (LAPESC/CCA/UFERSA), eviscerated, washed with chlorinated water (5 ppm), weighed, vacuum packed, frozen in an Ultra freezer at -30 °C, and stored in a freezer (-30 °C) until preparation of the product (7 days).

2.2 Production of the seasoned cobia sticks

The frozen cobia was thawed, weighed, washed with chlorinated water (5 ppm), cut into sticks (10 × 3 × 2cm | length x width x height), immersed in a cold brine (10% NaCl + 5% sodium tripolyphosphate + 2% monosodium glutamate – at 5 °C) for 30 minutes, drained and weighed. The sticks were then breaded (Figure 1) with a mixture of dehydrated spices (parsley, onion, garlic, tomato and bacon), provided by Aroma das Ervas Alimentos Ltda. (Campinas, SP, Brazil).

Figure 1. Seasoned cobia (*Rachycentron canadum*) sticks.

2.2.1 Proximate composition

Samples of cobia sticks (*in natura* and seasoned) were ground (to obtain a homogeneous sample) and submitted to an analysis of the proximate composition (moisture, crude protein, total lipid, ash), and chlorides, according to the official methodology (AOAC, 2011). All analyses were carried out in triplicate.

2.2.2 Microbial analysis

The microbiological analyses (total mesophilic count, total psychrotrophic count, coagulase positive *Staphylococcus*, *Salmonella* sp., and thermotolerant Coliforms at 45 °C) were carried out according to the Brazilian Official Analytical Methods (BRASIL, 2003).

2.2.3 Sensory analysis

The sensory analysis was applied using 50 untrained panelists and the Global Acceptance Test, with a nine-point structured hedonic scale ranging from "I liked it very much" to "I disliked it very much" (STONE; SIDEL, 2004; DUTCOSKY, 2007). The purchasing intention in relation to the products was also evaluated using a structured seven-point Attitude Scale Test that ranged from "I would always buy it" to "I would never buy it" (STONE; SIDEL, 2004). The acceptability index (AI) was calculated considering the maximum score reached as 100% and the decision criterion to be well accepted was at least 70% (TEIXEIRA; MEINERT; BARBETTA, 1987; DUTCOSKY, 2007). The seasoned cobia sticks were steamed for an average time of 30 minutes and served to the panelists.

2.3 Packaging and gas mixtures

Seasoned cobia sticks were packaged under vacuum and using MAP (Figure 2). The MAP gas mixtures used were: CONTROL (100% aerobic package – with the presence of atmospheric air), MAP1 (15% N_2 + 80% CO_2 + 5% O_2), and MAP2 (20% N_2 + 70% CO_2 + 10% O_2). The packed seasoned cobia sticks were stored at 5°C for 28 days.

2.5 Freshness evaluation

The hydrogen potential (pH) was measured using a digital pH meter (Hayonik® Model FTP905). The nitrogen of the total volatile bases (TVB-N) and trimethylamine (TMA-N) were determined following the LANARA protocols (BRASIL, 1981). All analyses were carried out in triplicate every 72 h.

2.6 Statistical analysis

The significance of the differences between the samples after each day of storage was determined by the analysis of variance (ANOVA) and the effects were considered significant at a p-value ≤ 0.05.

3. Results and discussion

3.1 Proximate composition

The results obtained for the proximate composition of the cobia sticks can be seen in Table 1. The significant increases (p <0.05) in the moisture, ash and chloride contents

Table 1. Proximate composition of the *in natura* and seasoned cobia sticks.

Parameters (%)	*in natura* cobia sticks	seasoned cobia sticks
Moisture	75.09 ± 0.45^a	78.20 ± 0.29^b
Crude protein	21.42 ± 0.16^a	17.12 ± 0.20^b
Total lipid	1.92 ± 0.16^a	2.73 ± 0.98^b
Ash	1.39 ± 0.03^a	1.78 ± 0.03^b
Chlorides	0.84 ± 0.14^a	1.12 ± 0.31^b

Different letters in the same line indicate significant differences (p <0.05).

Figure 2. Seasoned cobia sticks packaged under MAP.

could be due to the previous immersion in a cold brine (10% NaCl + 5% sodium tripolyphosphate). The increment in total lipid can be explained by the incorporation of the dried spices, and the decrease in crude protein due to the balance between the constituents. However, no data was found in the scientific literature regarding the influence of dried spices on the proximate composition of foods.

3.2 Microbial analysis

Similar values for the bacterial contents of all the packages were observed at the beginning of the experiment (zero time) (Table 2), since the same care was taken during the preparation of all products, i.e. hygiene and sanitization procedures were used. During storage, intense bacterial growth was observed in the samples packed in atmospheric air (control) reaching a maximum value of 4.8 x 10^3 CFU g^{-1} on the 28th day of storage. The growing rate for these microorganisms was 2.8% from the beginning to the end of the experiment; while the samples packed in MAP1 presented the lowest value of bacterial growth (2.8 x 10^3 CFU g^{-1}) and a reduced rate of 0.62%. Bono and Badalucco (2012) evaluated the efficiency of ozonated water (0.3 ppm) combined with a MAP system in the processing of striped mullet (*Mullus surmuletus*), and verified a reduction in bacterial growth, presenting low total mesophilic counts (2.5 log CFU g^{-1}) when compared to the control samples (3.7 log CFU g^{-1}).

A similar behavior was observed for the total psychrophilic count (Table 2) of the samples at zero time, independent of packaging, i.e., the same initial microbial counts. The bacterial growth in the samples packed in atmospheric air (control) reached a maximum value of 7.2 × 10^3 CFU g^{-1} on the 28th day of storage, with a bacterial growth rate of 14.9%. Samples packed in MAP1 presented a total psychrophilic count of 1.8 × 10^3 CFU g^{-1} for the same storage time and a reduced rate of 2.98%.

According to Table 2, samples packed in MAP1 showed similar results (reduced counts) for both the total mesophilic and total psychrophilic counts, during the 28 days of storage, and this was the most effective gas mixture for inhibiting these bacterial growths. Brazilian law (BRASIL, 2001) does not specify limits for the total mesophilic and psychrophilic counts of fish and fish products, but according to Agnese et al. (2001), values greater than 10^6 CFU g^{-1} are considered critical for fish quality. The values found in the present study, for these bacterial groups were below 10^3 CFU g^{-1} and could therefore be considered safe, but MAP1 was shown to be the most effective system, followed by MAP2 and vacuum packaging.

The bacterial growth in the atmospheric air package was the result of an inadequate air mixture as a form of food preservation, as evidenced by Poli et al. (2006) when studying the sensory, physicochemical and microbiological changes in sea bass (*European seabass*) packaged in a modified atmosphere and in atmospheric air. They found high levels of bacterial growth on the first day of storage, reaching values above 10^6 CFU g^{-1} on the 5th day of storage in atmospheric air, and on the 8th day of storage in the modified atmosphere packaging.

Table 2. Total mesophilic and psychrophilic counts (CFU g^{-1}) in the packed seasoned cobia sticks during cold storage (5 °C).

Total mesophilic count				
Days	**Air (control)**	**Vacuum**	**MAP1**	**MAP2**
0	$3.8\times10^{3\,a}$	$3.8\times10^{3\,a}$	$3.8\times10^{3\,a}$	$3.8\times10^{3\,a}$
7	$4.2\times10^{3\,b}$	$4.1\times10^{3\,b}$	$3.7\times10^{3\,a}$	$3.9\times10^{3\,a}$
14	$4.1\times10^{3\,b}$	$4.3\times10^{3\,c}$	$3.5\times10^{3\,b}$	$4.1\times10^{3\,b}$
21	$4.5\times10^{3\,b}$	$4.4\times10^{3\,c}$	$3.2\times10^{3\,b}$	$4.1\times10^{3\,b}$
28	$4.8\times10^{3\,c}$	$4.4\times10^{3\,c}$	$2.8\times10^{3\,c}$	$4.0\times10^{3\,b}$
Total psychrophilic count				
Days	**Air (control)**	**Vacuum**	**MAP1**	**MAP2**
0	$2.3\times10^{3\,a}$	$2.3\times10^{3\,a}$	$2.3\times10^{3\,a}$	$2.3\times10^{3\,a}$
7	$3.4\times10^{3\,b}$	$2.7\times10^{3\,b}$	$2.2\times10^{3\,a}$	$2.4\times10^{3\,a}$
14	$4.3\times10^{3\,c}$	$2.8\times10^{3\,b}$	$2.0\times10^{3\,b}$	$2.4\times10^{3\,a}$
21	$5.7\times10^{3\,d}$	$3.0\times10^{3\,c}$	$1.8\times10^{3\,b}$	$2.5\times10^{3\,b}$
28	$7.2\times10^{3\,e}$	$3.2\times10^{3\,c}$	$1.8\times10^{3\,b}$	$2.5\times10^{3\,b}$

CFU: colony forming units; MAP 1 (15% N_2 + 80% CO_2 + 5% O_2); MAP 2 (20% N_2 + 70% CO_2 + 10% O_2). Different letters in the same column indicate significant differences (p <0.05).

The reduction in microbial growth presented in Table 2, corroborates with the literature (DEBEVERE; BOSKOU, 1996; LÓPEZ-CABALLERO et al., 2001; HANSEN et al., 2007; HANSEN et al., 2016) and showed the efficiency of the MAP process in reducing bacterial multiplication (MANO et al., 2000). In fact, the CO_2 used in the MAP mixture has bacteriostatic properties by extending the bacterial lag phase and slowing growth (HANSEN et al., 2016; PERNA, 2016) and the bacterial growth rates were lower with increasing CO_2 concentrations (HANSEN et al., 2016). Similar results were obtained by Soccol et al. (2005) who observed a reduction in bacterial growth in samples of tilapia fillets packed in a modified atmosphere, providing better conservation; this phenomenon was also observed in sardine (LOPES et al., 2004). In agreement with these results, Ordoñez-Pereda et al. (2000), studying the microbiological and physicochemical changes in hake fillets (*Merluccius merluccious*) stored in atmospheres enriched with carbon dioxide, concluded that an atmosphere of 40% CO_2 + 60% N_2 is recommended for packaging hake under refrigeration (they observed an increase in the commercial life of up to 3 times, both from the microbiological and sensory points of view). In agreement with these observations, Parkin et al. (1981) also observed the inhibitory effect of an atmosphere with 80% CO_2 on the reduction of the mesophilic counts in fish fillets stored using the MAP system. Rodrigues et al. (2016) demonstrated that MAP (80% CO_2 + 20% N_2) in combination with UV-C radiation (80% CO_2 + 20% N_2;

106.32 mJ cm^{-2}) enhanced the shelf-life of rainbow trout fillets at least twice by retarding the microbial growth parameters and delaying chemical changes.

The inhibition of the growth of most spoilage bacteria, especially psychrotrophic species that can grow in different refrigerated foods, was observed at 5% CO_2. Several mechanisms for the action of CO_2 on microorganisms have been identified, such as changes in cell membrane function, including effects on nutrient uptake and absorption; direct enzyme inhibition or reduction in the rate of enzymatic reactions; cell membrane penetration, leading to intracellular pH changes; and direct changes in the physical-chemical properties of the proteins. A probable combination of these activities is likely to be responsible for the bacteriostatic effect of CO_2 (BLAKISTONE, 1999; KONTOMINAS, 2014).

According to Martin et al. (1978), the main habitat of *Staphylococcus aureus* is in the human skin, nasal mucosa and respiratory tract, and its presence indicates the improper handling of food, poorly sanitized equipment or contamination after processing. According to the Brazilian food microbiology law (BRASIL, 2001), the maximum permitted *Staphylococcus* count is 5x10^2 CFU g^{-1} for seasoned fish, but the microbiological examination for the existence of *S. aureus* in the seasoned cobia sticks packaged under vacuum and using MAP showed no-growth of this bacterium in all samples during the 28 days of storage. The results of this study were similar to those found by Wang and Ogrydziak (1986), who did not detect the presence of *S. aureus* in cod (*Gadus morhua*) packed in 80% CO_2 and stored at 4 °C.

According to Leitão (1977) the habitat of *Salmonella* sp. is in the intestinal tract, and its presence indicates probable fecal contamination from human or animal sources. Fish caught in unpolluted waters are free from *Salmonella* (since it is not part of the natural fish microflora) and their presence is derived mainly from handling or contact with improperly sanitized surfaces. Furthermore, these bacteria did not proliferate in foods containing other microorganisms, and the presence of *Salmonella* in a food is reason enough for it to be condemned. In the present study, the absence of *Salmonella* sp. was observed throughout the experiment, in agreement with other studies (PASSY et al., 1983; RANDELL et al., 1999) where the presence of this bacterium was also not detected in freshwater shrimp (*Macrobrachium rosembergii*), catfish (*Ictalurus punctatus*) or salmon (*Salmo salar*) after 2 weeks of storage in MAP.

Regarding thermotolerant coliforms, the most probable number values were <3 MPN g^{-1} at zero time, which is less than the value established by the Brazilian food microbiology law (BRASIL, 2001) and remained low throughout storage. Using 100% CO_2 Passy et al. (1983) inhibited the growth of coliforms in freshwater prawn (*M. rosenbergii*) during 11 days at 4 °C.

The low proliferation of microorganisms observed during the experiment was due to the good hygiene practices used during the processing of the products and remained in agreement the standards of current legislation.

3.3 Sensory analysis

The results of the sensory analysis (hedonic scale) demonstrated that the product was well accepted, with an average of 7.8±0.62 (scores between "I liked moderately" and "I liked very much"). The acceptability index was 86.2%, which shows good acceptance by the consumer (IA > 70%). The purchasing intent ranged from 54% of " I would certainly buy" to 4% of " I would probably not buy", indicating that the product was well accepted by consumers and has good commercial potential. Santos et al. (2007) stated that the presentation of seafood in a more elaborate way, associated with the use of some natural spices, i.e., adding value to the product, besides increasing its shelf life, makes it more appreciated by consumers, stimulating their consumption.

3.4 Freshness evaluation

Table 3 shows the pH values of the seasoned cobia sticks packed under vacuum and using MAP during the 28 days of storage at 5 °C. At the beginning of the

Table 3. The pH, TVB-N (mg 100 g^{-1}) and TMA-N (mg 100 g^{-1}) values found for the packed seasoned cobia sticks during cold storage (5 °C).

		pH		
Days	**Air (control)**	**Vacuum**	**MAP1**	**MAP2**
0	6.01 ± 0.03^a	6.01 ± 0.03^a	6.01 ± 0.02^a	6.01 ± 0.03^a
7	6.10 ± 0.02^b	6.06 ± 0.02^b	6.01 ± 0.02^b	6.02 ± 0.03^b
14	6.13 ± 0.02^{bc}	6.09 ± 0.01^b	6.02 ± 0.02^b	6.05 ± 0.02^b
21	6.18 ± 0.03^{cd}	6.11 ± 0.02^{cd}	6.05 ± 0.03^c	6.09 ± 0.01^c
28	6.21 ± 0.02^d	6.17 ± 0.03^d	6.08 ± 0.03^c	6.12 ± 0.03^c
		TVB-N (mg 100 g^{-1})		
Days	**Air (control)**	**Vacuum**	**MAP1**	**MAP2**
0	1.23 ± 0.01^a	1.23 ± 0.01^a	1.23 ± 0.01^a	1.23 ± 0.01^a
7	10.49 ± 0.50^b	5.57 ± 0.23^b	5.36 ± 0.23^b	5.32 ± 0.18^b
14	11.45 ± 0.61^b	7.30 ± 0.22^c	5.41 ± 0.56^b	6.09 ± 0.21^b
21	14.14 ± 0.43^c	8.30 ± 0.21^d	5.63 ± 0.51^b	6.34 ± 0.47^b
28	20.92 ± 0.53^d	8.59 ± 0.25^d	5.79 ± 0.62^b	6.92 ± 0.51^b
		TMA-N (mg 100 g^{-1})		
Days	**Air (control)**	**Vacuum**	**MAP1**	**MAP2**
0	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a	0.26 ± 0.01^a
7	2.49 ± 0.50^b	1.57 ± 0.23^a	1.36 ± 0.23^a	1.32 ± 0.18^a
14	3.45 ± 0.61^b	2.30 ± 0.22^b	1.41 ± 0.26^a	2.08 ± 0.21^b
21	5.14 ± 0.43^c	3.32 ± 0.21^c	1.63 ± 0.21^b	2.34 ± 0.27^b
28	5.92 ± 0.53^c	3.59 ± 0.25^c	1.79 ± 0.23^b	2.91 ± 0.33^c

MAP 1 (15% N_2 + 80% CO_2 + 5% O_2); MAP 2 (20% N_2 + 70% CO_2 + 10% O_2). Different letters in the same column indicate significant differences (p <0.05).

experiment, the pH value was the same for all the packaging conditions. On the 7th day of storage, a rapid increase was observed in the control group, and a slow increase amongst the other groups, which did not interfere with sample quality. This slight oscillation and stabilization of the pH values was also observed by Lalitha et al. (2005), Hansen et al. (2016), and Silbande et al. (2016), and can be explained by the diffusion of CO_2 into the fish tissue, generating the formation of carbonic acid, but at the same time the production of volatile bases acts as a barrier against the increase in pH (DEBEVERE; BOSKOU, 1996; LOPES et al., 2004; PERNA, 2016). Bono and Badalucco (2012) studied the effectiveness of ozone associated with MAP in different species, and verified that the pH did not vary according to the species.

The values obtained for the nitrogen of the total volatile bases (TVB-N) in the seasoned cobia sticks packed under vacuum and using MAP during the 28 days of storage at 5 °C, can be seen more clearly in Table 3. All treatments showed values below those established by Brazilian legislation (30 mg 100 g^{-1}). Initially higher values were observed for the control group (atmospheric air condition), and according to Cobb et al. (1974) and Yeh et al. (1978) the initial increase in TVB-N (mainly the ammonia content) can be counterbalanced by leaching, especially if the surface area is very large (fillets, steaks, etc.), but after a few days the increase intensifies and generally coincides with the increasing pH value. The more alkaline the medium is the more active is the enzyme deaminase.

As from the 7th day of storage, samples kept under atmospheric air conditions (control) reached a higher value (10.49 mg TVB-N 100 g^{-1}) as compared with the other atmospheres, and continued increasing during the storage period, reaching the highest value on the 28th day of storage (20.92mg TVB-N 100 g^{-1}). The variation in TVB-N values can be explained by bacterial action in the conversion of trimethylamine oxide (TMAO), abundant in most fish of marine origin, into trimethylamine (TMA), one of the main substrates for the production of volatile bases, thus having a direct correlation with the TVB-N values (HOWGATE, 2010a, 2010b). This result should reflect the increase in protein degradation and consequent increase in the production of volatile bases. For the other groups (vacuum and MAP) the TVB-N increase during the first 7 days of storage was slight and continued thus during the storage period, with small fluctuations and constant values, but below the legal limits for freshness. MAP1 showed better results as compared to MAP2, in agreement with the pH and microbial results. Bono et al. (2016) found higher TVB-N values (33.5 to 42.0) in shrimp stored in a modified atmosphere.

At the start of the experiment, the nitrogen from the trimethylamine (TMA-N) was 0.26 mg TMA-N 100 g^{-1} regardless of the treatment. The values found for the control group exceeded the sensory limit of 4mg TMA-N 100 g^{-1} as from the 21st day of storage (5.14 mg TMA-N 100 g^{-1}), while the other treatments were within the limits proposed by other studies (HANSEN et al., 2007; HOVDA et al., 2007; ÖZOGUL et al., 2004), where the fish packaged in modified atmospheres promoted lower values for TMA-N when compared to the control group. Differently, Bono and Badalucco (2012) considered the limit of acceptability for fish freshness to be a TMA-N value of 5 mg N 100 g^{-1}. These results (TVB-N and TMA-N, Table 2) corroborate with those found by Silbande et al. (2016) for vacuum and MAP (70% CO_2 + 30% O_2) packed yellowfin tuna.

The combination of O_2 with the inhibitory effect of CO_2 on microbial growth (which promotes degradation) is the best way to maintain the TMAO levels (HANSEN et al., 2007). López-Caballero et al. (2001) reported that the presence of O_2 is as important as CO_2, due to its inhibition of the reduction of TMAO, regardless of the concentration of *S. putrefaciens* (HOVDA et al., 2007). This fact was visible in the values recorded for TMA-N in the seasoned cobia sticks packed under MAP1 (10% O_2) during the 28 days of storage at 5°C, as compared with the values recorded in those packed in atmospheric air (control) and under vacuum, and even with smaller amounts of O_2 (i.e. MAP2 with 5% O_2), in particular after 14 days of storage, coinciding with the reduction of O_2 and the formation of an anaerobic atmosphere. Working with Chinese shrimp (*Fenneropenaeus chinensis*) packed in modified atmospheres with different gas concentrations (40% CO_2:30% O_2:30% N_2 and 100% CO_2) and pre-treated with ozone, Lu (2009) showed good acceptability up to the 21st day of storage.

4 Conclusions

The results of this study showed that the seasoned cobia sticks were well acceptance by the consumers, and remained safe and with good microbiological, physical and chemical quality for a longer period when packed in a modified atmosphere. The vacuum packaging showed good performance in prolonging the shelf life, although less than the atmospheres enriched with CO_2, and was not therefore the best indication as a conservation method for this product. The samples packed in modified atmospheres showed longer shelf lives than those packed in atmospheric air (control), being effective in maintaining the quality parameters proposed by the legislation. Of the two atmospheres evaluated, MAP1 (15% N_2 + 80% CO_2 + 5% O_2) showed the best performance in maintaining the physical, chemical and microbiological parameters, being the most suitable system and demonstrating feasibility in ensuring quality and a longer shelf life (28 days).

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