THE USE OF FILTERED AIR CURTAINS ALONG WITH ANTIMICROBIAL ICE IMPROVES THE QUALITY AND SHELF LIFE OF FRESH FISH IN REFRIGERATED OPEN DISPLAY CASES

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Abstract: *The shelf life of fresh chilled and ice-preserved fish is relatively short. For this reason, different techniques have been studied in order to maintain the freshness characteristics of the fish for as long as possible. The work that is now presented analyses the interest of using filtered air curtains through HEPA filters in combination with antimicrobial ice to maintain the freshness characteristics of fresh fish in open displays for longer. The sea bass preserved in the control conditions (without antimicrobial ice or air from HEPA filters) presented higher counts of Pseudomonas and total mesophilic aerobic microorganisms, than those preserved with antimicrobial ice (T1) or with control ice with a filtered air curtain (T2). We observed a synergistic effect of the use of antimicrobial ice and air curtain filtered in HEPA filters (T3), achieving a better control of the proliferation of the spoilage microbiology, and of the evolution of the freshness characteristics of the fish, in such a way that it could reach a shelf life of up to 23 days.*

Keywords: *Nanoencapsulated essential oils, sea bass, storage on ice, HEPA air filter, fish shop optimization.*

1. INTRODUCTION

The loss of freshness of chilled fish is mainly due to spoilage processes through complex biochemical and microbiological changes. Microorganisms such as *Pseudomonas* and *Achromobacter* are involved, which are present in the slime, gills and intestines of freshly caught fish [1]. Also, deterioration occurs due to the proliferation of other microorganisms, including anaerobic microflora, and oxidative processes of lipid components [2].

The enzymes that remain active after the death of the fish produce changes in the aroma that take place during the first days of conservation with ice, before the bacterial action takes place. Due to the high content of polyunsaturated fatty acids (PUFAs), lipid oxidations are frequent, significantly reducing quality. Sensory deterioration of fresh fish occurs through unwanted changes in taste, color and texture, and unpleasant odors may appear due to rancidity and the formation of trimethylamine nitrogen (TMA-N) by the action of spoilage bacteria [3].

The preservation of fresh fish in ice is a widely used method, especially when it comes to whole fresh fish. It has a good efficiency in the cooling of the fish, the temperature is well controlled, and it maintains the humidity of the product well. Still, the shelf life of iced fish is relatively short. For this reason, several investigations have been carried out to improve this method of preserving fish on ice, by adding antimicrobials and antioxidants [4]. Thus, the direct addition to ice, or ice-making water, of essential oils (EOs) as antimicrobials has been proposed, but to achieve significant effects of increasing the shelf life of fish, it was necessary to use high doses of these EOs [4, 5]. To avoid the use of high doses of EOs that negatively alter the sensory quality of the fish, the use of ice containing nanoencapsulated essential oils (in β-cyclodextrins, forming inclusion complexes) has been proposed, achieving a very significant increase in quality and shelf life of fresh fish preserved in this type of ice [6].

In an attempt to further increase the quality and shelf life of fresh fish preserved in ice, this paper proposes the use of antimicrobial ice with nanoencapsulated EOs in combination with filtered air curtains from HEPA filters (high-efficiency particulate air filters, which retain particles of size equal to or greater than 0.5 μm) [7]. In this way, we want to show that the EO vapors that are released as the antimicrobial ice melts, and that envelop the surface of the fresh fish on the ice, are better controlled and applied more effectively on the surface of the fish, achieving greater efficiency in surface decontamination. With the use of these filtered air curtains from HEPA filters, it is also possible to isolate the surface of the fish from contaminants that come from the air, which can increase the microbial load of the product.

2. MATERIAL AND METHODS

Fresh sea bass (*Dicentrarchus labrax* L) from aquaculture and supplied by the company Servicios Atuneros del Mediterráneo, S.L. were used in the experiments (San Pedro del Pinatar, Murcia). The seabass were transferred to the Universidad Politécnica de Cartagena (UPCT) where they were placed in polystyrene boxes with normal crushed ice (Control) and in polystyrene boxes with crushed ice with a combination of essential oils (EOs) encapsulated in β-cyclodextrins (β-CD). The EOs used were carvacrol, bergamot and grapefruit (CBP) in a ratio (3:1:1, volume (*v*):*v*:*v*) encapsulated in β-CD and with a concentration of 100 mg kg^{-1} of ice. The polystyrene boxes were left at room temperature (19-20 ºC) both in the pilot plant and in the clean room with an air curtain filtered in HEPA filters (clean room class 10000, according to the FS 209 E standard) for 8 h (9:00-17:00 h), simulating a traditional open displays cases in a fish market. Subsequently, they were stored in the same polystyrene boxes with a polystyrene lid, in the cold rooms of the UPCT pilot plant at ± 2 ºC. The objective was to simulate a real fish market, so it was tried to respect the exposure times of the fish during its commercialization. The fish was kept in the UPCT Pilot Plant at room temperature for 8 days. In the morning, at 9 a.m., the fish was placed on a layer of ice inside polystyrene boxes at room temperature. The temperature was taken every hour on the surface, muscle and ice. At 5:00 p.m., the fish was stored in polystyrene boxes with ice in the cold rooms of the UPCT Pilot Plant at 2 °C, until the next day. Melted ice was replaced with crushed ice and excess water drained daily. The polystyrene boxes contained 10 sea bass per box and each day 3 sea bass were caught for daily sampling. In short, the treatments were the following: (i) Control: sea bass preserved with control ice and exposed without filtered air curtain; (ii) T1: sea bass preserved with antimicrobial ice and exposed without curtain of filtered air; (iii) T2: sea bass preserved with control ice and exposed in a clean room with air filtered in HEPA filters; and (iv) T3: sea bass preserved with antimicrobial ice and exposed in a clean room with filtered air in HEPA filters.

Figure 1. Simulation of fish market in the Pilot Plant of the UPCT (left) and detail of fish with ice-toping by crushed ice (right).

Microbiology and physical-chemical parameter analyzes were performed: pH, texture, TMA-N, water retention capacity (WRC) and color. In addition, a sensory analysis of the fish was made. Fish muscle carvacrol residues were analysed. Temperatures of fish, air and ice were also recorded throughout the storage period at room temperature.

For the microbiological analysis, 25 g of muscle were taken from the dorsal region of the fish and diluted in 225 mL of sterile buffered peptone water (Scharlau Che; Seward Medical, London, UK) for two minutes. Serial dilutions in peptone water were made, which were cultured in different media and microbial counts were made. The microorganisms analyzed were: total mesophilic aerobes, psychrophiles, enterobacteria and *Pseudomonas*. The results were expressed as logarithms of colony-forming units per gram (log CFU g^{-1}). Shelf life was determined by regression using a linear fit, Y=a+bX, where "Y" represents the count of mesophilic aerobic microorganisms and "X" represents the storage time on ice.

For the pH determination, 5 g of fish were taken, which were mixed with 10 mL of sterile distilled water and subjected to constant stirring with a glass rod for 2 minutes. It was left to stand for 5 min and the pH was measured using a pH-meter (Basic 20; Crison, Barcelona, Spain). Measurements were performed in triplicate. To determine the evolution of the firmness of the fish meat during refrigerated storage, a texturometer (TA- XT plus; Stable Micro Systems, Godalming, UK) was used with a cylindrical compression probe of 50 mm diameter at 50% compression. The samples were cut into 2-3 cm squares, as homogeneous as possible. Firmness was expressed as the maximum force (N) necessary for compression. Three pieces of muscle were measured for each treatment of the dorsal part and the mean was calculated.

For the analysis of the WRC, 0.3 g of fish muscle sample, previously minced, were weighed. The sample was placed in the center of the filter paper (Whatman No. 540), previously weighed, between two petri dishes. A 1-kg weight was then gently placed on the top plate and left for 10 min, after which the weight was removed. The WRC was calculated as follows, expressed as a percentage: WRC = 100 - % free water.

TMA-N was determined using the picric acid method (AOAC, 1998). TMA-N content was expressed in mg N 100 g^{-1} of fish muscle. Determinations were performed in triplicate. The method of Ke et al. [8], with slight modifications, was used to analyze possible residues of β-CD-encapsulated EOs remaining in fish preserved with the antimicrobial ice.

The sensory attributes of the fish were evaluated by several panelists each sampling day. Fish assessment was carried out using the Tasmanian Food Research Unit system modified by Erikson et al. [9], and developed as a quality index (IQ) method. The panelists evaluated the parameters of three fish for each treatment. On the day of each analysis, the mean score of the panelists for each parameter was obtained. The panelists evaluated the following IQ attributes of the fish: appearance of the skin (0: bright and iridescent pigmentation, 1: slightly shiny, 2: rather dull, fading, 3: green, yellowish, mainly near the abdomen 4: pale, dull); belly and operculum (0: gray, silvery, 1: gray, yellowish spots, 2: gray and brown spots); skin odor (0: fresh seaweed, 1: neutral, 2: cucumber, metal, 3: sour, dishcloth, 4: rotten); skin texture/

firmness (0: firm, 1: fairly smooth, 2: very smooth, 3: misshapen); mouth resistance (0: very, 1: little, 2: no resistance); mouth color (0: pink, 1: yellowish); cornea of the eyes (0: clear, translucent 1: opaque and/or red, 2: slightly milky, 3: milky gelatinous); eye pupils (0: black, shiny metallic, 1: slightly milky, 2: milky, opaque 3: white, dull); eye shape (0: convex, 1: flat, 2: sunken, 3: deformed); gill color (0: bright red, 1: pale red, pink, 2: light brown, 3.): brown, gray); gill odor (0: fresh, seaweed, neutral, 1: metallic, grassy, 2: sour, moldy, dishcloth, 3: rotten); gill mucus (0: absent, 1: clear, 2: milky, coagulated, 3: brown, coagulated); anal area: mucus (0: absent, 1: slightly, 2: present); anal area: appearance (0: closed, 1: slightly open, 2: open). Consequently, the modified total IQ score ranged from 0 (very fresh fish) to 40 (spoiled fish). In addition, the panelists also performed a sensory evaluation for the muscle of the cooked fish (5 min in the microwave at 900 W). Panelists were asked to rate the smell, taste, and texture of the fish using a descriptive hedonic scale of 0-10 [10].

Data was analyzed with a unidirectional analysis of variance (ANOVA) computed in R studio. Tukey HSD test at a 95% confidence level was assessed (statistical significance $p = 0.05$). Results were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

Figure 2 shows the evolution of temperatures of the air, ice, surface, and fish muscle. The mean air temperature was 22.8 °C throughout the storage time. The average ice temperature during the fish-shop simulation period was -0.15 °C. Mean fish surface and muscle temperatures were 4.0 °C and 2.5 °C, respectively.

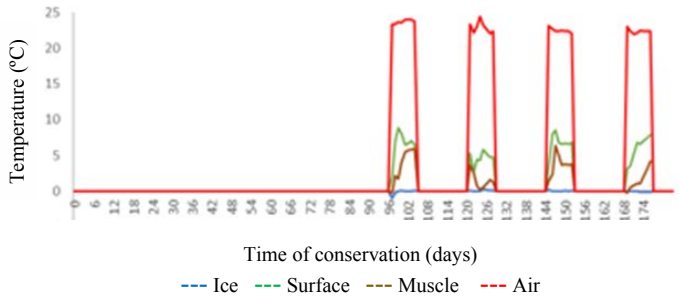

Figure 2. Evolution of the temperature of the air surrounding the product, the ice, the surface and the muscle of the fish during the conservation period, with intervals at room temperature and at 2°C in the chamber (at night). Only the last 4 days of temperature records are shown for the ice, and the fish muscle and surface.

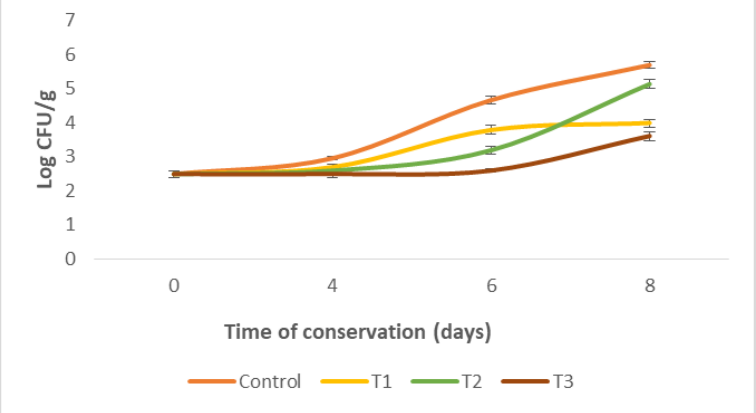

Figure 3. Counts (log CFU g^{-1}) of total mesophilic aerobes of sea bass during the period of conservation in ambient air simulating the period of exposure in a fish market and its conservation overnight at 2 °C in cold storage (mean (n=3) ± standard deviation).

The initial microbial load was 2.5 log CFU g^{-1} of total mesophilic aerobic microorganisms (Figure 3). From day 4, an increase in the count of these microorganisms begins to be observed. Papadopoulos et al [10] also observed an increased total mesophilic aerobes count in sea bass stored on ice. On day 6, significant differences were observed between the different treatments. At the end of the conservation period, we observed that the sea bass preserved with control ice and without filtered air (Control) presented a much higher count than the rest of the conditions studied. Antimicrobial ice is shown to reduce the growth of spoilage microorganisms. In addition, if we compare the counts in the fish with control ice and unfiltered air (Control) and the fish with filtered air curtain (T2), we also observe a reduction of 0.6 log CFU g^{-1} due to air filtration. The synergistic effect of the use of antimicrobial ice in combination with filtered air curtains is demonstrated, since the fish preserved in this way has a lower microbial count (2 log CFU g^{-1}) than the fish preserved under control conditions. This implies an increase in shelf life of 12 days (possibly up to 23 days) compared to fish kept in ice under normal conditions. In the evolution of *Pseudomonas* counts, a behavior similar to the evolution of total mesophilic aerobes was observed (data not shown). No significant differences were found between different treatments for the rest of the microorganisms studied: psychrophiles and enterobacteria (data not shown).

Regarding the evolution of TMA-N (Figure 4), the fish presented an initial content of 0.31 mg TMA-N 100 g^{-1}. During storage, there was a notable increase in TMA values in Control fish, reaching values of 2.22 mg TMA-N 100 g^{-1} at the end of the storage period. Papadopoulos et al. [10] also obtained low levels of TMA-N at the beginning of the conservation of sea bass in ice, values that increased during storage until they exceeded mg TMA-N 100 g^{-1} at the end of it, which indicates an incipient alteration and fish damage [11]. The other treatments T1, T2 and T3 did not exceed the value of 1 mg TMA-N 100 g^{-1}. It is shown that the use of antimicrobial ice achieves less deterioration of the fish during the conservation period (T1 and T3). As we saw with the results obtained in the spoilage microbiology of fish, we observed a synergistic effect between the use of antimicrobial ice and filtered air curtains (T3).

Sea bass preserved in antimicrobial ice without HEPA filters (T2) showed a firmness value of 30 N at the end of storage, demonstrating that the use of antimicrobial ice maintains a better texture of sea bass muscle during storage on ice (data not shown). As in the previously studied parameters, a synergistic effect of the use of antimicrobial ice with filtered air curtains was observed, since the values obtained were higher than in the rest of the conditions studied (35 N). For the rest of the physical-chemical parameters studied: pH, color and WRC, no significant differences were observed between the different conservation treatments studied (data not shown). However, regarding sensory analysis (Figure 5), there were significant differences. At the end of preservation, fish preserved in control ice and without filtered air (Control) obtained the worst scores, while fish preserved in antimicrobial ice and with filtered air (T3) was the best scored. Again, a synergistic effect of the combination of antimicrobial ice and HEPA-filtered air was observed.

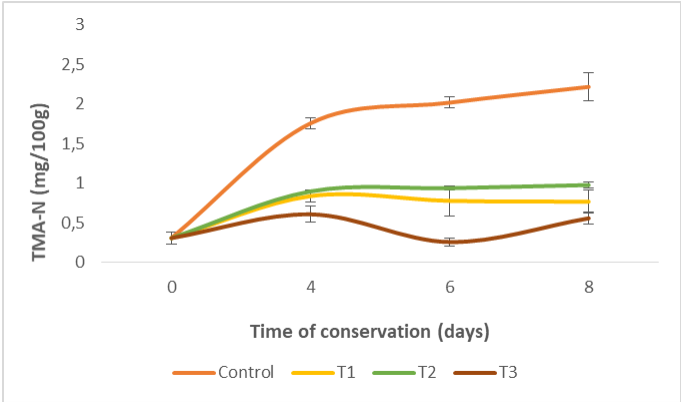

Figure 4. Evolution of the TMA-N content (mg 100 g^{-1}) during the storage period at room temperature simulating the period of exposure of sea bass in a fish market and its storage overnight at 2 ºC in a cold room (mean (n=3) ± standard deviation).

Regarding the results of carvacrol residues in fish muscle, it was found that the content of carvacrol in all samples was lower than the limit of quantification and the limit of detection. The other components of the EO pool (as limonene) were not detected either, so their concentration was <1 ppm.

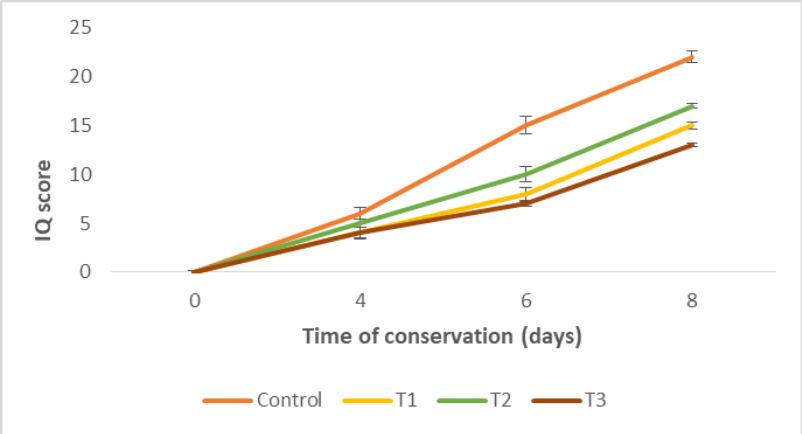

Figure 5. Sensory evaluation of sea bass during its conservation simulating the period of exposure of sea bass in a fish market and its conservation overnight at 2 ºC (mean (n=5) ± standard deviation).

4. CONCLUSIONS

The combined use of antimicrobial ice and filtered air curtain in HEPA filters proved to have a synergistic effect on the preservation of fresh fish (sea bass, in this case), since it reduced the content of TMA-N, which is an indicator parameter of the alteration of the fish. In addition, it significantly improved the maintenance of the visual characteristics of the fish freshness, such as the transparent cornea for a longer time, which is related to the control of the microbial load of the fish that causes the development of the processes of quality loss. On the other hand, the panellists did not detect off-odors or off-flavors due to the use of EOs, so the preservation of the fish with antimicrobial ice did not affect its sensory quality. In addition, no residues of EOs were found in the muscle of the fish.

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