EVALUATION OF AN OZONE- SLURRY ICE COMBINED REFRIGERATION SYSTEM FOR THE STORAGE OF FARMED TURBOT (*Psetta maxima*)

Carmen A. Campos[a,b], Vanesa Losada[c], Óscar Rodríguez[a], Santiago P. Aubourg[c], and Jorge Barros-Velázquez[a,*]

[a]*Department of Analytical Chemistry, Nutrition and Food Science, School of Veterinary Sciences, University of Santiago de Compostela, E-27002 Lugo, Spain;* [b]*Department of Industries, School of Exact and Natural Sciences, University of Buenos Aires, Ciudad Universitaria (1428) Buenos Aires, Argentina;* [c]*Department of Seafood Chemistry, Institute for Marine Research (IIM-CSIC), C/ Eduardo Cabello 6, E-36208 Vigo, Spain*

Corresponding: Tel.:+34.600.942264; Fax:+34.982.252195; E-mail: jbarros@lugo.usc.es

ABSTRACT

The use of ozonised slurry ice was investigated as a new refrigeration system for the storage of farmed turbot (*Psetta maxima*), a non-fat fish species of high commercial value. With this purpose in mind, an ozone generator device was coupled to a slurry ice system working subzero at -1.5ºC. The ozone concentration was adjusted to a 700 mV of redox potential, and the slurry ice biphasic mixture was prepared at a 40% ice/60% water ratio and a 3.3% of salinity. Microbiological, chemical and sensory analyses were carried out throughout a storage period of 35 days. Although certain biochemical parameters indicative of fish freshness –such as the rate of nucleotide degradation or TMA-N formation– were not significantly affected by the presence of ozone in the slurry ice mixture, storage in ozonised slurry ice significantly slowed down of mechanisms responsible for lipid hydrolysis –as determined by the release of free fatty acids– and lipid oxidation –evaluated by the peroxide value, the thiobarbituric acid-index and by the rate of browning reactions determined at 450/400 nm– in farmed turbot. Storage in ozonised slurry ice also led to significant ($p<0.05$) lower counts of both total aerobes and psychrotrophic bacteria in both turbot muscle and skin, as compared with the control batch stored without ozone. Sensory analyses confirmed an extended shelf life of turbot specimens stored in ozonised slurry ice, these maintaining "A" sensory quality up to day 14, while counterpart batch stored in slurry ice kept this quality only up to day 7. The combination of ozone and slurry ice may be recommended for the chilling and storage of farmed turbot with a view to extend its shelf-life.

Keywords: Slurry ice, Ozone, Turbot, Shelf life, Chilled storage, Quality

Running title: Storage of turbot in ozonised slurry ice

1. INTRODUCTION

Slurry ice –also known as flow ice, fluid ice, slush ice or liquid ice– consists of an ice-water suspension at a subzero temperature, and represents a relatively novel refrigeration system for the chilled storage and distribution of commercial fish species. Two remarkable features inherent to this advanced storage system are: its faster chilling rate –as compared to traditional chilling systems based on flake ice or chilled water–, and the reduced physical damage caused to seafood products by its spherical microscopic particles, as compared with the aciculate crystals of conventional flake ice (Piñeiro, Barros-Velázquez & Aubourg, 2004). Nevertheless, and despite the theoretical advantages of slurry ice, few empirical data about the experimental use of slurry ice-based chilling systems have been reported for the storage and distribution of commercial fish species. A previous report by Chapman (1990) found that finfish stored in slurry ice on board had a better quality as compared with other chilling methods, similar results being obtained for albacore (Price, Melvin & Bell, 1991) and shrimp (Huidobro, López-Caballero & Mendes, 2002). Another remarkable feature of slurry ice systems is that such mixtures can be pumped, thus implying: (i) a more hygienic fish handling, (ii) process automatisation, and (iii) its potential combination with other additives that may contribute to the preservation of fish quality.

On the other hand, ozone has been traditionally used as a disinfectant for fresh water aquaculture systems, and its applications for improving the sensory quality and shelf life of fish have been described recently (Kötters et al., 1997; Kim, Yousef and Dave, 1999; Kim et al., 2000). It is well known that molecular ozone and its decomposition products destroy microorganisms due to its effects on microbial intracellular enzymes, nucleic acids and other cell components. On contrast to these

positive effects, the possible pro-oxidant effect of ozone on fish constituents has not been extensively studied up to now, although previous reports have shown a potential negative effect on phospholipids, polyunsaturated fatty acids (PUFAs) and membrane proteins (Fukunaga, Suzuki & Takama, 1991; Takigi-Endo et al., 2002).

Turbot (*Psetta maxima*) represents a flatfish of remarkable commercial value (Person-Le Ruyet, 1990). In recent years, expanded production of this fish species as an aquaculture product has made them more available to consumers from Spain and other European countries. In this study we report on the application of an advanced refrigeration system –resulting from the combination of ozone and slurry ice– to farmed turbot. The physico-chemical nature of the slurry ice allowed a strict control of the ozone concentration in the liquid phase, such concentration being adjusted by means of the redox potential. The evaluation of the effects of ozonised slurry ice on the quality maintenance of turbot was carried out by biochemical, sensory and microbial analyses, the results being compared with a control batch stored in slurry ice studied in parallel.

2. MATERIALS AND METHODS

2.1. Refrigeration systems

Slurry ice was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). The composition of the flow ice binary mixture was 40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was -1.5ºC. The injection of ozone in one of the two slurry ice batches was accomplished with a prototype provided by Cosemar Ozono (Madrid, Spain), the redox

potential being adjusted to 700 mV (0.2 mg ozone/l) in the liquid phase. The other slurry ice batch was not ozonised and was considered as a control batch.

2.2. Fish material, processing and sampling

Two-year old farmed turbot (*Psetta maxima*) specimens were obtained from Stolt Sea Farm, S.A. (Carnota, Spain). Each batch of turbot specimens was sacrificed in the farm by immersion in slurry ice –either ozonised or not– and immediately transported in the same refrigerated system to our laboratory. The fish specimens were not headed nor gutted. The weight range was 1'3-1'5 kg. The fish specimens were surrounded by slurry ice –either ozonised or not– at a fish:ice ratio of 1:1, and stored for up to 35 days in a refrigerated room at 2ºC. Sampling times were established for both batches at day 0 (initial), and at days 7, 14, 21, 28 and 35. All experiments were done by triplicate. A third control batch of turbot specimens stored in flake ice was considered as an additional reference batch for sensory analysis. In this case, flake ice was prepared with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy). When required, the ice mixtures were renewed.

2.3. Chemical analyses

Analysis of the rate of the nucleotide autolytic degradation pathway was carried out by HPLC as described elsewhere (Ryder, 1985). The K value was calculated according to the following concentration ratio: K value = 100 x (hypoxanthine + inosine) / (adenosine triphosphate + adenosine diphosphate + adenosine monophosphate

+ inosine monophosphate + inosine + hypoxanthine). The evolution of pH values in turbot muscle along the storage time was determined by means of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

Total volatile base-nitrogen (TVB-N) contents were measured as described elsewhere (Aubourg et al., 1997). Briefly, fish muscle (10 g) was extracted with 6% perchloric acid and brought up to 50 ml. TVB-N contents were determined, after steam-distillation of the acid extracts rendered alkaline to pH 13 with 20% NaOH, by titration of the distillate with 10 mM HCl. The results were expressed as mg TVB-N/100 g muscle. Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described (Tozawa et al., 1971). This involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g /25 ml). Results were expressed as mg TMA-N/100 g muscle.

The lipid fraction was extracted using the Bligh and Dyer method (Bligh & Dyer, 1959). The peroxide value (PV) was determined according to the ferric thiocyanate method (Chapman & McKay, 1949), the results being expressed as milliequivalents of oxygen/Kg lipids. The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). Results were expressed as mg malondialdehyde/Kg fish sample. Browning development was assessed on the lipid extract at 400 and 450 nm according to Hassan, Khallaf, Abd-El Fattah & Yasin (1999). Results were expressed as the 450nm/400nm absorbance ratio. The free fatty acid (FFA) content was determined by the Lowry and Tinsley method, based on complex formation with cupric acetate-pyridine (Lowry & Tinsley, 1976). The results were expressed as g FFA/100 g lipids.

2.4. Sensory analyses

Sensory analyses were conducted in whole fish by a panel consisting of five experienced judges, according to official guidelines (Table 1) concerning fresh and refrigerated fish (Council Regulation, 1990). Four categories were ranked: highest quality (E), good quality (A), fair quality (B), and unacceptable quality (C). Sensory assessment of the fish included the examination of the following parameters: skin, external odor, gills, consistency and flesh odor. (Eliminé lo de promediar los panelistas)

2.5. Microbiological analyses

Fish skin sections of 5 cm^2 were swabbed with sterile 0.1% peptone water (Oxoid Ltd., London, UK) and the microbial load was resuspended in 10 ml of 0.1% peptone water (Difco, Michigan, IL). Samples of 5 g of fish muscle were also dissected aseptically in parallel from skinned chilled specimens, mixed with 45 ml of 0.1% peptone water, and homogenised in a stomacher (Seward Medical, London, UK). In both cases, serial dilutions from the skin or muscle microbial extracts were prepared in 0.1% peptone water. Total aerobic and psychrotrophic bacteria from surface and muscle turbot samples were investigated in Plate Count Agar (PCA, Oxoid) after incubation at 30°C for 48 h or at 7-8°C for 10 days, respectively, as described elsewhere (Ben-Gigirey et al., 1998, Rodríguez et al., 2004).

2.6. Statistical analyses

A multivariate analysis was performed to study the effect of each refrigeration system on the microbiological and chemical parameters. One-way analysis of variance

(ANOVA) was also used to explore the significance of differences among microbiological and chemical parameters throughout storage for each refrigeration system. Multiple comparisons between parameters were carried out by the Scheffé and DMS tests. All tests were carried out using the SPSS software (SPSS Inc., Chicago, IL). A confidence interval at the 95% level ($p<0.05$) was considered in all cases.

3. RESULTS AND DISCUSSION

3.1. Chemical analyses

The results of the investigation of chemical parameters in turbot muscle along storage are shown in Figures 1-3. Nucleotide autolytic degradation was studied by means of the K value (Figure 1A). In the present experiment, the K value rose to values slightly above 50 after 7 days of storage, no significant ($p>0.05$) differences being observed between the ozonised and the non-ozonised slurry ice batches. Accordingly, no inhibitory neither any enhancement effect of ozone on the autolytic degradation mechanisms could be assessed.

The formation of TVB-N was assessed in both batches, no significant ($p>0.05$) difference being observed between both storage systems. Thus, the TVB-N concentrations were in all cases below 25 mg/100 g muscle (data not shown). The evolution of TMA-N content in both batches is shown in Figure 1B. No significant differences were observed among batches up to day 21 neither with respect to the initial counterparts at day 0. However, after that time a sharp increase in the TMA-N content was observed in turbot muscle stored in slurry ice, a result that was not observed to such an extent in the batch stored in ozonised slurry ice. Nevertheless, it should be stressed that the TMA-N contents determined in this study were remarkably low in both batches,

this suggesting a very limited growth of TMA-producing bacteria. Unlike the results obtained in our study for turbot in slurry ice –either ozonised or not–, previous works relative to other small and medium-sized commercial fish species have reported sharp increases in the TMA-N content after 9-12 days of storage in flake ice (Pérez-Villarreal & Pozo, 1990; Fernández-Salguero & Mackie, 1987; Ruíz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2002), these results not being observed in our study with slurry ice-based systems.

Primary lipid oxidation was evaluated by means of the PV. The results indicated a slightly lower lipid oxidation in the batch stored in ozonised slurry, as compared with the batch stored in slurry ice, although the differences were not found to be significant ($p>0.05$) (Figure 2A). Secondary lipid oxidation was followed by the TBA-i. Interestingly, the presence of ozone slightly decreased the formation of TBA-reactive substances, this result being more remarkable after 21 days of storage (Figure 2B). Moreover, the rate of browning reactions involved in tertiary lipid oxidation, was found to be significantly ($p<0.05$, DMS test) lower in the ozonised slurry ice batch –even after only seven days of storage– this result indicating a significant reduction of the rate of lipid oxidation mechanisms in such batch (Figure 2C).

Lipid hydrolysis was determined according to the FFA assessment. In the present experiment, significant ($p<0.05$, DMS test) differences were observed between batches throughout storage (Figure 3). Accordingly, an inhibitory effect of ozonised slurry ice on lipid hydrolysis was concluded, this indicating a better protection of turbot muscle against lipid hydrolysis mechanisms as compared with the slurry ice batch. Although the release of FFA itself does not imply significant nutritional loss of quality, its evaluation was considered to be relevant since it has been proved that the accumulation of FFA is related to some extent to lipid oxidation enhancement

(Miyashita & Takagi, 1986; Yoshida, Kondo & Kajimoto, 1992) and to texture deterioration by interacting with proteins (Mackie, 1993; Sikorski & Kolakowska, 1994). In this sense, and according to the above-cited results concerning lipid hydrolysis and oxidation, the combination of ozone with slurry ice interestingly reduced the rate of lipid damage in turbot muscle.

With respect to the pH evolution in turbot muscle, no significant ($p>0.05$) effect that could be derived from the presence of ozone was observed. Thus, initial values of 6.3 slightly increased in both batches and reached final values of 6.6 after 35 days of storage. This type of behavior suggests a limited growth of alkalinising bacteria in both batches, probably derived from the subzero storage inherent to slurry ice in both batches.

In sum, the refrigeration and storage of turbot in the proposed ozonised slurry ice system significantly slowed down FFA formation and browning reactions, this indicating a better control of mechanisms involved in lipid damage. No negative chemical effect of ozone was observed as it can be derived from K value, TVB-N and TMA-N data.

3.2. Sensory analyses

The results of the sensory analyses are shown in Table 2. It can be observed that turbot specimens refrigerated by the combined use of slurry ice and ozone retained a good quality (E and A categories) up to day 21 and were unacceptable on day 28. However, when the slurry ice was used alone, the good quality was only retained up to day 7, although the product was unacceptable after 28 days of refrigerated storage (Table 2). The appearance of the gills was the first parameter that limited fish acceptability. A third control batch, stored in flake ice, was also considered as an

additional reference during the sensory assessment. Such batch exhibited a “B” score in four of the six sensory parameters evaluated even at day 7, the batch resulting to be unacceptable –as regards external odor, gills and general aspect– on day 14. Nevertheless, we should remark that the shelf lives of the turbot specimens stored of this study were not as long as those previously reported for other turbot specimens of considerably larger size (Rodríguez et al., 2003; Aubourg et al., 2005). To overcome this potential lack of comparability, an additional batch of turbot stored in flake ice was evaluated at the sensory level in parallel, thus allowing us to achieve a comparison of the potential benefits of the ozonised slurry ice system evaluated in this work, not only with respect to slurry ice, but also with respect to traditional flake ice.

From the results obtained, it can be concluded that the use of slurry ice produced a significant increase in turbot shelf life as compared with flake ice, this trend being enhanced in the case of ozonised slurry ice. The application of ozone has been previously reported to extend the shelf life of rockfish (*Sebastes* spp.) (Kötters et al., 1997), and catfish (*Ictalurus punctatus*) fillets (Kim et al., 2000). Likewise, other authors have described benefits as regards the discoloration of minced horse mackerel washed with ozonised water (Chen, Chiu & Huang, 1997), and a better maintenance of the sensory quality of scad (scad es lo mismo que horse mackerel) (*Trachurus trachurus*) treated on-board with gaseous ozone (Da Silva, Gibbs & Kirby, 1998). However, to our knowledge, our study represents the first report of the monitored application of ozone in the liquid phase of a subzero biphasic ice-water mixture for the storage of a non-fat fish species of remarkable commercial value, such as farmed turbot.

3.3. Microbiological analyses

The comparative evolution of microbial growth in turbot skin and turbot muscle along refrigerated storage in the slurry ice and ozonised slurry ice systems is presented in Figures 4 and 5, respectively. As can be seen for both microbial groups investigated, the use of ozone coupled to slurry ice significantly ($p<0.05$, Scheffé test) slowed down the microbial growth in the surface of turbot as compared to slurry ice (Figure 4). Thus, the counts of total aerobes determined in the surface of turbot stored in ozonised slurry ice were 1.23, 1.63 and 1.79 log CFU/cm^2 lower than the counterpart specimens stored for 14, 21 and 28 days, respectively, in slurry ice (Figure 4A). With respect to the effect of ozonised slurry ice on the surface growth of psychrotrophic bacteria, significant ($p<0.05$, Scheffé test) differences were also observed between both batches during the first 28 days of storage. Thus, the ozonised slurry ice batch exhibited microbial concentrations 0.21, 1.79 and 1.79 log CFU/cm^2 lower than the counterpart turbot specimens stored for 14, 21 and 28 days, respectively, in slurry ice alone (Figure 4B). The differences obtained in the results obtained for both storage systems proved to be statistically-significant ($p<0.05$, Scheffé test). It should be stressed that the significant differences determined for both microbial parameters coincided with the most remarkable differences between batches according to sensory analyses (Table 2). Thus, it seems that the less intense microbial growth in turbot surface obtained in the ozonised slurry ice batch coincided with an extended shelf life of this batch, as compared to slurry ice alone.

The bactericidal effect of ozone in fish has been previously described. Thus, Chen, Chang & Ing (1987) found that ozone, when added to water or to a NaCl solution, effectively inactivated microorganisms such as *Vibrio cholerae, Vibrio parahaemolyticus, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus.* Treatment of the skin of gutted fish with a 3% NaCl solution containing 0.6

ppm of ozone decreased the bacterial numbers by a factor of 2 to 3 log units, thus increasing shelf life up to a factor of 60%. More recently, Da Silva, Gibbs & Kirby (1998) reported the bactericidal effect of gaseous ozone on both total viable counts and H_2S-producing bacteria in fresh scad (*T. trachurus*). Different possible theories have been proposed to explain the ability of ozone to inactivate seafood surfaces. On one hand, some authors have suggested that ozone decreases the surface contaminants of fish during refrigerated storage (Dondo et al., 1992; Da Silva, Gibbs & Kirby, 1998). On the other hand, other authors defend that ozone inactivates microorganisms less effectively when they are on food surfaces as compared to low ozone-demand liquid media (Kim, Yousef and Dave, 1999). The results obtained in our study clearly indicated a significant reduction of the microbial populations present on turbot surface due to ozone, which, in our opinion, may support the former statement.

The evolution of microbial growth in turbot muscle along refrigerated storage in the slurry ice and ozonised-slurry batches is presented in Figure 5. As can be seen for total aerobes, the use of ozonised slurry ice allowed a significant ($p<0.05$, Scheffé test) better microbiological control in turbot muscle as compared with the slurry ice batch. Thus, the effect of ozone in the liquid phase of the slurry ice system implied reductions in the microbial numbers by a factor of 1.08 and 1.18 log CFU/g after 14 and 21 days of storage, respectively (Figure 5A). Moreover, total aerobes reached levels above 10^6 CFU/g after 28 days of storage in the slurry ice batch, while the numbers were below10^5 CFU/g at that sampling time in the muscle of turbot stored in the novel ozonised slurry ice system. Likewise, the numbers of the psychrotrophic microorganisms were significantly ($p<0.05$, Scheffé test) lower in turbot muscle from specimens stored in ozonised slurry ice batch than in the slurry ice batch during the first 28 days of storage. Thus, the counts of psychrotrophes were 1.20 and 1.25 log CFU/g lower in the batch

stored in the combined system, after 14 and 21 days of storage, respectively (Figure 5B). According to these results, the combination of ozone and slurry ice directly affected the microbial growth of microorganisms able to grow at low temperatures, a result that underlines the potential usefulness of the proposed combined system for the refrigerated storage of commercial fish species.

4. FINAL REMARKS

Storage of farmed turbot, a flat fish species of high commercial value in Europe, in the novel refrigeration system –developed by combining an ozone generator with a slurry ice system– evaluate in this work, allowed a better maintenance of the sensory and microbiological quality, these results implying a significant extension of its shelf-life. Biochemical analyses also confirmed that the presence of ozone did not imply any negative effect on fish quality, and even allowed the slowed down of certain mechanisms involved in lipid hydrolysis and oxidation. On the basis of the results obtained, the combined use of ozone and slurry ice may be recommended for the refrigerated storage of turbot and other flat fish species.

ACKNOWLEDGEMENTS

This work was supported by a project granted by the Secretaría Xeral de I+D from the Xunta de Galicia (Project PGIDT01MAR40202PR). The authors wish to thank KINARCA S.A.U. (Vigo, Spain) for providing the slurry ice equipment, and CoseMar Ozono (Madrid, Spain), for providing the ozone generator. Dr. C.A. Campos is a

recipient of a Visiting Research Fellowship from the Secretaría Xeral de I+D, Xunta de Galicia.

REFERENCES

Aubourg, S., Sotelo, C., & Gallardo, J. (1997). Quality assessment of sardines during storage by measurement of fluorescent compounds. *Journal of Food Science*, 62, 295-299.

Aubourg, S., Piñeiro, C., Gallardo, J., & Barros-Velázquez, J. (2005). Evolution of biochemical changes related to the quality loss in farmed turbot (*Psetta máxima*) during chilled storage. *Food Chemistry*, 90, 445-452.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M., & Vidal-Carou, M. (2002). Chemical and sensory changes in Mediterranean hake (*Merluccius merluccius*) under refrigeration (6-8ºC) and stored in ice. *Journal of Agricultural and Food Chemistry*, 50, 6504-6510.

Ben-Gigirey, B., Vieites Baptista de Sousa, J.M., Villa, T.G., & Barros-Velázquez, J. (1998). Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. *Journal of Food Protection*, 61, 608-615.

Bligh, E., & Dyer, W. (1959). A rapid method of total extraction and purification. *Canadian Journal of Biochemistry and Physiology,* 37, 911-917.

Chapman, L. (1990). Making the grade. Ice slurries get top marks for quality products. *Australian Fisheries*, July, 16-19.

Chapman, R., & McKay, J. (1949). The estimation of peroxides in fats and oils by the ferric thiocyanate method. *Journal of the American Oil Chemists' Society*, 26, 360-363.

Chen, H.C., Chang, S.O., & Ing, S.T. (1987). A study on the sterilization effect of ozone and its application for marine foods processing. *Journal of the Fisheries Society of Taiwan*, 14, 79-89.

Chen, H.H., Chiu, E.M., & Huang, J.R. (1997). Color and gel forming properties of horse mackerel (*Trachurus japonicus*) as related to washing conditions. *Journal of Food Science*, 62, 985-991.

Council Regulation (1990). *Official Journal of the European Communities*, No. C 84, 19 February, p. 69.

Da Silva, M.V., Gibbs, P.A., & Kirby, R.M. (1998). Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). *Journal of Applied Microbiology*, 84, 802-810.

Dondo, A.C., Nachtman, C., Doglione, L., Rosso, A., & Genetti, A. (1992). Foods: their preservation by combined use of refrigeration and ozone. *Ingenieria Alimentaria y Conserve Animale*, 8, 16-25.

Fernández-Salguero, J., & Mackie, I. (1987). Comparative rates of spoilage of fillets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of non-volatile and volatile amines. *International Journal of Food Science and Technology* 22, 385-390.

Fukunaga, K., Suzuki, T., & Takama, K. (1991). Effect of ozone exposure on the compositions of gill and erythrocyte membrane lipids and proteins of Japanese charr (*Salvelinus leucomaenis*). *Comparative Biochemistry and Physiology B*, 100B, 481-487.

Hassan, I., Khallaf, M., Abd-El Fattah, L. & Yasin, N. (1999). Quality criteria period and marketing loss estimations of pre-treated and cold stored mullet fish. *Grasas y Aceites* 50, 208-217.

Huidobro, A., López-Caballero, M. & Mendes, R. (2002). Onboard processing of deepwater pink shrimp (*Parapenaeus longirostris*) with liquid ice: Effect on quality. *European Food Research and Technology*, 214, 469-475.

Kim, J., Yousef, A., & Dave, S. (1999). Application of ozone for enhancing the microbiological safety and quality of foods: A review. *Journal of Food Protection*, 62, 1071-1087.

Kim, T.J., Silva, J.L., Chamul, R.S., & Chen, T.C. (2000). Influence of ozone, hydrogen peroxide or salt on microbial profile, TBARS and color of channel catfish fillets. *Journal of Food Science*, 65, 1210-1213.

Kötters, J., Pradur, A., Skura, B., Rosenthal, H., Black, E.A., & Rodrigues-Lopez, J. (1997). Observations and experiments on extending shelf life of rockfish (*Sebastes* spp) products with ozone. *Journal of Applied Icthiology*, 13, 1-8.

Lowry, R., & Tinsley, I. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society*, 53, 470-472.

Mackie, I. (1993). The effects of freezing on flesh proteins. *Food Reviews International*, 9, 575-610.

Miyashita, K. & Takagi, T. (1986). Study on the oxidative rate and prooxidant activity of free fatty acids. *Journal of the American Oil Chemists' Society*, 63, 1380-1384.

Pérez-Villarreal, B., & Pozo, R. (1990). Chemical composition and ice spoilage of albacore (*Thunnus alalunga*). *Journal of Food Science*, 55, 678-682.

Person-Le Ruyet, J. (1990). Sole and turbot culture. In Barnabe, G. (Ed.), *Aquaculture* vol. 2 (pp. 687-727). London (UK): Elliswood.

Piñeiro, C., Barros-Velázquez, J., & Aubourg, S.P. (2004). Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake ice chilling methods. *Trends in Food Science and Technology*, 15, 575-582.

Price, R., Melvin, E., & Bell, J. (1991). Postmortem changes in chilled round, bled and dressed albacore. *Journal of Food Science*, 56, 318-321.

Rodríguez, O., Barros-Velázquez, J., Ojea, A., Piñeiro, C., & Aubourg, S. (2003). Evaluation of microbial changes and identification of major proteolytic bacteria in farmed turbot (*Psetta maxima*) during chilled storage. *Journal of Food Science*, 68, 2764-2771.

Rodríguez, O., Losada, V., Aubourg, S., & Barros-Velázquez, J. (2004). Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International*, 37, 749-757.

Ruíz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International*, 34, 441-447.

Ryder, J. (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 33, 678-680.

Sikorski, Z., & Kolakowska, A. (1994). Changes in protein in frozen stored fish. In Sikorski, Z, Sun Pan, B., & Shahidi, F. (Eds.), *Seafood Proteins* (pp. 99-112). New York (USA): Chapman and Hall.

Takigi-Endo, M., Ono, K., Nakagawa, K., Yotsu-Yamashita, M., & Miyazawa, T. (2002). Ozonation of PC in ethanol: separation and identification of a novel ethoxyhydroperoxide. *Lipids*, 37, 1007-1012.

Tozawa, H., Erokibara, K., & Amano, K. (1971). Proposed modification of Dyer's method for trimethylamine determination in codfish. In R. Kreuzer (Ed.), *Fish Inspection and Quality Control* (pp. 187-190). London (UK): Fishing News Books Ltd.

Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette Seifen Anstrichm*, 72, 1084-1087.

Yoshida, H., Kondo, I., & Kajimoto, G. (1992). Participation of free fatty acids in the oxidation of purified soybean oil during microwave heating. *Journal of the American Oil Chemists' Society*, 69, 1136-1140.

FIGURE CAPTIONS

Figure 1: Comparative evolution of the: **(A)** nucleotide degradation rate, as determined by the K value, and **(B)** TMA-N formation, in turbot muscle during storage in ozonised slurry ice (grey square) or slurry ice (white square).

Figure 2: Comparative evolution of lipid oxidation mechanisms in turbot muscle stored in ozonised slurry ice (grey square) or slurry ice (white square). **(A)** Primary lipid oxidation, as determined by the peroxide value (PV); **(B)** secondary lipid oxidation, as determined by the thiobarbituric acid index (TBA-i), and **(C)** tertiary lipid oxidation as determined by the rate of browning reactions measured at 450 and 400 nm.

Figure 3: Comparative release of free fatty acids (FFA) in turbot muscle during storage in ozonised slurry ice (grey square) or slurry ice (white square).

Figure 4: Evolution of surface microbial growth on turbot skin during storage in ozonised slurry ice (grey square) or slurry ice (white square). **(A)**: total aerobes; **(B)**: psychrotrophes.

Figure 5: Evolution of microbial growth on turbot muscle during storage in ozonised slurry ice (grey square) or slurry ice (white square). **(A)**: total aerobes; **(B)**: psychrotrophes.

TABLE 1

Scale employed for evaluating the sensory quality of turbot batches

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
External odor	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Incipiently sour and putrid	Sour and putrid
Gills	Brightly red; without odor; lamina perfectly separated	Rose colored; without odor; lamina adhered in groups	Slightly pale; incipient fishy odor; lamina adhered in groups	Grey-yellowish color; intense ammonia odor; lamina totally adhered
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh odor	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Incipiently sour and putrid	Sour and putrid

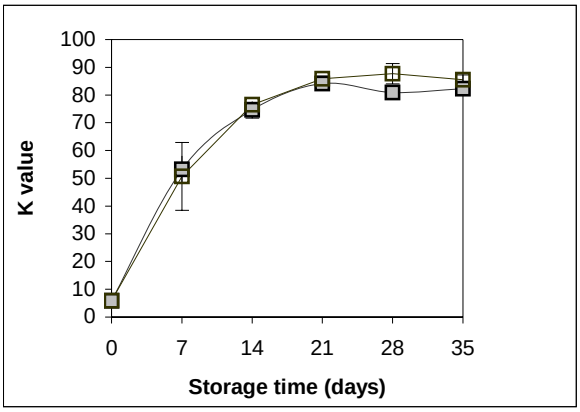
TABLE 2

Comparative sensory acceptability of turbot batches.

	Ozonised slurry ice (days of storage)						Slurry ice (days of storage)				
	0	**7**	**14**	**21**	**28**	**35**	**7**	**14**	**21**	**28**	**35**
Skin aspect	E	A	A	A	A	A	A	A	A	A	A
External odor	E	A	A	A	B	C	A	A	B	C	C
Gills	E	A	A	B	C	C	A	B	B	C	C
Consistency	E	A	A	A	A	A	A	A	A	A	A
Flesh odor	E	A	A	B	B	C	A	A	B	C	C
General aspect	E	E	A	A	B	C	A	A	B	B	C

FIGURE 1

A


K value
100
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
35
Storage time (days)

B

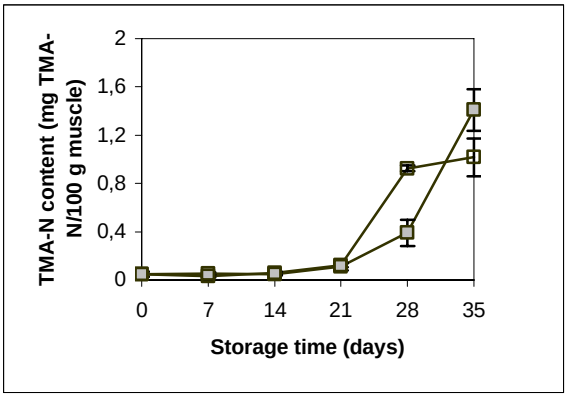
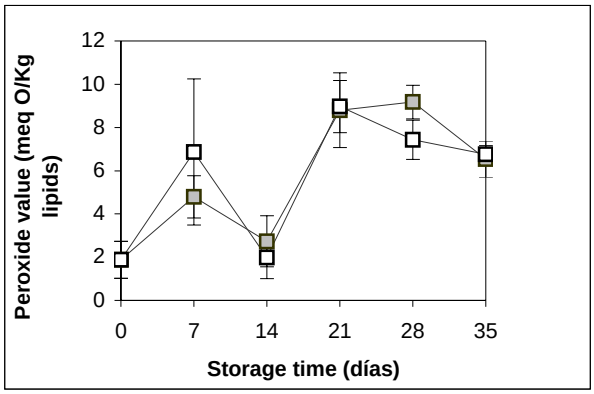
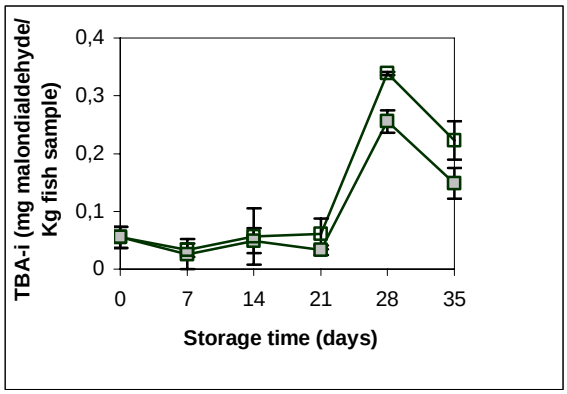

TMA-N content (mg TMA-N/100 g muscle)
2
1,6
1,2
0,8
0,4
0
0
7
14
21
28
35
Storage time (days)

FIGURE 2

A


Peroxide value (meq O/Kg lipids)
12
10
8
6
4
2
0
0
7
14
21
28
35
Storage time (días)

B


TBA-i (mg malondialdehyde/ Kg fish sample)
0,4
0,3
0,2
0,1
0
0
7
14
21
28
35
Storage time (days)

C

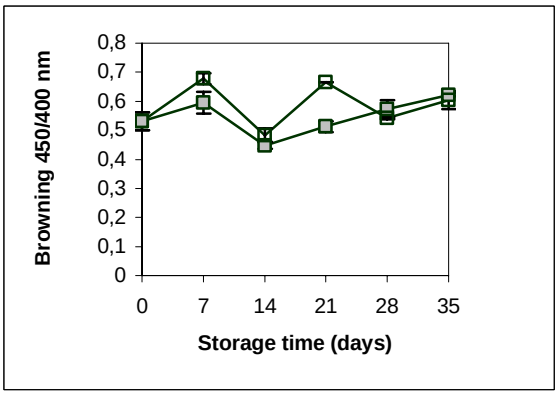

Browning 450/400 nm
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
7
14
21
28
35
Storage time (days)

FIGURE 3

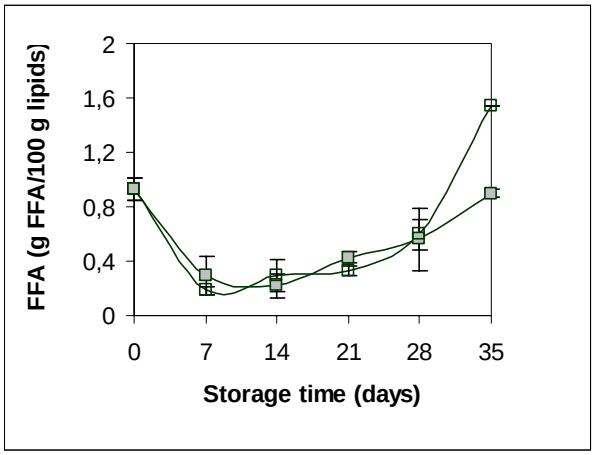
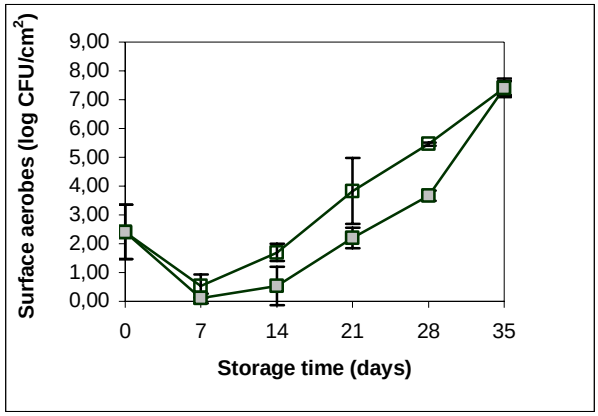

FFA (g FFA/100 g lipids)
2
1,6
1,2
0,8
0,4
0
0
7
14
21
28
35
Storage time (days)

FIGURE 4

A


Surface aerobes (log CFU/cm2)
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
35
Storage time (days)

B

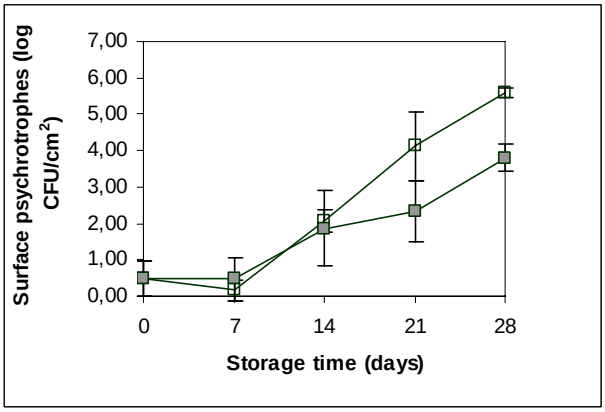
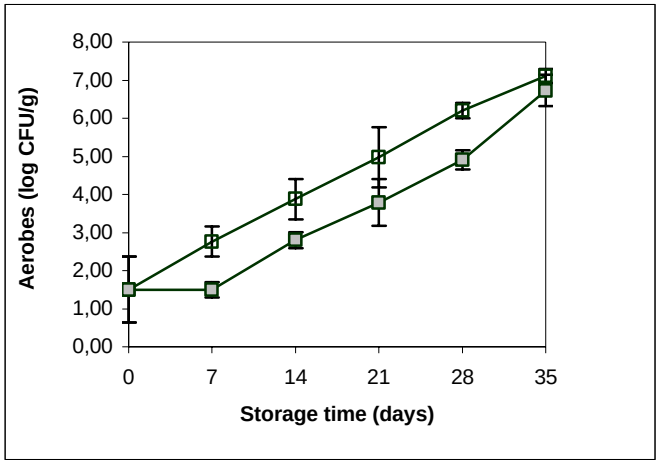
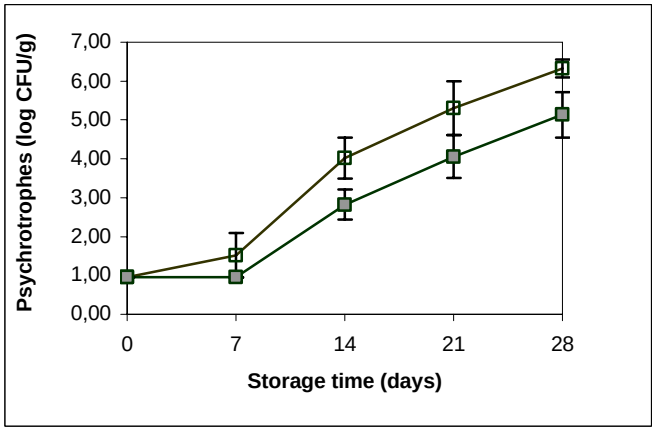

Surface psychrotrophes (log CFU/cm2)
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
Storage time (days)

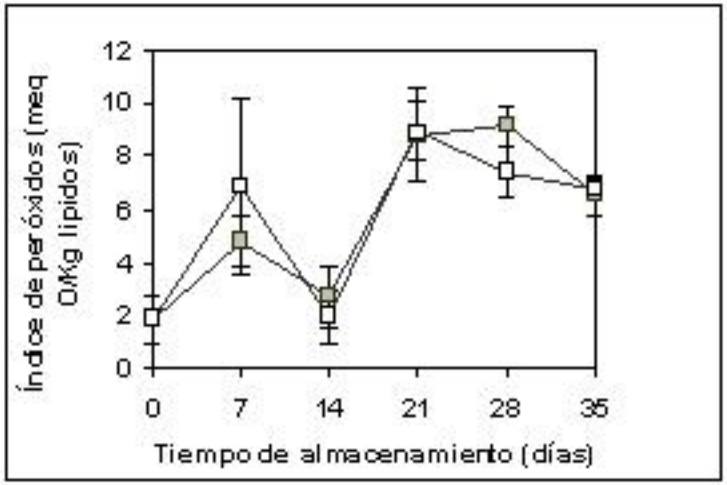
FIGURE 5

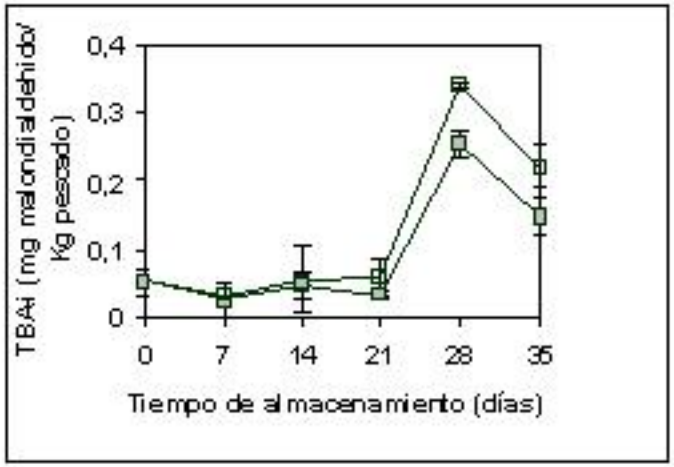
A

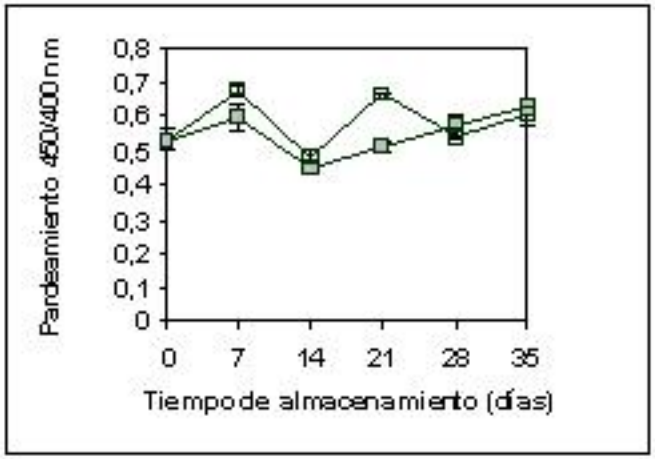

Aerobes (log CFU/g)
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
35
Storage time (days)

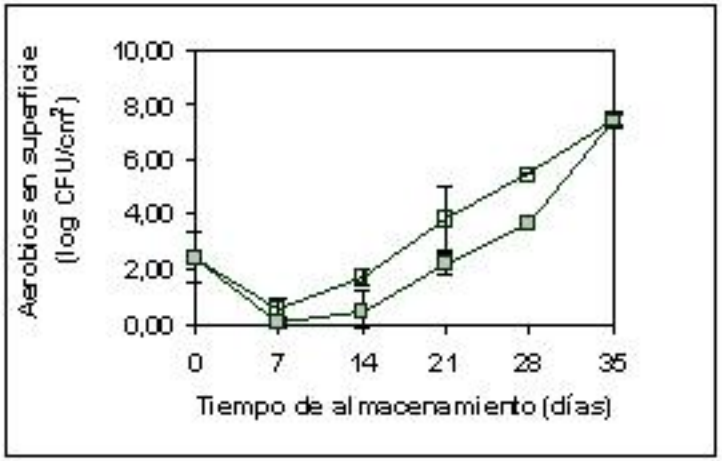
B

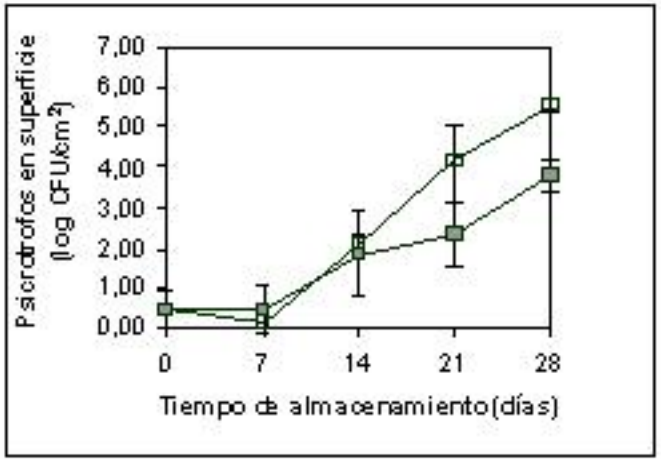

Psychrotrophes (log CFU/g)
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
Storage time (days)

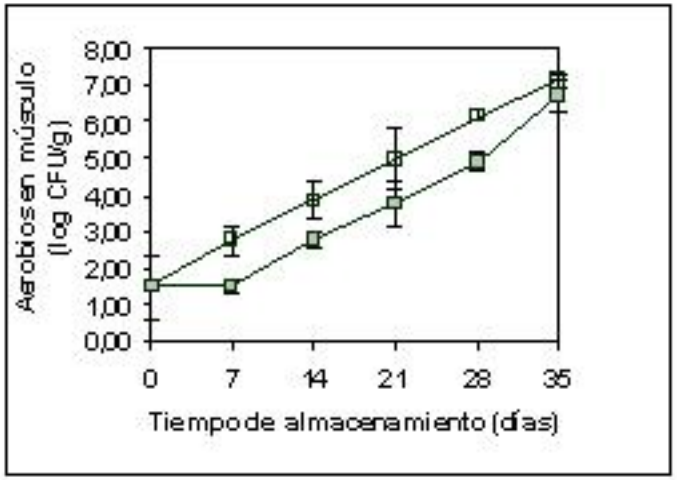

Índice de peróxidos (meq O/Kg lípidos)
12
10
8
6
4
2
0
0
7
14
21
28
35
Tiempo de almacenamiento (días)

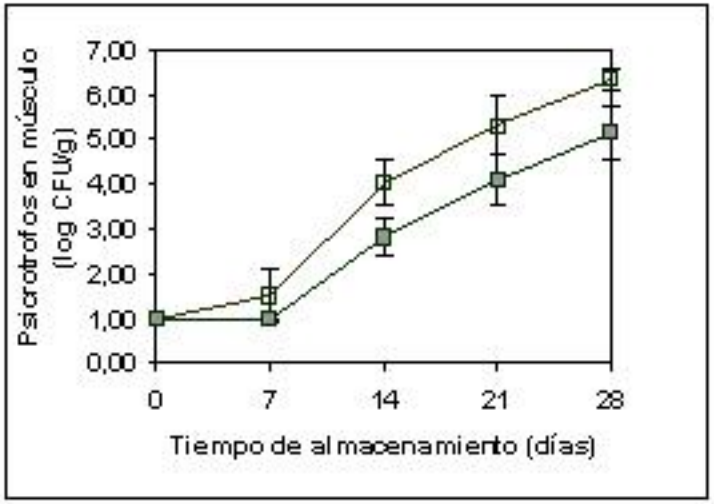

TBAi (mg malondialdehido/ Kg pescado)
0,4
0,3
0,2
0,1
0
0
7
14
21
28
35
Tiempo de almacenamiento (días)


Pardeamiento 450/400 nm
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
7
14
21
28
35
Tiempo de almacenamiento (días)


Aerobios en superficie
(log CFU/cm²)
10,00
8,00
6,00
4,00
2,00
0,00
0
7
14
21
28
35
Tiempo de almacenamiento (días)


Psicrotrofos en superficie (log CFU/cm²)
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
Tiempo de almacenamiento(días)


Aerobios en músculo
(log CFU/g)
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
35
Tiempo de almacenamiento (días)


Psicrotrofos en músculo (log CFU/g)
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
0
7
14
21
28
Tiempo de almacenamiento (días)