Title: Sensory, microbial and biochemical effects of a novel ozonised-slurry ice system for the on-board storage of megrim (*Lepidorhombus whiffiagonis*)

On-board quality preservation of megrim (*Lepidorhombus whiffiagonis*) by a novel ozonised-slurry ice system

Authors: Óscar Rodríguez[1], Vanesa Losada[2], José M. Miranda[1], Santiago P. Aubourg[2], and Jorge Barros-Velázquez[1,*]

Affiliations: [1]Laboratory of Food Technology, LHICA, Department of Analytical Chemistry, Nutrition and Food Science, School of Veterinary Sciences, University of Santiago de Compostela, E-27002 Lugo, Spain, and [2]Institute for Marine Research (IIM), Higher Council for Scientific Research (CSIC), Eduardo Cabello 6, E-36208 Vigo, Spain

Running head: On-board storage of megrim in ozonised slurry ice

On-board megrim preservation by ozonised slurry ice

***Author for correspondence:** Professor Jorge Barros-Velázquez, Laboratory of Food Technology, Department of Analytical Chemistry, Nutrition and Food Science, School of Veterinary Sciences, University of Santiago de Compostela, E-27002 Lugo, Spain. Tel: 34.600.942.264; Fax: 34.986.649266; E-mail: jbarros@lugo.usc.es

1 **Abstract**

2

3

4

5 **Key words:** on-board storage, megrim, slurry ice, ozone, shelf-life, freshness

Introduction

Marine species provide highly perishable products whose quality and freshness rapidly declines *post-mortem* due to a variety of microbial and biochemical degradation mechanisms. These undesirable events make mandatory the efficient refrigeration of marine food products on-board in the fish vessels immediately after the catch and the imposition of hygienic handling and storage conditions, since these will directly affect both the fish quality and its commercial shelf-life [1, 2].

Traditionally, fish species have been preserved by cooling and storing in flake ice [3], refrigerated seawater [4], or by exposure to the action of chemical preservation agents [5, 6]. In the last decade, slurry ice-based cooling and storage methods have been introduced in the fish sector [for an updated review: 7]. Slurry ice consists of a biphasic system composed by microscopic spherical ice crystals dispersed in refrigerated seawater cooled at subzero temperature in the range of -0.5ºC to -1.5ºC. Slurry ice systems exhibit several technical features of applied interest, among them: (i) its higher heat-exchange capacity as compared to flake ice, this reducing the cooling times of the fish catch, (ii) the reduced physical damage caused to the fish surface by the microscopic spherical particles as compared with flake ice aciculate crystals, (iii) the slowing down of a wide variety of chemical and enzymatic degradation mechanisms due to the subzero temperature imposed, and (iv) the fluid nature of slurry ice, which allows its pumping and a more hygienic handling of the fish specimens[7].

Pioneer works by Chapman [8] demonstrated a better maintenance of quality of finfish stored on-board in slurry ice as compared with other more traditional chilling technologies. Similar good conclusions were raised when slurry ice was used for the on-board storage of albacore tuna [9] and as a pre-cooling method [10]. Evidences of the

advantages of slurry ice systems for the management of shellfish batches have also been reported, both for inland storage [11], and, more recently, for on-board storage [12].

In our laboratory, refrigeration and subzero storage in slurry ice has proven to slow down microbial growth in lean [13], medium-fat [14] and fat [15] fish species. Likewise, refrigeration and storage in slurry ice have also been described to decrease the rates of a variety of biochemical mechanisms involved in quality loss in lean fish species [16], medium-fat [17] or fat [18] fish species. As a consequence of these effects, better sensory quality and extended shelf-lives have been reported for such fish species stored in slurry ice, as compared with traditional flake ice [13-18].

A technical relevant feature of slurry ice is that it may be combined with other agents, such as ozone, to achieve an antiseptic surface effect, or melanosis inhibitors, to prevent browning reactions in shellfish [12]. We have demonstrated the benefits of a novel ozonised-slurry ice combined system for the inland storage of sardine [14, 18] and farmed turbot [19]. However, such system has not been installed in a fishing vessel for its evaluation in the on-board storage of fish material. Thus, the main goal of this work was to combine a slurry ice prototype and an ozone generator for the on-board refrigeration and storage of megrim (*Lepidorhombus whiffiagonis*). This flat fish species represents the most fished species in the Gran Sol North Atlantic Fishing Bank, exploited by a number of European countries [20]. The capture of megrim in such a distant fishing bank usually means that the time elapsed between the catch and arrival at destiny varies from 10 to 15 days, this fact underlining the need to optimise refrigeration parameters in order to provide consumers with fish of the highest quality possible, especially in the case of fish specimens caught during the first days of the fishing run.

Materials and methods

Slurry ice system used

A combined slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) provided with an ozone generator (Cosemar Ozono, Madrid, Spain) was installed in the ship Cantábrico, based on Vigo fishing harbour (Northwestern Spain). The composition of the slurry ice binary mixture was 40% ice and 60% water, prepared on-board with filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was -1.5ºC. Flake ice was prepared using freshwater with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy).

Fish material, processing and sampling

Two batches of megrim specimens were prepared in flake ice (FI) and slurry ice (SI). Another two batches were prepared with ozonised slurry ice (oSI batches). In such batches, the injection of ozone in the slurry ice mixture was accomplished with the ozone generator (Cosemar Ozono), the redox potential being adjusted to 600 mV (0.16 mg ozone/l) (oSI_{600} batch) or to 300 mV (0.08 mg ozone/l) (oSI_{300} batch), respectively. In both batches, the ozone concentration was monitored by readings of the redox potential in the liquid phase. The fish specimens were surrounded by either ozonised slurry ice, slurry ice or flake ice at a fish:ice ratio of 1:1, and stored on-board for 14 days in a refrigerated room at 2ºC. The temperature of megrim in the SI and oSI batches was in the range of -1˚C/-1.5˚C, while the temperature in the flake ice was in the range of 0˚C/+1ºC. When required, the ice mixtures were renewed on-board.

The fish specimens were gutted and not headed. The length of the megrim specimens was in the 15–20 cm range and their weight was in the 80-100 (¿tan poco?) g range.

Once the four batches were unloaded at Vigo fishing harbour, they were transported to the laboratory of IIM (Vigo, Spain) and kept in an isothermal room in each type of ice at +2ºC for up to 6 days. Sensory, microbiological and biochemical analyses were performed at days 0, 2 and 6 after unloading, these corresponding to 14, 16 and 20 days after the catch. All analyses were performed in triplicate.

Sensory analyses

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to traditional guidelines concerning fresh and refrigerated fish [21]. Four categories were ranked: highest quality (E), good quality (A), fair quality (B), and unacceptable quality (C). Sensory assessment of the fish included the following parameters: external odour and appearance, gill cavity and flesh odour.

Microbiological analyses

Samples of 10 g of fish muscle were dissected aseptically from chilled hake specimens, mixed with 90 ml of 0.1% peptone water (Oxoid Ltd., London, UK), and homogenised in a stomacher (Seward Medical, London, UK) as previously described [22, 23]. In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes were investigated by surface inoculation in plate count agar (PCA, Oxoid) after incubation at 30ºC for 72 h. Psichrotrophes were also investigated in PCA (Oxoid) but incubation was carried out at 7-8ºC for 10 days. *Enterobacteriaceae* were investigated in Crystal Violet Neutral Red Bile Glucose Agar (VRBD Agar, Merck, Darmstadt, Germany) after incubation at 37ºC for 24 h. Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium [24] after incubation at 30ºC for 48 h, as previously described [25].

Chemical analyses

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

Total volatile base-nitrogen (TVB-N) values were measured as described elsewhere [26]. Briefly, fish muscle (10 g) was extracted with 6% perchloric acid and brought up to 50 ml -the TVB-N content being determined, following 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 [27]. This technique involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g /25 ml). The results were expressed as mg TMA-N/100 g muscle.

Lipid hydrolysis was evaluated by the free fatty acid (FFA) content, determined by the Lowry and Tinsley [28] method based on complex formation with cupric acetate-pyridine. Results were expressed as g FFA/100 g lipids. Lipid oxidation was evaluated by estimating the formation of fluorescent compounds with a Perkin Elmer LS 3B fluorimeter. Measurements were performed at 393/463 nm and 327/415 nm as previously described [26]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/mL in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463}$ nm/$RF_{327/415}$ nm. The FR value was determined in the aqueous phase resulting from the lipid extraction [30].

Statistical analyses

Bacterial counts were transformed into log CFU/g before undergoing statistical analysis. The SPSS 11.5 for Windows software (SPSS Inc., Chicago, IL) was used to explore the statistical significance of the results obtained, this including multivariate contrasts and multiple comparisons by the DMS test. A confidence interval at the 95% level ($p<0.05$) was considered in all cases.

Results and discussion

Sensory analyses

The megrim specimens stored on board in any of the two oSI systems were classified into the A category at unloading (Table 1). After this sampling time, quality decreased to B category in the batch oSI_{300} on day 6, this was 20 days after the catch, while the oSI_{600} batch maintained the A quality at that time. On contrast, megrim specimens kept on boar in FI or SI exhibited B category when unloading, although the latter batch exhibited a better sensory quality than the former. The quality of the FI batch after unloading decreased rapidly and, unlike the SI batches, was not acceptable on day 20 (Table 1).

The main negative aspect related to quality loss in the batches was both the gills and external odour. No significant alteration in the eye appearance was observed in any of the four batches studied this not being in agreement with the results obtained for seabream stored in slurry ice by other authors [31]. According to our results, on-board storage of megrim in ozonised slurry ice (oSI_{600} batch) allowed a significant extension of its shelf-life, such batch maintaining the A quality even after 20 days of storage. This result confirms at the sensory level previous works performed with oSI batches [15, 19],

1 although the present work represents the first scientific evaluation of an oSI system for
2 the on-board storage of a commercial fish species.
3
4 *Microbiological analyses*
5 Initially, one-way ANOVA was carried out considering aerobes, psychrotrophes,
6 *Enterobacteriaceae* and proteolytic bacteria as dependent variables, and time as the
7 factor. *Post-hoc* analyses were performed by means of the DMS test. Table 2 compiles
8 the average counts and ranges determined for the above-cited microbial groups in the
9 FI, SI, $oSI_{300,}$ oSI_{600} batches after 14, 16 and 20 days of storage. Statistically significant
10 ($p<0.05$) lower microbial numbers were determined for all four above-cited microbial
11 groups in the oSI_{600} with respect to the FI and SI batches.
12 With respect to the counts of total aerobes, the average difference between oSI_{600}
13 batch and FI and SI batches was 0.469 and 0.525 log units, respectively (Table 2).
14 However, the bacterial counts reached levels slightly above 10^4 CFU/g only in the FI
15 and SI batches, these numbers being considerably below those considered to be required
16 for the spoilage of fish stored aerobically [32]. These results are in agreement with the
17 significantly lower counts determined for aerobes in sardine [15] and turbot [19] muscle
18 stored in ozonised slurry ice with respect to FI and SI, although the latter two works
19 were not performed on-board.
20 The numbers determined for psychrotrophic bacteria in megrim specimens stored in
21 the oSI_{600} batch were also significantly ($p<0.05$) lower than those determined in the FI
22 and SI batches, with average differences of 0.445 and 0.446, respectively (Table 2), this
23 revealing a significant slow down of the growth of this bacterial group in the oSI_{600}
24 batch. However, and as in the case of aerobes, the numbers of psychrotrophes in all four
25 batches were not high, being in all cases below 10^6 CFU/g. More intense growth

reductions had been determined for psychrotrophes in sardine [15] and turbot [19] muscle stored in ozonised slurry ice.

With respect to the development of *Enterobacteriacea* in megrim muscle stored in either slurry ice or flake ice, significant ($p<0.05$) differences were observed between the oSI_{600} batch and both FI and SI batches, as in the case of aerobes and psychrotrophes (Table 3). However, the average counts were so low that the contribution on this bacterial group to megrim spoilage must be discarded.

The greatest differences between the oSI batches and both FI and SI batches were determined for proteolytic bacteria. This bacterial group may lead to the formation of microbial metabolites such as peptides or amino acids, derived from protein hydrolysis, thus contributing to undesirable sensory changes in seafood products [33-35]. As can be observed in Table 2, significant ($p<0.05$) lower counts of proteolytic bacteria were observed in the oSI_{600} and oSI_{300} batches than in the FI and SI batches. The greatest average difference (1.463 log CFU/g units) was found between oSI_{300} and FI batches. These results confirm a previous work indicating a significantly slower growth of proteolytic bacteria in sardine stored in ozonised slurry ice [15].

Chen *et al.* [36] reported that the presence of ozone in water or in NaCl solutions were effective for the inactivation of a wide variety of Gram-negative and Gram-positive bacteria. Although the present study was performed with slurry ice prepared with marine water, and not with a NaCl solution, the results described in our study and those described by Chen *et al.* [36] were in agreement. It should also be underlined that the results obtained in the microbiological analyses in the present study, correlated well with the differences observed in the sensory analyses, again confirming the better maintenance of quality in the megrim specimens stored in the oSI_{600} batch with respect to the other three batches.

Chemical analyses

It is widely accepted that pH increase denotes the accumulation of undesirable alkaline compounds, such as ammonia and TMA, which are mostly derived from microbial action [37]. It has been suggested that pH values above 7 may limit the consumption of certain lean fish species such as hake [38]. In our study regarding pH, significant ($p<0.05$) differences were determined between the FI batch on one hand, and the other SI-based batches, on the other (Table 3). Thus, at day 20 of storage the pH value of megrim muscle stored in FI resulted to be 7.38. On contrast, significantly ($p<0.05$) lower pH values of 7.07, 6.95 and 6.99 were determined in the SI, oSI_{300} and oSI_{600} batches, respectively. These results seem to be related to a more intense development of alkalinising microflora in the FI batch and are in agreement with previous reports that reported steady increases in pH for other lean fish species such as hake [38] and turbot [35], and a better control of the pH value as a consequence of their storage in slurry ice [13, 19] or in ozonised slurry ice [39].

As in the case of the pH values, TVB-N formation in megrim stored in FI was significantly ($p<0.05$) higher than in the other three batches (Table 3). Thus, TVB-N concentrations below 22 mg/100 g muscle were determined in the SI and oSI batches on day 14, while such values rose above 34 mg/100 g in the case of the FI batch. A similar trend was observed on days 16 and 20 (Table 3). Interestingly, the presence of ozone in the oSI batches implied the lowest TVB-N contents in megrim muscle, although such batches were not statistically different with respect to the SI batch. The FI batch would reach the legal limit of 35 mg/100 g set for TVB-N (Directive 95/149/EEC) on day 14, while such limit was not surpassed by the SI and oSI batches up to day 20. These results

are also in agreement with a previous study on farmed turbot stored in ozonised slurry ice, SI and FI [19].

A similar trend was also observed for TMA-N, another parameter that together with TVB-N has been previously proposed to estimate freshness in megrim [40]. Thus, TMA-N formation in megrim stored in FI was significantly ($p<0.05$) higher than in the case of the other three batches (Table 3). In example, TMA-N concentrations below 15 mg/100 g muscle were determined in the SI and oSI batches on day 16, while TMA-N reached values above 25 mg/100 g on that day. A similar trend was observed on day 20 (Table 3). As in the case of pH and TVB-N, the presence of ozone in the oSI batches implied lower TMA-N formation than in the SI batch, although all these three batches were not significantly ($p<0.05$) different. In this sense, the oSI_{600} batch was in the range of the legal limit of 12 mg/100 g set for TMA-N (Directive 91/493/EEC) on day 16, while the other three batches were above that limit. Our results clearly indicate a better control of TMA-N formation in the SI and oSI batches with respect to FI, a result that is in agreement with a previous study on farmed turbot [19].

Lipid hydrolysis was studied according to the FFA formation. A faster hydrolysis development was observed till day 16 for the FI treatment when compared to the three SI systems. At the end of the experiment, a high FFA formation was observed for the SI systems so that no differences ($p>0.05$) could be outlined among treatments. A strong inhibition on hydrolytic enzyme activity is inferred for the SI treatments according to previous research (jur2, sard2). Ozone presence has not afforded a different behaviour in the FFA production, according to previous research (sard2).

Interaction compounds produced between oxidised lipids and nucleophilic compounds present in the muscle were studied by fluorescence assessment. No differences could be outlined after the on-board storage. However, during the latest

storage period (days 16-20) a higher fluorescence development could be outlined, according to an increase in lipid oxidation. At day 20, all treatments showed a significant ($p<0.05$) increase, although no differences ($p>A0.05$) could be found among the SI systems.

Acknowledgements

The authors wish to thank KINARCA S.A.U. for providing the slurry ice equipment. This work was supported a project granted by the Secretaría Xeral de I+D from the Xunta de Galicia (Project XUNTA3XXXX). The authors also thank Mr. Marcos Trigo and Mr. Marcos Ojea for their excellent technical assistance.

References

[1]. Whittle, K., Hardy, R., & Hobbs, G. (1990). Chilled fish and fishery products. In Gormley, T., *Chilled foods: the state of the art*, (pp. 87–116). New York: Elsevier Applied Science.

[2]. Olafsdöttir, G., Martinsdöttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I., Henehan, G., Nielsen, J. & Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology, 8*, 258–265.

[3]. Nunes, M., Batista, I., & Morâo de Campos, R. (1992). Physical, chemical and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. *Journal of the Science of Food and Agriculture, 59*, 37–43.

[4]. Kraus, L. (1992). Refrigerated sea water treatment of herring and mackerel for human consumption. In Burt, J., Hardy, R., & Whittle, K., *Pelagic fish. The resource and its exploitation*, (pp. 73–81). Aberdeen, Scotland: Fishing News Books.

[5]. Ponce de León, S., Inoue, N., & Shinano, H. (1993). Effect of acetic and citric acids on the growth and activity (VB-N) of *Pseudomonas* sp. and *Moraxella* sp. *Bulletin of the Faculty of Fisheries of Hokkaido University, 44*, 80–85.

[6]. Hwang, K., & Regenstein, J. (1995). Hydrolysis and oxidation of mackerel (*Scomber scombrus*) mince lipids with NaCl and NaF treatments. *Journal of Aquatic Food Product Technology, 4*, 19-30.

[7]. PAPER TRENDS

[8]. Chapman, L. (1990). Making the grade. Ice slurries get top marks for quality products. *Australian Fisheries, 7*, 16–19.

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

[10]. Harada, K. (1991). How to handle albacore. *Australian Fisheries, 2,* 28–30.

[11]. Chinivasagam, H. N., Bremner, H. A., Wood, A. F., & Nottingham, S. M. (1998). Volatile components associated with bacterial spoilage of tropical prawns. *International Journal of Food Microbiology, 42,* 45–55.

[12]. 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.

[13]. MERL1

[14]. JUREL1

[15]. SARD1

[16]. MERL2

[17]. JUREL2

[18]. SARD2

[19]. ROD5

[20]. DATOS DE LA FAO

[21]. DOCE (1989). Baremo de clasificación de frescura. Brussels: European Comission.

[22]. 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.

[23]. Ben-Gigirey, B., Vieites Baptista de Sousa, J. M., Villa, T. G., & Barros-Velázquez, J. (1999). Histamine and cadaverine production by bacteria isolated

from fresh and frozen albacore (*Thunnus alalunga*). *Journal of Food Protection, 62*, 933–939.

[24]. Phaff, H.J., Starmer, W. T., Lachance, M. A., & Ganter, P. F. (1994). *Candida caseinolytica* sp. nov., a new species of yeast occurring in necrotic tissues of *Opuntia* and *Stenocereus* species in the Southwestern United States and Baja California, Mexico. *International Journal of Systematic Bacteriology, 44*, 641–645.

[25]. Ben-Gigirey, B., Vieites Baptista de Sousa, J. M., Villa, T. G., & Barros-Velázquez, J. (2000). Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. *International Journal of Food Microbiology, 57*, 19–31.

[26]. 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.

[27]. Tozawa, H., Erokibara, K., & Amano, K. (1971). Proposed modification of Dyer's method for trimethylamine determination in codfish. In Kreuzer R., *Fish inspection and quality control*, (pp. 187–190). London, UK: Fishing News Books.

[28]. Lowry and Tinsley

[29]. Vyncke

[30]. Bligh and Dyer

[31]. Huidobro, A., Mendes, R., & Nunes M. L. (2001). Slaughtering of gilthead seabream (*Sparus aurata*) in liquid ice: influence on fish quality. *European Food Research and Technology, 213* (4–5), 267–272.

[32]. Gram, L. & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology, 33*, 121–137.

[33]. Shewan, J. M. (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In *Handling, processing and marketing of tropical fish*, (pp. 51–68). London, UK: Tropical Product Institute.

[34]. Makarios-Laham, I. K., & Lee, T. C. (1993). Protein hydrolysis and quality deterioration of refrigerated and frozen seafood due to obligately psychrophilic bacteria. *Journal of Food Science, 58*, 310–313.

[35]. Rodríguez, O., Barros-Velázquez, J., Ojea, A., Piñeiro, C., & Aubourg, S. (2003a). Evaluation of sensory and microbiological changes and identification of proteolytic bacteria during the iced storage of farmed turbot (*Psetta maxima*). *Journal of Food Science, 68 (8),* (in press).

[36]. Chen et al. 87

[37]. Hebard, C. E., Flick, G. J., & Martin, R. E. (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In R. E. Martin, G. J. Flick, C. E. Hebard, & D. R. Ward, *Chemistry and biochemistry of marine food products,* (pp. 149-304). Westport, Connecticut: Avi.

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

[39]. ROD3

[40]. Civera et al. 1995. Sciences des Aliments.

TABLE 1

1

Time (days)	Process	ODOUR		
		External	Gill cavity	Muscle
14	HT	B	B	A
	HL	B	A	A
	HL300	A	A	A
	HL600	A	A	A
16	HT	B	B	B
	HL	B	B	A
	HL300	A	A	A
	HL600	A	A	A
20	HT	C	C	B
	HL	B	B	A
	HL300	B	B	A
	HL600	A	A	A

2

TABLE 2

Irá una tabla con las medias y los rangos de la microbiología: aerobios mesófilos, psicrófilos, enterobacterias y proteolíticos

TABLE 3

(Eliminaría DMA-N y TBA-i)

Time (days)	Process	Chemical index					
		TVB-N	TMA-N	DMA-N	FFA	TBA-i	Fluoresc
14	FI	34.15 b (3.00)	8.03 b (0.97)	0.96 c (0.16)	5.01 b (1.18)	0.18 a (0.05)	2.14 a (0.28)
	SI	18.55 a (1.99)	2.39 a (1.09)	0.13 a (0.07)	1.62 a (0.55)	0.43 b (0.14)	1.97 a (0.45)
	SI-300	21.34 a (7.79)	5.02 ab (2.99)	0.29 ab (0.15)	1.97 a (1.00)	0.41 b (0.16)	2.05 a (0.21)
	SI-600	21.61 a (4.38)	7.69 b (1.53)	0.39 b (0.14)	2.44 a (1.37)	0.24 ab (0.04)	1.32 a (0.71)
16	FI	45.04 b (3.17)	25.54 b (4.14)	1.35 b (0.10)	15.91 b (2.90)	0.18 a (0.09)	5.23 b (0.72)
	SI	29.67 a (1.75)	14.72 a (1.56)	0.85 a (0.10)	4.42 a (2.28)	0.25 a (0.07)	2.70 a (0.63)
	SI-300	28.49 a (2.75)	13.04 a (0.89)	0.71 a (0.11)	2.73 a (0.55)	0.14 a (0.03)	2.49 a (0.59)
	SI-600	26.16 a (3.60)	12.47 a (5.96)	0.68 a (0.21)	5.08 a (3.42)	0.26 a (0.15)	2.94 a (0.07)
20	FI	57.14 b (3.61)	39.76 b (4.60)	1.50 b (0.07)	18.78 a (5.88)	0.42 b (0.12)	7.11 b (1.09)
	SI	46.65 a (4.91)	23.65 a (4.51)	1.13 a (0.15)	14.38 a (1.33)	0.20 a (0.05)	4.48 a (0.37)
	SI-300	43.09 a (6.29)	27.40 a (2.18)	1.36 b (0.04)	13.60 a (1.80)	0.17 a (0.05)	3.07 a (0.74)
	SI-600	41.04 a (6.09)	28.07 a (4.08)	1.35 b (0.10)	19.41 a (3.89)	0.12 a (0.03)	4.21 a (1.21)

1