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Anchovy Shelf Life as Affected by Different Chilling Methods during Distribution

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ABSTRACT

Anchovies are a very labile fish and deteriorate fast under chilling conditions. In the South of Spain, fishing boats land their catches in wooden boxes with ice (12 to 14 kg). For some years now, fish processors have prepared this species for market distribution by placing about 7 kg fish in expanded polystyrene (EPS) boxes containing water and ice. Then, in the distribution market, boxes are dewatered and re-iced. Transportation of the fish in EPS boxes containing water and ice was recently forbidden on the grounds that boxes for transportation of fish in ice must have holes to let melted ice drain away. In this paper, the effect of preserving the anchovy in water and ice from landing to the distribution market was studied and compared with the more traditional methods of storing the fish in ice in either wooden or EPS boxes. Physical, chemical, microbiological, and sensory analyses were carried out over three different storage trials to account for the effect of seasonality. Little differences were found among lots, but some of the parameters showed that fish transported in water and ice did present less spoilage than fish stored in ice, especially when compared to the wooden boxes. According to these results, chilling of this fish in water and ice can be used as an alternative preserving method during transport.

Anchovies (*Engraulis encrasicholus*) are widely consumed in Spain. The total anchovy catch landed in 1998 was 18,348 tons at an average price of $2 per kg. Small pelagic species in general, and anchovies in particular, spoil faster than other fish species. This can be attributed to the higher surface/volume ratio than in larger species, spoilage processes occurring mainly on the surface *(31).* Also, they are highly susceptible to mechanical damage, exhibiting little resistance to skin breakage under the slightest pressure *(22).* Moreover, because of their biological makeup, these species have a very high metabolic rate and contain enzymes that remain active after death, causing protein and lipid hydrolysis as well as other changes that rapidly alter the sensory quality of these products *(32).* One of the problems associated with proteolytic degradation is the rapid rupture of abdominal walls, known as "belly burst." This problem is particularly acute at times of year when food is plentiful, when there is greater induction of digestive protease secretion *(17, 19),* a problem that is heightened if storage temperature is higher *(21).*

Proper storage of these fish is therefore essential from the time they are caught until they are prepared for consumption or subsequent processing in order to avoid rapid spoilage. Such storage entails chilling the fish at the earliest possible moment in conditions that will prevent physical damage and thus minimize the growth of microorganisms and an increase in enzymatic activity *(33).* Hansen and Jensen *(14),* for example, found that the shelf life of a pelagic species, sardine, was 4 days longer when chilled in ice immediately upon capture than when chilled 5 h after capture.

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In Spanish fisheries, the most common practice for small pelagic species is to chill on board with freshwater ice. The on-board chilled seawater (CSW) system has been used experimentally on these species by the Moroccan Mediterranean fleet *(4)* with good results. Several reviews *(17, 22, 23, 29, 33)* have reported that when cooling with refrigerated seawater or CSW, the washing is more efficient than with ice, making for less weight loss, less mechanical damage, and easier handling and also delaying the onset of rancidity. They also report drawbacks, such as temperature stratification at different points in the tank, and, in some species, increased salt content and loss of muscle firmness *(17).* For economical reasons, the CSW method as such has hardly been applied in the area. The introduction of CSW or refrigerated seawater on board in the Southern Spanish fleet would require alterations to the vessels, in accordance with directive 92/48/EEC *(11),* which are not practicable in the short to medium term in most of the cases.

For transportation, in compliance with European Union directive 91/493/EEC *(10),* the landed fish are generally iced in boxes from which melt water can easily drain off. Fishery companies claim that quality is better maintained by transportation in small containers with ice and water, since this makes for better appearance and less physical damage, but there is no explicit provision for using this system on land.

In view of the foregoing, it is necessary to gather some information that would help the Regulatory Authorities consider requiring that system fisheries companies actually use the European Union regulation or, on the contrary, continue the actions against this practice. Since the system has proven to be commercially advantageous, it would only be

needed to prove that it has technological or sanitary advantages or at least no drawbacks, compared to the already used systems.

There is little information in the literature regarding anchovy deterioration during chilled storage *(21, 25).* Most references to spoilage of anchovies relate to characteristics associated with the ripening process *(15, 18, 35)* or to conditions other than the usual ones *(36).* No references have been found as to the effect of storage in water and ice as opposed to storage in ice on parameters relating to sanitary and technological quality in this species, and only preliminary studies in some physical-chemical parameters were performed for other pelagic species *(24).*

The purpose of this study was to determine the effect of preserving the anchovy in water and ice in small containers from landing to the distribution market and to compare it with the more traditional methods of storing the fish in ice in either wooden or perforated expanded polystyrene (EPS) boxes. For that, physical, chemical, microbiological, and sensory analyses were carried out until spoilage over three different storage trials.

MATERIALS AND METHODS

Sample preparation. Anchovies (*E. encrasicholus*) were caught in February, May, and June 1999. For each storage experiment, the fish came from the same vessel and the same haul and were kept on board in wooden boxes with ice, each containing approximately 12 to 14 kg of product. The time elapse from catch to processing on land was between 2 and 7 h. Sample size was 30, 150, and 150 kg for the experiments performed in February, May, and June, respectively. Three lots were studied, consisting of anchovies stored in wooden boxes with ice (lot WB), anchovies stored in perforated EPS boxes (lot I), and anchovies stored during transportation in water and ice in watertight EPS boxes (lot IW). The processing and transportation conditions used currently in the area were reproduced in all cases as described below. Lot WB consisted of fish stored in wooden boxes on board and therefore required no preparation other than the addition of ice (approx. 1 kg). For lot I, 7 kg of fish was transferred from wooden boxes to perforated EPS boxes. One kilogram of ice was added to cover the fish. In lot IW, the contents of the wooden boxes were transferred to watertight EPS boxes. Three liters of water (previously chilled with ice) and 1 kg of ice were added to the contents of the watertight EPS boxes (7 kg). The various lots were freighted in an isothermal truck from the Port of Cadiz to the Madrid wholesale market, where they were collected approximately 20 h after preparation at the port and taken to the Instituto del Frío. On arrival, the watertight boxes were drained by making holes in the EPS boxes. All lots were re-iced and kept until they spoiled in a cold store at 1°C. The re-icing was performed periodically as required in each lot.

Analyses. Fish muscle temperature for the various lots was monitored at different points in the boxes (surface/bottom, center/ends) in different critical points (after landing the fish from the boats, during the preparation of the lots, at arrival in the distribution market, and throughout the storage in the chill room). In the measurements in port and distribution market, a digital thermometer with a steel sensor was used. In the chill room, temperature was measured with thermocouple probes connected to a temperature recorder (Model 3087, Yokogawa Hokushin Electric YEW, Tokyo, Japan). Weight gain was determined in fish kept in water and ice by placing a known amount of fish (approx. 2 kg) in a nylon net, which was weighed after 24 h in water and ice. Results were expressed as percentage weight gain with respect to the initial weight of the sample.

Percent fat *(5),* protein, moisture, and ash *(2)* were determined on portions taken from a homogenate of muscle belonging to at least 20 individuals.

The physicochemical analyses (except for determination of percent relaxation) were carried out in triplicate. A homogenate of muscle from at least 20 individuals per lot was used. The fish was gutted and headed, and the backbone, tail, and dorsal fin were removed. It was then lightly washed with water to remove any residue of blood, etc., and then ground for 1 min (Braun Control Plus 418 homogenizer, Esplugats de Llobregat, Barcelona, Spain). The resulting homogenate was kept in ice for use in measuring the pH and preparing extracts for determination of 2-thiobarbituric acid-reactive substances (TBARS), trimethylamine nitrogen (TMA-N), total volatile basic nitrogen (TVB-N), and proteolysis.

The pH was determined as recommended by Vyncke *(38).* TMA-N, TBA, and proteolysis were determined on 25 g of the muscle homogenate described earlier. This was ground with 50 ml of 7.5% trichloroacetic acid containing 0.1% propyl gallate and 0.1% EDTA for 1 min (Osterizer 867-50E homogenizer, Schaumburg, Ill.) at setting 2. The resulting suspension was centrifuged for 15 min in a desktop centrifuge (Sorvall RTB6000B, DuPont, Wilmington, Del.) at 1,240 $\times$ *g*. The supernatant was filtered through Whatman no. 1 paper. The filtrate was divided into aliquots in test tubes, and these were stored at −20°C for analysis (maximum 3 weeks). TMA-N was determined according to Dyer *(12),* as described in the Association of Official Analytical Chemists *(3),* and the results were expressed as TMA-N/100 g muscle. The TBARS were determined according to Vyncke *(37),* and the results were expressed as μmol TBARS/kg muscle. The degree of proteolysis was determined according to Chalmers et al. *(9),* and the peptides in the supernatant were determined by the method of Lowry et al. *(20).* The standard curve was plotted with tyrosine, and the results were expressed as μmol tyrosine/100 g muscle. Extracts for determination of TVB-N were obtained by homogenizing 10 g of ground muscle as described earlier in 90 ml of 6% perchloric acid in an Osterizer homogenizer. The resulting suspension was vacuum filtered (Whatman no. 1), and the filtrate was stored at −20°C until use (maximum 3 weeks). TVB-N was determined by the method of Antonacopoulus and Vyncke *(3)* in a "Tecator" distillator (Tecator Kjeltec System, Model 1002, Tecator Ab Höganäs, Sweden). The results were expressed as mg TVB-N/100 g muscle.

Percent relaxation of the fish following compression *(6)* was determined with a Universal Instron 4501 texturometer (Instron Corporation, Canton, Mass.), and analyses were performed with programs from the Instron Series IX (Automated Materials Testing System version 5). This was done directly on the medial dorsal part of individuals with a 14-mm-diameter cylinder exerting 2 mm compression for 1 min (initial rate 50 mm/min, 0.1 kN head). Samples were kept in ice until analysis. Ten replicates were performed for each lot and sampling day. Percent relaxation was calculated as % $(F_m - F_{60})/F_m$, where F_m is the maximum force, and F_{60} is the force after 60 s of compression.

Samples were taken in replicate for microbiological analysis in sterile conditions in a vertical laminar-flow cabinet (AV 30/70, Telstar, Terassa, Barcelona, Spain) following American Public Health Association *(34)* recommendations. Muscle (with skin) from 10 individuals was obtained by taking a total of 10 g and placing it in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK). This was homogenized with 90 ml of buffered peptone water

(Scharlau, Barcelona, Spain) in a "Stomacher" homogenizer (Stomacher Colworth 400, Seward, UK) for 2 min. Serial 10-fold dilutions were made (in duplicate) in the same dilutant to determine the following microorganisms. Pour plates on iron agar (Scharlau) were used to obtain the total viable count and H_2S-producing microorganisms after 72 h at 20°C *(13)*. Any microorganism-forming black colonies in this medium were assumed to be H_2S producers. Spread plates on starch glutamate agar (Scharlau) were used to obtain the psychrotrophic aerobic count after 5 days at 20°C *(30)*. Pour plates on violet red bile dextrose agar (Scharlau) subsequently overlaid with violet red bile dextrose agar were used to obtain the enterobacteria count after 48 h at 37°C *(26)*. Pour plates on a selective chromogenic medium, Coli ID (Biomérieux, Lyon, France), were used for detection and counting of coliforms *(28)* after 48 h at 37°C. All counts were carried out on plates containing between 25 and 250 colonies. Microbiological counts were expressed as log CFU/g of muscle.

Sensory inspection was performed following the Quality Index Method (QIM) developed for anchovy by Nielsen *(27)*, with a slight modification. The attribute "spinal column rupture strength" was replaced by "force required to separate bone from fillet" (Table 1). Except at time 0 (in port), on every day of analysis, QIM was carried out by a minimum of five inspectors, recruited from among the members of the Department. Ten specimens from each lot were scored separately by each assessor. The resulting score per inspector and lot was determined as the arithmetic mean of the demerit points given to the 10 fish. At time 0, the analysis was performed by only two inspectors, who traveled to the port to supervise preparation of the lots.

Initial exploratory statistical analyses (Descriptive Univariate Analysis and Saphiro-Wilk Distribution Test) were run for each of the variables to study the distribution of the data and correct any deviations from the norm if necessary. Two-way analyses of variance (ANOVAs) were carried out with storage time as covariant, in order to study the effect of the treatment (storage of fish in wooden boxes [WB], perforated EPS boxes [I], or EPS boxes with water and ice [IW]) and the trial. For each storage trial, a one-way ANOVA was run with storage time as covariant. ANOVA as a function of treatment with time as covariant assumes the absence of interactions between treatment and time; it also assumes a linear trend of the variables over time. Since the effect of storage time on some variables was nonlinear, regression analyses were run for each trial and treatment, with independent variables t and $(t)^2$, where t = storage time. For each storage trial, ANOVAs of the regression coefficients of each lot were carried out. Where there were significant differences, the level of significance of the curves was examined within the time range studied, for which purpose confidence intervals were calculated at each point from 0 to 14 days of storage. Only one-way ANOVAs of the physical, chemical, microbial, and QIM (total scores) are presented in a table, the other ANOVAs being cited in the text when needed. Only those regression analyses needed for comparison with ANOVA are presented in a table. The goodness of fit of the models was estimated by F-test. These analyses were performed using the programs BMDP (BMDP Statistical Software 7.1, 1995 executed on Open VMS) and SPSS 8.0 for Windows (SPSS Inc., Chicago, Ill.).

TABLE 1. *Quality Index Method (QIM) for whole anchovy (*Engraulis encrasicholus*)*

Parameter	Descriptors	Demerit points
General appearance		
Surface	Bright	0
	Less bright	1
	Dull	2
Firmness	Tense, firm, hard (rigor)	0
	Less tense, firm	1
	Flaccid, soft (postrigor)	2
Eyes		
Clarity (cornea)	Clear, transparent	0
	Central opacity	1
	Opaque, slightly yellowish	2
Pupil	Black and circular	0
	Gray	1
	Gray and distorted	2
Shape	Convex	0
	Plane, flat	1
	Concave, sunken	2
Gills		
Cover (bloodiness)	None	0
	Slight (<10%)	1
	Some (<50%)	2
	Bloody (>50%)	3
Color (internal)	Red	0
	Brownish-red	1
Slime	None	0
	Slight	1
	Slats stick together	2
Smell	Slight seaweedy, peppery	0
	Metallic, oil	1
	Metallic, acid, rancid	2
	Sour, stale blood	3
Abdomen		
Belly burst	Intact, firm	0
	Stretch marks	1
	Torn	2
Spinal column		
Strength to separate bone from fillet	Separates with force	0
	Separates with less force	1
	Comes apart easily	2
Muscle (filleted)		
Appearance and color	Fresh bloom, translucent	0
	Some opacity	1
	Dense, bloody	2
Total demerit points (0–25)		

RESULTS AND DISCUSSION

Initial analyses. Table 2 shows the size and weight of the anchovies as well as proximate analyses for the three experiments. These values were within normal limits for this species *(7)*. There were significant differences in percentage fat and percentage moisture between the first two experiments (February and May) and the third experiment (June). Preparation of the fish in water and ice (IW) entailed a weight gain of between 2 and 5% from water absorption.

Temperature measurements. The fish, when landed, did not always have sufficient ice to ensure temperatures

TABLE 2. *Weight (g) and size (cm) of anchovy (mean values of 20 individuals ± SE) and proximate analyses (%) of anchovy muscle (mean values ± SE of triplicates from a muscle homogenate from 20 individuals) in each storage experiment (February, May, and June)*

Experiment	Weight (g)	Size (cm)	% moisture	% fat	% protein	% ash
February	31.4 ± 0.2	—	77.4 ± 0.4	2.5 ± 0.2	18.8 ± 0.3	1.34 ± 0.02
May	12.9 ± 5.4	10.6 ± 1.2	77.6 ± 0.3	2.6 ± 0.1	18.3 ± 0.3	1.43 ± 0.06
June	16.9 ± 1.8	11.8 ± 0.5	76.0 ± 0.4	3.3 ± 0.3	19.2 ± 0.1	1.49 ± 0.03

around 0°C. In some cases, differences of as much as 7°C were found between the maximum and minimum temperatures in a single box. Preparation in EPS boxes, both with ice only and with water and ice, reduced the fish temperature in a short time and made for uniform temperatures at different points in the box. For all seasons, the IW lot reached the distribution market with sufficient ice, whereas this was not always the case with lots I and WB. In lot WB, temperatures close to 6°C were detected in the February experiment. The temperature of the fish from lot IW was consistently lower (0 to 1.5°C), whereas in lot I, temperatures sometimes approached those found in the fish kept in wooden boxes (5 to 6°C). In no case were any problems of temperature gradient found in the IW lots, which is unlike what happens when fish are kept on board in refrigerated seawater or CSW in larger containers *(17)*.

Physicochemical indices. TMA-N levels (Fig. 1) during storage differed significantly from one storage experiment to another. Levels were within the range of values reported for this species by other authors *(25)*. In general, the TMA-N in lot IW changed more slowly than in lots I and WB for all three experiments. Similar results were found by Moral et al. *(24)* when preserving horse mackerel, bogue, and pickerel in CSW for 10 to 12 h and then boxing in ice. ANOVA showed only significant differences between lots for the experiment performed in June (Table 3). Regression analysis detected differences between lots for all three experiments (Table 4). For practical purposes, these significant differences need to occur within the time interval during which the product was fit for consumption. The confidence intervals calculated from the regression equations indicated significant differences between days 5 and 8 for the June experiment, whereas for the February and May experiments, the differences were found on days 11 and 9, respectively, which is near the end of the product's shelf life.

TVB-N (Fig. 1) differed significantly in relation to experiment and storage time, but not treatment, according to ANOVA (Table 3). The maximum levels were higher in the spring-summer months and were within the range reported by Moral et al. *(25)* for anchovy stored in freshwater ice in a cold store at 2°C. Regression analysis (Table 4) revealed significant differences between lots, TVB-N being lower in lot IW than in lots I or WB. The confidence intervals calculated for each time on the basis of regression analysis showed significant differences between lots for the third

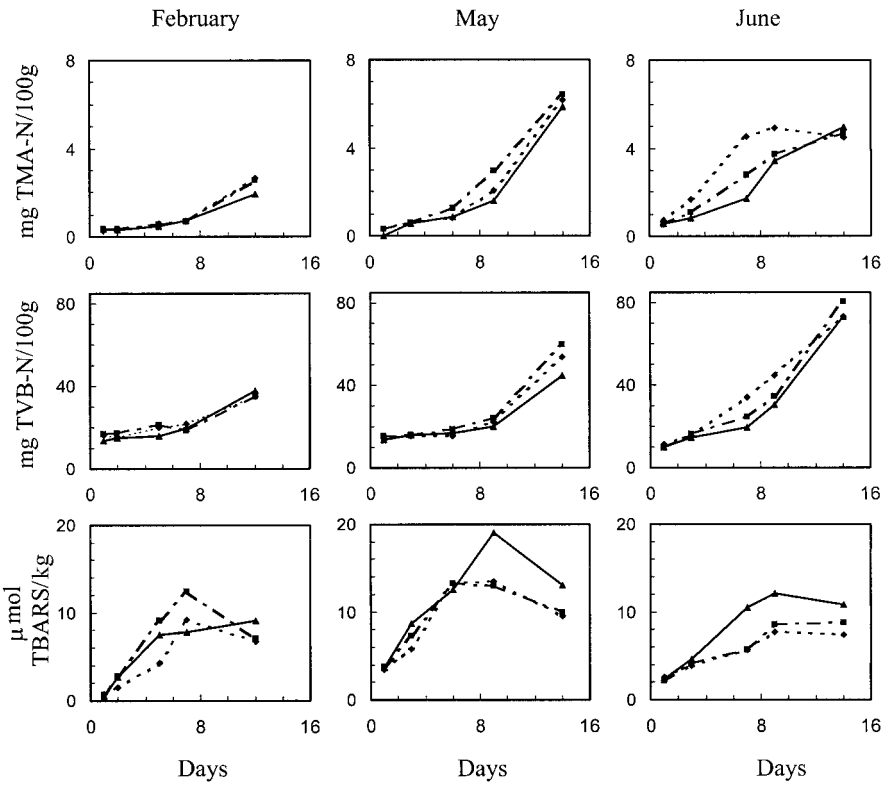

FIGURE 1. *Changes in trimethylamine nitrogen (TMA-N), total volatile basic nitrogen (TVB-N), and thiobarbituric acid-reactive substances (TBARS) of anchovies stored in ice; in wooden boxes (◆); in perforated EPS boxes (■); and preserved in water and ice for 20 h in EPS boxes, dewatered, and re-iced (▲). Left to right, experiments of February, May, and June.*

TABLE 3. *One-way ANOVAs as a function of treatment (fish stored in ice in wooden boxes [WB], perforated expanded polystyrene boxes ([I], and in water and ice in expanded polystyrene watertight boxes [IW]) with time as covariate of the variables listed below for the experiments performed in anchovy stored in ice in February, May, and June; significance established at 0.05 (*), 0.01 (**), and 0.001 (***) levels; NS, nonsignificant model*

Variable	February		May		June	
	Treatment	Time	Treatment	Time	Treatment	Time
Proteolysis	*	***	NS	***	**	***
Thiobarbituric acid-reactive substances	NS	***	NS	***	***	***
Total volatile basic nitrogen	NS	***	NS	***	NS	***
Trimethylamine nitrogen	NS	***	NS	***	***	***
pH	**	***	*	***	NS	***
% relaxation	NS	***	***	***	NS	***
Coliforms	***	***	***	***	***	***
Enterobacteria	NS	***	***	***	***	***
Total viable counts	*	***	***	***	*	***
H_2S producers	***	***	NS	***	**	***
Psychrotrophs	*	***	***	***	**	***
Quality Index Method	—	—	NS	***	NS	***

TABLE 4. *(A) Regression coefficients (intercept, time, and (time)^2) for the variables and treatments listed below and (B) ANOVAs and confidence intervals (CIs) of the regression coefficients as a function of treatment in each of the experiments (February, May, and June); significance established at 0.05 (*), 0.01 (**), and 0.001 (***) levels; NS, nonsignificant model; treatment abbreviations as in Table 3*

Variable	Treatment	A (regression coefficients)			B (ANOVA)	
		Intercept	Time	(Time)^2	*P* (model)	CI
February						
Thiobarbituric acid-reactive substances	WB	−2.00	2.20**	−0.12**		±4.20
	I	−3.58	3.94**	−0.25**	*	±3.72
	IW	−1.17	2.07**	−0.09*		±3.42
Total volatile basic nitrogen	WB	15.96	−0.02	0.13**		±4.00
	I	18.19	−0.85	0.19**	*	±4.86
	IW	15.00	−0.94	0.23**		±3.34
Trimethylamine nitrogen	WB	0.37	−0.11**	2.47×10^{-2}**		±0.09
	I	0.49	0.15**	2.7×10^{-2}	**	±0.09
	IW	0.37	-7.7×10^{-2}	1.75×10^{-2}**		±0.14
May						
Thiobarbituric acid-reactive substances	WB	−0.34	3.11**	−0.17**		±2.49
	I	0.87	2.81**	0.16**	**	±1.79
	IW	−0.30	3.60**	−0.19**		±3.11
Total volatile basic nitrogen	WB	18.04	−2.77**	0.38**		±3.52
	I	19.17	−2.80**	0.40**	**	±3.97
	IW	16.92	−1.82**	0.27**		±3.77
Trimethylamine nitrogen	WB	0.65	−0.23**	0.04**		±0.66
	I	0.28	−0.005	0.032**	**	±0.48
	IW	0.47	−0.22*	0.042**		±0.62
June						
Thiobarbituric acid-reactive substances	WB	1.54	0.93**	−0.04*		±1.87
	I	1.18	1.01**	−0.03	**	±2.23
	IW	−0.36	2.27**	−0.10**		±1.97
Total volatile basic nitrogen	WB	7.16	2.92**	0.13**		±2.17
	I	12.93	−1.38*	0.44**	**	±4.95
	IW	13.64	−2.19**	0.46**		±4.36
Trimethylamine nitrogen	WB	−0.50	1.02**	−0.05**		±0.83
	I	−0.12	0.51**	−0.01**	**	±0.40
	IW	0.19	0.22*	0.01		±0.78

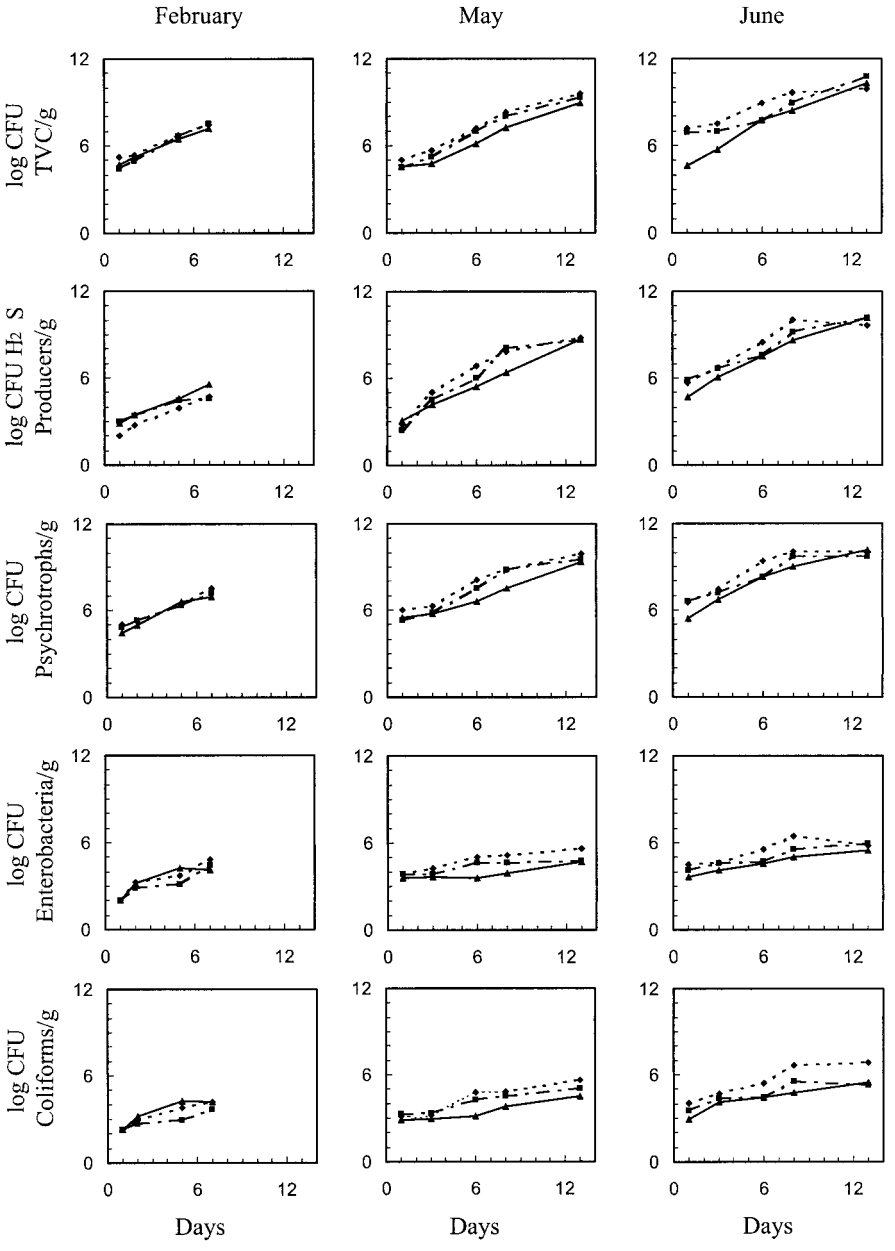

FIGURE 2. *Changes in total viable count (TVC), H_2S producers, psychrotrophs, enterobacteria, and coliforms of anchovies stored in ice; in wooden boxes (◆); in perforated EPS boxes (■); and preserved in water and ice for 20 h in EPS boxes, dewatered, and re-iced (▲). Left to right, experiments of February, May, and June.*

season between days 5 and 9. There were no significant differences for the first trial, and differences occurred only on day 9 for the experiment performed in May.

Because of the highly unsaturated lipid content of species of this kind and the loss of balance between oxidation activators and inhibitors following their death, these fish are apt to present problems of oxidative rancidity *(16).* This is reflected in the development of the TBARS (Fig. 1). A two-way ANOVA showed significant differences relating to storage trial, treatment, and storage time. Changes in time are expected from this parameter, which is measuring secondary products of lipid oxidation and may present a maximum value *(8).* Significant differences between lots were only found in the June experiment by both ANOVA and analysis of the confidence intervals calculated from the regression equations (Tables 3 and 4), where it was observed that TBARS increase was faster and reached higher values in lot IW. This same tendency was observed in May but was not confirmed by the results from the first experiment. The data for this experiment must, however, be treated with caution, since the sample size was smaller than in the other experiments. The occurrence of greater oxidative rancidity has been found in horse mackerel, pickerel, and bogue stored in similar conditions *(24).* However, it is known that when the water-ice system is not combined with subsequent storage in ice but is used until the product is consumed, less oxidative rancidity occurs *(17).* This higher rancidity in our study could be attributed to the combined effects of storage in water and ice and subsequent storage in ice only. First, in ice-water, the diffusion of prooxidant compounds in the medium is facilitated; then, in ice, the oxygen is allowed to come into contact with the prooxidants and lipids in the fish. This factor could be readily corrected, either by adding antioxidants or by storing the product with water and ice throughout its shelf life.

Other parameters measured, such as proteolysis, per-

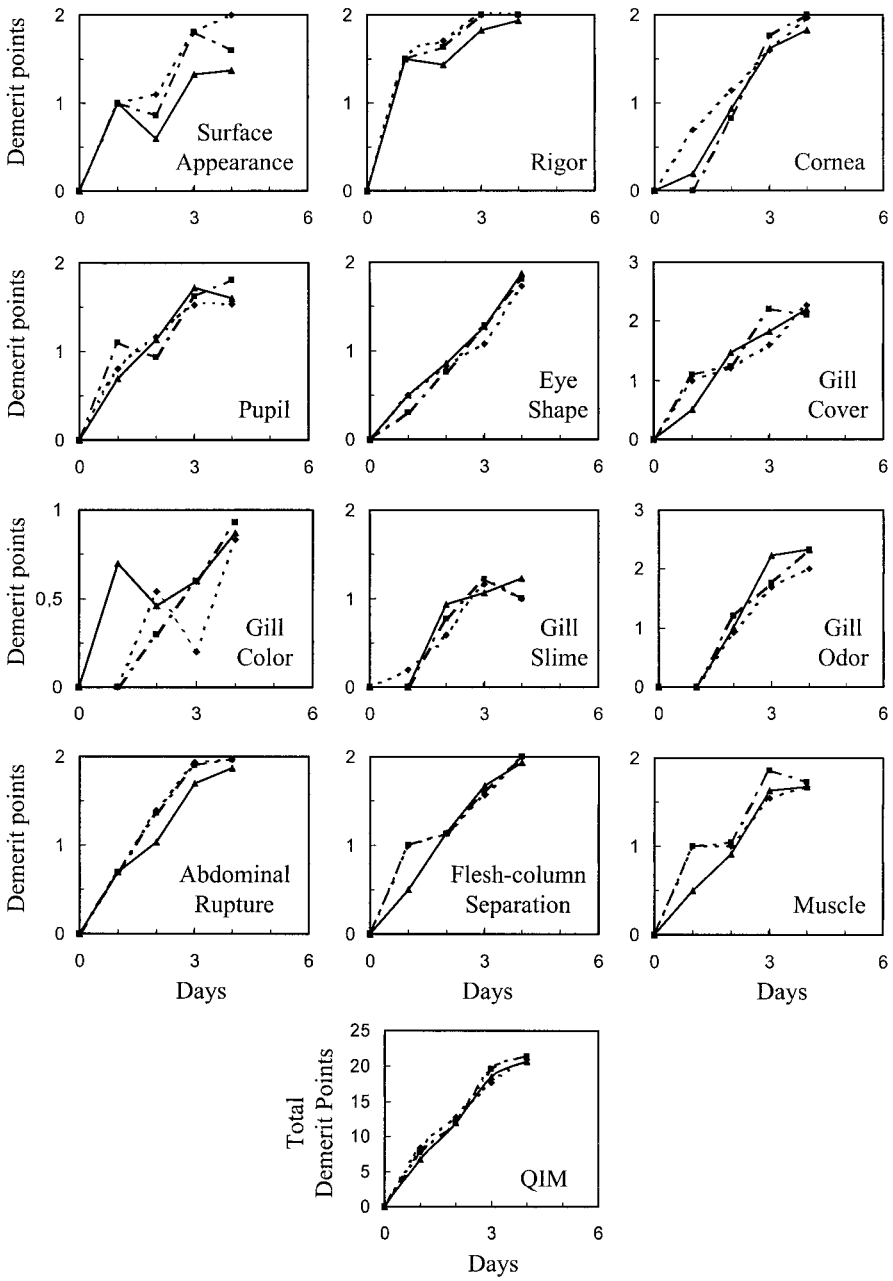

FIGURE 3. *Quality Index Method (QIM) total scores and QIM attributes scores of anchovies stored in ice; in wooden boxes (◆); in perforated EPS boxes (■); and preserved in water and ice for 20 h in EPS boxes, dewatered, and re-iced (▲). Fish caught in May.*

cent relaxation, and pH, varied significantly with storage trial and time (Table 3), but no consistent effect on treatment was found (figures not shown).

Microbiological analyses. Microbiological counts (Fig. 2) were generally dependent on the experiment and storage time. There were significant differences between lots depending on the storage experiment. This was the case, for example, in the total viable count, where the differences were greater in June (Table 3). In lot IW, the initial values were constant for all three seasons, whereas they varied in lots I and WB. In the H_2S-producing microorganism counts, which are considered to be specific to fish spoilage *(13),* there were differences relating to season and storage time and, also, differences relating to treatment when each season was analyzed separately (Table 3). Lot IW presented lower initial values than the other two lots only in the third season. In the February experiment, the H_2S counts were higher in lot IW, while in the May and June experiments, the opposite occurred. As mentioned earlier, this first experiment must be treated with caution in view of the small sample size. Psychrotrophic microorganisms (Fig. 2) presented the highest counts, this being the predominant flora in chilled conditions. There were significant differences in relation to storage trial, treatment, and storage time (Table 3). In the experiments of February and May, values tended to be lower in lot IW than in lot WB, while lot I presented intermediate values. The initial enterobacteria and coliform counts were lower in lot IW in the third trial (Fig. 2). Significant differences were found among treatments in all cases, except in February (Table 3), and represented the greatest differences between lots IW and WB for this species.

Sensory inspection. QIM was performed in the last two storage trials. Figure 3 shows an example of the total

QIM scores and separate attributes with storage time (experiment from May). No significant differences were observed between lots with respect to the QIM general scores (Table 3). A separated analysis by attributes showed significant differences in surface appearance, which was brighter in lot IW than in lots I or WB. Lot IW also presented a significantly smaller number of individuals with belly burst than did lots I and WB in May, but not in June. One aspect noted by the panel and not included as an attribute in the QIM table was that the anchovies kept in water and ice looked rounder, whereas the fish kept in ice, and particularly in wooden boxes, were flattened and deformed. These aspects were in agreement with the opinion of better appearance given by fish processors and distributors. It has been suggested that the lowering of temperature and the attenuating effect of the water can reduce mechanical damage and belly burst *(7, 17, 21),* but in certain cases, these factors appear to have no effect on this attribute. It is therefore probably necessary to look at other procedures, such as lowering the pH of the water and ice, as suggested by some authors *(21).*

In conclusion, results show that the effect of treatment was consistent among experiments for all the parameters. The experiment-dependent differences in the initial levels and evolution over time can be attributed both to the physiology of the fish and to environmental or processing aspects. All results, except for TBARS, showed lower or equal spoilage rates for the water and ice-stored anchovies compared to the anchovies stored in ice. The fish stored in ice and water had the advantage over the other methods (WB and I) of better maintaining the cold chain, and sensory inspection corroborated that the external appearance was better. Therefore, anchovies prepared on land in small containers of water and ice for transport, drained after 20 h, and subsequently stored in ice can be considered at least as fit for consumption as fish stored in ice. For the purpose of standardized use of this system, it may be necessary to establish certain specifications as to the proportion of water/ice/anchovy and the temperature of the added water.

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