Journal of Food Protection, Vol. 65, No. 6, 2002, Pages 1024–1032

Influence of Chilling Methods on the Quality of Sardines (*Sardina pilchardus*)

RAUL GARCÍA AND MERCEDES CARECHE*

Instituto del Frío, Consejo Superior de Investigaciones Científicas, José Antonio Novais, 10, Ciudad Universitaria, E28040 Madrid, Spain

MS 01-282: Received 21 August 2001/Accepted 7 December 2001

ABSTRACT

The aim of this study was to determine for sardines (*Sardina pilchardus*) the effect of (i) chilling in ice and water in small expanded polystyrene boxes during distribution and in ice thereafter and (ii) chilling in ice and water for the entire storage period. These storage methods were compared with storage of the fish in ice in wooden boxes or in expanded polystyrene boxes. Three storage experiments were performed to take into account the variability in handling conditions and seasonality. There were significant ($P < 0.05$) differences in the results of some of the sensory, physical, chemical, and microbiological analyses performed that showed that the fish were of better quality when preserved in water and ice than when preserved in ice alone. The effects of dewatering of the fish after transport and subsequent storage in ice were also significantly different ($P < 0.05$) from those of storage in ice, with the former storage method resulting in better sensory and microbial indices. However, oxidative rancidity, as measured by thiobarbituric acid–reactive substances, was higher for the dewatered fish than for fish preserved in water and ice throughout the storage period. It is concluded that for sardines, storage in water and ice, either only during transport or throughout the storage period, can be useful as an alternative preservation method during distribution and retail sale.

Sardines (*Sardina pilchardus*) are the most heavily consumed small pelagic species in Spain. Quayside commercial sardine production (excepting that for the Canary Islands) in 1998 totaled 55,282 metric tons, at an average price of US $0.6/kg. Like anchovies and other allied species, sardines have weak skin and fragile musculature, which means that they must be stored in such a way as to avoid friction and crushing *(17)*.

Storage of sardines in chilled-seawater (CSW) and refrigerated-seawater (RSW) systems is partially effective in overcoming these problems and offers some advantages over storage in ice only *(5, 10, 13, 21)*. Despite the advantages of CSW and RSW storage, Spanish fishing boats have not incorporated these systems for socioeconomic reasons. As previously discussed *(9)*, processors have proposed an alternative chilling method for use on land that consists of transferring the fish from the ice in which they are kept in the wooden boxes aboard the fishing boats to a mixture of ice and water in small containers. Once in the distribution market, the fish would be dewatered and returned to ice. However, regulatory authorities do not allow the use of this system because there is no explicit provision for its use on land *(3)*. When this system was considered for inclusion in the EU regulations, it was first assessed for anchovies *(9)*. The results of this assessment showed that the spoilage rate for anchovies stored according to this system was similar to, or in some cases slower than, that for anchovies stored in ice; however, oxidative rancidity was shown to proceed faster for the fish stored in water and ice but was not considered a commercial problem for anchovies.

The higher rancidity values for fish stored in water and ice and dewatered after 20 h *(9)* contrast with those found for fish stored in CSW and RSW for small pelagic species, for which less oxidative rancidity has been reported *(14, 15, 23, 24)*. The higher rancidity found when refrigeration with ice and water is combined with subsequent refrigeration with ice has been attributed to the diffusion of prooxidant compounds in water and ice under conditions in which there is little oxygen available, which will accelerate lipid oxidation when the system is allowed to come into contact with oxygen, as happens with the subsequent storage in ice *(9)*. Sardines have a higher fat content than anchovies and display more extensive seasonal variations in chemical composition. Fat content may range between 1 and 23% for sardines and between 1 and 8% for anchovies *(8)*. In the summer months, when sardines display a high fat content *(8)*, they will be more susceptible to rancidity, and therefore it may be more convenient to maintain the sardines in water and ice throughout the storage period rather than to use refrigeration in water and ice only during distribution.

The aim of this study was to find alternative chilling methods for sardines. For this purpose, the effects of (i) storing sardines in water and ice in small polystyrene containers during transport and in ice thereafter and (ii) storing sardines in water and ice in small polystyrene containers for the entire storage period were determined. These storage systems were compared with storage in ice in wooden boxes and in polystyrene boxes.

* Author for correspondence. Tel: 34 915492300; Fax: 34 915493627; E-mail: mcareche@if.csic.es.

MATERIALS AND METHODS

Sardines were caught in March, July, and September for the first, second, and third experiments, respectively. 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 elapsed from catch to processing on land was 2 to 7 h. For each experiment, three lots were studied, one containing sardines stored in ice in wooden boxes (lot WB), one containing sardines stored in ice in perforated expanded polystyrene (EPS) boxes (lot I), and one containing sardines stored in salt water with ice in EPS boxes during transportation and subsequently stored in ice (lot IW). Processing and transportation conditions were as previously described *(9)*. For the July and September experiments, an additional lot was introduced. Some of the fish transported in water and ice were kept in a chill room without dewatering (a storage method resembling the on-board CSW system) until they spoiled. Periodically, ice was added to these boxes if needed. This lot containing the fish stored thusly was called lot ND. In the July experiment, some of the fish were dewatered after 48 h and then stored in ice (lot E48). Samples sizes were 120, 150, and 150 kg for the March, July, and September experiments, respectively.

Fish muscle temperatures for the various lots were monitored at different spots in the boxes (surface and bottom, center and ends) at different critical points (after coming off the boats, during the preparation of the lots, on arrival in the distribution market, and throughout storage in the chill room). For the measurements at the port and in the distribution market, a digital thermometer with a steel sensor was used. In the chill room, the temperature was measured with thermocouple probes connected to a model 3087 temperature recorder (Yokogawa Hokushin Electric YEW, Tokyo, Japan). Weight gain and the percentages of moisture, proteins, fat, and ashes for the lots stored in wooden boxes were determined as previously described *(9)*.

pH, trimethylamine nitrogen (TMA-N), thiobarbituric acid reactive substances (TBARS), proteolysis, total volatile basic nitrogen (TVB-N), total viable counts, H_2S producers, psychrotrophic aerobic counts, enterobacteria, and coliforms were determined according to procedures described previously *(9)*. For sensory analysis, the quality index method (QIM) described by Nielsen *(19)* for sardines was used with the following modifications. The firmness of the muscle was assessed when the fish was filleted rather than for intact muscle. Some of the attribute descriptions were changed slightly (e.g., "gray and distorted pupil" was changed to "gray and eccentric," and "clarity of cornea, opaque" was changed to "opaque or bloody"). The parameter "spinal column easiness to break" was changed to "column-flesh separation strength" (Table 1). Except at time 0 (at the port), on every day of analysis the QIM was applied 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 per lot was determined as the arithmetic mean of the demerit points given to the 10 fish. At time 0, the analysis was performed by two inspectors who traveled to the port to supervise preparation of the lots.

Initial exploratory statistical analyses (the descriptive univariate analysis and the Saphiro-Wilk distribution test) were carried out for each of the variables to study the distribution of the data and to correct any deviations from the norm if necessary. Two-way analyses of variance (ANOVA) were carried out with storage time as the covariate in order to study the effect of the treatment (storage of fish in wooden boxes [lot WB], in perforated EPS boxes [lot I], or in EPS boxes with water and ice during distribution and in ice thereafter [lot IW]) and the experiment (March, July, or September). For each storage experiment, a one-way ANOVA was carried out with storage time as the covariate. ANOVA as a function of treatment with time as the covariate assumes the absence of interactions between treatment and time; it also assumes a linear trend for the variables over time. Since the effect of storage time on some variables was nonlinear, re-

TABLE 1. *Quality Index Method (QIM) for whole sardines (*S. pilchardus*)*

Parameter	Descriptors	Demerit points[a]
General appearance		
Surface	Very bright, iridescent	0
	Bright	1
	Less bright	2
	Slightly dull	3
Rigor	Prerigor	0
	Rigor	1
	Resolving rigor	2
	Postrigor	3
Eyes		
Clarity (cornea)	Clear, transparent	0
	Central opacity	1
	Opaque or bloody	2
Pupil	Black and concentric	0
	Black eccentric	1
	Gray and eccentric	2
Shape	Slightly convex	0
	Flat	1
	Sunken	2
Gills		
Cover (bloodiness)	None	0
	Slight (<10%)	1
	Some (<50%)	2
	Bloody (>50%)	3
Color (internal)	Bluish red	0
	Brownish red	1
	Faded	2
Smell	Fresh, marine, oily	0
	Oily or musty, neutral	1
	Rancid, sour	2
	Rancid, acrid, putrid	3
Abdomen		
Belly burst	Intact	0
	Stretch marks	1
	Torn	2
Filleting		
Column-fillet separation rupture	Difficult	0
	Easy	1
	Very easy	2
Muscle appearance	Fresh bloom, translucent	0
	Opaque	1
	Dense or bloody	2
Firmness	Firm, elastic	0
	Firm, hard	1
	Springless, soft	2
	Very soft, "paste-like"	3

[a] Total demerit points possible: 29.

TABLE 2. *Weights and sizes of sardines (mean values for 20 individuals ± SE) and proximate analysis results for sardine muscle (mean values ± SE for triplicate analyses of a muscle homogenate from 20 individuals) for each storage experiment (March, July, and September)*[a]

Experiment	Weight (g)	Size (cm)	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
March	45.7 ± 12.3	15.1 ± 1.5	75.61 ± 0.53	4.88 ± 0.04	19.07 ± 0.45	1.82 ± 0.08
July	47.4 ± 15.9	15.1 ± 1.5	70.93 ± 0.16	8.04 ± 0.28	18.82 ± 0.16	1.35 ± 0.17
September	30.9 ± 8.4	14.6 ± 0.5	71.03 ± 0.02	9.12 ± 0.34	18.54 ± 0.47	1.33 ± 0.03

[a] Sardines were stored for 20 h in wooden boxes.

gression analyses were carried out for each storage experiment and treatment with independent variables t and t^2, where t is storage time. For each storage experiment, ANOVA of the regression coefficients of each lot were carried out. When there were significant differences, the level of significance of the curves within the time range studied was examined; for this purpose, confidence intervals were calculated at each point from 0 to 13 days of storage. In the third experiment, the effect of lot ND was included, and a one-way ANOVA was carried out as a function of treatment with storage time as the covariate. Only some one-way ANOVA and regression analyses are presented in the tables; the other analyses are cited in the text as necessary. The goodness of fit of the models was estimated with an F test. Significance was established at P levels of 0.05, 0.01, and 0.001. These analyses were performed with the programs BMDP (BMDP Statistical Software 7.1, 1995, executed on Open VMS) and SPSS 8.0 for Windows (SPSS Inc., Chicago, Ill.).

RESULTS

Initial analyses and temperature variation. For each experiment, Table 2 shows the weights and sizes of sardines along with proximate analysis results. For the July and September experiments, the fat content was higher. There was a weight gain of 5 to 6% for lot IW.

Average temperatures for the sardines in wooden boxes immediately after landing were 3.5°C (range, 2.3 to 6°C) for the March experiment, 8.1°C (range, 6.1 to 11.2°C) for the July experiment, and 10.3°C (range, 1.6 to 18.8°C) for the September experiment. During processing on land, the cooling rate was found to be higher for lot IW. For example, for the experiment performed in July, average temperatures after 15 min of treatment were 8.0°C (range, 4.2 to 11.3°C) for lot WB, 2.3°C (range, 0.5 to 5.8°C) for lot I, and 0°C (range, 0 to 0°C) for lot IW. The lots differed with regard to the temperature of the fish on arrival at the wholesale market. The average temperatures of the fish in wooden boxes were 1.8°C (range, 1 to 2.3°C) in March, 3.8°C (range, 2.5 to 5.4°C) in July, and 7.7°C (range, 5.3 to 10.9°C) in September. The fish kept in water and ice (lots IW and ND) and in ice in EPS boxes (lot I) presented a uniform near-zero temperature for all three experiments. The temperature of the fish was 0°C for all lots during subsequent storage in the laboratory chill room.

Quality of fish kept in water and ice during transportation and subsequent storage in ice: effect of dewatering after 20 h. Figure 1 shows changes in the physicochemical variables over time. Two-way ANOVA as a function of treatment and storage experiment with time as the covariate for TMA-N, TVB-N, proteolysis, and pH showed significant differences related to experiment and storage time. There were no differences as a function of treatment, nor were such differences found with one-way ANOVA (Table 3). The confidence intervals derived from the regression equations confirmed the absence of any differences among lots between 0 and 13 days (Table 4). The increase in TMA-N and TVB-N levels during storage was faster in the experiment in which fish had the lowest fat content (March). TCA-soluble peptides, which indicate proteolysis, differed widely from one experiment to another in terms of both initial values and variation over time (Fig. 1). These results did not allow the identification of any clear trend that might be related to seasonality. pH increased with storage time (from 5.8 to 6.4), but no consistent season-related trend was observed (data not shown). TBARS exhibited faster evolution in lot IW, and significant differences were found with regard to experiment, treatment, and storage time by both ANOVA (Table 3) and regression analysis (Table 4).

Microbial counts during storage are shown in Figure 2. Two-way ANOVA showed that these counts were dependent on experiment, storage time, and treatment, although they were dependent on treatment for all variables in all experiments (Table 3). Total viable counts and H_2S producer counts generally indicated a smaller initial load for lot IW. There were significant differences between lots in all experiments for coliforms, enterobacteria, and psychrotrophs, with lot IW exhibiting the lowest growth levels. In the third experiment, the differences between lots were greater, with lot IW exhibiting less contamination.

Figure 3 shows the total QIM scores and the scores for separate attributes along with storage time for the experiment from September. There were significant differences related to time, treatment, and experiment. In the March experiment, no significant differences between lots were observed (Table 3), while in the July and September experiments there were significant differences between lots IW and WB, and there were significant differences between lots IW and I only in the September experiment. An attribute analysis (Fig. 3 and Table 5) indicated a better surface appearance and less belly burst for lot IW in both the July and the September experiments. In the September experiment, there were also significant differences in other attributes, such as evolution of rigor, eye shape, gill cover bloodiness, odor, strength of separation of bone from fillet, and firmness of fillet.

Quality of fish kept in water and ice during transportation and subsequently stored in ice: effect of de-

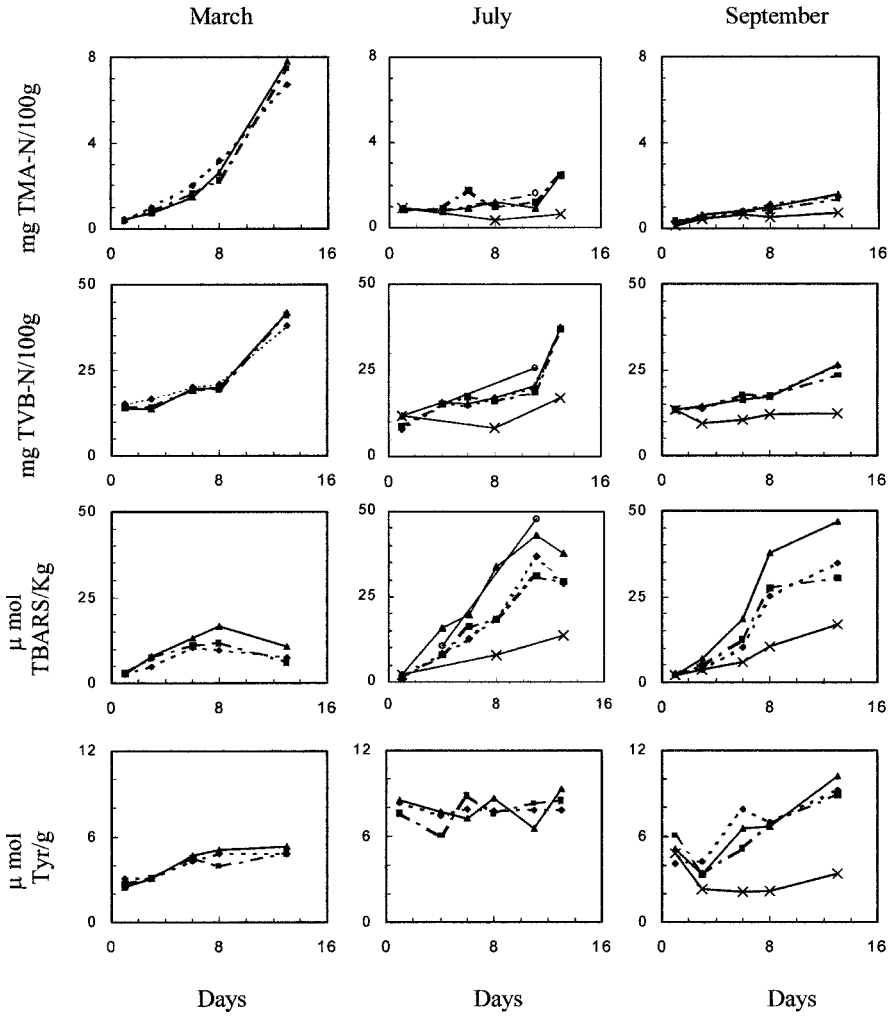

FIGURE 1. *Changes in TMA-N, TVB-N, TBARS, and proteolysis of sardines stored in ice in wooden boxes (♦); in ice in perforated EPS boxes (■); in water and ice for 20 h in EPS boxes, with subsequent dewatering and a return to ice (▲); in water and ice for 48 h, with subsequent dewatering and a return to ice (o); and in water and ice for the entire storage period (×). Experiments conducted in March, July, and September are presented from left to right.*

watering after 48 h. Although in most cases dewatering is performed in <24 h, there may be circumstances in which fish are retained for longer with this system, for example, if a given batch of fish is not transported on the day of processing. In the second experiment, one box of fish was dewatered after 48 h and stored in ice (lot E48). After 2 and 9 days in ice, the TMA-N, TVBN, and TBARS levels (Fig. 1) of the fish in lot E48 were similar to or even slightly higher than those of the fish in lot IW at similar storage times.

TABLE 3. *Results of one-way ANOVA as a function of treatment*[a] *with time as the covariate of the variables listed for experiments performed in March, July, and September*[b]

	March		July		September	
Variable	Treatment	Time	Treatment	Time	Treatment	Time
Proteolysis	NS	***	NS	NS	NS	***
TBARS	*	***	***	***	NS(limit)	***
TVB-N	NS	***	NS	***	NS	***
TMA-N	NS	***	NS	***	NS	***
pH	NS	***	NS	***	NS	***
Coliforms	***	***	**	***	***	***
Enterobacteria	***	***	**	***	***	***
Total viable counts	NS	***	NS	***	*	***
H_2S producers	NS	***	NS	***	NS	***
Psychrotrophs	*	***	**	***	***	***
QIM	NS	***	*	***	***	***

[a] Treatments included sardines stored in ice in wooden boxes (lot WB), in ice in perforated polystyrene boxes (lot I), and in water and ice during distribution (20 h) and in ice thereafter (lot IW).

[b] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, nonsignificant.

TABLE 4. *Regression coefficients for the variables and treatments[a] listed and ANOVA results with confidence intervals (CI) of the regression curves as a function of treatment for each of the experiments (March, July, and September)[b]*

		Regression coefficients			ANOVA	
Variable	Treatment	Intercept	Time	Time^2	P (model)	CI
March						
TBARS	WB	−0.01	1.20***	−0.07***		±1.35
TVB-N	I	−0.02	1.53***	−0.01***	**	±0.87
	IW	−0.71	1.98***	−0.12***		±1.21
	WB	16.34	−0.64	0.17***		±3.63
	I	15.39	−1.10*	0.24***	*	±3.28
	IW	14.76	−0.97*	0.23***		±3.66
TMA-N	WB	0.25	−0.10	0.05***		±0.84
	I	0.71	−0.20*	0.06***	**	±0.68
	IW	0.70	−0.22***	0.06***		±0.38
July						
TBARS	WB	−3.04	3.76**	−0.16**		±3.46
TVB-N	I	−1.78	2.91**	−0.04	**	±4.94
	IW	−4.00	5.72**	−0.19**		±7.50
	WB	8.94	0.03	0.16**		±4.72
	I	9.85	0.09	0.14**	NS	±5.48
	IW	13.58	−1.06*	0.22**		±3.72
TMA-N	WB	0.69	0.09	0.001		±0.58
	I	0.84	0.02	0.005	NS	±0.58
	IW	0.99	−0.10*	0.01**		±0.34
September						
TBARS	WB	−1.63	2.41*	0.03		±8.44
	I	−7.68	6.26**	−0.24	*	±15.6
	IW	−1.79	3.15**	0.05		±3.88
TVB-N	WB	13.33	−0.003	0.08**		±1.52
	I	13.05	0.42	0.03	**	±1.74
	IW	13.75	−0.21	0.09**		±1.32
TMA-N	WB	0.37	0.11**	−0.002		±0.16
	I	0.42	0.06**	0.008	*	±0.14
	IW	0.28	0.12**	−0.001		±0.26

[a] Treatment abbreviations as in Table 3.
[b] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, nonsignificant.

Quality of fish stored in water and ice until the end of shelf life. Preliminary data from the July experiment showed that the physicochemical indices for the fish stored in water and ice for the entire storage period (lot ND) evolved more slowly than did those of the fish stored in ice (lots WB, I, and IW) (Fig. 1). This finding was confirmed in the September experiment. ANOVA for TMA-N, TVB-N, TBARS, and proteolysis revealed significant treatment- and time-dependent differences. It was noted that the TBARS level in lot ND was lower than those in the other lots.

Lot ND also exhibited a significantly lower initial level of microorganisms and a lower growth rate (Fig. 2). The time required to reach a total viable microorganism count of 10^6 CFU/g for lot ND was twice the time required for lot I.

The QIM showed significant differences between the fish stored in ice (lot I) and the fish stored in water and ice (lots IW and ND) (Fig. 3). An attribute analysis (Fig. 3 and Table 6) showed better surface appearance, eye shape, gill cover throughout storage, and gill odor for ND lots only at the end of the experiment. One negative factor observed for the fish stored in water and ice for the entire storage period was that their gills were more discolored than were those of fish in the other lots because of the washing out of the gills, but the overall impression of the inspectors was that the fish of lot ND had the best appearance.

DISCUSSION

The possible advantages and disadvantages of the different systems studied in this work were evaluated as a function of (i) maintenance of temperature during storage, (ii) physicochemical parameters, (iii) microbiology, and (iv) sensory qualities. The experiment was repeated three times in order to take into account the effects of season and/or day-to-day handling and environmental variability so that the experimental conditions would resemble actual working conditions. The weights, sizes, and proximate analysis values were within the normal limits for sardines *(8)*. The higher fat content found in the July and September experiments is consistent with the increase in reserve fats exhibited by sardines in the summer months and after spawning

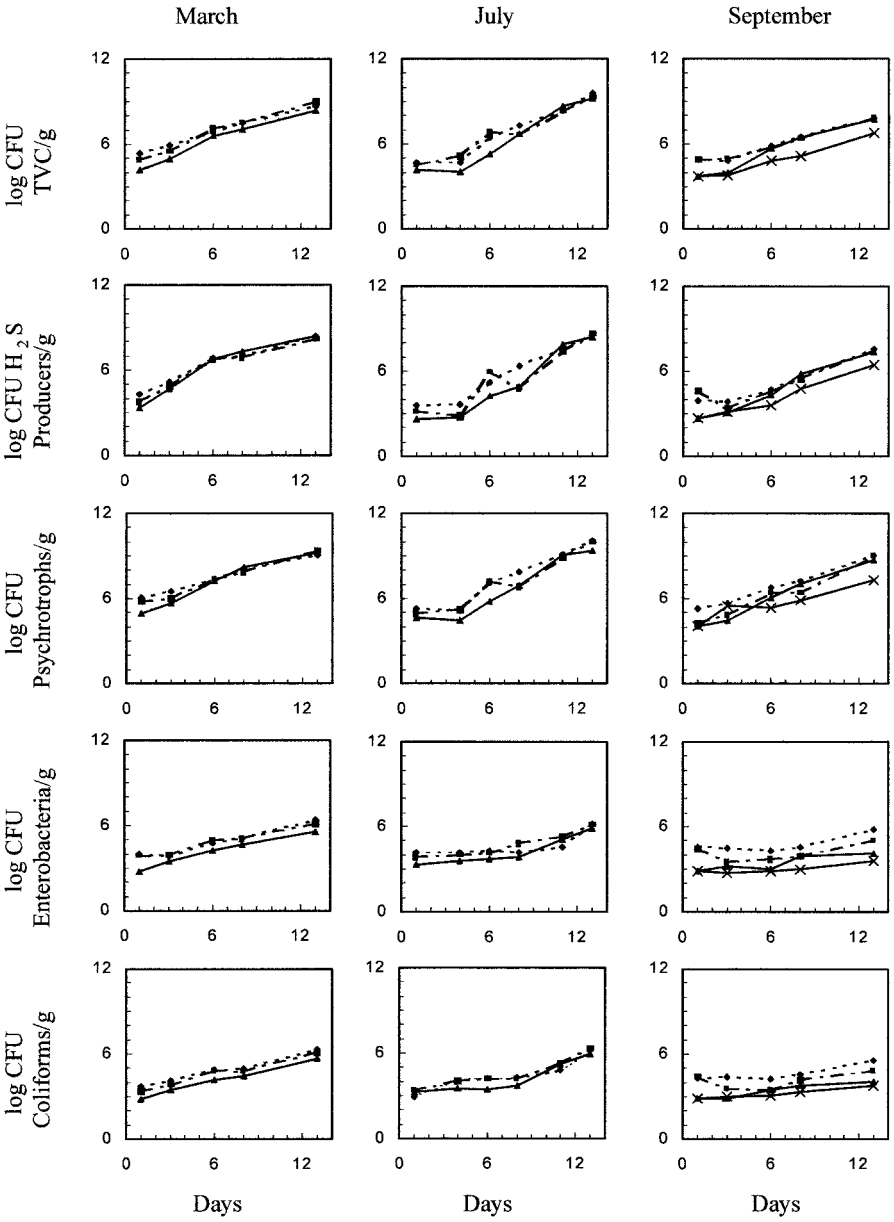

FIGURE 2. *Changes in total viable counts (TVC) and in counts of H_2S producers, psychrotrophs, enterobacteria, and coliforms for sardines stored in ice in wooden boxes (♦); in ice in perforated EPS boxes (■); in water and ice for 20 h in EPS boxes, with subsequent dewatering and a return to ice (▲); and in water and ice for the entire storage period (×). Experiments conducted in March, July, and September are presented from left to right.*

(20, 25). The weight gain exhibited by sardines in the IW lot was normal for fish stored under these conditions *(23).*

As previously observed for anchovies *(9),* the ice in which the fish were stored when the boat landed was not sufficient to ensure low enough temperatures to minimize spoilage processes. Again as with anchovies *(9),* the lot temperatures differed on arrival at the wholesale market. In contrast to fish stored in wooden boxes, it was shown that both the fish cooled and transported in water and ice (lot IW) and the fish stored in ice in insulated containers (lot I) arrived in the distribution market at near 0°C.

The physicochemical indices for the fish stored in water and ice only during transportation (20 h) showed no differences between the various systems except in the TBARS levels. The amounts of TMA-N and TVB-N produced during storage for all lots were similar to those found by other authors *(1, 11).* Moreover, pH levels were similar to those reported in the literature *(11).* Lower TBARS levels in the March experiment than in the July and September experiments may be attributed to a lower fat content for sardines in the winter. Because of a higher fat content, the TBARS differences relating storage time and treatment were greater for sardines than for anchovies *(9).* TBARS was the only parameter for which lot IW presented higher values than lot WB and lot I, indicating a higher rate of oxidative rancidity for lot IW. This high rancidity value further increased after 9 days' storage in ice for lot E48. The higher rancidity for lots IW and E48 was interpreted as a consequence of pro-oxidant contact with substrate that occurs when fish are stored in the presence of oxygen (ice) after being stored under conditions in which pro-oxidant compounds are released into the water-and-ice medium *(9).* Studies on herring indicated that fish stored in RSW exhibited low levels of oxidative rancidity, but when these fish were placed in oxygen-permeable containers, the process progressed faster than it did with systems involving ice only *(7).*

Comparable results for microbial counts were obtained

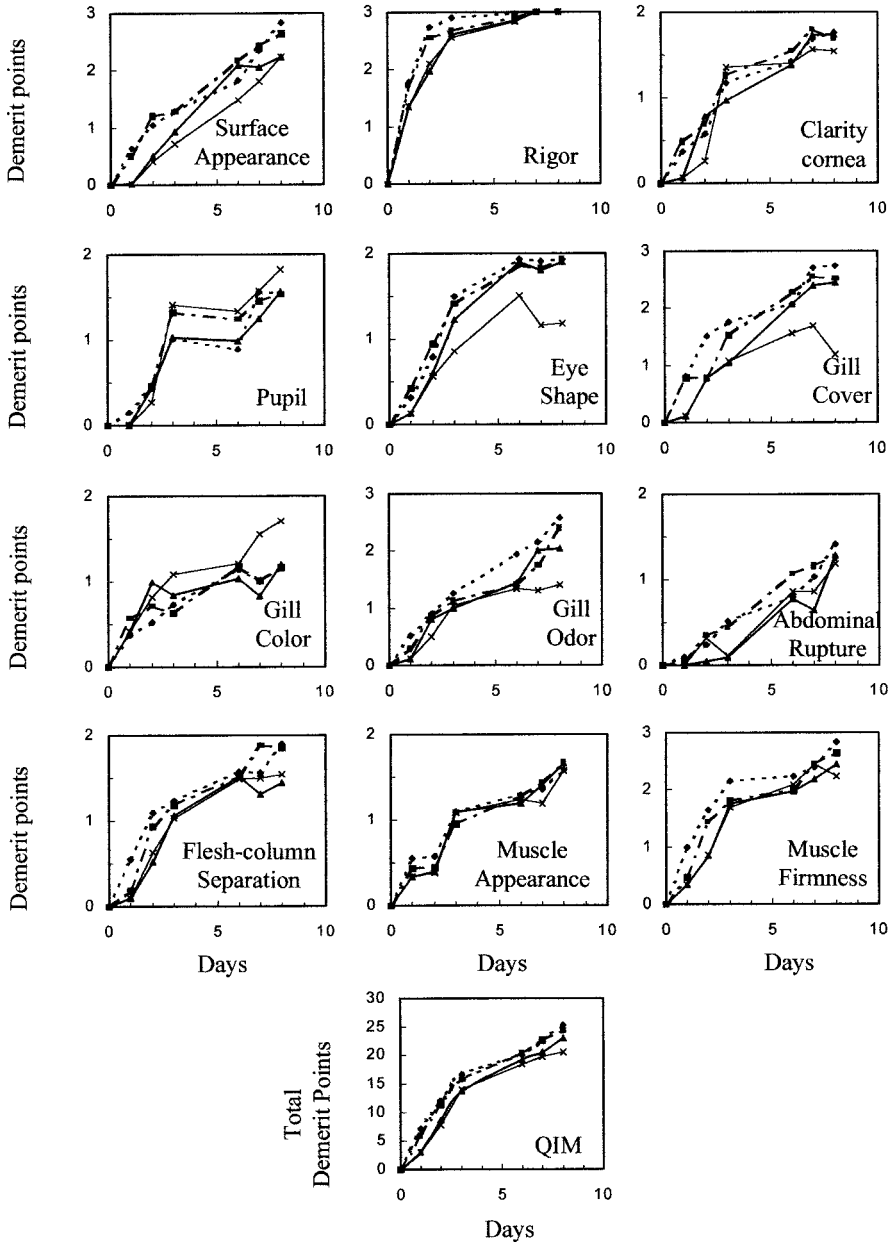

FIGURE 3. *Total QIM scores and QIM attribute scores for sardines stored in ice in wooden boxes (♦); in ice in perforated EPS boxes (■); in water and ice for 20 h in EPS boxes, with subsequent dewatering and a return to ice (▲); and in water and ice for the entire storage period (×). Fish were caught in September.*

in previous studies on anchovies stored under similar conditions *(9)*. The lower microbial content in lot IW than in lot WB was attributed to better maintenance of the cold chain and to the washing-out effect of the system *(9)*.

The changes in total QIM scores over time were similar to those found by other authors *(2)*. The better surface appearance and lower degree of belly burst in lot IW in both the July and the September experiments is consistent with reports for other fish species. It is believed that chilling in water and ice causes rapid temperature reduction, and the water-and-ice medium, being fluid, causes less mechanical damage, thus giving the product a better appearance *(8, 9, 14, 16)*. Fish inspection performed with the QIM demonstrated the advantages of the use of water and ice in the summer months, in accordance with the view of fish processors and retailers. It was also observed that differences in most indices were more extensive in September. These differences cannot be attributed to fat content, since the fat contents of sardines are similar in July and September. Temperatures of fish coming off the boats were higher in September, which suggests that the advantages of chilling in water and ice are clearer when handling is carried out less carefully on board fishing boats.

When the water-and-ice system was used until the end of storage (lot ND), microbiological, sensory, and physicochemical parameters indicated that the spoilage rate was slower than it was when fish were stored in ice alone. The lower rates of TMA-N and TBV-N formation can be interpreted as a consequence of lower growth rates for the organisms responsible for the formation of these compounds. The low TBARS levels found for samples stored in water and ice throughout the storage period are also consistent with findings for other species *(6, 13, 18)* and have been attributed to the fact that conditions in water and ice quickly become anaerobic because of the low solubility of oxygen in water *(14, 23)*. The absence of oxygen prevents oxidation

TABLE 5. *Results of one-way ANOVA as a function of treatment[a] with time as the covariate of the variables listed for experiments performed in July and September[b]*

Variable	July		September	
	Treatment	Time	Treatment	Time
Surface appearance	***	***	**	***
Rigor	NS	***	*	***
Clarity of cornea	NS	***	NS	***
Pupil	NS	***	NS	***
Eye shape	NS	***	*	***
Gill cover	NS	***	***	***
Gill color (internal)	NS	***	NS	***
Gill smell	NS	***	**	***
Abdomen rupture	***	***	**	***
Column-fillet separation rupture	NS	***	**	***
Muscle appearance	NS	***	NS	***
Muscle firmness (filleted)	NS	***	***	***

[a] Treatment descriptions as in Table 3.
[b] * $P < 0.05$; *** $P < 0.01$; *** $P < 0.001$. NS, nonsignificant.

of the lipids so that nondewatered lots exhibit lower oxidative rancidity values *(14, 15)*.

Moreover, lot ND exhibited a lower microbiological load than did lot IW. The lower microbial counts that resulted when fish were kept in water and ice for the entire storage period have been attributed to rapid initial chilling when the fish are caught and to improved maintenance of the temperature during storage *(12, 15)*. Other studies dealing with the storage of sardines in CSW indicate a qualitative change in the nature of the spoilage flora due to the combined action of bacterial genera like *Pseudomonas, Vibrio, Flavobacterium,* and *Aeromonas (22)*. In Italy, a high concentration of *Vibrio* spp. in commercial fishery products was found *(4)* and was attributed to contamination

TABLE 6. *Results of one-way ANOVA as a function of treatment[a] with time as the covariate of the variables listed for experiments performed in September[b]*

Variable	Treatment	Time
Surface appearance	***	***
Rigor	NS	***
Clarity of cornea	NS	***
Pupil	NS	***
Eye shape	***	***
Gill cover	***	***
Gill color (internal)	***	***
Gill smell	**	***
Abdomen rupture	*	***
Column-fillet separation rupture	NS	***
Muscle appearance	NS	***
Muscle firmness (filleted)	NS	***

[a] Treatments included sardines stored in ice in perforated polystyrene boxes (lot I), in water and ice during distribution (20 h) and in ice thereafter (lot IW), and in water and ice throughout the whole storage period (lot ND).
[b] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, nonsignificant.

of the seawater. When fish are processed on land, the ND system uses not seawater but tap water, which reduces the incidence of contamination from *Vibrio*. From a practical point of view, however, it is not easy to check retailers' control of their products, so problems arising from the occurrence of pathogenic, anaerobic microorganisms may not be traced properly. On the other hand, some of the advantages of the ND system were also observed for fish stored in ice after a certain number of hours in water and ice, although this method does have the drawback of increased oxidative rancidity. However, this problem can possibly be overcome by adding antioxidants to the water-and-ice medium.

The results presented in this paper confirm those found for anchovies with regard to the effect of storage of fish in water and ice during distribution. It can be concluded that both the IW and the ND systems offer advantages over storage in ice when fish handling on board has not been performed in the optimum manner, although the IW system should be improved for sardines to minimize oxidative rancidity. In all cases, but especially for the ND system, care should be taken to maintain temperatures at 0°C for the entire storage period, from transport to retail sale.

ACKNOWLEDGMENTS

This work was financed by Asociación Nacional de Poliestireno Expandido (Spain). We thank Prof. A. J. Borderías for his valuable comments, Ms. L. Barrios for the statistical analysis, and the members of the Departamento de Ciencia y Tecnología de la Carne y Productos Cárnicos y del Pescado y Productos de la Pesca for performing the QIM inspection.

REFERENCES

1. Abdalla, M. A., I. M. Hassan, A. R. Shalaby, and K. Naguib. 1989. Physicochemical and bacteriological changes occurring during storage of sardine fish. Aceites Grasas 6:389–398.
2. Andrade, A., M. L. Nunes, and I. Batista. 1997. Freshness quality grading of small pelagic species by sensory analysis, p. 333–338. *In* G. Olafsdottir, J. Luten, P. Dalgard, M. Careche, V. Verrez-Bagnis, E. Martinsdottir, and K. Heia (ed.), Methods to determine the freshness of fish in research and industry. International Institute of Refrigeration, Paris.
3. Anonymous. 1991. Directive 91/493/EEC: directive of 22 July 1991 laying down health standards for production and marketing of fishery products. Off. Diary L286:15–34.
4. Baffone, W., A. Pianetti, F. Bruscolini, E. Barbiei, and B. Citterio. 2000. Occurrence and expression of virulence-related properties of *Vibrio* species isolated from widely consumed seafood products. Int. J. Food Microbiol. 54:9–18.
5. Barhoumi, M. 1981. Pruebas de conservación de la sardina en agua de mar enfriada, p. 143–151. *In* D. G. Ordenación Pesquera (ed.), Consulta técnica sobre utilización de las especies pelágicas en el área mediterránea. Subsecretaría de Pesca, Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain.
6. Barhoumi, M., A. A. Faye, F. Teutscher, C. A. M. Lima-dos-Santos, and E. Vike. 1981. Storage characteristic of sardine (*Sardine pilchardus*) held in ice and chilled seawater. Refrig. Sci. Technol. 4: 243–249.
7. Bilinski, E., R. E. E. Jonas, and Y. C. Lau. 1978. Chill stowage and development of rancidity in frozen Pacific herring, *Clupea harengus pallasi*. J. Fish. Res. Board Can. 35:437–477.
8. Burt, J. R., and R. Hardy. 1992. Composition and deterioration of pelagic fish, p. 115–141. *In* J. R. Burt, R. Hardy, and K. J. Whittle (ed.), Pelagic fish. The resource and its exploitation. Fishing News Books, Oxford.
9. Careche, M., R. García, and J. Borderías. 2002. Anchovy shelf life

as affected by different chilling methods during distribution. J. Food Prot. 65:353–361.
10. Dora, K. C., and G. G. Hiremath. 1987. Effect of chilled seawater storage of oil sardine (*Sardinella longiceps*) on quality changes during frozen storage. Fish. Technol. 24:109–111.
11. El Marrakchi, A., M. Bennour, N. Bouchriti, A. Hamama, and H. Tagafait. 1990. Sensory, chemical and microbiological assessments of Moroccan sardines (*Sardina pilchardus*) stored in ice. J. Food Prot. 53:600–605.
12. Gennari, M., S. Tomaselli, and V. Cotrona. 1999. The microflora of fresh and spoiled sardines (*Sardina pilchardus*) caught in the Adriatic (Mediterranean) Sea and stored in ice. Food Microbiol. 16:15–28.
13. Hansen, P., and J. G. Jensen. 1982. Bulk handling and chilling of large catches of small fish, part 1. Quality and storage life. Infofish Market Dig. 6:26–28.
14. Huss, H. H. 1988. Quality and storage life of chilled fish, p. 77–102. *In* FAO (ed.), Fresh fish-quality and quality changes. A training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control. FAO Fisheries Series, Rome.
15. Jeyasekaran, G., and K. V. Saralaya. 1991. Influence of fish chilling methods on the quality of white sardine (*Kowala coval*). Fish. Technol. 28:55–58.
16. Martinez, A., and A. Gildberg. 1988. Autolytic degradation of belly tissue in anchovy (*Engraulis encrasicholus*). Int. J. Food Sci. Technol. 23:185–194.
17. Moral, A. 1985. Report of the second technical consultation on the utilization of small pelagic species in the Mediterranean area. FAO Report no. 331. General Fisheries Council for the Mediterranean Area, Zadar, Yugoslavia.
18. Moral, A., J. Almazán, M. Arranz, A. Beltrán, R. Gómez, and A. Miyar. 1989. Réfrigération á bord dans de la glace ou de l′eau de mer du chinchard, du picarel et du bogue. Rev. Gen. Froid 79(7, July–August):333–338.
19. Nielsen, J. 1992. Quality management of the raw material in the food fish sector based on standardized sensory method & physical dimensions of the raw fish. FAIR contract no. UP-2-452. European Commission, Brussels.
20. Nunes, M. L., I. Batista, and R. Morao de Campos. 1992. Physical, chemical and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. J. Sci. Food Agric. 59:37–43.
21. Perigreen, P. A., P. S. Ayyappan, P. K. Surendran, and T. K. Govindan. 1975. Studies on preservation of fish in refrigerated sea water. Fish. Technol. 12:105–111.
22. Shetty, T. S., and T. M. R. Setty. 1990. Bacteriology of Indian oil sardine (*Sardinella longiceps Valenciennes*) stored in chilled sea water. Fish. Technol. 27:141–144.
23. Shetty, T. S., T. M. R. Setty, and C. N. Ravishankar. 1991. Quality changes in Indian oil sardine (*Sardinella longiceps*) during storage in chilled sea water. Fish. Technol. 28:51–54.
24. Smith, J. G. M., R. Hardy, I. McDonald, and J. Templeton. 1980. The storage of herring (*Clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. J. Sci. Food Agric. 31:375–385.
25. Varela, M. G. 1985. Valores nutricionales de la sardina, p. 107–121. *In* FROM, Ministerio de Agricultura y Alimentación (ed.), La sardina un tesoro de nuestro mar. Lunwerg Editores, Sociedad Anónima, Spain.