JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Freshness and Quality Criteria of Iced Farmed Senegalese Sole (*Solea senegalensis*)

AMPARO C. GONÇALVES,* SOFIA E. ANTAS, AND MARIA L. NUNES

Department of Technological Innovation and Upgrading of Fish Products, National Research Institute on Agriculture and Fisheries, INIAP/IPIMAR, Avenida Brasília, 1449-006 Lisboa, Portugal

Senegalese sole (*Solea senegalensis*) is a high-value commercial species with increasing importance in aquaculture. The aim of this work was to study the quality changes of this species during chilled storage under refrigeration, through sensory and chemical methods. In particular, the optimization of a quality index method (QIM) scheme was proposed as well as the definition of sensory and chemical quality criteria. A shelf life of 15 days was reported, and a QIM scheme based on a total of 22 demerit points (dp) was proposed. Biogenic amines were never detected, and the usual spoilage indicators, such as TVB-N and TMA-N, were not significantly produced during the period of sensory-acceptable quality (15 days). On the basis of the significant correlations ($p < 0.001$) between sensory data and K_i values, a quality index (QI) $\leq$ 7 dp and a $K_i \leq 12\%$ correspond to a fresh fish, whereas a QI $>$ 19 dp and a $K_i > 40\%$ indicate unacceptable quality of iced Senegalese sole.

KEYWORDS: Quality index method; freshness; sensory evaluation; chilled storage; K_i value; biogenic amines; Senegalese sole

INTRODUCTION

The type and rate of fish quality loss during postharvest handling and storage are specific for each species and depend on several conditions, such as the nutritional status of the fish, its biological state, and the temperature of storage (*1*). Therefore, knowledge of the specific deterioration pattern of each species is essential for better fish handling and the establishment of quality criteria. The commercial value of a fish species is associated with its excellence, which is regarded as freshness quality. The freshness of fish is related to all sensory properties (flavor, odor, texture), but the general raw appearance is extremely important because it determines its marketability and price. For that reason objective and harmonized criteria, as well as experienced panelists in fish assessment, are required to yield more objective, simple, and rapid evaluation schemes. Such schemes are particularly needed at auctions and in the fish-processing sector, as well as in the retail market, to substitute the European Union (EU) freshness grade actually used (*2*).

The quality index method (QIM), first developed by the Tasmanian Food Research Unit (*3*), is the latest scheme to overcome these difficulties and has been applied to iced whole and gutted fish, and also to other fishery products, including cephalopods, raw fillets, frozen/thawed fish, and cooked fish/shrimp (*4*, *5*). QIM is a sensory scheme based on well-defined changes of fishery products during storage. Each attribute (quality parameter), such as the appearance (format/brightness of eyes, gill color in the case of fresh fish) and odor, is described by a maximum of four descriptors, and respective scores are associated (*5*). The descriptors that correspond to high freshness are scored with 0, whereas higher scores correspond to lower quality, at a maximum of 3 demerit points (dp). The descriptors should be very simple and clear to avoid misunderstandings. The scores of all attributes are summed to give an overall score, which is the quality index (QI). As a result, the theoretical evolution of the QI initiates at 0 or at low values and increases during storage, achieving the maximum when the fish should be rejected (*3*, *5*).

One difference between the QIM and other traditional sensory schemes is that the first is based in specific descriptors for each product or species, which implies the development and optimization of one particular scheme for each fish species or product in specific storage conditions (chilled, frozen) (*3*, *5*). In parallel, the assessment of cooked fish is needed to establish the fish rejection and estimate the shelf life in the specific storage conditions (*5*).

In the case of iced fish, the linear relationship of the QI with storage time permits the prediction of the number of days that the fish was in ice [predicted storage time (PST)]. Because the shelf life in ice [estimated shelf life (ESL)] was estimated on the basis of the assessment of cooked fish, it will be possible to estimate the remaining shelf life (RSL), defined as the time in which the fish is still acceptable for human consumption (RSL = ESL − PST) (*3*, *5*). It is important to note that the estimation of RSL is affected by some uncertainty due to the effect of several factors such as fish handling and storage conditions, in particular, the temperature.

QIM schemes have already been developed for several farmed fish species (*4*, *7*), including some flatfish such as brill (*Scophthalmus rhombus*), turbot (*Psetta maximus*), and sole

* Author to whom correspondence should be addressed (telephone +351213027036; fax +351213015948; e-mail amparo@ipimar.pt).

10.1021/jf0632942 CCC: $37.00
Published on Web 04/07/2007

(*Solea solea*) (*5*). Nevertheless, development and optimization for other species are still needed. Senegalese sole (*Solea senegalensis*) is an emergent species in aquaculture, and little information is available regarding the quality changes during chilled storage, even for wild species. Although the production of this species is still not well-established, it has a high commercial value due to its delicate texture and flavor. For that reason, the primary aim of the present work was to study the sensory changes of Senegalese sole during ice storage to optimize a QIM scheme for this species. Considering the usefulness of some chemical methods as freshness/quality indicators (*1*, *6*), the nucleotide degradation products, the volatile nitrogen compounds (TVB-N, TMA-N), and biogenic amines were also evaluated to contribute to a full characterization of the quality changes of this species and to establish some chemical freshness/quality indicators.

MATERIALS AND METHODS

Fish Source. Three different batches of Senegalese sole, fasted for 24 h before slaughter, were obtained from two Portuguese fish farms, in three different periods: July, August, and October 2005. Average length and weight were, respectively, 29.3 ± 1.8 cm and 304.4 ± 55.4 g. The fish were slaughtered by hypothermia, in an ice−water slurry. After death, fish were packed in expanded polystyrene boxes, covered by a thin plastic film, with crushed ice on the top, and transported to the laboratory.

Storage Experiments and Sampling. The delivery of fish to the laboratory occurred approximately 18 h after slaughter. Fish were conditioned in polypropylene boxes with perforated bottoms, covered by a thin plastic film with crushed ice on top. The boxes were stored under refrigerated conditions at 1.5 ± 0.8 °C, and the ice was replenished as necessary.

During the chilled storage samples were taken for sensory assessment as follows: July, August, sampling after 1, 3 or 4, 7, 9, 11, 14, and 16 days in ice; October, sampling after 1, 4, 7, 11, 15, and 17 days in ice. Samples for proximate composition were taken only at the reception day (1 day in ice). Sampling for other chemical analyses was done initially (1 day in ice) and after 4, 9, and 16 or 17 days of storage, except in the case of volatile compounds and biogenic amines, for which samples were collected initially and when the fish was sensory rejected.

Chemical Analyses. All chemical analyses were done on the fish muscle (without skin), previously minced. A minimum of three and up to five fish samples per batch were used in each sampling day.

Proximate Composition. The ash, moisture, crude protein, and fat contents were determined at day 1 by the reference methods, described in AOAC (*8*). *Ash* was determined by incineration of fish muscle (previously dried) in a furnace at 500 ± 25 °C until to constant weight (method 942.05). *Moisture* was determined by the air-drying method, during 12−16 h at 105 ± 2 °C in an air oven (method 950.46). *Crude protein* was determined by the Kjeldahl method, and the percentage of protein was calculated as total nitrogen × 6.25 (method 981.10). *Total fat* was extracted with diethyl ether solvent in a Soxhlet apparatus (method 991.36). Sample was refluxed during 7 h at adjusted temperature. Fat was determined by weight after drying to constant weight in a 105 ± 2 °C air oven.

Total Volatile Base Nitrogen (TVB-N), Trimethylamine Nitrogen (TMA-N), and Trimethylamine Oxide Nitrogen (TMAO-N). Volatile compounds were determined in extracts from 25 g of fish mince homogenized with 50 mL of 10% trichloroacetic acid (TCA) by a microdiffusion method (*9*).

Biogenic Amines. Determination was done in the previous TCA extracts by high-performance liquid chromatography (HPLC) (*10*), in a LKB Biochromma LC system (Pharmacia), on a reverse phase column (Spheri-5 RP18 5 μm, 220 × 4.6 mm) by a gradient elution (two sodium acetate buffers, 0.1 and 0.2 M, pH 4.5; flow rate = 1.0 mL/min), followed by postcolumn derivatization with *o*-phthaldialdehyde. Detection was made in a fluorescence spectrophotometer (Perkin-Elmer LC-1) with excitation at 340 nm and emission at 455 nm. The system permits detection of histamine (Him), cadaverine (Cad), putrescine (Put), tyramine (Tyr), and agmatine (Agm). Identification and quantification were done by comparison with specific standards (Sigma) through calibration curves, using the software EZChrom Chromatography Data System, version 6.7 (Scientific Software).

Nucleotides and Nucleotide Catabolites. Extracts were prepared from 5 g of fish mince homogenized with 25 mL of 0.6 M perchloric acid, and the separation was done by HPLC in an Agilent 1100 Series LC System, as described in ref *11* on a reverse phase column (Lichrosorb 100 RP-18 10 μm, 250 × 4.6 mm). Phosphate buffer (0.1 M, pH 6.95) composed the mobile phase; elution was isocratic (flow rate = 1.6 mL/min), and detection was done at 254 nm. Compounds were identified and quantified by comparison with specific standards (Sigma) through calibration curves, using the Agilent ChemSation software G2170AA (Agilent Technologies).

Free amino acids (FAA) were extracted with a solution of 10% TCA in a ratio of 1:10 (fish muscle/TCA) followed by a centrifugation step (*12*). FAA were separated by ion exchange liquid chromatography in an automatic analyzer Biochrom 20 (Amersham Pharmacia Biotech AB) equipped with a Peek polymeric cation exchange column (200 × 4.6 mm), using three sodium citrate buffers (pH 3.20, 4.25 and 6.45; Amersham Biosciences) and three different temperatures (50, 58, and 95 °C). The detection of amino acids was done at 440 and 570 nm after reaction with ninhydrin reagent (Amersham Biosciences). Amino acid identification and quantification were done by comparison with specific standards (Sigma) through calibration curves, using the software EZChrom Chromatography Data System, version 6.7 (Scientific Software).

Sensory Evaluation. The sensory evaluation was carried out in a special room equipped with individual booths by a sensory panel composed of four to six experienced assessors. A minimum of three and up to five fish samples per batch were used in each sampling day. In total, 24, 24, and 25 fish samples were assessed in the trials of July, August, and October, respectively.

Raw Assessment and QIM Optimization. Each panelist assessed one fish (whole raw), but also observed the other fish samples in order to be familiar with the fish variability. Despite the experience of the panelists in sensory assessment of fish, in particular using QIM schemes, a preliminary experiment was done to train the panel on the quality parameters and descriptors for Senegalese sole and also for some adjustments in the original QIM scheme, which was first developed for sole (*Solea solea*) (*5*).

Sensory Assessment of Cooked Fish. After the raw assessment, the fish were gutted, headed, and washed with tap water. Each fish sample was wrapped with perforated aluminum foil and cooked for 7−8 min at 100 °C in a saturated steam oven (Rational Combi-Master CM6 Cross Kuchentechnik GmbH, Landsberg a. Lech, Germany). Each panelist evaluated all of the fish samples and gave an average score. In October cooked fish was not assessed on days 4 and 17. The panel evaluated the odor and flavor using the Torry scale for lean species (*13*), which was also slightly modified (**Table 1**) during the preliminary experiment. In addition, the panelists were asked their opinion about the texture of the fish flesh (only in qualitative terms).

An average score of ≤5.5 for odor and flavor is usually used as the limit of acceptability (*5*), and the shelf life of Senegalese sole was defined on the basis of this sensory criteria together with the autolysis level of the viscera of whole raw fish.

Viscera Evaluation. The status of the viscera was qualitatively evaluated by three assessors immediately before the gutting of fish.

Statistical Analysis. The statistical treatment of the results was performed using Software Statistica, vers. 6.1 (Stat Soft, Inc., Tulsa, OK). ANOVA analysis and the Tukey multicomparison test of different batches and storage days were done. A time-dependent linear regression analysis was performed for each quality parameter/descriptor and QI. Comparison of the several regression equations was done according to the method described in ref *14*. Correlations between sensory and chemical variables were also done. Significance was tested at $\alpha = 0.05$ ($p < 0.05$).

RESULTS AND DISCUSSION

Proximate Composition. Significant differences among the three independent fish batches were detected only for fat content,

Table 1. Sensory Scores for the Assessment of Cooked Farmed Senegalese Sole [Modified from Howgate (*13*)]

odor	flavor	score
initially weak odor of sweet, starchy, followed by strengthening of these odors	insipid; initially no sweetness but flavors with slight sweetness may develop; slightly bitter/sour aftertaste	10
weak odor of sweet, boiled meat, slightly starchy	sweet, without bitter/sour aftertaste	9
loss of odor, neutral	sweet and characteristic flavors but reduced in intensity	8
woodshavings, musty	neutral	7
boiled potato	insipid	6
slightly sour	slight sourness, off-flavors traces	5
lactic acid, sour milk, fishy odor (TMA)	slight bitterness, sour, off-flavors	4
lower fatty acids (e.g., acetic or butyric acids), decomposed grass, soapy, turnipy, tallowy	strong bitterness, rubber, slight sulfide	3

Table 2. Proximate Composition of the Flesh of Raw Farmed Senegalese Sole[a]

proximate composition (g/100 g of flesh)	July	August	October
ash	1.31 ± 0.07	1.31 ± 0.01	1.32 ± 0.05
moisture	77.2 ± 0.5	74.8 ± 1.2	75.6 ± 0.7
fat	0.7 ± 0.2 a	2.4 ± 0.8 b	1.7 ± 0.1 b
protein	20.6 ± 0.3	20.3 ± 0.1	20.9 ± 0.3

[a] $N = 5$ fish analyzed. Values with different letters are significantly different ($p < 0.05$).

in which the fish of the later two experiments presented the highest fat level (**Table 2**). The influence of nutrition in the composition of fish flesh is well-known (*15*), and the proximate composition of marine farmed fish generally reflects the diet composition, in particular, the fat level and composition (*16*, *17*). Values in the range of 1.0−1.8% were reported for the muscle of farmed turbot fed different dietary fat levels (*16*), whereas levels within 0.5−9.6% were found for the muscle, fillet, or flesh of wild pleuronectiformes, turbot and Atlantic halibut (*18*).

Sensory Assessment of Cooked Fish. A gradual decrease of the odor and flavor scores was observed during the storage in ice, and significant differences among the three experiments were not observed (ANOVA two-way, $p > 0.05$); the storage time was responsible for the main differences reported. In general, slightly higher scores were reported for odor, but this attribute and the flavor were positively correlated ($r^2 = 0.8056$, $r = 0.8976$, $p < 0.001$), and a significant negative linear correlation between the average scores of odor/flavor and the storage time was reported (**Figure 1**). Initially (1 day in ice), the fish was very fresh (average scores of 9.8 for odor/flavor) and was characterized by a weak sweet odor, insipid to fairly sweet flavor, and slightly bitter/sour aftertaste (**Table 1**). During the further 4 days of storage a slight decline of freshness was observed, and an average score of 8.9 was reported on the fourth day. Differences between scores became significant only at the seventh day (ANOVA, $p = 0.0388$), and between days 7 and 11 the scores slightly decreased from 7.8 to 7.3, corresponding to acceptable grade of quality (regarded as musty odor and neutral flavor; **Table 1**). On days 14 and 15 the scores were very close to the limit of acceptability (5.5) (*3*), and slightly sour odor/flavor and off-flavor traces were perceived by the panelists; 75% of the scores reported on the 14th day were ≤6.5, of which 50% were ≤6.0, whereas on the 15th day 75% of the scores did not exceed 5.9, of which 50% were ≤5.5. On day 16 the average score was 5.2, and 50% were below 4.8; undoubtedly, the fish was rejected. In October the panelists rejected the fish on day 15 due to its raw appearance and on the 17th day cooked fish was not assessed.

The flavor was the attribute that most influenced the rejection of fish, although the texture (qualitatively assessed) was also an important trait for the sensory panel. Generally, the texture was considered to be somewhat mushy, and this feature became more intense during the storage, in particular in the August trail, which corresponded to the fish with the highest fat content (**Table 2**). The available data about the effects of dietary fat on the organoleptic properties of fish flesh are somewhat contradictory, and some authors did not report any effect of dietary fat level on the texture of farmed turbot (*16*). In the present work, such a study was not done, but the difference reported for fat content between the three fish batches suggests that influence could be imperceptible. On the 15th and 16th days the fish presented a very soft flesh and the brownish coloration along the fins evidenced some oxidation (which was also noted by a fairly rancid odor). At this time, the tenderness of fish flesh was visible even in the raw fish because in some individuals the bones were easily separated from the muscle.

These results are concordant with the viscera evaluation. At the beginning of storage the viscera were individualized and bright, but after 4 days they lose some brightness. The first signal of autolysis was noted on days 14 and 15, and on the 17th day the viscera were moderately dissolved.

Considering all of the above results, in particular the sensory assessment, a shelf life of 15 days was defined for Senegalese sole stored in ice at proper conditions (temperature below 3 °C). Similar results were found for other flatfish species, and shelf lives of 13, 14, and 15 days in ice were estimated, respectively, for whole turbot, brill and sole (*5*), and gutted wild turbot (average weight of 525.93 g) (*19*). On the contrary, a higher shelf life of 19 days was reported for iced farmed turbot, for which weight ranged between 1400 and 1900 g (*20*−*22*).

Sensory Assessment of Raw Fish−QIM Optimization. Despite the low numbers of fish assessed in the preliminary experiment, it allowed the first modifications to be introduced to the original QIM scheme, developed for sole (*5*), mostly related with the texture, gill coloration, and flesh color. This modified scheme (**Table 3**) was used in the further three independent storage experiments, and additional changes were made as was necessary to adjust the scheme. **Figure 2** illustrates the evolution of all the quality parameters during the chilled storage. In general, the majority of the selected parameters were useful in reflecting the quality changes of Senegalese sole, and significant linear correlations with the storage time were observed for most of them. Significant differences between the regression slopes were found only for gill odor of August and October, but it was mainly related with the high variability reported in the former experiment. An enhancement of the adjustment of the linear model to the data (higher coefficient of determination, r^2) was observed for some quality parameters during the three experiments. This was the case for the

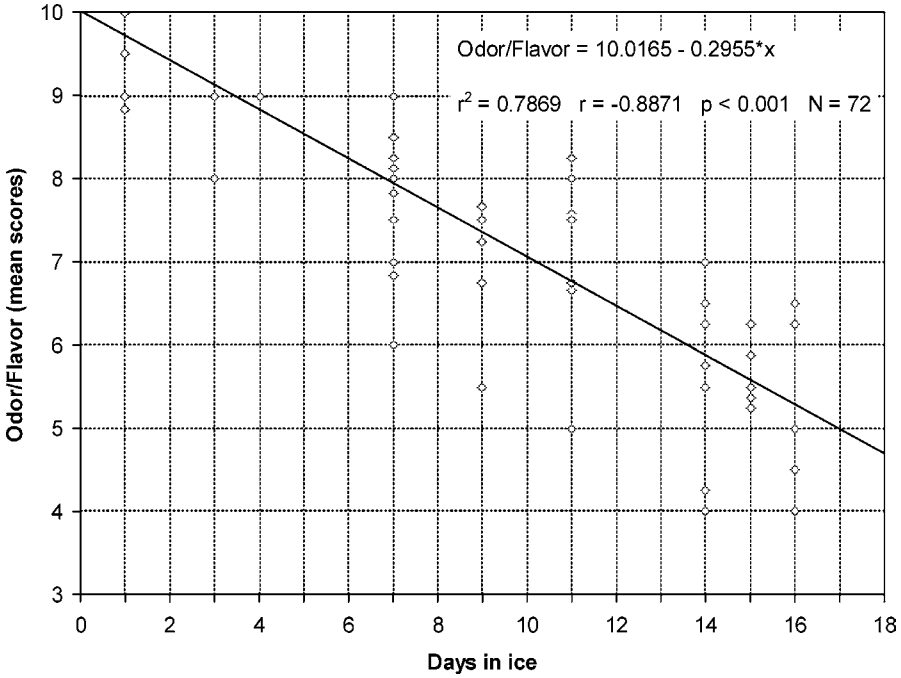

Figure 1. Linear relationship between the average scores of odor/flavor (cooked fish) and the storage time in ice for whole raw farmed Senegalese sole based on the data of three independent storage experiments (1.5 ± 0.8 °C). Score 10, very fresh fish; score 3, putrid fish.

Table 3. Initial Quality Index Method (QIM) for Freshness Assessment of Whole Raw Farmed Senegalese Sole during Iced Storage at 1.5 ± 0.8 °C [Modified from (*5*)]

quality parameters		descriptors/demerit points			
		0	1	2	3
skin appearance	*ocular side*	fresh, bright	rather dull or pale, somewhat shrunken skin	dull, pale, some green or purple discoloration	dull, green and purple discoloration, very shrunken skin
	blind side	bright white	some purple discoloration at the edges of the fins	dull, purple, yellow discoloration at fins and in the middle	yellow and purple discoloration
	mucus	clear, not clotted	slightly clotted and milky	clotted and slightly yellow	yellow and clotted
texture (ocular side)		firm, elastic	less firm, elastic	soft	very soft
eyes	*form*	flat, eye socket convex	slightly sunken, eye socket shrunken	sunken and or swollen, eye socket shrunken	
	brightness	black and clear, golden rim around the pupil	rather matte, faint golden rim around the pupil	matte, purple/ reddish	
gills	*odor*	fresh, seaweedy	neutral, metallic, rubbery	musty, sour	rotten, sour, sulfurous
	color	bright, light red	slightly discolored	discolored	yellowish, green/blue discoloration
	mucus	no mucus	clear	milky, slightly clotted	yellow, thick, clotted
flesh	*color*	translucent, bluish	slightly yellowish	yellow, discolored	waxy, opaque, yellow
quality index (QI) range: 0–28 demerit points					

appearance of the blind side and the body mucus, for which low r^2 and slopes were reported for the first two experiments. Generally, the mucus was more visible in the blind side due to its white coloration.

In the case of flesh texture, although some improvement was observed, a satisfactory linear correlation was not achieved (low values of r^2 for two experiments). This parameter evolves slowly, and some variability was reported (**Figure 2**), even after the adjustment of the maximum score to 2 dp (October). Further evaluations will demonstrate the essentialness of this characteristic in the QIM scheme.

The earliest and most evident changes were found in the skin appearance (in particular the ocular side) as well as in the appearance/form of the eyes (**Figure 2**), which were the attributes that better correlated with the storage time (highest r^2 and slopes). The maximum scores were attained at the rejection time of cooked fish (15 days in ice). The skin depression along the spinal column (**Table 4**) was visible in an earlier stage of storage (after 3−4 days in ice), and this descriptor was consolidated in the two last experiments. Another useful descriptor was the blue/whitish spots that were particularly visible in very fresh fish. During the storage these spots became less visible, and at the same time the skin lost the brownish pigmentation and became pale and purple discolored (**Table 4**).

Some fluctuation in the gill odor scores was observed (**Figure 2**), and the panel did not identify the descriptor "seaweed odor"; instead, they considered the fish to have an initial neutral odor

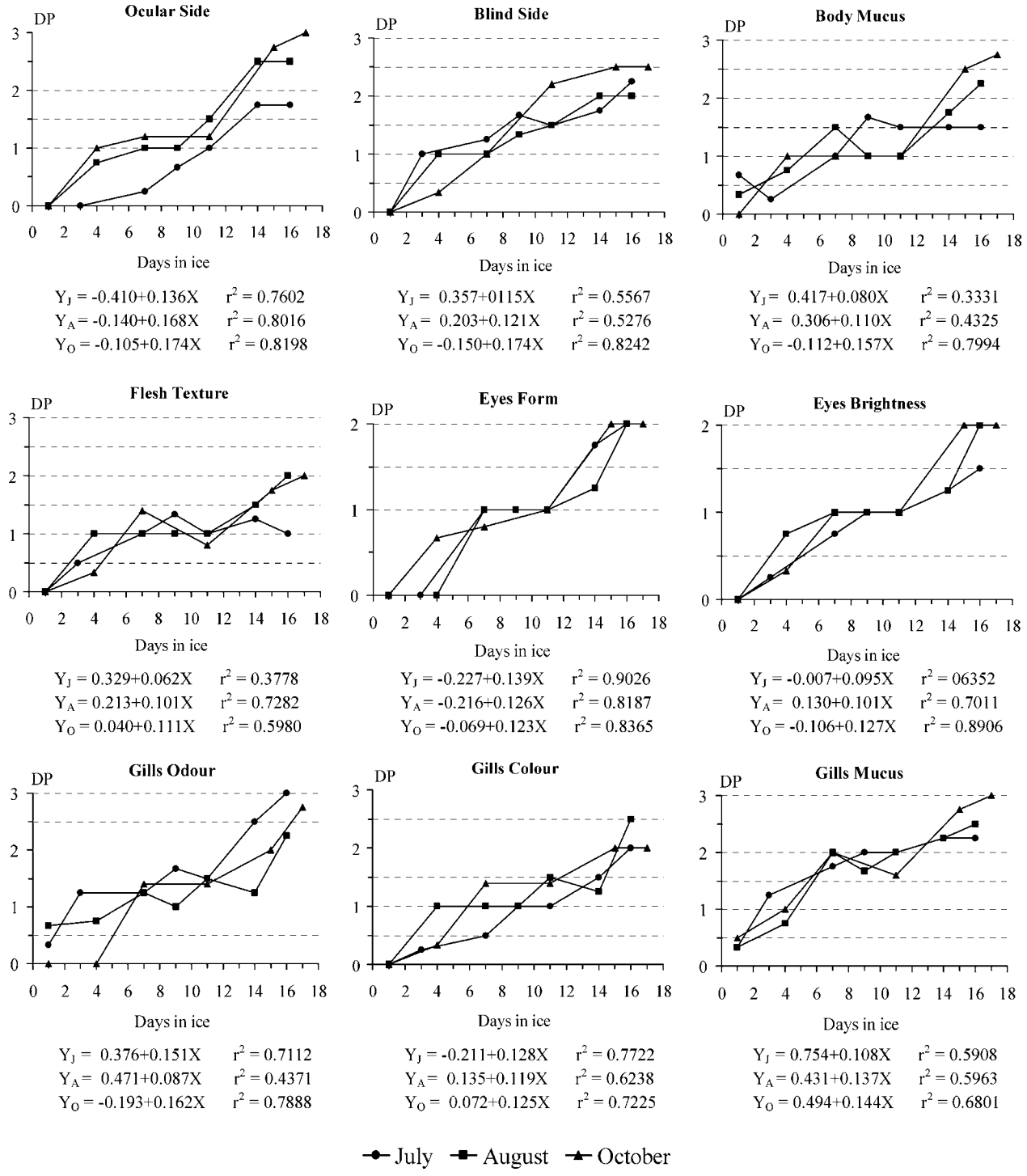

Figure 2. Evolution of raw quality parameters of farmed Senegalese sole during the iced storage at 1.5 ± 0.8 °C. Each data point is the mean value of three to five fish assessed (standard deviation ≤ 1.0). DP, demerit points; score 0, high freshness; score 3, unacceptable quality; Y_J, Y_A, Y_O, regression equations for the experiments of July, August, and October, respectively.

or a somewhat "earth odor". As a consequence, the descriptors were adjusted as presented in **Table 4**.

Regarding the color of gills, although changes were evident, the mean scores never attained the maximum of 3 dp, even after 17 days of storage (**Figure 2**). The mucus seems to be the mainly responsible for the bad appearance of gills because it was always scored with higher values than the color (**Figure 2**). Even at the rejection time of cooked fish (15th day) the maximum scores reported for the gill color were 2 dp. Therefore, the maximum score was changed to 2 dp (**Table 4**).

The flesh color (**Table 3**) was excluded from the QIM after the July experiment, in which the maximum score was not attained even after the rejection of cooked fish (results not shown). Furthermore, the evaluation of this parameter requires the cutting of the fish surface, which compromises the sale of fish in a real market.

The successive modifications introduced in the QIM scheme allow a better adjustment of QI. In October a value around 20 dp was reported at the 15th day (rejection of cooked fish), in a total of 23 dp (**Figure 3**). On the contrary, in the previous experiments QI values close to 19 and 20 dp were obtained, in a total of 28 and 25 dp, respectively, for July and August. Nevertheless, significant differences among the three QI were not observed, which reflects well the loss of freshness through-

Table 4. Final Quality Index Method (QIM) Scheme for Freshness Evaluation of Iced Whole Raw Farmed Senegalese Sole

quality parameters		descriptors/demerit points			
		0	1	2	3
skin appearance	*ocular side*	bright brown pigmentation, blue/whitish spots evident	rather dull or pale, somewhat shrunken skin (fair skin depression a long the spinal column)	dull, pale, slight depigmentation, some purple discoloration at the edges of dorsal and anal fins, shrunken skin evident	pale, extensive depigmentation and purple discoloration at the edges of fins, much shrunken skin
	blind side	bright white	some purple discoloration at the edges of the fins	dull, purple/ yellow discoloration at fins and in the middle	yellow and purple discoloration
	mucus	abundant and clear, not clotted	slightly clotted, milky	clotted and slightly yellow	yellow and clotted
texture (ocular side)		firm, elastic, pressure signs disappear immediately	less firm, elasticity reduced	soft, presence of pressure signs	
eyes	*form*	eye socket convex	slightly sunken, eye socket shrunken	sunken and/or swollen, deformed	
	clarity/brightness	clear, black pupil, golden rim around the pupil	rather matte, faint golden rim around the pupil	matte, purple/reddish, milky pupil	
gills	*odor*	neutral or earth odor, fresh	metallic, rubbery	musty, sour	rotten, sulfurous
	color	bright light red, lamina perfectly separated	slightly discolored	discolored, yellowish, lamina adhered	
	mucus	no mucus	clear	milky, slightly clotted	yellow, thick, clotted
quality index (QI) range: 0–22 demerit points					

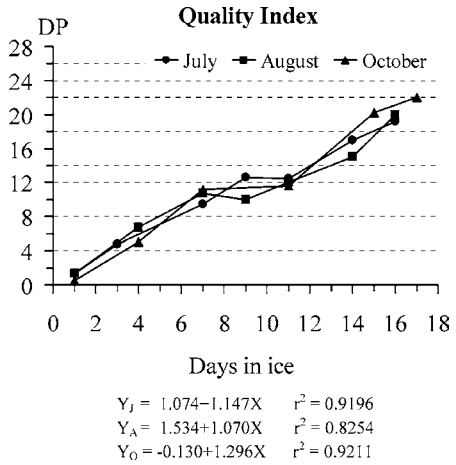

Figure 3. Quality index evolution of whole raw farmed Senegalese sole during the iced storage at 1.5 ± 0.8 °C. Each data point is the mean value of three to five fish assessed (standard deviation ≤ 1.0). DP, demerit points; score 0, high freshness; score 28, unacceptable quality; July, total 28 DP; August, total 25 DP; October, total 23 DP; Y_J, Y_A, Y_O, regression equations for the experiments of July, August, and October, respectively.

out the rise of total scores. A significant linear correlation between the QI and storage time was observed, and the three regression equations were coincidental (identical slopes and intercepts, $p < 0.05$).

Therefore, the best estimation of the quality changes of raw fish using the QI is given by the model that considers the data from the three independent storage experiments (**Figure 4**). These results are quite similar to those obtained for sole ($r^2 = 0.91$, QI = 0 + 1.85 × days in ice), brill ($r^2 = 0.872$, QI = 0 + 1.3108 × days in ice), and turbot ($r^2 = 0.890$ QI = 0 + 2.205 × days in ice) (*5*).

The PST (number of days that the fish was in ice) can be predicted through the equation PST = (QI − 0.8354)/1.1405. This prediction could be done at 95% confidence level through the association of confidence limits, and the PST ($PSTL_1$ and $PSTL_2$) would be calculated by the equation (23, 342−344)

$$X_{\text{Mean}} + [b(Y_{\text{iMean}} - Y_{\text{Mean}})/K] \pm (t/K)\sqrt{(S^2_{Y.X})'[(Y_{\text{iMean}} - Y_{\text{Mean}})^2/\Sigma x^2 + K(1/m + 1/n)]}$$

where X_{Mean} and Y_{Mean} are the mean values of days in ice (X data) and QI (Y data) from the regression model, b is the slope of the regression equation, Y_{iMean} is the mean QI value of m fish assessed, n is the number of data points comprising the regression model, m is the number fish assessed, $t = t_{\alpha(2)}$, $(n + m - 3)$ is the critical value of t Student distribution at α level of significance (usually 0.05) and $(n + m - 3)$ degrees of freedom, $K = b^2 - t^2(S^2_b)'$, $(S^2_b)'$ and $(S^2_{Y.X})'$ are the variances, respectively, of the slope and Y values (residual mean square), Σx^2 is the sum of squared deviations from the X_{Mean}, and $PSTL_1$ and $PSTL_2$ are, respectively, the minimum and maximum days in ice estimated.

As a result of the application of confidence limits to the PST, the remaining storage days in ice (defined as RSL = ESL − PST) is obtained by

$$15 - \text{PSTL}_2 \le \text{RSL} \le 15 - \text{PSTL}_1$$

These limits could be updated when new data are available, and then its usefulness could be truly tested.

The QI significantly correlated with the changes of odor/flavor of cooked fish ($r^2 = 0.8317$, $r = -0.9120$, $p < 0.001$), which evidence the utility of the QIM scheme. Moreover, ANOVA analysis demonstrated significant differences for QI values sooner than for Torry scores (respectively after 4 and 7 days of storage, ANOVA $p = 0.015$ and $p = 0.0388$). This result suggests a higher efficacy of QIM to detect the earlier storage alterations than the assessment of cooked fish.

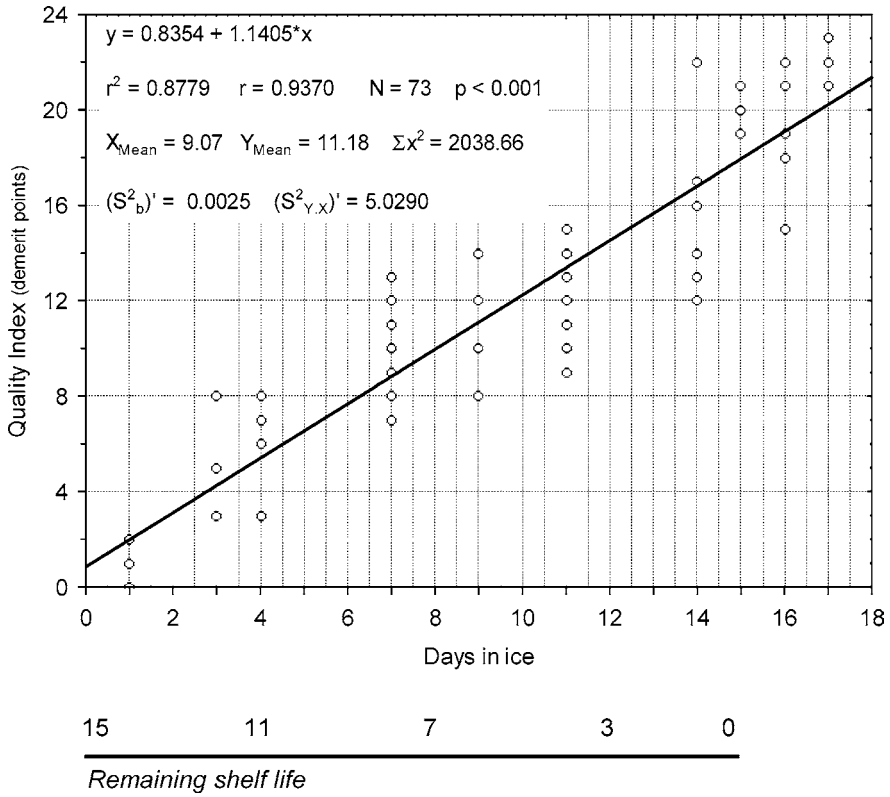

Figure 4. Linear relationship between the quality index and the storage time in ice for whole raw farmed Senegalese sole, based on the data of three independent storage experiments (storage at 1.5 ± 0.8 °C). Score 0, high freshness; score 24, unacceptable quality.

Table 5. Chemical Changes of Whole Raw Farmed Senegalese Sole during Iced Storage at 1.5 ± 0.8 °C

	days in ice							
	1		4		9		16–17	
chemical parameters	*N*[a]	mean ± SD[b]	*N*	mean ± SD	*N*	mean ± SD	*N*	mean ± SD
nucleotides (μmol/g of flesh)								
IMP	10	6.5 ± 0.8 a	3	6.9 ± 0.8 a	3	6.1 ± 0.2 a	6	3.7 ± 1.2 b
Ino	10	0.0 ± 0.0 a	3	0.4 ± 0.0 b	3	0.8 ± 0.2 c	6	0.9 ± 0.3 c
Hx	10	0.1 ± 0.1 a	3	0.4 ± 0.0 ab	3	0.7 ± 0.1 b	6	1.1 ± 0.3 c
K_i value (%)	10	2.3 ± 2.4 a	3	10.8 ± 1.4 b	3	20.0 ± 1.9 c	6	35.3 ± 4.6 d
volatile compounds (mg of N/100 g of flesh)								
TVB-N	12	11.9 ± 2.7		na[c]		na	8	12.7 ± 3.2
TMA-N	12	<0.7[d]		na		na	8	<0.7[d]
TMAO-N	12	2.9 ± 2.1		na		na	8	4.0 ± 3.2
biogenic amines (mg/kg of flesh)	6	<1.1[d]		na		na	6	<1.1[d]
free amino acids (g/100 g of flesh)								
histidine	4	0.04 ± 0.01		na	3	0.03 ± 0.01	3	0.04 ± 0.00
lysine	4	0.02 ± 0.01		na	3	0.01 ± 0.01	3	0.01 ± 0.00
arginine	4	0.01 ± 0.00		na	3	0.01 ± 0.00	3	0.01 ± 0.00
other FAA	4	0.26 ± 0.02		na	3	0.26 ± 0.01	3	0.23 ± 0.01
ΣFAA	4	0.34 ± 0.05		na	3	0.31 ± 0.03	3	0.30 ± 0.02

[a] Fish samples analyzed. [b] SD, standard deviation. In each row, values with different letters are significantly different ($p < 0.05$). [c] Not analyzed. [d] Detection limit.

Nucleotide Degradation and K_i Value. The changes of nucleotide amounts and degradation products are shown in **Table 5**. The differences reported were mainly due to the effect of storage time (ANOVA two-way), and therefore the mean values of the three experiments are presented. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were not detected, and this result is related to the rapid post-mortem dephosphorylation and deamination of adenine nucleotides throughout inosine monophosphate (IMP) by autolytic process (*1*, *6*).

The initial IMP content was is in the range of 6−13 μmol/g reported for several wild and farmed fish species (*7*, *24*−*26*). Lower initial IMP values (1.0−4.0 μmol/g) were reported for wild flatfish species from the Gulf of Alaska (*27*). The high variability for initial nucleotide contents is associated with differences among species, season, catching gear, stress during fish death, water temperature, and the time elapsed between catch/slaughter and storage (*1*, *6*).

During the chilled storage, the IMP levels of Senegalese sole progressively decreased, attaining significantly lower contents

Table 6. Quality Criteria Proposed for Whole Raw Chilled Farmed Senegalese Sole

	criteria	
quality grade	quality index[a] (0–22 dp)	K_i value[b] (%)
very fresh	≤3	≤5
fresh	4–7	≤12
acceptable	8–14	
borderline	15–18	
unacceptable	>19	>40

[a] Demerit points. [b] K_i value (%) = {[Ino] + [Hx]} × 100/{[IMP] + [Ino] + [Hx]}.

when the fish was already sensory rejected (16−17 days, **Table 5**). A significant production of the IMP breakdown products, inosine (Ino) and hypoxanthine (Hx), was reported during the storage, and values around 1.0 μmol/g of flesh were reported for both compounds at the end of the storage period. Slightly lower Hx values were found by other authors, for iced farmed and wild sea bass and sea bream, at the limit of sensory acceptability, but higher amounts of Ino (around 3.0 μmol/g flesh) were reported (*7*, *25*).

The rate of nucleotide degradation is usually shown by the *K* value (%) = {[Ino] + [Hx]} × 100/{[ATP] + [ADP] + [AMP] + [IMP] + [Ino] + [Hx]} (*28*) because it reflects the formation of Hx and Ino and the decrease of the nucleotide levels, giving in general a good freshness indicator. However, other ratios such as K_i, G, P, H, and Fr have been also used (*7*, *25*) due to their good correlation with fish freshness. In the present work K_i, H, and Fr ratios were calculated because ATP, ADP, and AMP were not detected, but the evolution of the K_i index (%) = {[Ino] + [Hx]} × 100/{[IMP] + [Ino] + [Hx]} (*29*) was the most reliable. In fact, despite the variability in the initial values (**Table 5**), mainly due to the variability of Hx levels, the K_i index significantly increased during the entire storage period ($r^2 = 0.9597$, $r = 0.9796$, $p < 0.001$, $K_i = 0.4273 + 2.1228 \times$ days in ice), attaining a value around 35% after 16−17 days in ice. Other researchers found analogous initial values for the *K* index of farmed turbot (*21*, *22*), but much higher values (around 70%) were found for rejected fish, after 19−20 days, and considerable changes were not observed until the end of iced storage (30−40 days). Similar K_i and *K* values (close to 80%) were also reported for unacceptable wild turbot, after 15 days of iced storage (*19*). Values in the range of 60−100% were obtained for some wild flatfish species and wild ground and pelagic fish, after 7 and 15 days of ice storage, respectively (*27*, *24*). These results suggests a slow degradation from IMP to Hx [mainly due to microbial action (*1*, *6*)] during the edible period of iced Senegalese sole stored at temperatures below 3 °C.

The evolution of the K_i index follows the loss of freshness, as evidenced by the significant correlations between this index and sensory data ($r = -0.9638$, $p = 0.0005$; $r = 0.9883$, $p = 0.00003$, respectively, for odor/flavor and QI). In particular, both QI and K_i values significantly increased after 4, 9, and 16−17 days (**Figure 3**; **Table 5**), which allows the proposal of some freshness/quality criteria present in **Table 6**.

Volatile Nitrogen Compounds (TVB-N/TMA-N). TVB-N and especially TMA-N are associated with seafood spoilage, and in general they are considered unreliable for the measurement of spoilage during the first 10 days of chilled storage of several fish species (*1*, *6*). The determination of these compounds at the time of sensory rejection appears to be adequate to evaluate the spoilage level of fish. In the present work, significant formation of these volatile compounds was not observed (**Table 5**). In particular, the nonformation of TMA-N [which is a result of the bacterial reduction of TMAO (*1*, *6*)] is corroborated with the constancy of TMAO-N values (**Table 5**) and with sensory assessment, because the panel did not perceive the typical fishy odor (associated with high TMA-N levels of spoiled fish) in any of the three experiments. Levels around 10−15 mg of TMA-N/100 g of flesh were used as the limit for fresh fish aerobically stored (*30*), and the TVB-N limits were fixed in the range of 25−35 mg of N/100 g for some fish species (*31*); in particular, the limit of 30 mg of N/100 g of flesh was established for the Pleuronectidae family [with the exception of halibut (*Hippoglossus* spp.)].

These results suggest a limited spoilage of farmed Senegalese sole during a period of 17 days in ice, at appropriate conditions, and are in accordance with others obtained for farmed turbot, in which significant TVB-N/TMA-N formation was not observed within the sensory acceptable period, and even after 40 days in ice the TMA-N contents were below 4 mg/100 g (*20*, *22*). The same pattern was found for other farmed species such as sea bass (*32*).

In further experiments, it will be interesting to evaluate if the limited spoilage of Senegalese sole is due to the low levels of TMAO in its flesh [values of 38−64 mgN/100 g of flesh were found for turbot and lemon sole (*33*)] or to the presence of specific spoilage bacteria other than *Shewanella putrefaciens*, which is associated with the reduction of TMAO to TMA in marine fish species (*34*) or a combination of both factors.

Free Amino Acids (FAA). Low amounts of FAA were reported (**Table 5**); in particular, the precursors of biogenic amines, such as lysine, histidine, and arginine, were below 0.05 g/100 g of flesh over the storage period.

Biogenic Amines. These chemical compounds are the result of the decarboxylation of FAA, mainly through decarboxylase enzymes of bacterial origin (*1*, *35*). Like TVB-N and TMA-N, the formation of biogenic amines is measurable mainly during the later stages of chilled storage and is usually used as a seafood quality indicator (*1*, *35*). In this work their levels were below the detection limit (**Table 5**), probably due to the low amounts of FAA. Another reason could be the bacteria profile of Senegalese sole (which was not studied in the present work). Other researchers reported the production of biogenic amines during the chilled storage of wild turbot and farmed sea bass (*19*, *36*). In the former, concentrations of 19 and 14 mg/100 g were reported, respectively, for Put and Cad, at the limit of acceptability (15 days), whereas lower values (3 mg/100 g for Put and 6 mg/100 g for Cad) were found for unacceptable sea bass (15−16 days in ice).

In conclusion, a shelf life of 15 days was defined for whole raw farmed Senegalese sole stored in ice at proper conditions (up to 3 °C). The optimized QIM scheme contains a total of 22 demerit points and several quality parameters to discriminate the freshness of this species and to predict its remaining shelf life in ice. The K_i value significantly correlated with sensory data and appears to be a useful chemical freshness indicator of iced Senegalese sole. Periods longer than 16−17 days seem to be needed for the spoilage of this species. Further studies should be done for a better comprehension of the spoilage pattern of this species during the chilled storage and to validate the results obtained in this work.

ACKNOWLEDGMENT

We thank the farms Aqualvor (Algarve, Portugal) and Rio Alto (Viana do Castelo, Portugal) for the supply of Senegalese sole. We thank Manuel Pires, Helena Silva, Rogério Mendes, Irineu Batista, and Leonor Nunes for their availability for sensory assessment and also Bárbara Fonseca for her technical assistance on the chemical analyses.

LITERATURE CITED

(1) Huss, H. H. *Quality and Quality Changes in Fresh Fish*; FAO Fishery Technical Paper 348; FAO: Rome, Italy, 1995; 195 pp.

(2) Council Regulation (EC) 2406/96. Common marketing standards for certain fishery products. *Off. J. Eur. Communities* **1996**, *L 334*, 1−15.

(3) Bremner, H. A.; Olley, J.; Vail, A. M. A. Estimating time−temperature effects by a rapid systematic sensory method. In *Seafood Quality Determination, Developments in Food Science*; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1987; Vol. 15, pp 413−435.

(4) Barbosa, A.; Vaz-Pires, P. Quality index method (QIM): development of a sensorial scheme for common octopus (*Octopus vulgaris*). *Food Control* **2004**, *15*, 161−168.

(5) QIMEUROFISH. QIM−your ideal tool for quality determination of fish freshness; available at http:// www.qim-eurofish.com/index7/index.htm, accessed Sept 2006.

(6) Ólafsdóttir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I. M.; Henehan, G.; Nielsen, J.; Nielsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997**, *8*, 258−265.

(7) Alasalvar, C.; Taylor, K. D. A.; Öksüz, A.; Shahidi, F.; Alexis, M. Comparison of freshness quality of cultured and wild sea bass (*Dicentrarchus labrax*). *J. Food Sci.* **2002**, *67*, 3220−3226.

(8) AOAC. *Official Methods of Analysis of AOAC International*, 16th ed., 4th rev.; Association of Official Analytical Chemists International: Gaithersburg, MD, 1998; CD-ROM.

(9) Cobb, B. F., III; Alaniz, I.; Thompson Jr., C. A. Biochemical and microbial studies on shrimp: volatile nitrogen and amino nitrogen analysis. *J. Food Sci.* **1973**, *38*, 431−436.

(10) Veciana-Nogues, M. T.; Hernandez-Jover, T.; Marine-Font, A.; Vidal-Carou, M. C. Liquid chromatographic method for determination of biogenic amines in fish and fish products. *J. AOAC Int.* **1995**, *78*, 1045−1050.

(11) Ryder, J. M. Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. *J. Agric. Food Chem.* **1985**, *33*, 678−680.

(12) Gras, J.; Gudefin, Y.; Chagny, F. Free amino acids and ninhydrin-positive substances in fish: I. Muscle and skin of rainbow trout (*Salmo gairdnerii* Richardson). *Comp. Biochem. Physiol.* **1978**, *60B*, 369−372.

(13) Howgate, P. *Approaches to the Definition and Measurement of Storage Life of Chilled and Frozen Fish*; Torry Research Station: Aberdeen, U.K., 1985; 12 pp.

(14) Zar, J. H. Comparing simple linear regression equations. In *Biostatistical Analysis*, 4th ed.; Prentice Hall: Englewood Cliffs, NJ, 1999; pp 360−376.

(15) Lie, Ø. Flesh quality−the role of nutrition. *Aquac. Res.* **2001**, *32*, 341−348.

(16) Regost, C.; Arzel, J.; Cardinal, M.; Robin, J.; Laroche, M.; Kaushik, S. J. Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*). *Aquaculture* **2001**, *193*, 291−309.

(17) Fountoulaki, E.; Alexis, M. N.; Nengas, I.; Venou, B. Effects of dietary arachidonic acid (20:4n-6), on growth, body composition, and tissue fatty acid profile of gilthead bream fingerlings (*Sparus aurata* L.). *Aquaculture* **2003**, *225*, 309−323.

(18) Ruff, N.; Fitzgerald, R. D.; Cross, T. F.; Kerry, J. P. Comparative composition and shelf life of fillets of wild and cultured turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*). *Aquac. Int.* **2002**, *10*, 241−256.

(19) Özogul, Y.; Özogul, F.; Kuley, E.; Özkutuk, A. S.; Gökbulut, C.; Köse, S. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the black sea, during chilled storage. *Food Chem.* **2006**, *99*, 752−758.

(20) Rodríguez, Ó.; Barros-Velázquez, J.; Ojea, C.; Piñeiro, C.; Aubourg, S. P. Evaluation of sensory and microbiological changes and identification of proteolytic bacteria during the iced storage of farmed turbot (*Psetta maxima*). *J. Food Sci.* **2003**, *68*, 2764−2771.

(21) Aubourg, S. P.; Piñeiro, C.; Gallardo, J. M.; Barros-Velázquez, J. Biochemical changes and quality loss during chilled storage of farmed turbot (*Psetta maxima*). *Food Chem.* **2005**, *90*, 445−452.

(22) Rodríguez, Ó.; Barros-Velázquez, J.; Piñeiro, C.; Gallardo, J. M.; Aubourg, S. P. Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chem.* **2006**, *95*, 270−278.

(23) Zar, J. H. Simple linear regression equations. In *Biostatistical Analysis*, 4th ed.; Prentice Hall: Englewood Cliffs, NJ, 1999; pp 324−359.

(24) Gonçalves, A.; Pereira, T.; Bettencourt, F.; Nunes, M. L.; Quinta, R. Nucleotide degradation as an indicator of freshness in groundfish and small pelagic fish species. In *Methods To Determine the Freshness of Fish in Research and Industry*; Olafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., Heia, K., Eds.; International Institute of Refrigeration: Paris, France, 1997; pp 273−276.

(25) Alasalvar, C.; Taylor, K. D.; Shahidi, F. Comparative quality assessment of cultured and wild sea bream (*Sparus aurata*) stored in ice. *J. Agric. Food Chem.* **2002**, *50*, 2039−2045.

(26) Grigorakis, K.; Alexis, M.; Gialamas, I.; Nikolopoulou D. Sensory, microbiological and chemical spoilage of cultured common sea bass (*Dicentrarchus labrax*) stored in ice: a seasonal differentiation. *Eur. Food Res. Technol.* **2004**, *219*, 584−587.

(27) Greene, D. H.; Babbitt, J. K.; Reppond, K. D. Patterns of nucleotide catabolism as freshness indicators in flatfish from Gulf of Alaska. *J. Food Sci.* **1990**, *55*, 1236−1238.

(28) Saito, T.; Arai, K. I.; Matsuyoshi, M. A new method for estimating the freshness of fish. *Bull. Jpn. Soc. Sci. Fish.* **1959**, *24*, 749−750.

(29) Karube, I.; Matsuoka, H.; Suzuki, S.; Watanabe, E.; Toyama, K. Determination of fish freshness with an enzyme sensor system. *J. Agric. Food Chem.* **1984**, *32*, 314−319.

(30) Connell, J. J. Methods of assessing and selecting for quality. In *Control of Fish Quality*; Fishing News (Books): Surrey, U.K., 1975; pp 107−132.

(31) Commission Decision 95/149/EC. Total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. *Off. J. Eur. Communities* **1995**, *L 097*, 84−87.

(32) Castro, P.; Padrón, J. C. P.; Cansino, M. J. C.; Velázquez, E. S.; Larriva, R. M. Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control* **2006**, *17*, 245−248.

(33) Oehlenschläger, J. Volatile amines as freshness/spoilage indicators. A literature review. In *Seafood from Producer to Consumer, Integrated Approach to Quality. Developments in Food Science*; Luten, J. B., Børresen, T., Liston, J., Oehlenschläger, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1997; Vol. 38, pp 571−586.

(34) Gram, L.; Huss, H. H. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol.* **1996**, *33*, 121−137.

(35) Rawles, D. D.; Flick, G. J.; Martin, R. E. Biogenic amines in fish and shellfish. *Adv. Food Nut. Res.* **1996**, *39*, 329−365.

(36) Paleologos, E. K.; Savvaidis, I. N.; Kontominas, M. G. Biogenic amines formation and its relation to microbial and sensory attributes in ice-stored whole, gutted and filleted Mediterranean sea bass (*Dicentrarchus labrax*). *Food Microbiol.* **2004**, *21*, 549−557.

Received for review November 4, 2006. Revised manuscript received March 1, 2007. Accepted March 2, 2007. This work was financially supported by EU-QCA III-MARE/FEDER: Project "Quality and Innovation of Fishery Products".

JF0632942