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Chemical and Sensory Changes in Mediterranean Hake (*Merluccius merluccius*) under Refrigeration (6–8 °C) and Stored in Ice

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Fresh Mediterranean hake (*Merluccius merluccius* var*. mediterraneus*), a species mainly caught off the shores of Spain, was stored at usual temperatures: in ice (commercial chain) and under refrigeration (home). Sensory and chemical analyses were performed throughout the storage time to determine the changes that took place and evaluate the effect of the storage temperature. Storage in ice resulted in a slight accumulation of volatile and biogenic amines in hake. When it was stored at 6−8 °C, a significant production of both trimethylamine and total volatile basic nitrogen (TVB-N) was observed, and biogenic amines were formed. Sensory analysis revealed that hake stored in ice was inedible after 29 days, the figure for refrigerated hake being 20 days. There was a nonsignificant correlation ($p > 0.05$) between TVB-N values and sensory score in hake stored at 0 °C. In all other cases, a significant correlation ($p < 0.001$) between volatile parameters and sensory analysis was found.

KEYWORDS: Hake; trimethylamine; total basic volatile nitrogen; biogenic amine; putrescine; histamine

INTRODUCTION

Fish muscle is highly susceptible to spoilage during storage, mainly due to the growth and activity of Gram-negative aerobic bacteria (*1*, *2*). Fish freshness is generally assessed by sensory methods based on changes in its appearance, odor, color, flavor, and texture. Speed, simplicity, and low cost are the main advantages of these methods. However, sensory analyses are inherently subjective, even when panel members have received extensive training (*3*). For this reason, chemical methods have been developed to measure the amounts of breakdown products derived from either bacterial or endogenous enzymatic activity. In particular, chemical parameters, such as volatile and non-volatile amine levels, have been used to assess fish freshness.

The occurrence of volatile basic compounds, such as ammonia, trimethylamine (TMA-N), and dimethylamine (DMA-N), is one of the characteristic features attributed to chemical changes occurring in marine fish muscle during spoilage. The analytical parameter, which includes these volatile compounds, is the total volatile basic nitrogen (TVB-N), and this is widely considered to be a useful index of fish freshness (*4*). In fact, there is an European Union regulation (*5*) that considers a TVB-N value of 30−35 mg/100 g as the limit above which fish is not acceptable for human consumption. The main volatile compound accumulated in fish stored in ice is TMA-N, originating from the bacterial reduction of trimethylamine oxide (TMAO), and this is considered to be the main reason for the off-odors of fish products (*3*). Therefore, TMA-N has also been widely used as a freshness grading criterion (*6*) and as a chemical indicator of marine fish spoilage at both refrigerated and room temperatures (>8−10 °C) (*7*). A TMA-N limit has yet to be officially established in the European Union. Most authors suggest that TMA-N levels should be <10−15 mg/100 g, although a maximum TMA-N level of 5 mg/100 g has been proposed specifically for hake (*8*).

Biogenic amines are nonvolatile amines, and measuring their levels has been proposed as another way for assessing fish hygienic quality. These compounds are found at very low levels in fresh fish, and their accumulation is associated with bacterial spoilage, biogenic amines being the result of microbial amino acid decarboxylation (*9*). In addition to the issue of hygiene, biogenic amines are of concern due to their potential toxic effect. Adverse effects, such as histamine intoxication, hypertensive crises, and food-induced migraines, have been related to food containing relatively high levels of some biogenic amines, especially the aromatic amines (histamine, tyramine, and phenylethylamine). However, even low amine levels may be hazardous for some groups of people, for instance, patients under monoamine oxidase inhibitor (MAOI) drug therapy (*10*) or individuals with a genetic diamino-oxidase deficiency (*11*). In these cases, it is highly recommended to reduce, or even avoid, the consumption of food containing biogenic amines, even at low concentrations. Moreover, particularly diamines (such as putrescine and cadaverine), but also polyamines (such as

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spermidine and spermine), can boost the toxic effect of aromatic amines through a competitive mechanism that favors their intestinal absorption. Thus, the relevance of studying levels of biogenic amines in food also extends to food containing these compounds at low concentrations. The most extensively studied biogenic amine, especially in pelagic fish, is histamine. In fact, histamine is the only biogenic amine that has a legally established regulation level: the European Union (EU) has set the maximum average content at 100 mg/kg for fresh or canned products (*12*). The U.S. Food and Drug Administration lowered the histamine defect action level from 100 to 50 mg/kg (*13*), recommending that not only histamine but also other biogenic amine contents had to be taken into account. Moreover, some studies have reported the accumulation of biogenic amines during the storage and/or spoilage of nonpelagic fish (*14−16*). Thus, mainly histamine but also other biogenic amines have been proposed as potential markers for evaluating fish freshness (*17−20*).

Volatile and nonvolatile amine accumulation during fish storage depends very much on the temperature and time of storage. These two factors influence biogenic amine accumulation through their effect on both the growth and enzymatic activity of aminogenic microorganisms. In a previous study (*21*), the accumulation of diamines in hake was related to abusive storage temperatures, although less is known about the production of biogenic amines when this fish is stored at the temperatures usually applied by the fishing industry, along the commercial chain and at home. Therefore, the present study aimed to comparatively assess the influence of different storage conditions on the accumulation of both volatile and nonvolatile amines in Mediterranean hake, which is a widely processed and consumed species in Spain but scarcely studied in this respect. The potential use of each type of volatile and nonvolatile amine as an indicator of hake freshness and/or spoilage was studied under two particular and commonly used storage conditions: ice (reproducing the commercial distribution channel conditions) and refrigeration at 6−8 °C (reproducing the storage temperature usually achieved in domestic refrigerators at home). In addition to the measurement of the volatile and nonvolatile amines, sensory analysis were also performed as a reference criterion to asses the freshness degree of samples.

MATERIALS AND METHODS

Samples and Sample Preparation. *Samples.* Approximately 40 kg of Mediterranean hake (*Merluccius merluccius* var. *mediterraneus*) was obtained directly from the fishing port. Fish were caught in June within the following geographical area: latitude between 41° 7′ 85″ and 41° 13′ 99″ north and longitude between 2° 27′ 53″ and 2° 12′ 65″ east, at a distance of 6−13 mi from the Catalonian coast and a depth of between 167 and 300 m.

The average weight of each individual used in this study was 337.0 g [standard deviation (SD) = 92.1], and the average length was 36.2 cm (SD = 3.8). Ungutted fish samples (n = 108) were divided into two batches, which were stored in ice (∼0 °C) and under refrigeration at 6−8 °C in a domestic refrigerator distributed in self-draining boxes. The temperature of the refrigerator was monitored daily. During storage, two or three fish were taken at the following sampling times: 0, 1, 3, 4, 6, 8, 10, 13, 15, 20, 22, 24, 27, and 29 days. Determinations were done at least in duplicate.

Sample Preparation. Muscle tissue from the fish sample was minced, and a 10 g aliquot was blended for 20 min with 7 mL of 0.6 N perchloric acid (PCA) (Panreac, Barcelona, Spain). After centrifugation (20 min at 10000 rpm), supernatant was filtered through a Whatman no. 1 filter (Whatman, Kent, U.K.). The pellet was again homogenized with 7 mL of 0.6 N PCA, centrifuged, and filtered. This operation was repeated once more, the three filtrates were combined, and the volume was adjusted to 25 mL with 0.6 N PCA. The extract was frozen at −20 °C until the chemical analyses of TVB-N, TMA-N, and biogenic amines were performed.

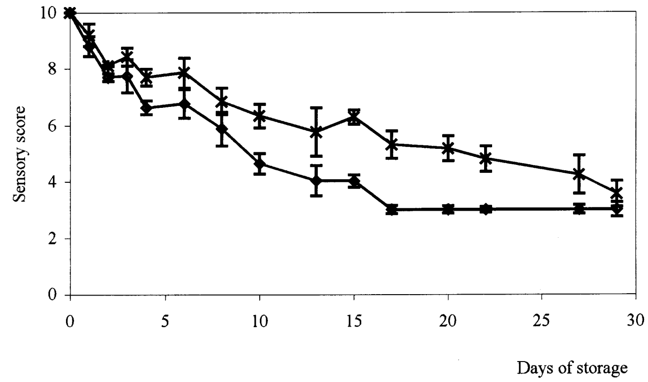

Figure 1. Evolution of overall sensory score of hake (*M. merluccius*) stored in ice (∗) and at 6–8 °C (◇).

Analytical Methods. *Sensory Analysis.* The Torry sensory score scheme for white fish (*22*) was used. Six to eight panel members were previously trained through several sessions using fresh and spoiled fish to familiarize themselves with the fish attributes and to establish a uniform degree of sensory evaluation. The appearance of the skin, eyes, gills, and internal organs was assessed in raw fish. For the sensory assessment of cooked fish, portions were covered with a perforated plastic film and cooked by microwave (500 W, 3 min) before being immediately presented to the panelists. Odor, taste, and texture/appearance were evaluated. Frozen fish (−18 °C) was used as a control sample for the sensory analysis of cooked hake. The points system used ranged from 10 (excellent quality) to 3 (spoiled) for both raw and cooked fish.

Determination of TVB-N. The semimicro steam distillation method was applied according to the EU procedure (*5*).

Determination of TMA-N. A gas chromatography (GC) procedure previously described and validated by Veciana-Nogués et al. (*23*) was used. The method includes a first step of deproteinization with 0.6 N PCA, alkalinization with 65% (w/w) KOH (Panreac), and finally volatile amine extraction with toluene. A Silcosteel packed column (Hewlett-Packard, Avondale, PA) of 200 cm of 4% Carbowax 20M + 0.8% KOH on Carbopack B 60/80 and a flame ionization detector were used.

Determination of Biogenic Amines. Biogenic amines were analyzed after PCA extraction by applying the ion-pair high-performance liquid chromatographic (HPLC) method described by Veciana-Nogués et al. (*24*). The ion pairs between biogenic amines and octanesulfonic acid were separated using a reverse phase column C_{18}, and after postcolumn derivatization with *o*-phthalaldehyde, amines were detected spectrofluorometrically.

Statistical Analysis. Statistical analysis of the data was carried out using SPSS 9.0 for Windows software (SPSS Inc., Chicago, IL). A nonparametric test was applied when data were not normally distributed (significant Shapiro−Wills test). Least-squares of linear regression analyses of chemical and sensory parameters versus time of storage were applied. Differences between the chemical index of hake samples stored in ice and at 6−8 °C were examined by the nonparametric U-Mann−Whitney test. Pearson's regression analysis was performed to determine the correlation between volatile amines and sensory analyses.

RESULTS AND DISCUSSION

Sensory Results. Figure 1 shows the changes in the overall sensory score found in hake samples stored in ice and at 6−8 °C. Odor, taste, and texture scores decreased versus time of storage, giving correlation coefficients of $r = 0.972$ ($p < 0.001$) for ice storage and $r = 0.933$ ($p < 0.001$) for 6−8 °C storage. From the first day of the study the sensory score of hake stored at 6−8 °C was always lower than that for hake stored in ice. Using a sensory score value of 3 as the rejection point (*22*),

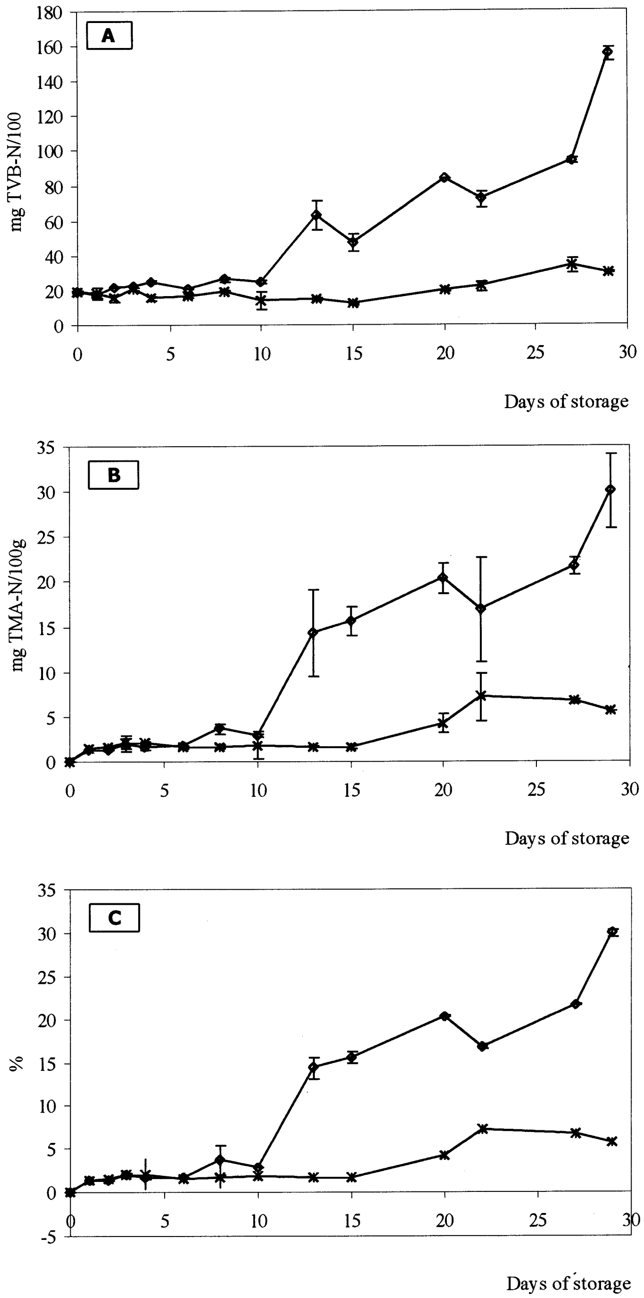

Figure 2. Evolution of TVB-N (A), TMA-N (B), and *P* ratio (C) values in hake (*M. merluccius*) stored in ice (∗) and at 6–8 °C (◇).

hake stored at 6−8 °C would be inedible after 20 days of storage, whereas hake stored in ice was considered inedible after 29 days. The assigned shelf life using the Torry sensory scheme was not in agreement with the subjective opinion of the panelists. As consumers, they were asked about hake acceptance and always refused fish samples earlier than when the objective rejection value of 3 was reached. It is important to note that it might be appropriate to develop a more specific and exigent sensory grading score for the evaluation of Mediterranean hake, as has been already pointed out by Simeonidou et al. (*25*).

Volatile Amines Results. Levels of TVB-N (**Figure 2A**) were initially near 20 mg/100 g, as has been reported by other authors for fresh hake (*26*−*28*). A latent period was observed in TVB-N accumulation in hake samples stored both in ice and at 6−8 °C. Significant differences ($p < 0.05$) between TVB-N levels for ice and 6−8 °C storage were already found by day 10, and much greater differences were observed at the end of the study. Thus, TVB-N levels clearly increased after 10 days of storage at 6−8 °C, whereas they remained constant in ice storage. Applying the EU TVB-N limit of 30−35 mg/100 g, hake would still edible after almost 1 month of ice storage and after 10−13 days of storage at 6−8 °C. Kairiyama et al. (*29*) and Pastoriza et al. (*30*) found a similar TVB-N behavior in hake stored under refrigeration.

Figure 2B shows TMA-N evolution with respect to the storage of fresh hake at both temperatures. TMA-N was not detected in the initial hake samples, 6−8 h after being caught, suggesting excellent quality according to the classical Castell's criterion (*6*). Few changes were observed in TMA-N content during storage in ice. Only after 20 days of storage did the TMA-N value rise to 4 mg/100 g. Koutsoumanis et al. (*2*) also reported that concentrations of TMA-N in Mediterranean nonpelagic fish species remained very low, or were even undetected, during the first days of storage in ice. In contrast, earlier and much higher production of TMA-N was observed in hake samples stored at 6−8 °C, which contributed to the negative effect on the organoleptic quality of the fish. After 10 days at 6−8 °C, spoilage of fish started to become significant with a sharp increase in TMA-N content, producing values of up to 15 mg/100 g at day 15 and double this at the end of the study. These values are consistent with those previously reported for hake under refrigeration (*31*, *32*). TMA-N production followed an exponential curve, with $r^2 = 0.885$ ($p < 0.0001$), a finding consistent with its bacterial origin. Before day 10, the content of TMA-N was not significantly different ($p > 0.05$) between the two storage temperatures. However, significant differences ($p < 0.05$) were subsequently found in the TMA-N content of hake stored in ice and at 6−8 °C. Taking into account the suggested TMA-N limit of 5 mg of N/100 g, above which fish should not be accepted for human consumption (*8*), samples stored in ice would be rejected after 20 days of storage, whereas hake stored at 6−8 °C would be rejected at day 10. Chemical indexes, both TMA-N and TVB-N, were not consistent with the hake rejection time and were always longer for the TVB-N criterion. TMA-N seems to be an acceptable parameter for evaluating fish quality during refrigerated storage and has been shown to be correlated with storage time (*33*, *34*) and sensory changes (*35*). Rodriguez et al. (*36*) argue that the quality evaluation by TMA-N measurement is particularly suitable for the species studied here. However, due to its exponential behavior, there is a controversy over the real value of TMA-N as a measure of the loss of fish freshness because any increase is not evident enough during the first days of storage. Thus, Ryder et al. (*37*) concluded that TMA-N has no value in monitoring the loss of freshness in hoki (family Merlucciidae) stored at 6−8 °C. Our results suggest that TMA-N would be better used as an indicator of spoilage, rather than freshness, in hake. However, even if it is not considered to be the optimal alternative indicator, TMA-N could still be an additional complementary tool in the sensory assessment of hake.

Malle and Poumeyrol (*38*) proposed the *P* ratio parameter (calculated as the quotient between TMA-N and TVB-N) as a useful index of seafood quality. The *P* ratio seems to minimize the effect of the different factors influencing both TMA-N and TVB-N contents and normally provides more comparable values among different fish species. Changes in *P* ratio during storage showed a peak evolution profile at both the temperatures studied: a gradual increase was followed by a decrease, this occurring at day 22 and day 15 in ice storage and at 6−8 °C, respectively (**Figure 2C**). The observed *P* ratio profile suggests it is difficult to use as an indicator because a hake sample could

Table 1. Correlation between Sensory Score (SS) and Chemical Parameters during Ice and Refrigerated Storage of Mediterranean Hake (*M. merluccius*)

	ice storage		refrigerated storage	
	r	*p*[a]	*r*	*p*[a]
SS and TMA-N	–0.770	<0.0001	–0.859	<0.0001
SS and TVB–N	–0.434	0.093[b]	–0.766	<0.001
SS and *P* ratio	–0.792	<0.0001	–0.862	<0.0001

[a] Significance level. [b] Nonsignificant, $p > 0.05$.

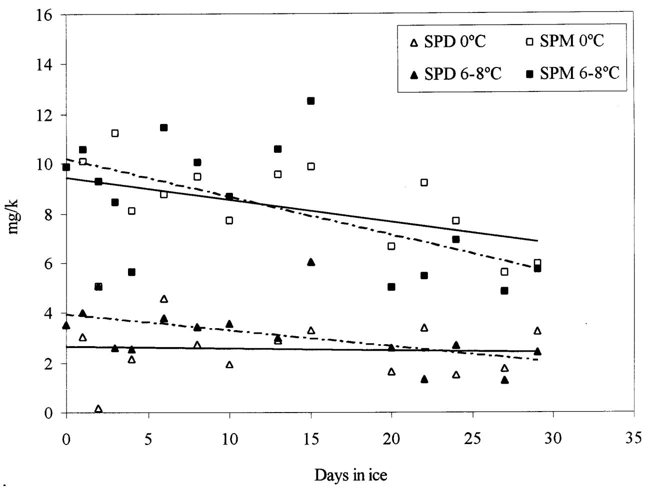

Figure 3. Evolution of SPD and SPM content in hake samples (*M. merluccius*) during storage in ice and at 6–8 °C: regression lines of both amines versus time of storage in ice (– - –) and at 6–8 °C (—).

have a low *P* ratio value even when spoiled; however, it could be used for the first few days of storage. Therefore, as in a previous study (*33*), we suggest that the *P* ratio is a suitable indicator of the degree of freshness in hake, especially during the early storage period. Civera et al. (*4*) also reported the usefulness of the *P* ratio as an indicator of fish quality.

A better correlation between TMA-N and TVB-N was observed in hake samples stored at 6−8 °C than in samples stored in ice, with correlation coefficients of $r = 0.932$ ($p < 0.001$) and $r = 0.762$ ($p < 0.002$), respectively. **Table 1** shows the correlation between volatile and sensory parameters for both ice and refrigerated storage conditions. In all cases, the correlation coefficients obtained for hake stored at 6−8 °C were higher than those obtained for hake stored in ice. Ice storage seems to limit the bacterial growth responsible for TMAO reduction and, therefore, the accumulation of TMA-N and TVB-N in fish. Ice storage was able to delay rejection times until approximately the 25th day of storage, this being double the shelf life of hake stored at 6−8° C. Therefore, these results confirm that ice storage seems to be much more appropriate than refrigeration for preserving fish quality.

Biogenic Amines Results. The only biogenic amines found in the initial hake samples (time zero) were spermidine and spermine, both of these being naturally occurring polyamines. Their physiological nature also explains their variability among samples. **Figure 3** shows spermidine and spermine contents over storage time. There were no significant changes ($p > 0.05$) in the average values of either spermidine or spermine found in samples, irrespective of storage conditions. Spermidine remained constant, showing an average value of 2.87 mg/kg (SD = 1.18). In contrast, a slight decrease ($p < 0.01$) was observed for spermine, from an initial value of 9.88 mg/kg (SD = 1.32) to 5.83 mg/kg (SD = 0.16). The decrease in polyamine content could be explained by the fact that these compounds can be used by microorganisms as a nitrogen source (*39*), although it could also be due to desamination reactions (*9*). **Table 2** shows putrescine (PU), cadaverine (CA), agmatine (AGM), tyramine (TY), and histamine (HI) evolution during storage in ice and at 6−8 °C. Putrescine was also found in initial samples, although at <0.1 mg/kg. Low levels of putrescine have been described in fresh fish (*20*, *21*, *24*), and such an occurrence may be considered as natural or endogenous, because putrescine is the physiological precursor of the above-mentioned polyamines. However, an increase in putrescine levels was observed during hake storage, the final putrescine content in hake stored at 6−8 °C being 2.5-fold higher than that stored in ice. In addition, when ice and refrigeration temperatures were compared, the same values of putrescine observed in hake stored in ice at day 29 (3.1 mg/kg) were found in those stored at 6−8 °C after only 10 days of storage.

Agmatine values were maintained at ∼1 mg/kg, and no significant differences ($p > 0.05$) were observed between its average values in hake stored in ice (0.85 mg/kg) and at 6−8 °C (1.17 mg/kg). However, agmatine showed a particular profile during storage of hake samples under both conditions, a maximum peak value being observed before the end of the study. Hake stored in ice showed the maximum agmatine value on day 20, whereas in hake stored at 6−8 °C this occurred after 15 days of storage. A similar agmatine profile, but with higher and earlier levels, has also been observed in hake stored at 8−10 and 18−20 °C (*21*), as well as in other fish species, such as tuna and anchovy (*40*). This peak profile could be explained by the fact that agmatine may be formed as an intermediate metabolite in the putrescine production pathway from arginine by some microorganisms (*35*). Cadaverine was detected after 4 days of storage at 6−8 °C and increased significantly ($p < 0.05$) over the storage time. In contrast, hake samples stored in ice did not show cadaverine until day 20 and its values never exceeded 1 mg/kg. The low initial amounts of cadaverine found in fresh fish has been associated with endogenous enzyme activity before the animal's death, but its formation during the storage of fish seems to result only from bacterial lysine-decarboxylase activity (*35*).

In terms of aromatic biogenic amines, accumulation of tyramine and histamine was observed only in hake stored at 6−8 °C and after 6−8 days of storage, whereas phenylethylamine and tryptamine were never detected. Levels obtained for tyramine were not important in comparison with other foods, such as fermented foods, but its occurrence in hake indicates a certain degree of deterioration or an initial stage of decomposition. Moreover, tyramine levels are important from a toxicological point of view. Intakes of 100−125 mg of tyramine have been related to migraines, and severe hypertensive crises due to interaction with classical (nonreversible) MAOI drugs have been reported after a total ingestion of only 6 mg of tyramine (*10*). According to our results, tyramine does not seem to be dangerous for healthy individuals, but the consumption of 70 g of hake stored at 6−8 °C for 8 days could provoke hypertensive crises in patients taking classical MAOI drugs. Histamine values obtained after 29 days of storage at 6−8 °C were 14.30 mg/kg, which is far below of the limit established by the U.S. FDA (*13*). According to the observations of other researchers, histamine occurrence is extremely variable and its production is a function of time, temperature, and the microflora present during fish storage (*3*, *41*). Fletcher et al. (*41*) argued that histamine could be used as an indicator of spoilage, because histamine formation was observed only when TMA-N levels

Table 2. Biogenic Amine Content (Milligrams per Kilogram) during Storage of Mediterranean Hake (*M. merluccius*) in Ice and Refrigerated Conditions

	PU		AGM		CA		TY		HI	
days	0 °C	6–8 °C	0 °C	6–8 °C	0 °C	6–8 °C	0 °C	6–8 °C	0 °C	6–8 °C
0	0.20[a] (0.31)[b]	0.20 (0.31)	nd[c]	nd	nd	nd	nd	nd	nd	nd
1	0.41 (0.12)	0.41 (0.11)	nd	0.48 (0.67)	nd	nd	nd	nd	nd	nd
2	0.61 (0.83)	0.22 (0.07)	nd	0.23 (0.32)	nd	nd	nd	nd	nd	nd
3	0.46 (0.16)	0.50 (0.13)	0.32 (0.45)	0.58 (0.81)	nd	nd	nd	nd	nd	nd
4	0.09 (–)	0.75 (–)	0.83 (–)	1.00 (–)	nd	0.25 (–)	nd	nd	nd	nd
6	0.40 (0.04)	0.95 (0.07)	nd	0.81 (0.17)	nd	0.06 (0.08)	nd	nd	nd	nd
8	0.40 (0.01)	1.34 (0.47)	nd	1.00 (0.43)	nd	0.68 (0.87)	nd	0.70 (0.23)	nd	0.36 (0.48)
10	0.61 (0.50)	1.28 (0.42)	0.64 (0.89)	0.57 (0.59)	nd	0.28 (0.39)	nd	nd	0.03 (0.04)	nd
13	0.45 (0.06)	3.34 (0.75)	0.11 (0.09)	1.03 (0.22)	nd	3.90 (1.47)	nd	1.28 (0.42)	0.05 (0.07)	4.23 (2.34)
15	0.45 (0.01)	3.22 (0.93)	nd	2.06 (2.03)	nd	1.57 (1.12)	nd	0.66 (0.16)	nd	0.48 (0.18)
20	0.95 (0.68)	1.25 (0.77)	1.76 (0.11)	1.40 (1.56)	0.21 (0.30)	0.72 (0.39)	nd	nd	nd	0.38 (0.53)
22	1.52 (0.57)	1.55 (0.76)	0.72 (0.83)	1.08 (0.33)	0.97 (1.37)	1.52 (0.69)	0.30 (0.42)	0.48 (0.22)	nd	0.59 (0.10)
24	0.72 (0.23)	4.12 (0.49)	0.10 (0.14)	1.33 (0.12)	nd	3.52 (0.04)	nd	0.72 (0.11)	nd	1.91 (0.21)
27	1.37 (0.18)	8.60 (4.79)	nd	1.65 (0.57)	0.03 (0.04)	7.98 (3.68)	nd	3.07 (2.62)	nd	4.10 (5.13)
29	3.10 (0.08)	8.24 (2.57)	nd	1.12 (0.11)	0.92 (0.28)	24.71 (10.23)	nd	9.92 (9.46)	nd	14.30 (11.69)

[a] Mean value. [b] Standard deviation. [c] Not detected.

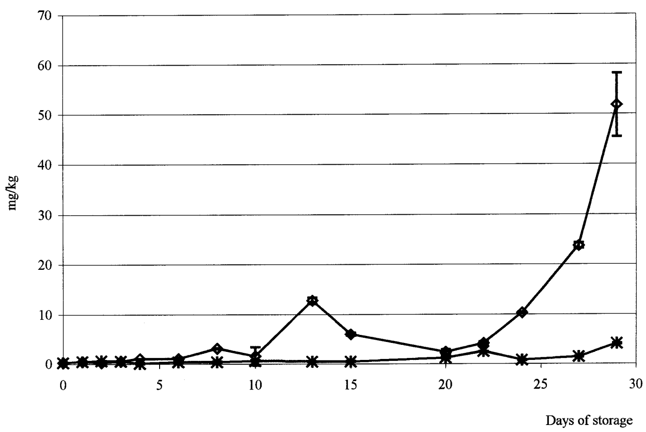

Figure 4. Evolution of index results from the sum of PU, CA, TI, and HI in hake samples stored in ice (∗) and at 6–8 °C (◇).

reached 6 mg/100 g of fish. However, in contrast to what happens in pelagic fish, the accumulation of histamine seems to be quantitatively less important in hake.

Putrescine and cadaverine contents have been suggested as freshness indicators for several fish species (*18*, *42*, *43*), because these diamines are the most important amines found during the decomposition of fish. Although cadaverine usually starts to increase later than putrescine, its levels at the end of the storage are generally higher.

Biogenic amines levels at day 10 were still <2 mg/kg, as they were initially. Thus, any individual biogenic amine would be suitable for evaluating hake freshness and, probably, the use of an index involving several amines would be the most appropriate. The biogenic amines index (BAI) proposed by Mietz and Karmas (*17*) is the classical criterion for evaluating fish on the basis of biogenic amine contents, and it simultaneously considers increases in putrescine, cadaverine, and histamine levels with decreases in spermidine and spermine. The value of 10 mg/kg proposed by Mietz and Karmas (*17*) as the limit of fish acceptability was not reached in samples stored at 6−8 °C at the end of storage. The accumulation of tyramine observed in our study suggests that this amine could be used in the evaluation of fish quality, like the BAI proposed by (*19*) for tuna fish, which is based on the sum of putrescine, cadaverine, histamine, and tyramine levels. **Figure 4** shows the evolution of this index applied to our samples of hake stored in ice and at 6−8 °C. As with biogenic amine accumulation during storage, no changes in the BAI were observed in hake samples stored in ice, whereas those stored at 6−8 °C showed a clear increase. However, the guiding limit for tuna acceptance, 50 mg/kg, was not surpassed until day 29 of storage, when fish was clearly refused by both sensory and chemical criteria. Thus, it seems necessary to establish a new limit for lean fish or maybe a new combination of the biogenic amines included.

As was expected, the shelf life of hake stored in ice (commercial chain) is higher than that stored at 6−8 °C, which would be the conditions usually used at home. Great differences were observed between the rejection times suggested by chemical and sensory parameters. Therefore, it might be useful to describe a more specific and exigent grading sensory score for the evaluation of Mediterranean hake, as has already been pointed out by Simeonidou et al. (*25*).

The TMA-N parameter could be useful for evaluating the quality of refrigerated fish, but not iced fish, and the suggested limit of 5 mg/100 g seems to be adequate for indicating whether fish is fit for human consumption. This parameter could be a complementary tool in the inspection of fish. However, it would be appropriate to optimize its analysis in terms of speed, cost, and simplicity. *P* ratio calculation seems to be adequate for categorizing fish quality, but further studies are necessary before strong conclusions with respect to hake can be drawn.

Nonsignificant quantitative accumulation of biogenic amines was observed in hake samples stored both in ice and at 6−8 °C. Although putrescine and cadaverine increased over the storage time, it does not seem feasible to use the level of only one of those biogenic amines as a freshness index in hake stored in ice or at 6−8 °C. Levels obtained for tyramine and histamine were relatively low, but their occurrence in hake could indicate a degree of spoilage. The presence of high amounts of these compounds is related to inappropriate storage conditions, such as a break in the cold chain (*21*). Levels of biogenic amines in hake would be important, in terms of their toxic effects, only in certain groups of people who are sensitive to these compounds.

ABBREVIATIONS USED

DMA-N, dimethylamine; TMA-N, trimethylamine; TVB-N, total volatile basic nitrogen; TMAO, trimethylamine oxide.

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