

Comparative Quality Assessment of Cultured and Wild Sea Bream (*Sparus aurata*) Stored in Ice

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Comparative quality assessment of cultured and wild sea bream stored in ice for up to 23 days was achieved by the monitoring of sensory quality, levels of nucleotide, nucleotide breakdown products, and texture by a texturometer. The changes in sensory quality of both raw and cooked fish were assessed using the modified Tasmanian and Torry schemes, respectively. K and related values (freshness indicators), namely, K , K_i , G , P , H , and F_r , were calculated. Linear increases ($r^2 \geq 0.99$) in K , K_i , G , and P (and a decrease in F_r) values for cultured sea bream and in the H value for wild sea bream with increasing storage periods were observed. The limit for acceptability of cultured and wild sea bream stored in ice was ~16–18 days (average K , K_i , G , and P values: ~35–40%; H values: ~5% for cultured and 10% for wild; and F_r values: ~65–70%). The texture of cultured and wild sea bream decreased throughout the storage period, and they were not significantly ($p > 0.05$) different until after day 16 when the wild sea bream was significantly softer than the cultured. The sensory score of both cultured and wild raw fish showed a good relationship with some freshness and texture indicators over the entire storage period (r^2 values ≥ 0.99). These indicators were K , K_i , G , P , and F_r values for cultured and H value for wild fish.

KEYWORDS: Cultured and wild sea bream; nucleotides; freshness indicators; K , K_i , G , P , H , and F_r values; sensory assessment; texture

INTRODUCTION

Aquaculture production of gilthead sea bream (*Sparus aurata*) has increased considerably in recent years, 59 577 metric tons in 1999 (wild capture production, 5730 metric tons) (1). However, the fresh aroma of wild sea bream is superior to that of its cultured counterpart (2). Nonetheless, there are no research results currently available on the aroma of cultured and wild sea bream following storage in ice. Therefore, it is of interest to compare the quality of cultured and wild sea bream during handling, distribution, and storage in ice.

The freshness of fish is the single most important attribute when assessing the quality of such products. Sensory methods are still the most satisfactory way of assessing the freshness quality of fish in terms of consumer expectation (3). The Tasmanian Food Research Unit (TFRU) (4, 5) and the European Union (6, 7) schemes for raw fish and the Torry scheme (8) for cooked fish are the most commonly used methods for the assessment of freshness quality in the inspection service and fishing industries. However, when chemical methods are being used for assessing the freshness quality of fish, sensory methods

should be conducted to ensure that these results show good agreement with the chemical procedures employed.

Fresh quality of muscle food is related to biochemical changes taking place during the postmortem period. Among the chemical methods, the concentrations of adenosine 5'-triphosphate (ATP) and its breakdown/degradation products, adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), respectively, are used as indices of freshness in a wide variety of fish (9–12). The pattern and rate of nucleotide degradation differ among species, with body location (dark or white muscle), antemortem condition, stress during capture, handling, season, and storage conditions (13–15). These significant variations, both between and within species, influence the practical usefulness of measuring the ratios of the concentration of these breakdown compounds rather than use of single ATP derivatives. Therefore, K , K_i , G , P , H , and F_r values have been considered to be the most reliable and useful indicators of freshness quality in a wide variety of fish by several researchers (16–22). Depending upon ATP breakdown compounds, one value can be superior or more reliable than other values within species.

The F_r (or IMP ratio) value may serve as a good indicator for freshness of many fish species, namely, those in which the breakdown of IMP occurs gradually, but may not be applicable

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to some species, such as cod (23), in which IMP levels drop rapidly in the early stage of ice storage. It has been reported that values of *G* and *P* are most useful with lean fish. In fatty fish, however, factors such as the development of rancidity may render the product undesirable before meaningful *G* and *P* values can be obtained (21). ATP degrades to IMP very soon after death and, consequently, the K_i value (which does not involve determination of ATP, ADP, or AMP) may be used. However, it should be noted that ATP, ADP, and AMP remain in some species of fish even after 2 weeks (17), and so in such cases, the *K* value can be superior to the other values. Burns et al. (18) have reported that for North Atlantic cod, which can accumulate Ino very rapidly, the K_i value is inadequate as a quality indicator and instead recommended that the *G* value be used. Pacific cod quality has been assessed using the *H* value (20).

Texture is another important attribute of the freshness quality of fish. In the fish market and fishing industry, texture assessment is performed by touch to decide firmness or softness of the flesh. Numerous attempts have been made to replace sensory assessments by instrumental methods (24–27). However, it is important to use instrumental parameters that have been validated against sensory testing.

The goal of the study was to investigate the shelf life and freshness of cultured vs wild sea bream. Specific objectives were to determine the most reliable freshness indicator(s) for assessing sea bream quality and evaluate the relationships between sensory, freshness, and texture results over the storage period in ice.

MATERIALS AND METHODS

Materials. Cultured gilthead sea bream, *S. aurata*, (average weight and length: 418 ± 71 g and 250 ± 12 mm, respectively) used in this study were cultivated in net cages (located in Messolongi lagoon, Greece) and harvested (~1 year old) in November 1999. The commercial feed (LAKY, Nea Kerasounta, Prevezis, Greece) used contained 46% protein, 20% fat, 17.6% carbohydrate, 1.2% crude fiber, 8% moisture, and 7.2% ash. Wild sea bream (average weight and length: 407 ± 70 g and 265 ± 13 mm, respectively) were caught in the lagoon of the Aegean Sea. The time of harvest was the same for both fish; all other factors during capture were not controlled nor assessed. All chemicals were obtained from Sigma-Aldrich-Fluka Company Ltd. (Fancy Road, Poole, Dorset, U.K.), unless otherwise specified.

Sample Preparation and Storage Conditions. Cultured gilthead sea bream were slaughtered by immersing in ice cold water (hypothermia) and dispatched (packed into an insulated polystyrene box with ice) by TNT World Wide Expresses to the Food Research Center, University of Lincoln, U.K., within 1 day of harvest. Wild sea bream were also dispatched at the same time in a similar manner. Six cultured or wild fish were immediately sampled (day 1), while the rest (whole fish) were repacked separately (cultured and wild) with flake ice into polystyrene boxes provided with holes for drainage. Boxes were stored in a cold room (2–4 °C) for up to 23 days from the time of harvest at a fish-to-ice ratio of 2:1 (w/w), maintained throughout the storage period. Chemical, sensory, and texture analyses were performed on days 1, 5, 9, 12, 16, 19, and 23. Samples of white muscle from each of three fish for both cultured and wild sea bream were analyzed on each occasion.

Proximate Analysis. The fish samples were analyzed for proximate composition: moisture by air-drying (method 950.46), total fat by acid hydrolysis (method 948.15), protein by Kjeldahl (method 981.10), and ash by direct analysis (method 938.08), according to the official methods of the Association of Official Analytical Chemists (28).

Sensory Assessment. The sensory assessment of raw sea bream was conducted using the modified TFRU scheme (29). The panel consisted of at least five or six regular contributors, each of whom was trained in fish quality assessment. Each contributor was given up to four simple

descriptors, scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and any higher score indicated poorer quality. The scores for the separate characteristics were summed to give an overall sensory score. This system gave score 0 (or near 0) for very fresh fish while increasingly larger values were obtained as fish deteriorated. Therefore, minor variations in scoring individual attributes had little influence on the overall score.

The measurements of freshness of cooked sea bream (odor, flavor, and texture) were conducted according to the modified Torry scheme (29). A hedonic scale from 10 to ≤3 was used, 10 representing absolutely fresh and representing ≤3 completely putrid or spoiled fish. Fish fillets (each sampling day) were cooked in a microwave for 3 min and then served to the panelists after 5 min. The fish were assessed for odor, flavor, and texture. Mean values of combined assessments for odor, flavor, and texture were calculated and used for correlation with sensory data, as explained elsewhere (29).

ATP Breakdown Compounds. ATP-related compounds were determined according to the high-performance liquid chromatography (HPLC) procedure of Ryder (30). The HPLC system consisted of a Merck Hitachi L-6000 pump, PU-4020 UV detector, and Elonex PC 466/1 Computer. A 5 μL sample was injected after filtration through a 0.45 μm filter. Nucleotides were separated by a 5 μm 100 RP C18 column (250 mm × 4 mm ID). The mobile phase was 0.04 M potassium dihydrogen phosphate (KH₂PO₄) and 0.06 M dipotassium hydrogen phosphate (K₂HPO₄) dissolved in purified HPLC water. The buffer solutions were prepared on a daily basis (pH 7). The flow rate was 2 mL/min, and the wavelength for monitoring ATP breakdown products was set at 254 nm.

The *K*, K_i , *G*, *P*, *H*, and *F_r* values were calculated according to Saito et al. (16), Karube et al. (17), Burns et al. (18), Shahidi et al. (21), Luong et al. (20), and Gill et al. (19), respectively.

$$K (\%) = \left[\frac{(\text{Ino} + \text{Hx})}{(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx})} \right] \times 100$$

$$K_i (\%) = \left[\frac{(\text{Ino} + \text{Hx})}{(\text{IMP} + \text{Ino} + \text{Hx})} \right] \times 100$$

$$G (\%) = \left[\frac{(\text{Ino} + \text{Hx})}{(\text{AMP} + \text{IMP} + \text{Ino})} \right] \times 100$$

$$P (\%) = \left[\frac{(\text{Ino} + \text{Hx})}{(\text{AMP} + \text{IMP} + \text{Ino} + \text{Hx})} \right] \times 100$$

$$H (\%) = \left[\frac{(\text{Hx})}{(\text{IMP} + \text{Ino} + \text{Hx})} \right] \times 100$$

$$F_r (\%) = \left[\frac{(\text{IMP})}{(\text{IMP} + \text{Ino} + \text{Hx})} \right] \times 100$$

Texture Measurement. The hardness of fish muscle was measured according to Sigurgisladottir et al. (25) as modified by Alasalvar et al. (29), using a TA.XT2 Texture Analyzer (Stable Micro System, Surrey, U.K.). A flat-ended cylinder that simulates the human finger was applied. Three sampling points were selected in each fillet [dorsal, tail (10 mm from the edge of tail), and between dorsal and tail]. Double compression was applied to construct the texture profile analysis parameters. The flat-ended cylinder (20 mm diameter) approached the sample at the speed of 2 mm/s and penetrated 2.5 mm into the fillet (this penetration depth was selected as the maximum distance that could be applied without breaking the muscle fibers and leaving a mark on the fillet). Six fillets from three fish were used for analysis.

Statistical Analysis. SigmaStat was used to normalize the data, analysis of variance was performed, and differences in mean values were determined using Tukey's procedures of statistical analysis system (31).

RESULTS AND DISCUSSION

Proximate Analysis. The proximate analysis of the sample conducted on day 1 is shown in **Table 1**. As compared to wild

Table 1. Proximate Analysis (%) of Cultured and Wild Sea Bream^a

	protein	fat	moisture	ash
cultured	18.1 ± 0.7	9.8 ± 1.4	71.2 ± 2.5	1.4 ± 0.1
wild	20.1 ± 2.3	1.2 ± 1.0	78.1 ± 1.8	1.5 ± 0.1

^a Data are expressed as mean ± standard deviation ($n = 3$) on a fresh weight basis.

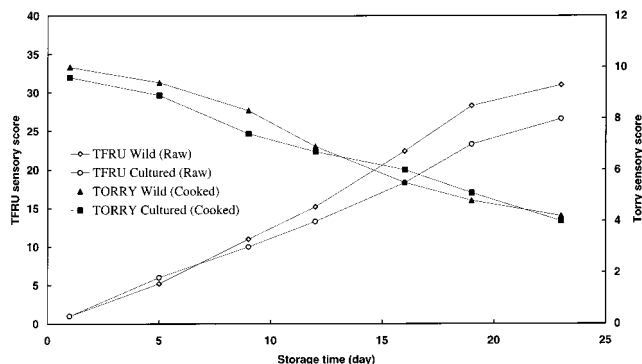


Figure 1. Changes in sensory quality of raw and cooked cultured and wild sea bream stored in ice. Torry (cooked fish): score 10, absolutely fresh; score 0, completely putrid. TFRU (raw fish): score 0, absolutely fresh; score 38, completely spoiled. The r^2 values of linear regression for TFRU are 0.99 (cultured and wild) and for Torry are 0.99 and 0.98 (cultured and wild, respectively), with time. Each point represents the mean value from five trained panelists' assessment. Average relative standard deviation (RSD), 8.47%.

sea bream, cultured sea bream possessed a significantly ($p < 0.01$) higher fat ($9.8 \pm 1.4\%$; $1.2 \pm 1.0\%$ in wild) and lower moisture ($71.2 \pm 2.5\%$; $78.1 \pm 1.8\%$ in wild) content. This may be due to the high dietary fat level in the feed (20%) and reduced activity of cultured fish. Protein and ash contents did not differ significantly ($p > 0.05$) between the two fish.

Sensory Assessment. Cultured and wild sea bream have fundamental external differences in their morphology. As compared to cultured sea bream, wild sea bream had a more bleached greenish appearance, sharper dorsal fins, more scales, and sharper teeth with bigger height and conical edge, smaller bellies, and shorter tails. They also have a golden tape between the eyes and a reddish patch on the surface of the gill cover.

The changes in the TFRU sensory score of raw cultured and wild sea bream over the 23 day storage in ice are shown in **Figure 1**. In this scheme, 0 represents absolutely fresh fish and 38 represents completely spoiled fish; a score of ~ 20 – 22 coincided with the level at which the fish were considered unacceptable by the members of the panel. The sensory scores of both cultured and wild sea bream increased linearly ($r^2 = 0.99$ and 0.99 , respectively) with storage time. The limit for acceptability of cultured and wild sea bream stored in ice was ~ 16 – 18 days. However, the perceived level of acceptability of both fish freshness quality depends on the particular buyer, user, or regulatory agency. Although the initial sensory score of both fish was the same on day 1, this score for wild sea bream was significantly higher than that for cultured fish on days 16, 19, and 23 ($p < 0.05$). This could possibly be explained by a higher proportion of highly unsaturated fatty acids in the wild sea bream (32) that might cause faster deterioration of its desirable flavor despite a lower fat content. Some other factors, such as higher capture stress and/or higher numbers of initial microbial flora (not measured), in the wild sea bream may also possibly result in the more rapid spoilage.

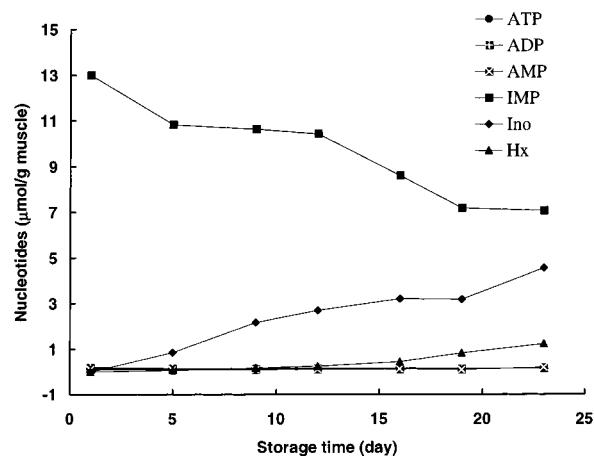


Figure 2. Changes in concentration of ATP breakdown compounds in raw cultured sea bream stored in ice. Each point represents the mean value of three determinations. Average RSD, 8.42%. Symbols for ATP, ADP, and AMP are superimposed.

Figure 1 also shows the changes in sensory quality (odor, flavor, and texture) of cooked cultured and wild sea bream through 23 days of storage. The Torry score of cooked cultured and wild sea bream gradually decreased ($r^2 = 0.99$ and 0.98 , respectively) during storage. In this scale, 10 showed absolutely fresh fish ("strong seaweedy odors"; "fresh sweet flavor characteristic"; "dry"; and "crumbly with short fibrous texture") and ≤ 3 showed completely putrid or spoiled ("composted grass" and "boiled clothes-like odors"; "strong bitter", "rubber", "slight sulfide", and "putrid flavor"; "much less succulent" and "soft texture"). A score of 4 ("lactic acid" and "sour milk odors"; "slight bitterness", "sour off flavors"; "less succulent" and "softer texture") was considered unacceptable by the members of the panel. No significant differences ($p > 0.05$) existed between the cooked cultured and the wild sea bream over the storage period.

The fresh flavor characteristic of both cultured and wild sea bream was strong for 1–5 days, slowly decreasing in intensity to a bland, relatively flavorless stage by 9–12 days. Off flavors were evident by days 16–18. As spoilage progressed, the off flavors increased in intensity and changed in character, until the fish became unacceptable on days ~ 19 – 23 . Although both cultured and wild sea bream were unacceptable on days 16–18, using the modified TFRU sensory score, cooked fish were considered to be of acceptable quality (**Figure 1**), characterized by "boiled potatoes", "caramel toffee-like odor", and with "slight sourness" but not "off flavors". The reason for this could be explained in that cooking may mask undesirable changes observed in fish provided that these changes are not extreme or it may possibly remove some of the volatile "off" odor. Similar results were also found in mackerel stored in ice for 12 days (33).

ATP-Related Compounds. The pattern of ATP breakdown products in ice-stored cultured and wild sea bream over 23 days is illustrated in **Figures 2** and **3**, respectively. Different postmortem patterns were observed in the various ATP breakdown compounds between the two fish. The main changes occurred in IMP, Ino, and Hx, whereas the concentration of ATP, ADP, and AMP remained unchanged (superimposed symbols) at a very low concentration ($< 0.2 \mu\text{mol/g}$). Following the death of fish, the conversion of ATP to IMP is usually complete within 1 or 2 days and this is presumed to be a totally autolytic process (34, 35). The subsequent breakdown of IMP to Hx is rather slow in ice-stored fish and caused by both

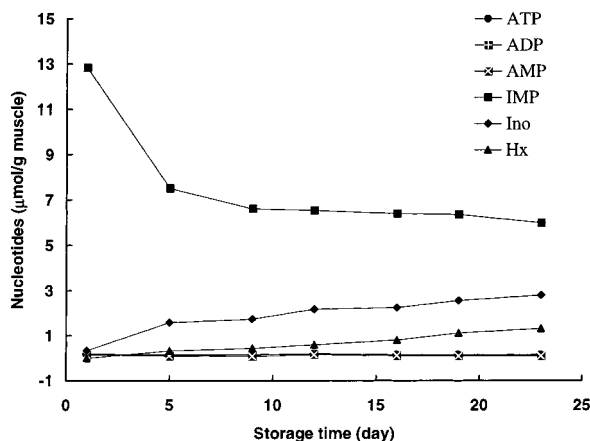


Figure 3. Changes in concentration of ATP breakdown compounds in raw wild sea bream stored in ice. Each point represents the mean value of three determinations. Average RDS, 9.62%. Symbols for ATP, ADP, and AMP are superimposed.

autolytic and microbial enzymes (9, 36). However, an initially large variation may occur in the level of ATP breakdown compounds due to the duration of antemortem struggling and onset of death.

The initial level of IMP in cultured sea bream was 13 $\mu\text{mol/g}$ and decreased steadily to 7.1 $\mu\text{mol/g}$ on day 23. One of the main differences in ATP breakdown compounds between the cultured and the wild sea bream was that the initial level of IMP in wild fish (12.8 $\mu\text{mol/g}$) decreased sharply to 7.5 $\mu\text{mol/g}$ by day 5 and then decreased slowly until day 23 (6 $\mu\text{mol/g}$), whereas in cultured fish it was 10.8 $\mu\text{mol/g}$ by day 5 and decreased fairly steadily throughout the storage period. This could possibly be related to capture stress affecting IMP, thus causing its rapid decrease in the early storage of fish, or typical enzymatic activity of sea bream that is characteristic for the environmental and physiological differences between the two fish. ATP is an important nucleotide in resting muscle and undergoes enzymatic dephosphorylation to form ADP and AMP. Deamination of AMP by the tissue enzyme AMP deaminase produces IMP. These steps are fast and give rise to rapid accumulation of IMP (10, 52).

It has been reported that the breakdown rate of IMP to Ino varies from species to species (34). Ehira and Uchiyama (37) found that IMP in the muscle of Pacific cod and Alaska pollack decreased to less than 1 $\mu\text{mol/g}$ in only 2 days. In contrast, the IMP level was around 6 $\mu\text{mol/g}$ after 15 days of ice-stored albacore (38). Several researchers have also reported that the decrease or disappearance of IMP is correlated with the loss of fresh fish flavor in most species (39, 40). Among the ATP breakdown products, IMP is most desirable in fish since it is a flavor enhancer and a high level of IMP is a good indicator of fresh fish quality (41).

Ino and Hx levels, which cause off flavors, increased during storage in both cultured and wild sea bream. Hx, which contributes a bitter flavor in fish, was not detected in either fish on day 1. The level of Hx in cultured sea bream was negligible during the first 12 days (0.25 $\mu\text{mol/g}$) before starting to increase gradually, whereas a gradual increase was observed in wild sea bream throughout the storage period. The increase in the Hx level was dependent upon the decrease in IMP. A slow and relatively small increase of Hx was also reported for other fish species over the storage period (42–44).

Hx content has been reported by several researchers to be an accurate indicator of fish freshness in many fish species (45,

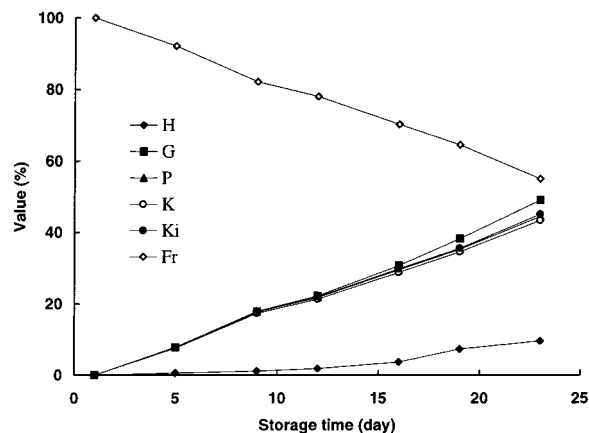


Figure 4. K , K_i , G , P , H , and F_r value changes of raw cultured sea bream stored in ice. The r^2 values of linear regression are 0.99 (K , K_i , G , P , and F_r) and 0.80 (H) with time. Each point represents the mean value of three determinations. Average RDS, 3.87%.

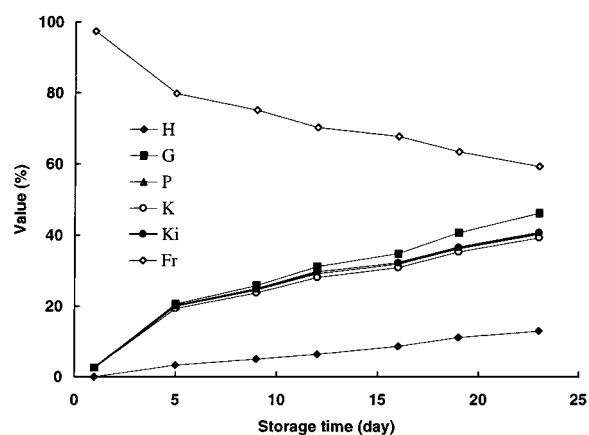


Figure 5. K , K_i , G , P , H , and F_r value changes of raw wild sea bream stored in ice. The r^2 values of linear regression are 0.79 (K), 0.78 (K_i and P), 0.86 (G), 0.99 (H), and 0.90 (F_r) with time. Each point represents the mean value of three determinations. Average RDS, 4.90%.

46). However, the formation of Hx has been reported to vary considerably both within a given species (47) and within an individual fish, as its formation may be greater in red muscle than in white muscle (48, 49). In addition, using Hx content without using other indices of freshness may be misleading whenever a fish has been processed during the latter stages of its storage life (22). This is due to possible breakdown of Hx to xanthine, which in turn can be oxidized to uric acid.

K and Related Values. The freshness indicators, namely, K , K_i , G , P , H , and F_r values, of cultured and wild sea bream were calculated from the concentrations of nucleotide. **Figures 4 and 5** exhibit changes of these values in both cultured and wild sea bream over the 23 days of storage, respectively.

K and related values increased (F_r decreased) linearly with storage time in cultured sea bream (**Figure 4**). Linear regressions (r^2) obtained from K , K_i , G , P , and F_r were all 0.99. The worst value obtained from the linear regression was 0.80 for H , which is expressed as the ratio of Hx to total concentrations of IMP, Ino, and Hx (20). During the first 12 days, no clear trend was evident in changes of the H value due to a steady increase in the concentration of Hx (**Figure 2**). This observation was in line with our previous study on cultured sea bream (29).

Figure 5 shows changes of K , K_i , G , P , H , and F_r values of wild sea bream over the entire storage period. In contrast to cultured sea bream, K , K_i , G , P , and F_r values did not correlate

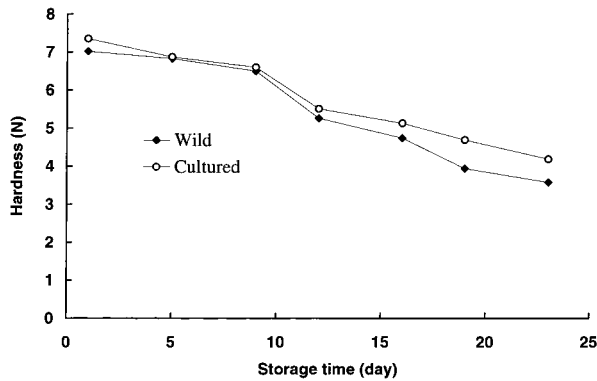


Figure 6. Texture analysis of raw cultured and wild sea bream stored in ice. The r^2 values of linear regression are 0.97 (cultured) and 0.95 (wild). Each point represents the mean of 18 determinations. Average RSD, 16.13%.

well ($r^2 = 0.79, 0.78, 0.86, 0.78,$ and $0.90,$ respectively) with storage time, but the H value did increase linearly ($r^2 = 0.99$). The reason for this could be explained through the initial sharp decrease of IMP in wild sea bream (**Figure 3**) causing a more rapid increase in the percentage of $K, K_i, G,$ and P values (decrease in F_r value). As mentioned earlier, either stress during capture or typical enzymatic activity for wild sea bream might be the cause for this effect.

When both cultured and wild fish were considered at the limit of acceptability by the members of the panel on day ~16–18, average $K, K_i, G,$ and P values were ~35–40%, H values were ~5% for cultured and 10% for wild, and F_r values were ~65–70% (**Figures 4** and **5**). However, the best indicators for freshness, i.e., good linear relationships with time, were $K, K_i, G, P,$ and F_r values for cultured fish and the H value for wild fish. Significant differences ($p < 0.05$) were observed in K and related values between the two fish over the storage period. The K value differs between species, for example, Lee et al. (50) found that the K value in rainbow trout increased from 3.4 (day 0) to 83% (day 6) during storage at 5 °C. Öksüz (51) found that the F_r value was higher than 20%, indicating the acceptability of rainbow trout stored in ice (day 9).

Texture. **Figure 6** shows the hardness of cultured and wild sea bream fillets measured by flat-ended cylinder. The initial hardness of both cultured and wild fish was over 7 N and decreased linearly ($r^2 = 0.97$ and $0.95,$ respectively) during the entire storage period (4.2 N for cultured and 3.9 N for wild on day 23), and no significant differences ($p > 0.05$) existed between the two fish until day 16. When both fish were rejected by the members of panel on day ~16–18, the hardness of cultured and wild sea bream was reduced to ~5 and ~4.5 N, respectively.

Relationship between Sensory Assessment, K and Related Values, and Texture. As the K and related values increased (F_r decreased), the sensory quality and hardness of sea bream decreased (**Figures 1, 4–6**). **Figure 7** shows the typical relationship between the K value and the TFRU sensory evaluation and between the hardness and the TFRU sensory evaluation of the cultured sea bream, with r^2 values of linear regression 0.99 and 0.97, respectively, over the entire storage period. Similar relationships were also obtained from $K_i, G, P,$ and F_r values, except the H value (data are not shown). In contrast, there were good relationships between the H value and TFRU sensory evaluation and between the hardness and the TFRU sensory evaluation of the wild sea bream ($r^2 = 0.99$ and $0.97,$ respectively). The rest of the values had poor relationships

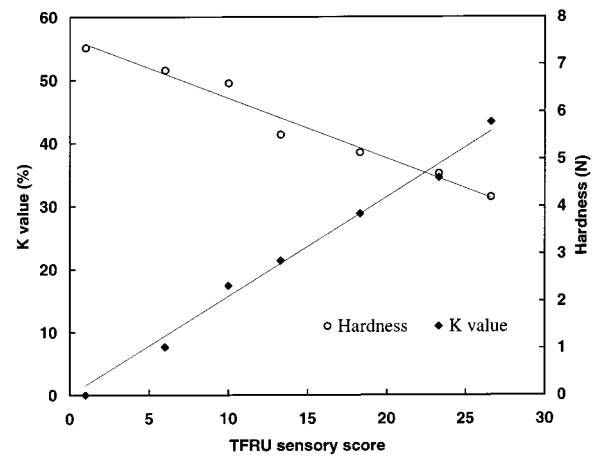


Figure 7. Typical relationship between the K value and the TFRU and between the hardness and the TFRU over the storage period. The r^2 values of linear regression are 0.99 (K value with TFRU) and 0.97 (hardness with TFRU).

between the TFRU sensory evaluation during the storage period (data are not shown).

Conclusions. These data provide evidence that the limit for acceptability of cultured and wild sea bream stored in ice is ~16–18 days (average $K, K_i, G,$ and P values: ~35–40%, H values: ~5% for cultured and 10% for wild, and F_r values: ~65–70%). The $K, K_i, G, P,$ and F_r values offered a better indication for cultured sea bream freshness than the H value, as a better linear relationship was obtained between these indicators, hardness, and storage of the fish up to 23 days. In contrast, the H value showed a better relationship than other values for wild sea bream. Generally, values that had good relationships ($K, K_i, G, P,$ and F_r for cultured and H for wild fish) with sensory and texture results over the storage period can be used as the most reliable freshness quality indicators.

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