

Comparison of selected methods of assessing freshness quality and remaining storage life of iced gilthead sea bream (*Sparus aurata*)

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Abstract

Quality changes of whole, iced gilthead sea bream were monitored by sensory evaluation, k_1 value, GR Torrymeter, and bacterial counts. The methods were tested for their suitability to determine freshness quality and remaining storage life in ice. Depending on the measured parameter, post-mortem age of the iced fish could be predicted with an accuracy of ± 1.5 – 3.6 days. Although assessment of cooked fish flavour is the underlying basis for establishing the state of the fish, the quality index method can be more effective for routine freshness evaluations. The k_1 value provides a useful means of monitoring early storage change, resulting primarily from autolytic reactions. Counts of sulphide-producing bacteria can be used to determine the time to rejection, while total counts at 20 °C are only poor measures of freshness quality. The GR Torrymeter offers a unique practical tool for assessing freshness quality and remaining storage life of iced gilthead sea bream.

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1. Introduction

The farming of gilthead sea bream (*Sparus aurata*) is a fast growing aquaculture venture in many Mediterranean countries and has the potential to become a major food producing industry in Southern Europe. At present, gilthead sea bream are marketed in the whole, ungutted condition (Smart, 2001). However, over the past years the demand for convenient, pre-packed products has increased significantly. Dressing or splitting the fish, and processing by curing, smoking and/or marinating will enable the fish industry to put a number of desirable, new products on the market. Reasonably precise information on the storage history of the raw material is a necessary adjunct to the processing of high quality products. However, little information is available on the most effective methods of assessing quality deterioration and storage stability of this highly priced euryhaline fish species. Changes in trimethylamine (TMA) during the first half of the edible storage life are insignificant and the value of total volatile bases (TVB)

measurements is limited by the fact that when significant amounts of these compounds have been produced, the fish is already in an incipient stage of spoilage (Kyranas, Lougovois, & Valsamis, 1997). The magnitude of change in hypoxanthine (Hx) levels is too small to yield a reliable indicator of post-mortem age, and deterioration of flesh lipids presents no serious problem during iced storage. The K value, defined as the ratio of non-phosphorylated ATP metabolites to the total ATP breakdown products (Saito, Arai, & Mutsuyoshi, 1959) and the modified k_1 value, equal to $[(\text{Ino} + \text{Hx})/(\text{IMP} + \text{Ino} + \text{Hx})] \times 100$ (Karube, Matsuoka, Suzuki, Watanabe, & Toyama, 1984), have been reported to have a potential as objective measurements of freshness quality and future storage life of gilthead sea bream (Alasalvar, Taylor, Öksüz, Garthwaite, Alexis, & Grigorakis, 2001; Huidobro, Pastor, & Tejada, 2001; Kyranas, 2001). A rapid freshness quality grading system, the quality index method (Hyldig & Nielsen, 1997; Luten & Martinsdottir, 1997), may also provide a reliable sensory tool for quality management purposes in the euryhaline fish processing industry (Huidobro, Pastor, & Tejada, 2000; Kyranas, 2001). The aim of this study was to compare different freshness/

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spoilage indicators, namely cooked fish flavour, quality index, Torrymeter measurements, k_1 value, total viable counts and selective counts of hydrogen sulphide-producing bacteria, as to their suitability to determine days in ice after harvest or remaining storage life of whole, iced gilthead sea bream.

2. Materials and methods

2.1. Storage conditions and sampling

Gilthead sea bream used in this study were obtained directly from the farm. A total of 96 commercial size fish (350–550 g) were used in the course of two independent storage trials. The fish were slaughtered by immersing in ice cold water (hypothermia), packed with flaked ice into polystyrene boxes provided with holes for drainage and delivered to the laboratory within 3–4 h of harvesting. The fish were held ungutted in a refrigerator set at 2 ± 2 °C and fresh ice was added as required. On the day of analysis six randomly chosen fish were removed from the batch held in ice and their raw sensory attributes and dielectric properties determined. Three fish were sampled for microbiological analyses and assays of nucleotide metabolites as described later. The remaining fish were filleted and the fillets were skinned and used for sensory evaluation of the cooked flesh. Sampling was continued over an 18-day storage period.

2.2. Sensory evaluation of raw and cooked fish

Sensory analyses were performed by a panel of five experienced assessors. Raw fish were evaluated using the quality index method (QIM) shown in Table 1. This structured category scale is based on the freshness quality grading system for whole iced gilthead sea bream developed by Kyrana (2001). The QIM involves specifying the characteristics of appropriate sensory attributes of the raw fish. Once the characteristic of a sensory attribute is determined, it is assigned a demerit score ranging from 0 to 3 (see Table 1). The scores for all characteristics are then summed to give an overall sensory score, the so-called quality index (Botta, 1995). The scale gives zero score for absolutely fresh fish, while increasingly larger totals result as fish deteriorate. The limit of acceptability for round gilthead sea bream on this freshness scale is 16.

Cooked fish were assessed according to the simplified Torry Sensory Scheme for white fish fillets (Whittle, Hardy, & Hobbs, 1990). In the preparation of cooked samples, small portions of skinned flesh were wrapped in aluminium foil and steam-cooked for 12 min. The scoring was carried out in individual booths and all samples were evaluated hot (60 °C) within 15 min of

Table 1
Quality Index Method for round gilthead sea bream (*Sparus aurata*)

Parameter being assessed	Defined characteristic	Demerit points
Appearance	Bright, shining, iridescent	0
	Less bright, some loss of iridescence	1
	Pale, dull	2
Body stiffness	Very stiff, hard (in rigor)	0
	Firm, elastic (post-rigor)	1
	Some softening	2
Odour	Fresh	0
	Neutral	1
	Slight off-odours	2
	Spoiled	3
<i>Eyes</i>		
Cornea	Clear, translucent	0
	Cloudy	1
	Opaque	2
Pupil	Black, bright, shiny	0
	Slightly greyish	1
	Grey - white	2
<i>Gills</i>		
Appearance	Uniformly dark red	0
	Brownish red	1
	Discoloured/faded	2
Odour	Fresh	0
	Neutral	1
	Slight off-odours	2
	Spoiled	3
Total demerit points		0–16

cooking. The simplified Torry scale consisted of 10, fresh, sweet flavours characteristic of the species; 9, some loss of sweetness; 8, slight sweetness and loss of flavours characteristic of the species; 7, definite loss of flavour but no off-flavours; 6, absolutely no flavour; 5, trace of off-flavours, some sourness but no bitterness; 4–3, increasing off flavours; 1, strong bitterness, but not nauseating, and 0, putrid flavours. The fish were judged unfit for consumption when the mean value for sensory score was below 5.5.

2.3. Microbiological analyses

One flesh sample was taken from the antero-dorsal region of each of three fish. Prior to sampling the skin was rinsed with 70% ethanol and removed aseptically. Approximately 20 g of the underlying flesh were sampled to the bone using sterile scalpels and forceps. Ten-fold dilutions in 0.1% peptone water were prepared from the flesh samples and 1 ml aliquots were plated in duplicate in Iron Agar (Gram, Trolle, & Huss, 1987). Total viable counts and selective counts of hydrogen

sulphide-producing bacteria were enumerated after 3 days incubation at 20 °C. Black colonies were recorded as sulphide-producers.

2.4. Torrymeter measurements

Changes in the dielectric properties of ice-stored gilthead sea bream were determined using the GR Torrymeter (Distell Industries Ltd., Scotland). A single measurement was obtained on each fish by applying the probe of the meter above and parallel to the lateral line, just behind the gill cover. The electrodes were cleaned in between measurements to remove scales and slime, and any remaining ice was cleared from the measuring surface. Instrument readings were read on a digital display.

2.5. Nucleotide metabolites

Hypoxanthine (Hx), inosine (Ino) and inosine-5-monophosphate (IMP) were assayed by the method of Kramer, Nordin, and Gardner (1977). Analyses were conducted on three fish per storage time (those already sampled for microbiological analysis) and carried out in duplicate. The antero-dorsal portion of the fillet was used for nucleotide extraction because it is the thickest section and provides the most homogenous sample for quantitative chemical analysis (Jones, Murray, Livingston, & Murray, 1964). Nucleoside phosphorylase (EC 2.4.2.1 from calf spleen, ca. 18 U mg⁻¹), 5-nucleotidase (EC 3.1.3.5 from *Crotalus adamanteus* venom, ca. 110 U mg⁻¹) and Hx were purchased from Sigma Chemical Co (St. Louis, MO, USA). Xanthine oxidase (EC 1.1.3.22 from buttermilk, ca. 1 U mg⁻¹) was obtained from Serva (Heidelberg, Germany), as were Ino and IMP (disodium salt). Spectrophotometric measurements were carried out in 10-mm silica cells against water, using a Unicam Helios γ UV-VIS spectrophotometer (Unicam, Dorset, UK).

2.6. Statistical analyses

Results from two trials were combined and analysed using linear regression techniques (Cheremisinoff, 1987). Means were submitted to Duncan's multiple range test for significant differences at $P < 0.05$. Pearson's correlation coefficients were used to compare physical, chemical, and microbiological measurements to sensory panel results.

3. Results and discussion

3.1. Sensory evaluation of cooked fillets

Mean scores for flavour of the cooked fillets are shown in Fig. 1. The deterioration of sensory quality was rather

slow compared with typical demersal fish such as cod (Huss, 1995; Whittle et al., 1990), but similar to that reported before for whole, iced gilthead sea bream (Kyrana et al., 1997). During the first half of the edible storage life there was a continuous loss of intrinsic fresh odours and flavours characteristic of the species, until the flesh became insipid, flavourless, by about 10–12 days. Slight sour and rotten off-odours and off-flavours were evident by 14–15 days but the cooked flesh was still palatable, on the Torry scale, at 19–20 days. The limit of acceptability on this freshness scale is 4.5, whilst score 5 is indicative of "poor quality" edible fish possessing trace off-flavours (Whittle et al., 1990). Such fish is not very acceptable to many consumers, so a rejection level of 5.5 was adopted in the present study. The maximum storage life of whole, iced gilthead sea bream, calculated from the equation of the regression line (Fig. 1) when the average score became 5.5, was 16.3 days.

3.2. Freshness assessment using the QIM

The quality index method has been considered a reliable sensory tool for assessing fish freshness in the fishery chain (Botta, 1995; Nielsen, 1997). A major feature of this rapid scaling method is that freshness quality, expressed as a demerit score, is linearly related to the length of time the fish are held in ice (Hyldig & Nielsen, 1997). However, a separate scheme should be developed for each species evaluated. The QIM scheme used in the present study (Table 1) consisted of seven freshness criteria representing a total of 16 demerit points. The maximum score for each criterion depended on the detectable variability of that criterion, and thereby determined its relative importance in the total quality index. The parameter of skin slime that had originally been included in the system (Kyrana, 2001) was removed, as storing the fish with a surplus of ice and replenishing melted ice daily has been observed to result in a lack of slime on the skin. The experimental data indicated that the suggested QIM scheme described successfully the different freshness quality levels of iced gilthead sea bream. On the basis of the QIM it was possible to develop a calibration curve of the total number of demerit points assigned to the fish (quality index) against time of storage in ice (Fig. 2). Mean values for quality index reached a maximum at 16.5 days and correlated significantly ($P < 0.001$) with time of storage in ice. Thus, the quality index curve could be applied to predict remaining storage life in ice and to calculate the maximum allowable number of index points to fulfil given freshness criteria when selecting gilthead sea bream for a certain type of product. The storage life of whole, iced gilthead sea bream, evaluated by sensory assessment of the raw fish, has been reported to be 15–17 days (Alasalvar et al., 2001; Huidobro et al., 2000; Kyrana et al., 1997).

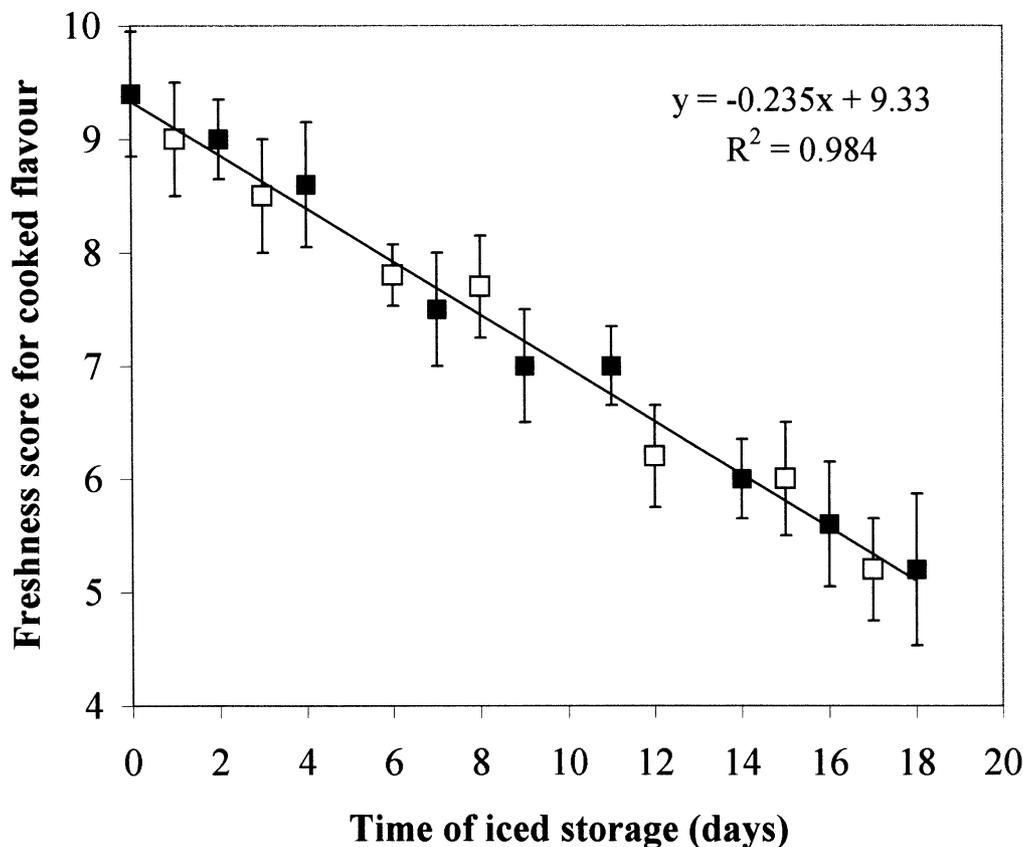


Fig. 1. Changes in freshness score of cooked fillets from whole, iced gilthead sea bream. Combined results from two trials. Each point represents the mean of 10 samples evaluated by individual assessors. Vertical bars denote standard deviation.

3.3. Changes in microbiological quality

Results from the microbiological analyses tented to confirm the findings of the sensory evaluation. Ice temperature prolonged the lag phase of most bacteria, thereby extending the storage life of the fish. The total viable count (TVC) on iron agar incubated at 20 °C for 3 days did not exceed spoilage levels of 10^7 CFU g^{-1} (IFST, 1999) until after 16 days of storage, reaching 10^8 – 10^9 CFU g^{-1} at the end of the trials (Table 2). Thus, the microbiological storage life of the fish was in good agreement with the results of the sensory evaluation. As bacterial loads in the fish muscle prior to day 10 remained rather low ($<10^5$ CFU g^{-1}), it was assumed that the early loss of flavour resulted primarily from autolytic reactions. Thus, the exclusion of bacteria from fresh gilthead sea bream would not prevent the product from rapidly becoming less acceptable.

Hydrogen sulphide-producing bacteria constituted a low proportion (less than 1%) of the total aerobic flora of fresh gilthead sea bream. This percentage increased to 6% by the end of the test period (day 18). Sulphide-producing bacteria (SPB) and species of *Pseudomonas* are commonly identified with spoilage of aerobically stored fish (Huss, 1995; ICMSF, 1998). Sulphide pro-

ducers are important in generating offensive fishy, rotten and H_2S -off-odours and off-flavours associated with the spoilage of marine, temperate water fish stored in melting ice, the predominant sulphide-producing bacterium being *Shewanella putrefaciens* (Gram, 1992; Gram et al., 1987). In warmer waters, *Pseudomonas* spp. can be the dominant spoilage bacteria (Koutsoumanis & Nychas, 1999, 2000). Fruity, rotten and sulphhydryl odours and flavours are more typical of the *Pseudomonas* spoilage of iced fish (Gram & Huss, 1996). Counts of sulphide-producers have been used as indicators of iced fish spoilage (Jørgensen, Gibson, & Huss, 1988), and the number of *S. putrefaciens* has been observed to be inversely linearly related to remaining storage life, irrespective of whether or not that organism is itself causing the rejection (Capell, Vaz-Pires, & Kirby, 1997). As high cell concentrations of more than 10^8 CFU g^{-1} of *S. putrefaciens* are normally required to cause spoilage of ice-stored fish (Gram & Huss, 1996), it was assumed that this organism was not a major spoiler in the present study. The low numbers of sulphide-producers were consistent with the lack of foul, H_2S -like off-odours and off-flavours in spoiling gilthead sea bream, as judged by sensory evaluation. Nonetheless, a highly significant correlation ($r=0.971$, $P<0.001$) existed

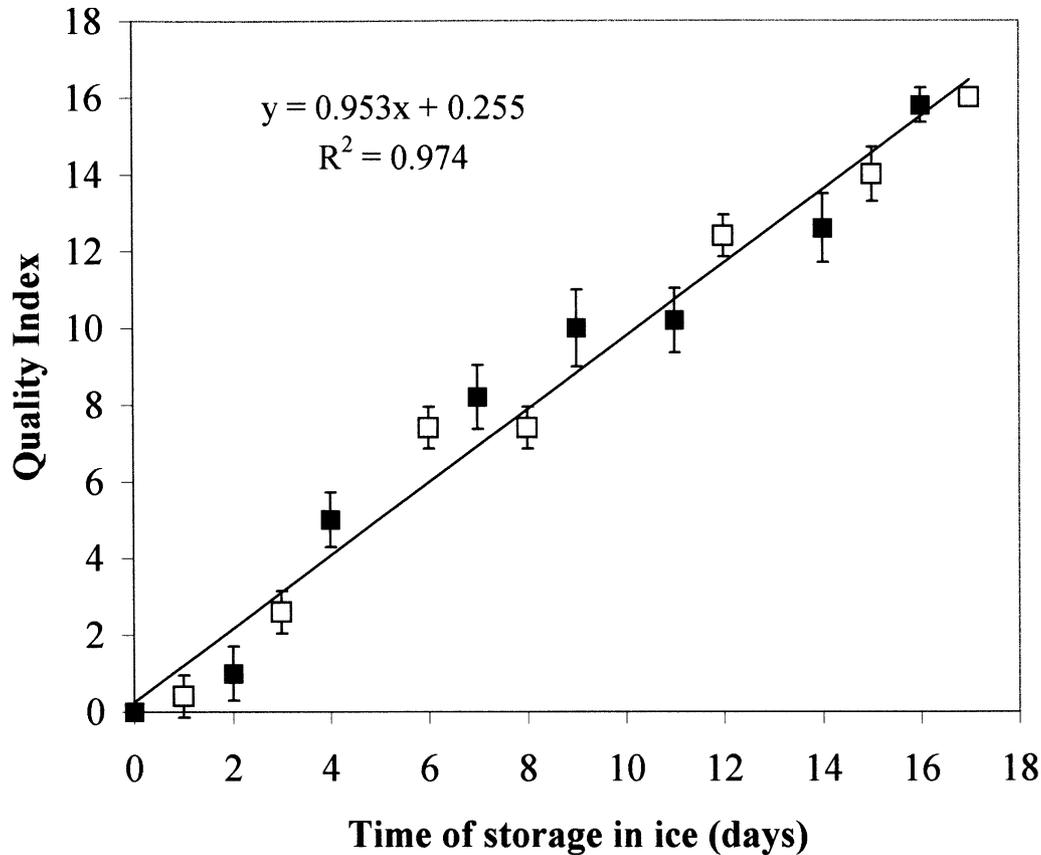


Fig. 2. Linear regression for quality index against time of storage, in whole, iced gilthead sea bream. Combined results from two trials. Means of six fish evaluated by individual assessors. Vertical bars denote standard deviation. Where bars are not visible, determination is within the size of the symbol.

Table 2

Changes in total viable count (TVC), selective count of hydrogen sulphide-producing bacteria (SPB) and nucleotide metabolites in whole, iced gilthead sea bream

Days in ice	Trial	TVC (log CFU g ⁻¹)	SPB (log CFU g ⁻¹)	IMP (μmol g ⁻¹)	Ino (μmol g ⁻¹)	Hx (μmol g ⁻¹)
0	a	ND	ND	ND	ND	ND
1	b	ND	ND	10.63 (0.62) A	0.30 (0.02) A	0.06 (0.01) A
2	a	3.0 (0.12) A	nd	9.75 (0.42) A, B	0.25 (0.03) B	0.05 (0.01) A
3	b	3.3 (0.26) A, B	nd	10.40 (0.47) A	0.52 (0.05) C	0.10 (0.01) B
4	a	3.0 (0.24) A	nd	9.00 (0.50) B, C	0.65 (0.04) D	0.17 (0.02) C
6	b	3.1 (0.20) A	1.0 (0.0) A	8.93 (0.46) C	0.95 (0.06) E	0.24 (0.02) D
7	a	ND	ND	7.23 (0.56) D, E	1.15 (0.11) F	0.30 (0.05) D
8	b	3.7 (0.16) B	1.7 (0.09) B	7.85 (0.45) D	1.25 (0.07) F	0.45 (0.02) E, G
9	a	4.5 (0.24) C	2.4 (0.17) C	ND	ND	ND
11	a	4.3 (0.10) C	2.5 (0.15) C	6.70 (0.35) E, G	1.50 (0.07) G	0.40 (0.05) E
12	b	5.2 (0.17) D	3.5 (0.17) D	6.05 (0.35) F, H	1.66 (0.08) H	0.58 (0.03) F
14	a	5.4 (0.18) D	4.0 (0.12) E	6.86 (0.32) G	1.80 (0.10) H, I	0.60 (0.03) F
15	b	6.3 (0.21) E	5.0 (0.24) F	5.57 (0.07) H, J	1.70 (0.08) H	0.50 (0.05) G
16	a	6.8 (0.22) F	5.6 (0.11) G	ND	ND	ND
17	b	7.6 (0.15) G	6.6 (0.12) H	4.80 (0.16) I	1.90 (0.12) I	0.80 (0.10) H
18	a	8.5 (0.21) H	7.2 (0.23) I	5.35 (0.17) J	2.00 (0.24) I	0.91 (0.10) H

Data are mean values; figures in parentheses are standard deviations, $n=3$. Means within the same column with different letters (A–J) are significantly ($P<0.05$) different. ND, not determined; nd, not detected.

between selective counts of H₂S-producing bacteria and freshness score for flavour. An average level of 10⁶ CFU g⁻¹ of sulphide-producers would be indicative of marginal quality gilthead sea bream (flavour score ≈5.5). Similar results have been reported for cultured Eur-

opean sea bass (*Dicentrarchus labrax*) stored in melting ice (Kyrana & Lougovois, 2002). Counts of sulphide-producers in the range of log 6–log 6.7 are normally present on rejectable fish from temperate and tropical waters (Capell et al., 1997).

3.4. Freshness assessment using the GR Torrymeter

The changes in dielectric properties of fish skin and fish muscle are closely related to spoilage rates and have been used as quality indicators since the first commercial version of the Torrymeter appeared in 1970 (Burt, Gibson, Jason, & Sanders, 1976; Storey & Mills, 1976). High correlation between Torrymeter values and sensory attributes has been reported for cod (Burt et al., 1976), herring (Damoglou, 1980), hake (Lupin, Gianini, Soule, Davidovich, & Boeri, 1980), blue whiting (Barassi et al., 1981), flounder and mackerel (Pivarnik et al., 1990), and a number of tropical fish (Hoffmann, 1981). Changes in Torrymeter values for whole, iced gilthead sea bream over the period of storage are shown in Fig. 3. Mean instrument readings declined in a linear fashion and were generally more variable during the second half of the edible storage life of the fish. Differences in chemical composition and physical damage to the skin and underlying muscle tissue may have accounted, to a certain degree, for the observed variability between individuals of equal time–temperature history. Cultured gilthead sea bream may possess an intramuscular lipid content of 3.3–10.6% and have a rather thin, fragile skin, constituting about 4.1% of the

total weight of the fish (Kyrana, 2001). Fat is known to have an effect on the dielectric properties of fish and tends to make observed Torrymeter values more variable (Pivarnik et al., 1990). On the other hand, loss of skin and/or muscle integrity and deterioration of the skin caused by bruising during harvesting and packing operations would result in lower and more variable Torrymeter values, thus indicating an apparently inferior product. This type of damage could result in accelerated spoilage, particularly if storage was not optimal. A highly significant linear relationship ($r=0.960$, $P<0.001$) was found between Torrymeter readings and sensory score for flavour indicating that the measurements were reflective of quality changes and could be used to make judgments as to fish freshness. Mean Torrymeter values ≥ 11 will indicate very fresh fish (no more than four days in ice), whereas a value of 6 will be indicative of marginal quality fish, assuming a storage life of 16 days for whole, iced gilthead sea bream.

3.5. Nucleotide degradation

Changes in the concentrations of nucleotide metabolites in the muscle tissue of gilthead sea bream are shown in Table 2. The concentration of IMP decreased

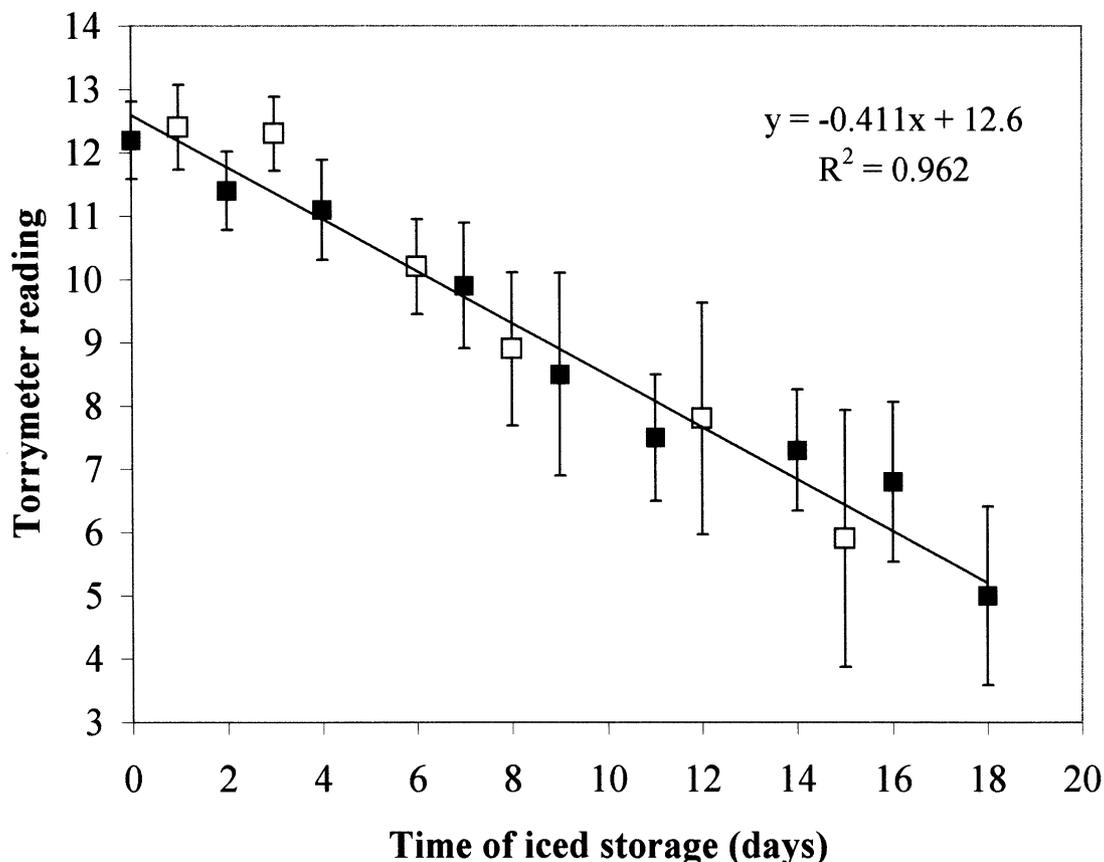


Fig. 3. Regression line for GR Torrymeter readings against time, in whole, iced gilthead sea bream. Combined results from two trials. Each point represents the mean of six fish. Vertical bars denote standard deviation.

rather slowly during storage in ice with less than 35% of peak levels metabolized by day 10. The breakdown of Ino to Hx was also slow and did not appear to increase during the period of proliferation of the spoilage microflora. The major IMP metabolite at the end of the storage trials was Ino, whilst Hx accounted for less than 12% of the total pool of purine derivatives. The substances that may accumulate in fish muscle as well as the rates at which they will increase or decrease in concentration will be determined by the relative activity of the different enzymes involved in the sequence of degradative changes. The activity of 5-nucleotidase varies considerably with species, whilst even greater variations occur in the rates of cleavage of Ino by muscle riboside hydrolases and phosphorylases (Kassemsarn, Perez, Murray, & Jones, 1963). The accumulation of Ino rather than Hx in conjunction with slow degradation of IMP, as observed in gilthead sea bream, is also characteristic of many fish popular for *sashimi* (Ehira & Uchiyama, 1987; Karube et al., 1984). The changes in nucleotide metabolites have been found to contribute directly to the sensory quality of fish, with IMP showing a distinct taste-enhancing effect, particularly in combination with glutamic acid, and Hx contributing bitterness to chill-stored fish that is nearing the limit of

edibility (Lindsay, 1994). In the present study, a highly significant correlation ($r=0.962$, $P<0.001$) existed between freshness score for flavour and IMP concentration and it was assumed that the slow degradation of the mononucleotide through autolytic reactions was implicated in the loss of desirable odours and flavours in the fresh fish. However, due to variations in the original amount of ATP present in the muscle of different fish, it is not likely that IMP alone could give precise information of the post-mortem age or remaining storage life of ice-stored gilthead sea bream. The k_1 value, primarily measuring the extent to which IMP is degraded, reduces variability and has been reported to be an appropriate freshness quality indicator for a number of fish species (Ehira & Uchiyama, 1987). In iced gilthead sea bream, k_1 value increased almost linearly during storage (Fig. 4) and correlated significantly ($r=0.990$, $P<0.001$) with flavour score. Since dephosphorylation of IMP was not complete within the period of edibility, the k_1 value appeared to have a predictive function throughout the trial and could provide a useful indicator of early change and remaining storage life. Very fresh gilthead sea bream will have a k_1 value lower than 10%, while fish at the end of its shelf life (day 16) will have a k_1 value of 33–35%. Alasalvar et al. (2001) reported that

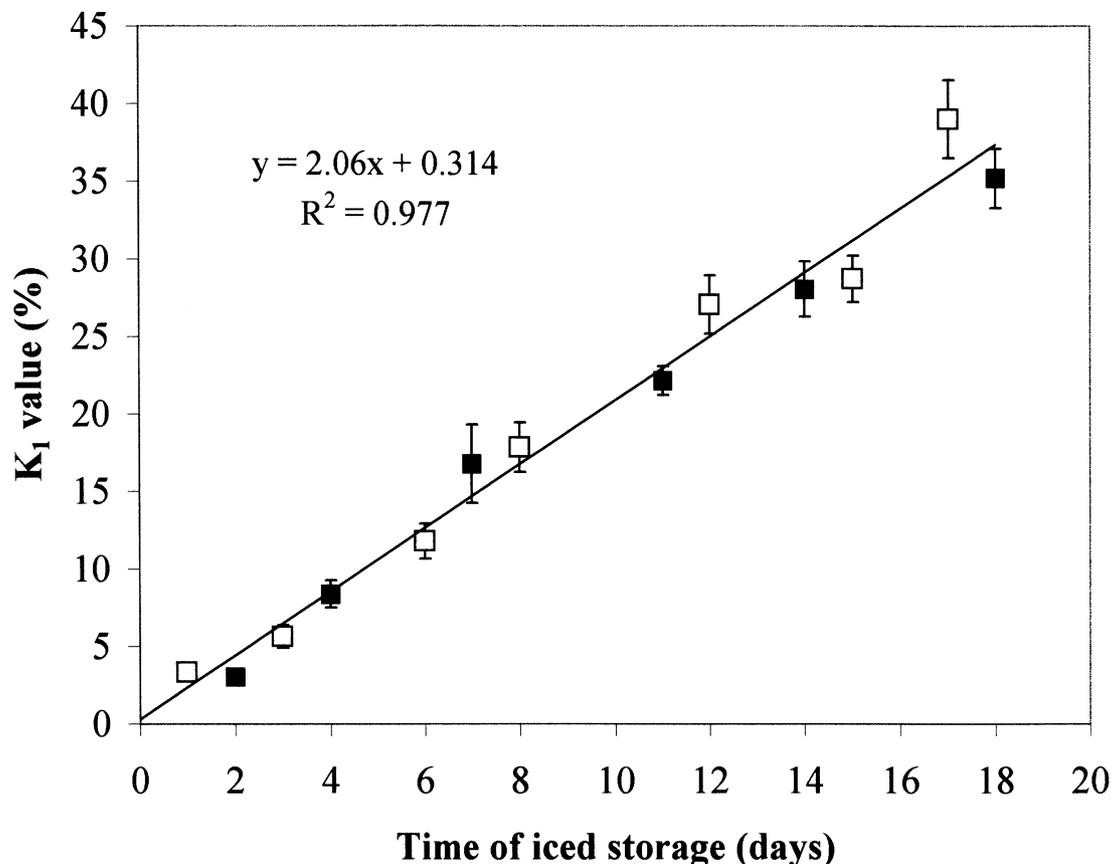


Fig. 4. Changes in k_1 value during iced storage of whole gilthead sea bream. Combined results from two trials. Each point represents the mean of three fish. Vertical bars denote standard deviation. Where bars are not visible, determination is within the size of the symbol.

when iced gilthead sea bream was considered unacceptable by the members of the taste panel on day 17, the k value (determined by HPLC) was 39%, which is not much different from the findings of the present study. Small differences in the experimental data could be due to natural variability, as well as to the different methodologies used in the two studies.

3.6. Comparison of freshness evaluation methods

Once the rate of change occurring in the freshness/spoilage indicators had been monitored, the next step was to develop models in which the measured parameters could be used to predict days in ice after harvest or remaining storage life. Assuming linear regression analysis, the equations for the prediction models were obtained and confidence intervals for 90% probability were determined (Table 3). Depending on the measured parameter, post-mortem age of the iced fish could be predicted with an accuracy of ± 1.5 –3.6 days.

Cooked fish flavour is an excellent indicator of freshness quality and may provide precise information on the time of storage following harvest. However, specially trained expert assessors are required for objective sensory evaluation (Botta, 1995) and this can be both expensive and inconvenient (Connell, 1995). For routine freshness evaluations, the quality index method could be a more effective sensory tool, as it is faster, non-destructive and requires less training than sensory evaluation of cooked fish flavour. Using the quality index, the post-mortem age of iced gilthead sea bream could be predicted to within a limit of less than two days, which would be an adequate measure for quality management purposes in the fishery chain.

The k_1 value provided an objective means of monitoring early storage change, being maximally sensitive before active bacterial spoilage could be detected. Changes in k_1 value are strongly influenced by the physiological effects of fish harvest and the death struggle (Lowe, Ryder, Carragher, & Wells, 1993).

Accordingly, the predictive function of this indicator will depend largely on the ability to maintain control over husbandry techniques, killing methods and immediate post-mortem handling of the cultured fish.

Counts of H_2S -producing bacteria, though constituted a small proportion of the total aerobic flora, provided a useful indicator of quality deterioration and could be used to determine the time to rejection, showing similar accuracy of prediction with k_1 value. Total numbers of bacteria correlated significantly ($r = 0.941$, $P < 0.001$) with flavour score, even though microbial action appeared to play a minor role in the deterioration of quality prior to day 10–12. However, based on total counts, post-mortem age of the iced fish could be predicted with an accuracy of approximately ± 4 days, which would be a rather poor measure of assuring freshness quality.

Chemical and microbiological methods of assessing freshness quality are useful for research or product development but are not practical for routine use, as they require expensive laboratory equipment and trained staff, are destructive, and can be both labour intensive and time consuming. For certain types of products, e.g. pre-packed chilled fillets that usually only carry a 3-day sell-by date from the time of packing, gilthead sea bream equivalent to 6 days in ice or better (flavour score ≥ 8) would be required. A fast, reliable method is therefore necessary for assuring freshness specification of the starting material and making sure that the product will not become stale when distributed and displayed. The GR Torrymeter offered a unique tool for indirectly measuring freshness quality of gilthead sea bream. Instrument readings were consistent with sensory assessment and provided a basis for estimating days in ice passed with an accuracy (± 2.2 days), which was not practically different from that provided by either k_1 value, quality index method or counts of sulphide-producing bacteria. Advantages of using the electrical tester include ease of use, immediate response, portability, and minimal training requirements.

Table 3
Post-mortem age prediction models for iced gilthead sea bream, based on different freshness/spoilage indicators^a

Freshness/spoilage indicator	Intercept (b_0)	Slope (b_1)	r^b	S^c	n^d	Accuracy of prediction (days) ^e	Rejection level ^f
Sensory score for flavour	39.2	-4.18	0.992	0.778	16	± 1.5 (0.03)	5.5
Quality index	-0.045	1.02	0.987	1.00	15	± 1.9 (0.05)	16
k_1 value	0.059	0.475	0.988	0.919	13	± 1.8 (0.05)	33
Torrymeter measurement	29.8	-2.34	0.981	1.18	15	± 2.2 (0.06)	5.9
SPB (log CFU g ⁻¹)	5.13	1.89	0.980	0.861	10	± 1.8 (0.07)	5.9

^a Days in ice after harvest = $b_0 + b_1 \times (\text{value of freshness/spoilage indicator})$.

^b Correlation coefficient.

^c Standard deviation of prediction.

^d Number of experiments.

^e Mean accuracy of prediction for 90% confidence level (figures in parentheses denote standard deviation).

^f Level of freshness/spoilage indicator at rejection.

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References

- Alasalvar, C., Taylor, K. D. A., Öksüz, A., Garthwaite, T., Alexis, M. N., & Grigorakis, K. (2001). Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. *Food Chemistry*, 72(1), 33–40.
- Barassi, C. A., Boeri, R. L., Crupkin, M., Davidovich, L. A., Giannini, D. H., Soulé, C. L., Trucco, R. E., & Lupin, H. M. (1981). The storage life of iced southern blue whiting (*Micromesistius australis*). *Journal of Food Technology*, 16(2), 185–197.
- Botta, J. R. (1995). *Evaluation of seafood freshness quality*. New York: VCH Publishers.
- Burt, J. R., Gibson, D. M., Jason, A. C., & Sanders, H. R. (1976). Comparison of methods of freshness assessment of wet fish. Part III. Laboratory assessments of commercial fish. *Journal of Food Technology*, 11, 117–122.
- Capell, C., Vaz-Pires, P., & Kirby, R. (1997). Use of counts of hydrogen sulphide producing bacteria to estimate remaining shelf life of fresh fish. In *Methods to determine the freshness of fish in research and industry. Proceedings of the final meeting of the concerted action "evaluation of fish freshness"* (pp. 175–182) AIR3CT94 2283, Nantes, 12–14 November 1997. Paris, France: International Institute of Refrigeration.
- Cheremisinoff, N. P. (1987). *Practical statistics for engineers and scientists*. Lancaster, USA: Technomic Publishing Company.
- Connell, J. J. (1995). *Control of fish quality* (4th ed.). Farnham, Surrey: Fishing News (Books).
- Damoglou, A. P. (1980). A comparison of different methods of freshness assessment of herring. In J. J. Connell (Ed.), *Advances in fish science and technology* (pp. 394–399). Farnham, Surrey, UK: Fishing News (Books).
- Ehira, S., & Uchiyama, H. (1987). Determination of fish freshness using the k value and comments on some other biochemical changes in relation to freshness. In D. E. Kramer, & J. Liston (Eds.), *Seafood quality determination* (pp. 185–207). Amsterdam: Elsevier Science Publishers.
- Gram, L. (1992). Evaluation of the bacteriological quality of seafood. *International Journal of Food Microbiology*, 16, 25–39.
- Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33, 121–137.
- Gram, L., Trolle, G., & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at (0 °C) and high (20 °C) temperatures. *International Journal of Food Microbiology*, 4, 65–72.
- Hoffmann, A. (1981). The use of the GR Torrymeter for the assessment of freshness of iced tropical fish from the Indian Ocean. *Tropical Science*, 23, 283–291.
- Huidobro, A., Pastor, A., & Tejada, M. (2000). Quality index method developed for raw gilthead seabream (*Sparus aurata*). *Journal of Food Science*, 65(7), 1202–1205.
- Huidobro, A., Pastor, A., & Tejada, M. (2001). Adenosine triphosphate and derivatives as freshness indicators of gilthead sea bream (*Sparus aurata*). *Food Science and Technology International*, 7(1), 23–30.
- Huss, H. H. (1995). *Quality and quality changes in fresh fish. FAO fisheries technical paper 348*. Rome: FAO.
- Hyldig, G. & Nielsen, J. (1997). A rapid sensory method for quality management. In *Methods to determine the freshness of fish in research and industry. Proceedings of the final meeting of the concerted action "evaluation of fish freshness"* (pp. 297–305) AIR3CT94 2283, Nantes, 12–14 November 1997. Paris, France: International Institute of Refrigeration.
- International Commission on Microbiological Specifications for Foods. (1998). *Microorganisms in foods (vol. 6). Microbial ecology of food commodities. 3 Fish and fish products*. London: Blackie Academic & Professional.
- IFST. (1999). *Development and use of microbiological criteria in foods*. London: Institute of Food Science & Technology (UK).
- Jones, N. R., Murray, J., Livingston, E. I., & Murray, C. K. (1964, November). Rapid estimations of hypoxanthine concentrations as indices of the freshness of chill-stored fish. *Journal of the Science of Food and Agriculture*, 15, 763–774.
- Jorgensen, B. R., Gibson, D. M., & Huss, H. H. (1988). Microbiological quality and shelf life prediction of chilled fish. *International Journal of Food Microbiology*, 6, 295–307.
- Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E., & Toyama, K. (1984). Determination of fish freshness with an enzyme sensor system. *Journal of the Agricultural and Food Chemistry*, 32(2), 314–319.
- Kassemsarn, B., Perez, B. S., Murray, J., & Jones, N. R. (1963). Nucleotide degradation in the muscle of iced haddock (*Gadus aeglefinus*), lemon sole (*Pleuronectes microcephalus*), and plaice (*Pleuronectes platessa*). *Journal of Food Science*, 28, 228–233.
- Koutsoumanis, K., & Nychas, G. J. E. (1999). Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7 and 10 °C. *Applied and Environmental Microbiology*, 65, 698–706.
- Koutsoumanis, K., & Nychas, G. J. E. (2000). Application of a systematic procedure to develop a microbial model for rapid fish shelf life predictions. *International Journal of Food Microbiology*, 60, 171–184.
- Kramer, D. E., Nordin, D. M. A. & Gardner, L. J. (1977). *A comparison of the quality changes of Alaska pollock and Pacific cod during frozen storage at –28 °C*. Fisheries and Marine Services technical report no. 753. Vancouver, BC: Vancouver Technological Research Laboratory.
- Kyranas, V. R. (2001). *Assessment of freshness quality and storage life of farm-raised gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax) by sensory, chemical, microbiological and physical methods*. Mphil thesis, Institute of Food Health Quality, University of Hull.
- Kyranas, V. R., & Lougovois, V. P. (2002). Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. *International Journal of Food Science and Technology*, 37(3), 319–328.
- Kyranas, V. R., Lougovois, V. P., & Valsamis, D. S. (1997). Assessment of shelf-life of maricultured gilthead sea bream (*Sparus aurata*) stored in ice. *International Journal of Food Science and Technology*, 32(4), 339–347.
- Lindsay, R. C. (1994). Flavour of fish. In F. Shahidi, & J. R. Botta (Eds.), *Seafoods—chemistry, processing technology and quality* (pp. 75–84). London: Blackie Academic & Professional.
- Lowe, T. E., Ryder, J. M., Carragher, J. F., & Wells, R. M. G. (1993). Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. *Journal of Food Science*, 58, 770–773,796.
- Lupin, H. M., Giannini, D. H., Soule, C. L., Davidovich, L. A., & Boeri, R. L. (1980). Storage life of chilled Patagonian hake (*Merluccius hubbsi*). *Journal of Food Technology*, 15(3), 285–300.
- Luten, J. B., & Martinsdottir, E. (1997). QIM: a European tool for fish freshness evaluation in the fishery chain. In *Methods to determine the freshness of fish in research and industry. Proceedings of the final meeting of the concerted action "evaluation of fish freshness"* (pp. 287–296) AIR3CT94 2283, Nantes, 12–14 November 1997. Paris, France: International Institute of Refrigeration.
- Nielsen, J. (1997). Sensory analysis of fish. In *Methods to determine the freshness of fish in research and industry. Proceedings of the final*

- meeting of the concerted action "evaluation of fish freshness" (pp. 279–286) AIR3CT94 2283, Nantes, 12–14 November 1997. Paris, France: International Institute of Refrigeration.
- Pivarnik, L. F., Kazantzis, D., Karakoltsidis, P. A., Constantinides, S., Jhaveri, S. N., & Rand, A. G. (1990). Freshness assessment of six New England fish species using the Torrymeter. *Journal of Food Science*, 55(1), 79–82.
- Saito, T., Arai, T., & Mutsuyoshi, M. (1959). A new method for estimating the freshness of fish. *Bulletin of the Japanese Society of Scientific Fisheries*, 24, 749–750.
- Smart, G. (2001). Problems of sea bass and sea bream quality in the Mediterranean. In S. C. Kestin, & P. D. Warriss (Eds.), *Farmed fish quality* (pp. 120–128). Oxford: Fishing News (Books)/Blackwell Science.
- Storey, R. M., & Mills, A. (1976). Instrumental techniques applied to the measurement of fish quality. *Process Biochemistry*, 11, 25–28.
- Whittle, K. J., Hardy, R., & Hobbs, G. (1990). Chilled fish and fish products. In T. R. Gormley (Ed.), *Chilled foods. The state of the art* (pp. 87–116). Essex, UK: Elsevier Applied Science.