Assessment of shelf-life of maricultured gilthead sea bream (*Sparus aurata*) stored in ice

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Summary Gilthead sea bream (*Sparus aurata*) were stored in melting ice (0 °C) for a period of 24 days from the time of harvest with sensory assessments of the whole raw fish and of the cooked fish flesh conducted at regular intervals. The ungutted fish was given an EC freshness grade E for up to 3 days, grade A for a further 7 days, and grade B for 4 more days after which it was graded as C (unfit). The sensory score for flavour of the cooked fillets decreased linearly with period of storage: fresh characteristic flavours were present for 2–4 days, decreasing to a relatively bland flavour after 10–12 days. Off flavours were evident by 13–15 days storage and by 18–19 days the flesh was unpalatable. With the possible exception of hypoxanthine, none of the chemicals investigated was particularly useful as an indicator of change. Changes in pH, trimethylamine and total volatile bases during the first half of the edible storage life were insignificant. Deterioration of flesh lipids, assessed by free fatty acid content and thiobarbituric acid value, appeared to present no serious problem during shelf-life. Proximate composition and sensory attributes, appropriate for routine inspection of gilthead sea bream were also determined.

Keywords Composition, fish, freshness indicators, quality assessment.

Introduction

Demand for quality chill-stored gilthead sea bream (*Sparus aurata*) on Greek and other European markets has increased significantly over the past decade. To meet the increasing demand, Greek aquaculture companies have expanded and in recent years production of the species has soared (Urch, 1994). Annual production increased from 1000 tons in 1990 to 9300 tons in 1995. Over the same period, exports to other EC countries increased tenfold, reaching 6500 tons in 1995 (Anonymous, 1996). Gilthead sea bream (*S. aurata*) belongs to the Sparidae family which includes a fair number of different genera and a number of well known commercial species. It has a white flesh and is a popular, high-valued fish.

Despite the commercial importance of the species, almost no studies have investigated the changes occurring in gilthead sea bream through typical handling, distribution and storage conditions. Ehira & Uchiyama (1974) included Japanese red and black bream (*Chrysophrys major* and *Mylio macrocephalus*, respectively) as part of their biochemical study of the freshness lowering rates of fish and concluded, from investigations of the nucleotide catabolism, that the rate of loss of freshness of sea breams was rather slow compared with that of cod. Boyd & Wilson (1976, 1977) and Fletcher & Hodgson (1988) studied the shelf-life of New Zealand snapper (*Chrysophrys auratus*). Curran *et al.* (1980, 1981) evaluated the shelf-life of iced stored gold-lined sea bream (*Rhabdosargus sarba*) and threadfin bream (*Nemipterus japonicus*). Amu & Disney

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(1973) and Diouf *et al.* (1982) studied changes in sensory, chemical and microbiological properties of red pandora (*Pagellus coupei*) stored in ice and chilled sea water, respectively, and more recently Civera *et al.* (1995) investigated total volatile bases and trimethylamine levels in cold stored saddled sea bream (*Oblada melanura*), bogue (*Boops boops*) and common pandora (*Pagellus erithrinus*).

This investigation was carried out as a first step towards the improvement in quality of maricultured gilthead sea bream (*S. aurata*), through the post-harvest sequence of handling, distribution and retail display. The aim of the study was to determine the rate and type of deterioration processes occurring during iced storage of ungutted fish, by use of sensory and chemical assessment. Further, it was intended to identify those analyses which could be used to monitor changes in gilthead sea bream during its shelf-life in ice.

Materials and methods

Storage conditions and sampling

Gilthead sea bream used in this study were cultivated in net cages and raised on pellets having the following proximate composition: crude protein, 45.0%; total lipids, 14%; moisture, 9.0%; ash, 9.0%; crude fibre, 2.0%. The fish were slaughtered by immersing in ice cold water (hypothermia) and delivered to the laboratory within 3 h of harvesting, packed into insulated containers with ice. Five fish were immediately taken to make the first sampling (day 0), while the rest were repacked ungutted with an equal volume of flaked ice into polystyrene boxes provided with holes for drainage. Boxes were stored in a refrigerator (0 °C) and the ice: fish ratio maintained throughout the trial. At set intervals, three randomly chosen fish were removed from ice, weighed and their raw sensory attributes determined. They were then beheaded, eviscerated, spray-washed with tap water and filleted. The fillets from one side of the fish were skinned and minced/mixed for the chemical analyses by passing three times through a meat grinder with 4 mm diameter holes, whilst the other side was used for cooked sensory assessment. Sampling in triplicate was continued over the 24-day storage period.

Sensory assessment

Sensory analyses were conducted by a taste panel consisting of five experienced judges on the whole raw fish according to the Multilingual Guide to EC Freshness Grades for Fishery Products (Howgate *et al.*, 1992), and on the cooked fish using the simplified Torry Sensory Scheme for cooked, white fish fillets (Whittle *et al.*, 1990). In the preparation of cooked samples, skinned fillets were steamed for 12 min in a household steam cooker and served hot. The scoring was carried out in individual booths.

Chemical analyses

Total nitrogen was determined by the semi-micro Kjeldhal procedure using potassium sulphate and copper(II) sulphate as the catalysts. Moisture content was determined by drying a portion of the prepared sample at 103 ± 2 °C for 24 h. The ash content was obtained by heating the residue from the moisture determination in a muffle furnace at 550 °C for 24 h, using magnesium acetate as an ashing aid. The non-protein nitrogen (NPN) content of the samples was determined according to the method described by Perez-Villarreal & Howgate (1987).

Total lipids were determined on a 20 g sample of the minced fillets using the extraction method of Bligh & Dyer (1959), as modified by Hanson & Olley (1963).

For the determination of free fatty acid (FFA) content, 20 mL of the chloroform extract were mixed with an equal volume of neutral alcohol and titrated with 0.01N NaOH, using phenolphthalein as indicator. FFA content was expressed as grams of oleic acid per 100 g lipid.

The pH measurements were carried out on a 5:1, water:fish homogenate, using a glass electrode at 20 °C.

Rancidity development was estimated by the thiobarbituric acid (TBA) value, according to the extraction method of Witte *et al.* (1970). Absorption values at 530 nm were measured in a Spectronic 20D spectrophotometer.

Determination of total volatile basic nitrogen (TVBN) levels was performed in perchloric acid extracts, according to the EC reference procedure (European Union, 1995). For the determination

of trimethylamine (TMA) content, the Dyer picrate method was used (AOAC, 1990). Trimethylamine oxide (TMAO) was determined as TMA after reduction with titanium (III) chloride.

Hypoxanthine (Hx) was assayed by the AMC method (Analytical Methods Committee, 1979). Xanthine oxidase (EC 1.1.3.22 from buttermilk, *c*. 1 U mg^{-1}) was obtained from Serva, and Hx was purchased from Sigma Chemical Co. Spectrophotometric measurements were carried out on a Hitachi U-3210 dual beam UV spectrophotometer. All chemical analyses were conducted on samples derived from a pool of three fish per storage time (except for day 0 when five fish were used) and carried out in triplicate. Reagents were of analytical grade.

Statistical analyses

Results were analysed using ANOVA and means were separated by the least significant difference test (Cheremisinoff, 1987) at $P < 0.05$. Linear regression was used on Hx concentration and sensory scores vs. storage period.

Results and discussion

Proximate analyses

In total 35 fish were used in this study (average weight 410 g, range 315–490 g). Proximate analyses conducted at day 0 are shown in Table 1. As would be expected, cultured gilthead sea bream possessed a considerably higher lipid level than that reported for wild fish (Torry Research Station, 1989), with a correspondingly lower moisture content. During storage in ice, moisture content increased from 70.30% at day 0 to a maximum of 73.55% at day 11, then fell to 72.40% at day 24 (Table 2). Changes in moisture content were followed by a reverse change in lipid content.

The total nitrogen content of the fillets was found to be higher than in most demersal white-fleshed teleosts (Analytical Methods Committee, 1973) resulting in a 'crude protein' level of 21.9% which is within the range of values (18.1–22.8%) reported for a number of *Sparus* spp. (Torry Research Station, 1989). The NPN-fraction was also high, constituting 11.7% of the total nitrogen content or 1.38% of the dry weight of the muscle.

Table 1 Proximate composition (w/w) of maricultured gilthead sea bream

Fat	7.69 (0.17)
Moisture	70.30 (0.30)
Total nitrogen (T_N)	3.50 (0.15)
Non-protein nitrogen (NPN)	0.41 (0.01)
Ash	1.32 (0.02)

Data are mean values; figures in brackets represent standard deviation, $n = 3$.
Samples derived from a pool of five fish.

Table 2 Changes in pH, moisture and fat content, free fatty acid (FFA) and thiobarbituric acid (TBA) value in gilthead sea bream over the period of iced storage

Days in ice	pH	Moisture[1]	Fat[1]	FFA[2]	TBA-value[3]
0[4]	6.20 (0.05)^{A}	70.30 (0.30)^{A}	7.69 (0.17)^{A}	ND	0.67 (0.05)^{A,C}
1	6.12 (0.04)^{B}	70.50 (0.51)^{A}	7.83 (0.16)^{A}	ND	0.67 (0.06)^{A,C}
3	6.10 (0.03)^{B}	71.85 (0.47)^{B}	6.58 (0.26)^{B,C}	2.19 (0.11)^{A}	0.79 (0.13)^{A,B,C}
6	6.10 (0.02)^{B}	ND	ND	ND	ND
7	ND	72.20 (0.26)^{B}	6.68 (0.08)^{B}	2.52 (0.13)^{B}	0.62 (0.05)^{A}
9	6.22 (0.05)^{A}	72.50 (0.34)^{B}	6.37 (0.06)^{C}	2.92 (0.03)^{C}	0.93 (0.11)^{B,D,F}
11	6.35 (0.05)^{C}	73.55 (0.22)^{C}	6.22 (0.19)^{C}	3.33 (0.34)^{C,D}	0.96 (0.08)^{B,F}
13	ND	ND	ND	ND	ND
14	6.45 (0.05)^{D}	72.00 (0.35)^{B}	6.72 (0.13)^{B}	3.67 (0.29)^{D}	0.76 (0.03)^{C,D}
17	6.50 (0.06)^{D,E}	ND	ND	ND	0.91 (0.04)^{B}
21	6.57 (0.05)^{E,F}	72.20 (0.18)^{B}	6.18 (0.02)^{C}	9.01 (0.10)^{E}	1.17 (0.03)^{E}
24	6.60 (0.03)^{F}	72.40 (0.40)^{B}	6.65 (0.09)^{B}	6.20 (0.18)^{F}	1.07 (0.03)^{F}

[1]g per 100 g flesh; [2]g oleic acid per 100 g fat; [3]mg malonaldehyde per kg flesh;
[4]approximately 4 h after harvesting.
Data are mean values; figures in brackets represent standard deviation, $n = 3$.
Means within the same column with different superscripts are significantly ($P < 0.05$) different.
ND = not determined.

However, TMAO, which represents a characteristic and important part of the NPN-fraction in most marine species, was present at a very low concentration, equivalent to 3.56 mgN/100 g sample (s.d. 0.17, $n = 3$). This was probably a reflection of the composition of the feed, which is generally formulated on a least-cost basis to include as little fish meal as practical. In this case the role of osmotic regulation that TMAO in part performs was probably taken over by free amino acids and other non-protein nitrogen compounds. The fact that the NPN-fraction was relatively high supports this idea.

Sensory assessment

Changes in the attributes of the raw fish during storage in ice were compiled (Table 3) using the descriptions given by the individual panel members. This table covers the range from freshly harvested to inedible fish. Changes occurring in the gills and skin of gilthead sea bream during storage have potential for use in the routine evaluation of whole, ungutted fish. Rigor mortis, metallic sheen and iridescence of the skin and glossy, bright red gills possessing seaweedy and shellfish odours should be considered as attributes of extreme freshness, whereas loss of brilliance and iridescence, fading of skin colours and bleaching of the gills in patches would indicate stale fish.

The sensory score for flavour of the cooked fillets decreased linearly with storage time (Fig. 1). The fresh flavour characteristic of the species was strong for 2–4 days, slowly decreasing in intensity to a bland, relatively flavourless stage by 10–12 days. Off-flavours, due to bacterial metabolites, were evident by 13–15 days. As spoilage progressed, the off-flavours increased in intensity

Table 3 Table of descriptive terms related to EC freshness grades for whole, round gilthead sea bream (raw fish) stored in ice

Days in ice	Skin	Outer slime	Eyes	Gills (appearance)	Gill and internal odours	EEC Grade
0	Bright; iridescent; metallic silver grey sheen; well differentiated colours	Glossy; thin; transparent	Bulging; convex lens; black bright pupil; translucent cornea	Glossy, bright pink or red; clear mucus	Fresh; iodine; seaweedy; shellfish odours	E
3			Convex lens; black pupil with slight loss of initial clarity		Less sharp seaweedy and shellfish odours	
6	Loss of brilliance of colour; very slight bleaching	Aqueous; transparent	Slight flattening of plane; loss of brilliance	Loss of gloss and brightness;	Freshly cut grass; weak seaweedy	A
10			Plane; slightly grey pupil; slight opacity of cornea	slight loss of colour; clear mucus	Slight musty, mousy, milk	
14	Dull with some bleaching; some loss of scales	Opaque and somewhat milky	Plane or concave; slight opacity and reddening of cornea	Bleached with some brown discolouration and cloudiness of the mucus	Musty; lactic; slight sour; boiled cabbage	B
17					Muddy; putrid; faecal; amines	B → C
21	Loss of differentiation; general fading of colours; overall dull greyish pigmentation	Yellowish-grey; clotted	Concave to sunken; grey pupil; opaque, red cornea	Brown or bleached; mucus yellowish-grey and clotted		C
24					Sour; faecal; acidic; sulphides	

and changed in character, until the fish became unpalatable by about 18–19 days.

Changes in indicator chemicals

Bases

During shelf-life, TMA-N concentration remained very low (Fig. 2). The level of 1 mg/100 g flesh which is thought to indicate the incipient spoilage in the teleost fish (Castell & Greenough, 1958) was reached after ≈19–20 days of iced storage, when fish had already been rejected by sensory assessment. The small increase in TMA over the storage period reflects the low starting level of TMAO in the flesh of maricultured gilthead sea bream and precludes the usefulness of this compound as a freshness indicator. Low TMA-N levels (1.8–4.8 mg/100 g flesh) at the point of rejection have been reported also for other Sparidae species (Civera *et al.*, 1995). The production of TMA and other volatile bases is due to the metabolism of bacteria and therefore is influenced by the particular microbial flora of the fish. In the case of cold water fish stored in ice, the main spoilage organism is *Shewanella putrefaciens* which has considerable ability to produce TMA. In warmer waters *Pseudomonas fragi* can be the dominant bacterial spoilage organism. This species does not produce TMA, so that spoilage can occur with little or no TMA production (Gram *et al.*, 1990; Gram & Huss, 1996). The microbial flora may depend on water temperature and hence vary during the year.

Total volatile basic nitrogen concentration determined at the start of the trial ranged from 25.4–26.8 mg per 100 g flesh (mean 26.0 mg/100 g flesh, s.d. 0.73) and remained at that level before rising almost exponentially to about 50 mg/100 g flesh between days 10 and 24 (Fig. 2). The concentration of TVB in freshly caught fish is typically between 5 and 20 mg TVBN/100 g flesh, whereas levels of 30–35 mg/100 g flesh are generally regarded as the limit of acceptability for iced stored cold-water fish (Connell, 1995). The high initial content of TVB may be attributed to the high level of NPN present in the flesh of gilthead sea bream. Breakdown of low molecular weight nitrogenous compounds occurs under the conditions of analysis, releasing volatile base nitrogen. However, due to the TMA levels remaining low throughout the trial, the increase in TVB during iced storage was slower than in typical demersal fish such as cod and hake (Whittle *et al.*, 1990; Perez-Villarreal & Howgate, 1987) and should be attributed essentially to ammonia produced from bacterial catabolism of nitrogen-containing compounds. Whatever the spoilage organisms were, they had a ready supply of nutrient material and possibly a substantial supply of glycogen (well fed fish). If so, the organisms would not need free amino acids and TVB would not be formed in the early stages of storage.

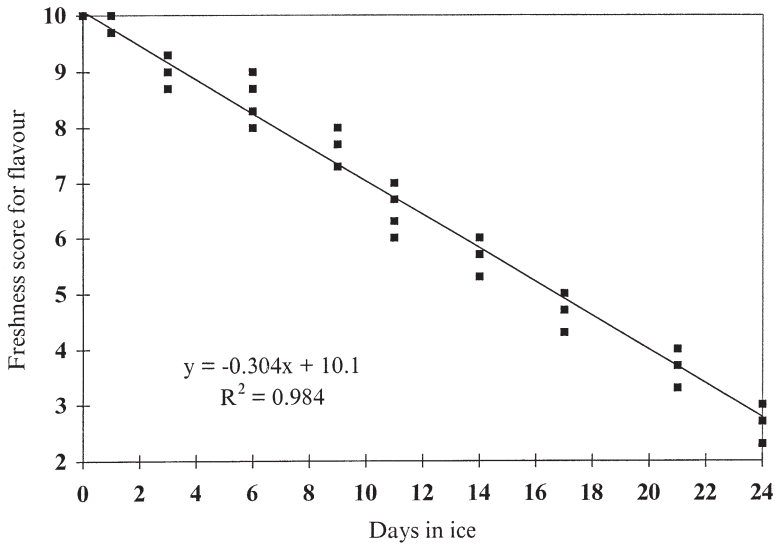

Figure 1 Changes in freshness score of cooked fillets from ungutted gilthead sea bream stored in ice. Each point represents the mean of three samples evaluated by individual panellists.

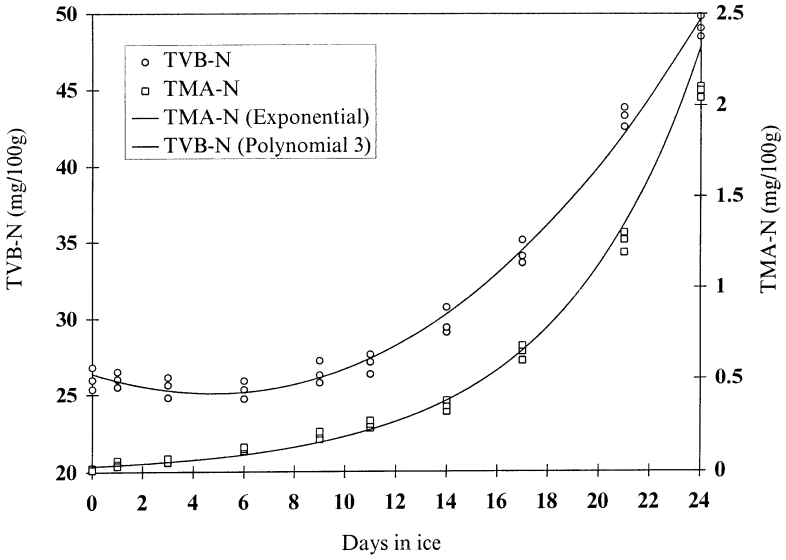

Figure 2 Changes in total volatile base (TVB) and trimethylamine (TMA) nitrogen levels in ungutted gilthead sea bream stored in ice. Three samples per storage time, assayed in duplicate.

Owing to the lack of significant change over the first half of the edible storage life of iced gilthead sea bream, TVB should be considered a very unreliable indicator of storage life, perhaps of potential use towards the end of the edible shelf-life of the fish (≈35 mg TVBN per 100 g flesh as the limit of acceptability). Factors such as age, locality and culture method may influence the content of non-protein nitrogenous compounds in fish muscle (Morishita *et al.*, 1989) and this could in turn affect TVB levels.

pH changes

Changes in pH over the period of iced storage are shown in Table 2. The low muscle pH early in the storage period reflected the good nutritional state of the fish. The low pH values encountered a few hours after harvesting may also indicate that the fish were not harvested in a rested state and that they had been stressed. The first pH measurements were made four hours after the death of the fish. The glycogen in the muscle would have been metabolised to lactic acid by then and would account for the low pH found. The typical pH of live fish muscle is ≈7.0. During the initial storage period the pH was consistently low (less than 6.2) and this may have contributed to the increased shelf-life of the fish used in this trial. However, at the end of the first week of storage pH started to increase, reaching a value of 6.6 by the end of the trial. The increase in pH values after day 7 reflected the production of alkaline bacterial metabolites in spoiling fish and coincided with the increase in TVBN.

Lipid changes

The oxidative and hydrolytic changes of muscle lipids during storage in ice are shown in Table 2. Icing of whole, round fish, tended to slow down the production of malonaldehyde, while it allowed more rapid hydrolysis of muscle lipids and accumulation of FFA. TBA value in the edible flesh remained low (less than 0.3 μmol g^{-1} lipid) and below the level (1–2 μmol g^{-1} lipid) at which rancid flavours may become evident in fish (Connell, 1995). In addition, none of the judges detected any rancidity in the cooked fillets during the edible shelf-life of the iced fish. Similar malonaldehyde concentrations have been reported for rainbow trout (Dawood *et al.*, 1986), spiny dogfish (Bilinski *et al.*, 1983) and roughhead grenadier (Botta & Shaw, 1975) stored in ice. Thus, according to the results obtained in this study, lipid oxidation does not appear to be a dominant spoilage process in ungutted gilthead sea bream stored in melting ice. This is in accord with the thesis that in wet fish storage, components introduced primarily by bacterial spoilage as well as by enzymic reactions contribute more to the flavour than those derived from lipid autoxidation (Hardy, 1980; Smith *et al.*, 1980, 1980a), even though in some species such as jack

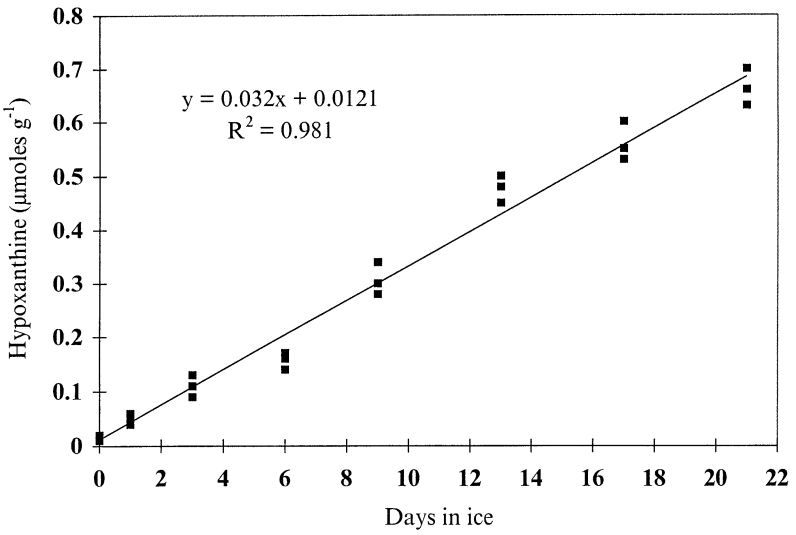

Figure 3 Linear regression for hypoxanthine (Hx) against time, in ungutted gilthead sea bream stored in ice. Three samples per storage time, assayed in duplicate.

mackerel and mullets which undergo rapid quality changes during iced storage, rancid flavours have been reported to affect acceptability and limit storage life (Ryder *et al.*, 1984; Lee & Toledo, 1984). The observed differences in susceptibility to oxidation between species may arise from the presence of higher concentrations of natural antioxidants in fish lipid as well as from a lower proportion of unsaturated fatty acids in the depot lipids. Exposure of the lipid to atmospheric oxygen, resulting from gutting of fish or skinning and mincing of fillets, also appears to accelerate oxidation.

The rate of lipid hydrolysis appeared to be more pronounced during the latter stages of storage, probably due to a greater diffusion of lipolytic enzymes from the viscera of spoiling fish, as well as to the intervention of bacterial lipases. However, FFA content did not appear to correlate with texture, taste, odour or overall acceptability of ungutted gilthead sea bream stored in ice. This result was not entirely unexpected as the consequences of lipolysis on the acceptability of fish and fish products are not so clear. In fact, although the terms rancid and soapy are often used as descriptors in taste panel score sheets, no correlation appears to have been established between the development of these flavours and fatty acid production (Hardy, 1980). Further breakdown of the fatty acids may have been brought about by bacterial lipoxidases, activating the fatty acid chain in a reaction with oxygen. Free fatty acids may also have been involved in reactions with other muscle constituents (e.g. proteins).

Hypoxanthine changes

Hypoxanthine concentration increased almost linearly over the storage period (Fig. 3). However, during the shelf-life the rate of increase was too low to be useful as an index of freshness. The slow build-up of hypoxanthine suggests that the complete ATP degradation cycle proceeds at a slower pace than in most species and puts gilthead sea bream in a category similar to jack mackerel (Ryder *et al.*, 1984), scad (Smith *et al.*, 1980b), witch flounder (Shaw *et al.*, 1977), japanese red bream (Ehira & Uchiyama, 1974) and a number of tropical fish (Bremner *et al.*, 1988) which are also slow to form Hx, averaging less than 1.0 μmol g^{-1} by day 10–12. The pattern of nucleotide metabolism in gilthead sea bream, particularly IMP and inosine levels and the potential use of these compounds as freshness indicators warrant a more fundamental study.

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