

Food Chemistry 72 (2001) 33-40

Food Chemistry

www.elsevier.com/locate/foodchem

Freshness assessment of cultured sea bream (Sparus aurata) by chemical, physical and sensory methods

C. Alasalvar^{a,*}, K.D.A. Taylor^a, A. Öksüz^a, T. Garthwaite^a, M.N. Alexis^b, K. Grigorakis^b

a Food Research Centre, University of Lincolnshire and Humberside, Nun's Corner, Grimsby, North East Lincolnshire, DN34 5AZ, UK ^bNational Centre for Marine Research, Laboratory of Nutrition, 16604 Agios Kosmas, Ellinikon, Athens, Greece

Received 29 February 2000; received in revised form 31 May 2000; accepted 31 May 2000

Abstract

The quality changes of cultured sea bream (Sparus aurata) stored in ice for a period of up to 23 days were determined by K and related values, sensory assessment and texture by texturometer. Sensory schemes, based on the Tasmanian Food Research Unit (TFRU) scheme for raw fish and on the Torry scheme for cooked fish were modified to be appropriate for whole cultured sea bream, according to the trained panellists' perceptions, during the storage period in ice. The TFRU sensory score of fish showed good agreement with K value and texture results throughout the storage period. The limit for acceptability of cultured sea bream stored in ice was about 17 -18 days. Generally, K, K_i and G values had good correlation with the degree of freshness and can be used as freshness indicators. \odot 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cultured sea bream; K value; Texture; Sensory assessment

1. Introduction

In Europe, the demand for fresh sea bream has increased significantly over the past decade due to its desirable aroma and quality, and consequently its high value has made the farming of the fish a profitable business. Many of the UK's big food stores sell this fish. To meet the increasing demand, many farmers on the Mediterranean coast have expanded their annual production (4620 Mt. in 1996; FAO, 1998), though it has been asserted that wild fish possess more aroma than their cultured equivalent (Kyrana, Lougovois & Valsamis, 1997). The increase in demand for this fish from Mediterranean coast to Northern European countries has resulted in a more competitive market. It is, therefore, of considerable interest to farming industries, retailers and consumers to investigate quality changes of cultured sea bream occurring during the handling, distribution and storage in ice.

The freshness is the single most important attribute when assessing fish quality. Although a variety of chemical and physical methods have been used to assess the freshness of fish during the storage, the main quality parameters for fresh fish are aroma, flavour, texture and sensory response.

The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius, 1990). Sensory methods, as opposed to non-sensory, offer the best opportunity of getting a valid idea of what the consumer wants because they provide immediate quality information (Connell, 1975). Although the sensory method is still the most satisfactory way of assessing the freshness of fish, it has limitations and therefore its use is potentially limited in fish processing and technology sites. However, most trade is based on sensory assessments, although measurements are not always objective and documented. Thus, when chemical and physical methods are being used for assessing the quality of fish, sensory evaluation should be conducted to ensure that those results show good agreement with the instrumental (objective) tests.

Various ratios of the concentration of ATP and its breakdown compounds have been widely used as

^{*} Corresponding author. Tel.: $+44-1472-874140$; fax: $+44-1472-$ 315099.

E-mail address: calasal.ast@gy.humber.ac.uk (C. Alasalvar).

^{0308-8146/00/\$ -} see front matter \odot 2000 Elsevier Science Ltd. All rights reserved. PII: S0308-8146(00)00196-5

chemical methods to estimate freshness quality in a wide variety of fish (Gill, 1990; Handumrongkul & Silva, 1994; Surette, Gill & LeBlanc, 1988). The pattern and rate of nucleotide degradation differs from one species to another, with body location, pre-death condition, handling, season, and storage conditions (Chiba, Hamaguchi, Kosaka, Tokuno, Asai & Chichibu, 1991; Luong & Male, 1992). Hence, one ratio is often more appropriate than others for a given species. For example, the G value of iced Atlantic cod (which rapidly accumulate Ino) has been reported to be much superior to the K_i value in North America (Burns, Kee & Irvine, 1985). Similarly, the H value of iced Pacific cod has been observed to be much superior to the K_i value (Luong, Male, Masson & Nguyen, 1992). These significant variations, both between and within species, obviously indicate the practical usefulness of measuring the concentration of a single nucleotide degradation product or a single nucleotide ratio to indirectly measure freshness quality (Greene, Babbitt & Reppond, 1990).

Texture of fish is commonly tested in the industry by the "finger method". A finger is pressed on the skin and firmness is evaluated as a combination of the hardness when pressed on fish and mark or hole left in the fish after pressing. This is not desirable to the consumers. This method depends to a large extent upon subjective evaluation of the person who is performing the measurements (Sigurgisladottir, Torrissen, Lie, Thomassen $&$ Hafsteinsson, 1997). Texture of raw fish can be measured by different methods using mechanical food testing equipment. The main techniques applied for fish are puncture, compression, shear and tensile stress. Among them, the shearing force and compression methods are recommended for use with fresh fish (Sigurgisladottir et al., 1999). When the texture of raw fish is measured, hardness and springiness are often the major variables (Botta, 1991).

This study was designed to investigate storage life and quality changes of cultured sea bream stored in ice, by using chemical, physical and sensory assessment. Further, it was intended to focus on sensory odours appropriate for cultured sea bream which could be used to monitor changes occurring during the storage period and determine shelf-life in ice.

2. Materials and methods

2.1. Materials

Cultured gilthead sea bream, Sparus aurata, (average weight and length: 375 g and 280 mm, respectively) used in this study were cultivated in net cages in a Greek farm and harvested in May 1999. All chemicals were purchased from Aldrich, Fluka and Sigma Chemicals Co., UK, unless otherwise indicated.

2.2. 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 DHL World Wide Expresses to the Food Research Centre, Grimsby, UK, within 1 day of harvesting. Six fish were immediately sampled (day 1), while the rest were repacked with an equal volume of flake ice into polystyrene boxes provided with holes for drainage. Boxes were stored in a cold store $(2\pm 2^{\circ}C)$ for a period of 23 days from the time of harvest and the ice: fish ratio was maintained throughout the trial. Chemical, physical and sensory analyses took place on days 1, 6, 10, 14, 17, 21 and 23. Sampling from white muscle in triplicate was continued over a 23 day storage period.

2.3. Proximate analysis

Moisture content was determined by drying a portion of prepared sample at $103 \pm 2^{\circ}$ C for 24 h. Total lipid was determined on a 20 g sample of the minced fillets using the extraction method of Bligh and Dyer (1959). Total crude protein was determined by the semi-micro Kjeldhal procedure using potassium sulphate and copper (II) sulphate as the catalysts (Egan, Kirk & Sawyer, 1981). 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.

2.4. Sensory assessment

The sensory assessment of fish was conducted using the Tasmanian Food Research Unit (TFRU), a system developed at CSIRO Division of Food Research (1984), Tasmanian Food Research Unit, Hobart. The panel normally consisted of at least five of 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 (see Table 2 below). The scores for the separate characteristics were summed to give an overall sensory score. This system gave score zero (or near zero) for very fresh fish while increasingly larger totals resulted as fish deteriorated. Minor variations in scoring individual attributes, therefore, had little influence on the overall score. Also, the original TFRU scheme was modified and scores developed to reflect the changes in cultured sea bream according to the panellists' perceptions during the storage period.

The measurements of freshness of cooked fish (odour, flavour and texture) were assessed (Howgate, 1982) according to the Torry scheme. A hedonic scale from 10

to \leq 3 was used, 10 showed absolutely fresh and \leq 3 completely putrid or spoiled. Fish fillets were cooked in a microwave for 3 min then served to the panellists after 5 min. The fish were assessed for odour, flavour and texture. The original Torry scheme was also modified for cultured sea bream according to the panellists' perceptions during the storage period.

2.5. ATP breakdown compounds

ATP-related compounds were determined according to Ryder (1985). The chromatography consisted of a Merck Hitachi L-6000 pump, PU- 4020 UV detector and Elonex PC 466 Computer. A 5 ul sample was injected after filtration through a 0.45 µm filter. Nucleotides were separated by a 5 μ m 100 RP C18 column 250 \times 4 mm ID. Mobile phase was 0.04 M potassium dihydrogen orthophosphate (KH_2PO_4) and 0.06 M dipotassium hydrogen orthophosphate (K_2HPO_4) dissolved in purified HPLC water. The buffer solutions

Table 1 Proximate analysis $(\%)$ of cultured sea bream^a

Protein	18.0 ± 1.19
Fat	6.53 ± 1.27
Moisture	74.74 ± 0.54
Ash	1.53 ± 0.05

^a Data are expressed as mean \pm S.D. (*n* = 5).

were prepared daily (pH 7). The flow rate was 2 ml/min . The monitoring wavelength was set at 254 nm for ATP breakdown compounds.

The K , K_i , G and H values were calculated according to Saito, Arai and Matsuyoshi (1959), Karube, Matsuoka, Suzuki, Watanabe and Toyama (1984), Burns et al. (1985) and Luong et al. (1992), respectively.

$$
K(\%)
$$

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

$$
K_{\text{i}}(\%) = \left[\frac{(\text{Ino} + \text{Hx})}{(\text{IMP} + \text{Ino} + \text{Hx})} \right] * 100
$$

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

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

where: ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; IMP, inosine 5'-monophosphate; Ino, inosine; Hx, hypoxanthine.

2.6. Texture measurement

Texture measurement was carried out according to Sigurgisladottir et al. (1999). The TA.XT2 Texture

Table 2 Modified Tasmanian Food Research Unit (TFRU) sensory assessment scheme for cultured sea bream

Parameters being assessed	Demerit points ^a					
	$\boldsymbol{0}$		$\overline{2}$	3		
Appearance	Very bright	Bright	Slightly dull	Dull		
Skin	Firm or elastic	Soft				
Slime	Absent	Slightly slimy	Slimy	Very slimy		
Stiffness	Pre-rigor	Rigor	Post-rigor			
Eyes						
Clarity	Clear	Slightly cloudy	Cloudy			
Shapeb	Normal	Flat sunken	Sunken or swollen			
Iris	Visible	Slightly visible	Not visible			
Blood	No blood	Slightly bloody	Bloody	Very bloody		
Gills						
Colour ^b	Dark red	Red	Brown	Dark brown or grey		
Mucus	Absent	Slight	Moderate	Excessive or sticky		
Smell	Neutral	Fishy	Stale	Spoilt		
Belly						
Discoloration	White iridescent	Some yellowish	Yellow	Excessive yellow		
Firmness	Firm	Soft	Sunken	Burst		
Vent						
Condition	Normal	Slight break and darkening	Excessive			
Smell	Fresh	Neutral	Fishy	Spoilt		

 $^{\rm a}$ Total demerit points (0–38).

^b Eyes shape and gills colour are not good indicators for cultured sea bream during storage period in ice.

Analyser was used (Stable Micro System, Surrey, UK). A flat-ended cylinder that simulates the human finger was applied. Constant penetration depth of 2.5 mm was applied on the fillets of around 10 mm thickness after testing penetrations in the range of $2-5$ mm. This penetration depth was selected as the maximum distance which could be applied without breaking the muscle fibres and affecting the muscle structure by erupting it and leaving a mark on the fillet. 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 analyses (TPA) 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. Then the force was reduced and the fillet was allowed to rebound 10 s with the cylinder just touching the surface. After this, the cylinder was pressed on the fillet a second time and TPA was obtained by analysing the force and time curve. The hardness was the height of the fist peak. Six fillets out of three fish were used for analysis.

2.7. Statistical analysis

Statistical significance was checked by using the two sample *t*-test, assuming equal variances.

3. Results and discussion

3.1. Proximate analysis

Proximate analysis conducted at day 0 is shown in Table 1. Initial experimental work carried out in this laboratory on wild and cultured sea bream showed that cultured sea bream possessed a considerably higher (5.5 times) lipid level than wild fish, with a correspondingly lower moisture content. This was probably due to the reduced activity of fish and fat level in the feed. Protein and ash contents were almost the same percentage in both fish.

3.2. Sensory assessment

The TFRU system was used in this study. Some of the descriptors on the original score sheet were not related to the cultured sea bream. Therefore, this original score sheet (CSIRO Division of Food Research, 1984) was modified to be appropriate for cultured sea bream according to the panellists' perceptions obtained during the storage period in ice (Table 2). The developers of the TFRU system observed that it was necessary to modify the general TFRU system for a "new" species (Botta, 1995). Some of the descriptions such as: "skin", "eyes shape", "gills colour" "mucus" and "smell", "belly discoloration" and "firmness", and "vent condition"

were modified. However, "eyes shape" and "gills colour'' which changed unevenly during the storage period, were found not to be good indicators of freshness for this fish. "Rigor mortis", "very bright appearance", "dark red gills" "possessing seaweedy" and "shell-fish odours'' should be considered as attributes of extreme freshness, whereas "loss of brilliance", "sunken and bloody eyes", "dark brown or grey gills colour" and "spoilt smell" and "excessive discoloration" in belly area would indicate stale fish. A list of descriptive terms obtained from the Modified TFRU sensory assessment scheme for cultured sea bream stored in ice is shown in Table 3. Botta (1995) said that whenever one intends to use the TFRU system for a "new" species, preliminary studies must be conducted to ensure that all the criteria and their corresponding defined characteristics incorporated in the grade standards are appropriate and will actually be used. The TFRU system has already been modified for iced anchovy, Atlantic cod, Atlantic herring, plaice, red fish, saithe and sardine (Botta, 1995).

The changes in TFRU sensory score of raw cultured sea bream during 23 storage days in ice are shown in Fig. 1. In this scheme, θ showed absolutely fresh fish and 38 completely spoiled, a score of about 20 coincided with the level at which the fish were considered unacceptable by the members of the panel. The sensory score increased linearly (r^2 = 0.99) with storage time. The limit for acceptability of cultured sea bream stored in ice was about 17-18 days. However, the perceived level of acceptability of sea bream quality depends on the particular buyer, user or regulatory agency.

The modified Torry scheme for cooked sea bream is shown in Table 4. Fig. 1 shows the changes in sensory quality (odour, flavour and texture) of cooked cultured sea bream through 23 days of storage. The characteristic odour of sea bream gradually decreased $(r^2 = 0.98)$ in intensity during storage. In this scale, 10 showed absolutely fresh fish ("strong seaweedy odours"; "fresh sweet flavour characteristic"; "dry" and "crumbly with short fibrous texture") and ≤ 3 showed completely putrid or spoiled ("composted grass" and "boiled clothes-like odours"; "strong bitter", "rubber", "slight sulphide" and "putrid flavour"; "much less succulent" and "softer texture"). A score of 4 ("lactic acid" and "sour milk odours"; "slight bitterness" and "sour off flavours"; "less succulent" and "softer texture") was considered unacceptable by the members of the panel.

The fresh flavour characteristic of the sea bream was strong for 1–6 days, slowly decreasing in intensity to a bland, relatively flavourless stage by $10-14$ days. Offflavours were evident by day 17. As spoilage progressed, the off-flavours increased in intensity and changed in character, until the fish became unacceptable at about $21-23$ days.

Although sea bream were unacceptable on day 17, using the TFRU sensory score, cooked sea bream was

Table 3 Table of descriptive terms obtained from Modified TFRU sensory assessment scheme for cultured sea bream stored in ice

-1	6	10	14	17	21	23
Very bright	Bright	Bright	Slightly dull	Slightly dull	Dull	Dull
Firm or elastic	Firm or elastic	Firm or elastic	Soft	Soft	Soft	Soft
Absent	Absent	Absent	Slightly slimy	Slightly slimy	Slimy	Very slimy
Rigor	Post-rigor	Post-rigor	Post-rigor	Post-rigor	Post-rigor	Post-rigor
Clear	Clear	Slightly cloudy	Slightly cloudy	Slightly cloudy	Cloudy	Cloudy
Normal	Normal	Flat sunken	Flat sunken	Flat sunken	Sunken	Sunken
Visible	Visible	Visible	Visible	Slightly visible	Slightly visible	Not visible
No blood	No blood	Slightly bloody	Slightly bloody	Slightly bloody	Slightly bloody	Bloody
Red	Red	Red	Red	Brown	Dark brown	Dark brown
Absent	Slight	Slight	Moderate	Moderate	Moderate	Moderate
Neutral	Fishy	Fishy	Stale	Spoilt	Spoilt	Spoilt
White iridescent	White iridescent	White iridescent	Some yellowish	Some yellowish	Some yellowish	Some yellowish
Firm	Firm	Soft	Soft	Soft	Soft	Sunken
Normal	Normal	Normal	Normal	Normal	Slight break	Slight break
Fresh	Neutral	Neutral	Fishy	Fishy	Spoilt	Spoilt

Fig. 1. Changes in sensory quality of raw and cooked cultured sea bream stored in ice. Torry (cooked fish): Score 10: absolutely fresh 0; completely putrid. TFRU (raw fish): Score 0: absolutely fresh; 38: completely spoiled. Data are mean of results from 5 trained panellists. Average relative standard deviation (RSD) fro TFRU: 8.5%; Torry: 5.5%. r² Values of linear regression are 0.99 (TFRU) and 0.98 (Torry) with time.

considered of acceptable quality (Fig. 1), characterised by "boiled potatoes", "caramel toffee-like odour" and "slight sourness", but not "off-flavours" (Table 4). The reason for this could be explained in that cooking would appear to either mask undesirable changes observed in fish provided these changes are not extreme, or, possibly remove some of the volatile "off" odours. Similar results were also found in mackerel stored in ice for 12 days (Alasalvar, Quantick & Grigor, 1999). Cooking can also quite significantly mask differences in precooked duckling with taste panellists having difficulty in distinguishing "relatively fresh" from "obviously deteriorated'' duckling post-cooking (J. Hanna, pers. comm., 1993).

3.3. ATP-related compounds

Fig. 2 depicts the changes in ATP, ADP, AMP, IMP, Ino and Hx concentrations in raw cultured sea bream stored in ice. The main changes occurred in IMP, Ino and Hx, whereas ATP, ADP and AMP remained approximately constant in very low concentration $(< 0.2$ umol/g) during the 23 day storage period. It has been reported that the conversion of ATP to IMP is usually complete within 1 day and is presumed to be totally autolytic (Hiltz, Dyer, Nowlan & Dingle, 1972; Jones, 1965). The level of IMP in fish flesh was over 8 μ mol/g on day 1 and then decreased steadily to 3.5 mmol/g on day 23. Ino and Hx levels increased during storage period. These increases were due to both autolytic and microbial enzymes (Surette et al., 1988), which caused spoilage.

It is recognised that Hx content in fish can be an accurate indicator of fish freshness in many fish species (Burns & Kee, 1985; Jacober & Rand, 1982; Zhang & Lee, 1997). Kyrana et al. (1997) studied the shelf-life of maricultured gilthead sea bream in ice and found that Hx concentration increased almost linearly over the storage period. Hx was not detected until after day 6 in this study. However, the formation of Hx has been reported to vary considerably both within a given species (Huss, 1988) and within an individual fish, as its formation may be greater in red muscle than in white muscle (Murata & Sakaguchi, 1986; Watabe, Kamal & Hashimoto, 1991). In addition, using Hx content without using any other index of freshness quality, may be misleading whenever a fish has been processed during

Fig. 2. Changes in concentration of ATP breakdown compounds in raw cultured sea bream stored in ice. Values shown are the mean of tripicate measurement. Avertage RSD: 8.2%.

the latter stages of its storage life (Botta, 1995). Hx can be oxidised to xanthine, which in turn can be oxidised to uric acid. It has been reported that, during chilled storage, Hx content can reach a maximum and can then begin to decline (Jacober & Rand, 1982). This study with cultured sea bream found that Hx level stopped increasing after day 17. Although Hx has been reported to be a good indicator of freshness during the storage period, it was not found to correlate with the freshness quality of cultured sea bream.

3.4. K and related values

There were good time-independent relationships between the K value and sensory evaluation by TFRU, with r^2 value of linear regression ($r^2 = 0.98$) over the

storage period (Fig. 3). K value concentration also increased linearly $(r^2 = 0.99)$ over the storage period (Fig. 4). As the K value increased, the sensory quality of sea bream decreased. When the fish was considered unacceptable by the members of the panel on day 17, the K value was 39% . This value differs between species; for example, Lee, Ohshima and Koizumi (1982) found that the K value in rainbow trout increased from 3.4% (day: 0) to 83% (day: 6) during storage at 5° C.

Changes in K and related values $(H, G \text{ and } K_i)$ of raw cultured sea bream stored in ice are illustrated in Fig. 4. These values showed linear increases with the storage time. Linear regressions (r^2) obtained from H, G, K and K_i were 0.84, 0.99, 0.99 and 0.99, respectively. The worst value obtained from the linear regression was for H, which is based on Hx. It was not detected on days 1 and 6. There were no significant $(P>0.05)$ differences between the K and K_i values during the storage period. ATP degraded to IMP very soon after death and, consequently, a K_i value may be used which does not involve determination of ATP, ADP or AMP. Where the K_i value is used, with some species it increases very rapidly and then remains constant. This is due to accumulation of a large quantity of Ino relative to the quantity of Hx produced. Whilst this gives a reasonable indication of freshness in terms of quality parameters (which may refer to raw seafood consumption), it does not accurately reflect the freshness of some species of fish in other parts of the world (Garthwaite, 1997). However, it should be noted that ATP, ADP and AMP remain in some species of the fish even after 2 weeks (Karube et al., 1984) so the K value can be superior to the other values. For cultured sea bream, K , K_i and G values can be used as reliable quality indicators.

Fig. 3. Time-independent relationship between the K value and TFRU, and between the hardness and TFRU over the storage period. r^2 Values of linear regression are 0.98 (*K*-value with TFRU), and 0.99 (Hardness with TFRU).

Fig. 4. H , G , K and K_i value changes of raw cultured sea bream stored in ice. Values shown are the mean of triplicate measurements, Average RSD: 3.6%. r^2 Values of linear regression are 0.84 (*H*), 0.99 (*G*), 0.99 (K) and 0.99 (K_i) with time.

3.5. Texture

Fig. 5 shows the hardness of sea bream fillets measured by flat-ended cylinder. There were statistically significant ($P < 0.05$) differences in hardness during the storage period. Initial hardness of fish was 7.4 N on day 1 and gradually decreased over the storage period. When the fish was considered unacceptable on day 17, hardness of fish was over 5 N . This value may differ between species. There was also a good time-independent relationship between the hardness and sensory evaluation by TFRU, with r^2 value of linear regression $(r^2 = 0.99)$ over the storage period (Fig. 3).

The thickness of the fillets varied from head to tail, from 12 mm at the front part to 7 mm thick at the tail. The results, using samples of equal thickness, showed that hardness increased from head to tail, with decreasing thickness of the original fillet. Sigurgisladottir et al. (1999) studied the textural properties of raw salmon fillets and found similar results. Measurement of hardness in

Fig. 5. Texture analysis of raw cultured sea bream stored in ice. Values shown are the mean of 18 measurements. Average RSB: 16.9%. r^2 Value of linear regression is 0.99.

fish can be used as a quality indicator and showed good correlation with sensory and chemical results (Fig. 3). Although the shearing force method is recommended for raw salmon fillets as against the compression method (Sigurgisladottir et al., 1999), the latter is less destructive than the shearing force.

4. Conclusions

The modified TFRU sensory score for sea bream had good time-independent correlations with K value $(r^2=0.98)$ and hardness $(r^2=0.99)$ results over the 23 day storage period (Fig. 3). The limit for acceptability of cultured sea bream stored in ice was about $17-18$ days. The K value reached 39% and hardness decreased to 5 N when fish were organoleptically rejected. Generally, K , K_i and G values had good correlation with the degree of freshness and can be used as freshness indicators.

Acknowledgements

The authors gratefully acknowledge funding from the European Union for this work.

References

- Alasalvar, C., Quantick, P. C., & Grigor, J. M. (1999). Effect of cooking on consumers perceptions of mackerel (Scomber scombrus) of poor quality. In Y. L. Xiong, C. T. Ho, & F. Shahidi, Quality attributes of muscle foods (pp. 211-218). New York: Kluwer Academic/Plenum Press.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911-917.
- Botta, J. R. (1991). Instrument for non-destructive texture measurement of raw Atlantic cod (Gadus morhua). Journal of Food Science, 65, 962-968.
- Botta, J. R. (1995). Sensory evaluation: Freshness quality grading. In J. R. Botta, *Evaluation of seafood freshness quality* (pp. 65–97). New York: VCH Publishers.
- Burns, G. B., & Kee, P. J. (1985). Liquid chromatographic determination of Hx in fish tissue. Journal of Association of Official Analytical Chemistry, 68, 444-447.
- Burns, G. B., Kee, P. J., & Irvine, B. B. (1985). Objective procedure for fish freshness evaluation based on nucleotide changes using a HPLC system. Canadian technical report of fisheries and aquatic science no. 1373.
- Chiba, A., Hamaguchi, M., Kosaka, M., Tokuno, T., Asai, T., & Chichibu, S. (1991). Quality evaluation of fish meat by phosphorusnuclear magnetic resonance. Journal of Food Science, 56, 660-664.
- Connell, J. J. (1975). Control of fish quality. London: Fishing News Books Ltd.
- CSIRO Division of Food Research (1984). Annual report. CSIRO Division of Food Research, Camberra, Australia.
- Egan, H., Kirk, R. S., & Sawyer, R. (1981). Pearson's chemical analysis of foods (8th ed.). London: Academic Press.
- FAO (1998). Fishery statistics: Capture production (Vol 82). Rome: Food and Agriculture Organisation of the United Nations.
- Garthwaite, T. (1997). Chilling and freezing of fish. In G. M. Hall, Fish processing technology (2nd ed.) (pp. 93–118). Glasgow: Chapman and Hall.
- Gill, T. A. (1990). Objective analysis of seafood quality. Food Reviews International, 6, 681-714.
- Greene, D. H., Babbitt, J. K., & Reppond, K. D. (1990). Patterns of nucleotide catabolism as freshness indicators in flat fish from the Gulf of Alaska. Journal Food Science, 55, 1236-1238.
- Handumrongkul, C., & Silva, J. L. (1994). Aerobic counts, colour and adenine nucleotide changes in $CO₂$ packed refrigerated striped bass strips. Journal of Food Science, 59, 67-69.
- Hiltz, D. F., Dyer, W. J., Nowlan, S., & Dingle, J. R. (1972). Variation of biochemical quality indices by biological and technical factors. In R. Kreuzer, *Fish inspection and quality control* (pp. $191-195$). London: Fishing News Books Ltd.
- Howgate, P. F. (1982). Quality assessment and quality control. In A. Aitken, I. M. Mackie, J. H. Merritt, & M. L. Windsor, Fish handling and processing (pp. 177-186). Edinburgh: HMSO Press.
- Huss, H. H. (1988). Fresh fish: Quality and quality changes. Rome: Food and Agriculture Organisation of the United Nations.
- Jacober, L. F., & Rand, J. A. G. (1982). Biochemical evaluation of seafood. In R. E. Martin, G. J. Flick, C. E. Hebard, & D. R. Ward, Chemistry $& biochemistry of marine food products (pp. 347–366).$ Wesport CT: AVI Publishing Company.
- Jones, N. R. (1965). Hypoxanthine and other purine-containing fractions in fish muscle as indices of freshness. In R. Kreuzer, The technology of fish utilisation (pp. 179-183). London: Fishing News Books Ltd.
- Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E., & Toyama, K. (1984) . Determination of fish freshness with an enzyme sensor system. Journal of Agricultural and Food Chemistry, 32, 314-319.
- Kyrana, 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, 339±347.
- Lee, E. H., Ohshima, T., & Koizumi, C. (1982). High performance liquid chromatographic determination of K value as an index of freshness of fish. Bulletin of the Japanese Society of Scientific Fisheries, 48, 255.
- Luong, J. H. T., & Male, K. B. (1992). Development of a new biosensor system for the determination of the hypoxanthine ratio, an indicator of fish freshness. Enzyme and Microbial Technology, 14, 125±130.
- Luong, J. H. T., Male, K. B., Masson, C., & Nguyen, A. L. (1992). Hypoxanthine ratio determination in fish extract using capillary electrophoresis and immobilised enzymes. Journal of Food Science, 57, 77-81.
- Murata, M., & Sakaguchi, M. (1986). Storage of yellowtail (Seriola quinqueradiata) white and dark muscle in ice: Changes in content of adenine nucleotides and related compounds. Journal of Food Science, 51, 321-326.
- Reineccius, G. (1990). Off-flavours in foods. Critical Reviews in Food Science and Nutrition, 29, 381-402.
- Ryder, J. M. (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry, 33, 678±680.
- Saito, T., Arai, K., & Matsuyoshi, M. (1959). A new method for estimating the freshness of fish. Bulletin of the Japanese Society of Scientific Fisheries, 24, 749-750.
- Sigurgisladottir, S., Hafsteinsson, H., Jonsson, A., Lie, O., Nortvedt, R., Thomassen, M., & Torrissen, O. (1999). Textural properties of raw salmon fillets as related to sampling method. Journal of Food Science, 64, 99-104.
- Sigurgisladottir, S., Torrissen, O., Lie, O., Thomassen, M., & Hafsteinsson, H. (1997). Salmon quality: methods to determine the quality parameters. Reviews in Fisheries Science, 5, $1-30$.
- Surette, M. E., Gill, T. A., & LeBlanc, P. J. (1988). Biochemical basis of postmortem nucleotide catabolism in cod (Gadus morhua) and its relationship to spoilage. Journal of Agricultural and Food Chemistry, 36, 19±22.
- Watabe, S., Kamal, M., & Hashimoto, K. (1991). Postmortem changes in ATP, creatine phosphate and lactate in sardine muscle. Journal of Food Science, 56, $151-153$.
- Zhang, H. Z. & Lee, T. C. (1997). Gas chromatography-mass spectrometry analysis of volatile flavour compounds in mackerel for assessment of fish quality. In F. Shahidi & K. R. Cadwallader, Fla vour and lipid chemistry of seafoods, (pp. 55-63) ACS Symposium Series 674. Washington DC: American Chemical Society.