# A Quality Index Method-based evaluation of sensory quality of red mullet (*Mullus barbatus*) and its shelf-life determination

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## Abstract

Every day the consumer must choose a food product rather than others based on its quality; for fishery products the main quality parameter is freshness. Implementation of the Quality Index Method (QIM) in the fish industry provides information on fish quality; therefore, it is important for effective quality and process management in the production of high-quality fish products. The present study aims to validate the shelf-life study of fresh red mullet (*Mullus barbatus*) stored in ice previously presented by Özyurt in 2009 through Torry scheme in cooked filet and microbiological evaluation. Next, this revised scheme was applied to determine the shelf-life. It included seven descriptors and varies from 0 to 15 points. The use of a modified QIM scheme showed a good correlation between the quality index and days of storage time, with a $R^2$ value of 0.9698. In fact, all the subjects examined reached the end of their commercial life, or the day of sensory rejection, in 9-10 days with a Quality Index of 13.83.

## Introduction

Red mullet (*Mullus barbatus*) is a bony fish, belonging to the order of *Perciformes.* It is widely spread in the Mediterranean Sea, the Black Sea and Western Atlantic (FAO). In Adriatic Sea (FAO area 37.2.1), there are two species of *Mullidae* Family: red mullet (*M. barbatus*) and stripped mullet (*M. surmuletus*) (Jardas, 1996). It is fished with trawl nets, generically defined as those gear whose fishing is determined by being "towed" (in mid-water or on the seabed) by hand or using a boat. It is fished all year around, especially in autumn. Indeed, in this period the juveniles of *M. barbatus* take refuge along the coast to find suitable conditions for growth. Therefore, the fishing boats, in this period are located closer to the coast and then they move away with the succession of months to follow the migrations of the mullet's shoal. Red mullet (*M. barbatus)* is usually eaten in the Adriatic region, and it is mainly marketed fresh or frozen. Sensory analysis has been shown to be an important tool to assess the quality of most food products. The definition of quality is very complex within the food industry, especially in seafood. Botta (1995) defined the main quality attributes for seafood: safety, nutritional characteristics, availability, convenience, integrity, and freshness. Freshness loss of seafood is the result of biochemical, physic-chemical, and microbiological post-mortem processes of each species that are also influenced by handling on board and technological processing. These changes are perceived and can be evaluated in sensory terms by sight, touch, odor, and flavor (Huidobro *et al.*, 2006). The most used method for quality assessment in the inspection service and fish industry is the EU Freshness Grading or EC scheme [Regulation (EC) No 2406/96]. Mullet species were citied in article 3, clause 1, letter "a" in fish saltwater fish list [Regulation (EC) No 2406/96].

The EC scheme for quality grading of fish products includes three levels: E (Extra, the highest quality), A (good quality), and B (satisfactory quality). Below level B (lower than B level is sometimes called unfit or C) fish is no longer acceptable for human consumption, thus it is rejected or discarded [Regulation (EC) No 2406/96: article 4].

However, this scheme is somehow limited when the quality assessment of some fish species is requested: it does not clarify differences between species that have different ethological behavior (*i.e.*, benthonic and pelagic species) and different *genera* (bony and cartilaginous fish: dogfish and mackerel). It only uses general parameters for describing changes of iced fish (Luten and Martinsdottir, 1997; Nilsen and Esaiassen, 2005). Therefore, it is important to have an effective method to assess the freshness of products in a fast, easy, and reliable way. The use of descriptive analysis, such as the Quality Index Method (QIM) is currently increasing for sensory analysis of marine products. The QIM is a descriptive, fast, and simple method to evaluate the freshness of seafood (Hylding and Green-Pettersen, 2005). This seafood freshness grading system is based on significant sensory parameters for raw fish with a scoring system from 0 to 3 demerit points. Since no excessive emphasis is attributed to a single parameter, a sample is not rejected based on a single criterion (Freitas *et al.*, 2021). Therefore, the minor differences in results for any criterion do not unduly influence the total QIM score (Nilsen and Esaiassen, 2005). Specifically, in each fish species, QIM evaluates sensory parameters and attributes that change significantly during the degradation processes (Huidobro *et al.*, 2006). The score for all characteristics is summarized in an overall sensory score that increases when quality decreases. However, the efficiency of QIM depends on sensory parameters, sampling size, sampling condition, assessors experience (Bernardo *et al.*, 2020). For continuous study and continuous research related to QIM schemes, it is possible to construct new schemes or validate existing ones by researchers who validate or increase their effectiveness and accuracy. The present study aims to validate the shelf-life study of fresh red mullet (*M. barbatus*) stored in ice previously presented by Özyurt (2009) through Torry scheme in cooked filet, sensory evaluation on the fresh products (Martinsdottir and Magnusson, 2001) and microbiological evaluation to obtain further information on products deterioration and shelf-life (Chytiri *et al.*, 2004).

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## Materials and methods

### Mullet samples

The fish were caught in winter, along the Adriatic coast of San Benedetto del Tronto (Marche region, Italy, FAO area 37.2.1). The fish were presented in 3 batches (70 whole red mullets every batch, 210 in total) measuring 470×253×86.5 mm. The fish weighed between 47±5 g and they were 13,5±0,5 cm long on average. Ungutted fish were placed in clean insulated containers, stored in flaked ice, and delivered to the laboratory of the University of Teramo, Italy (within 1 hour after harvest). The cold chain was always respected. For the storage, fish were stored in a refrigerator (0±2°C) with an appropriate quantity of flaked ice on top. During this time, every 24 hours the thawing liquid was removed, the fish were turned to avoid modification caused by ice and the melted ice was replaced. Two batches were used by the panel team for this evaluation, as performed by Ozyurt (2009) QIM scheme; one of them was used for the new QIM scheme based on evidence emerged from the previously mentioned batches.

### Sensory analyses

According to the guidelines for sensory evaluation proposed by Martinsdòttir *et al.* (2001) and Hyldig *et al.* (2004) for fish species, five people participated in the sensory analysis (two women and three men). All members had previous training in developing and using fish QIM schemes according to ISO 5492 and ISO 8586. Informed consent was obtained for 5 judges. In each session, each judge received a fish sample, randomly sampled and placed on white observation plates for 30 minutes before each sensory evaluation. Every sample was not washed with tap water before the presentation to panellists, since Huidobro *et al.* (2006) reported that it could influence the sensory quality of some species. A total of 140 ungutted red mullet (*M. barbatus*) were used in the validation of QIM scheme performed by Ozyurt *et al.* (2009): 70 to QIM validation, 35 Torry scheme and 35 for microbiological evaluation; 70 samples were used in the validation of revised QIM scheme and its application in the study of shelf-life.

### Sensory analyses QIM scheme performed by Ozyurt *et al.* (2009) in raw and cooked fish

The panel team conducted the sensory analysis on the raw fish using the scheme proposed by Ozyurt *et al.* (2009) to analyse the parameters change of red mullet during storage in ice, starting from the arrival in the laboratory (day 0) and, subsequently, every 24 hours until the evident end of the shelf-life (Table 1).

All observations of the red mullet were conducted under standardized conditions, with as little interruption as possible, at room temperature and under white, fluorescent light.

The evaluation of the cooked samples was carried out using modified Torry scoring system as proposed by Alasalvar *et al.* (2001) for cultured seabream (Table 2).

The Torry scheme used assigned a score to each sample according to a structured scale from 10 to 3. A score of 10-9 indicates absolute freshness, 8 indicates a good quality product, 7-6 indicates a tasteless or neutral product, 5-4 indicates the presence of slight extraneous odors and tastes (initial state of alteration), and 3 indicates the presence of severe extraneous odors and tastes (putrid product) (Table 2).

Samples were deemed unfit for consumption when the overall sensory score for the odor and flavor of the product was lower than 6.

After cooking, four judges evaluated the parameters of the flesh in independent conditions and with the right light and temperature conditions.

### Microbiological analysis

A total of 35 samples were collected after QIM evaluation to estimate total mesophilic counts (TMC) and total psychrophilic counts (TPC) dependent of storage day to evaluate the hygienic conditions as previously reported by Kuvei *et al.* (2019). Fish epiaxial muscles were collected, after aseptic removal of skin, under condition of sterility through the incision on the same side and section of the fish body by hand, with gloves. All employed tools were sterilized. Twenty-five grams of flesh were weighed in stomacher bags containing 225 mL of Maximum Recovery Diluent (MRD, Oxoid Ltd., UK) to obtain 10-fold dilutions. Mixing was performed in a stomacher for 2 minutes at room temperature. Additional 10-fold dilutions were made as needed. The TMC was done on Standard Plate Count Agar (PCA, Oxoid Ltd., UK) by the pour plate technique according to ISO 4833 (2013). The plates were incubated at 30°C for 48 h. TPC was done on Standard Plate Count Agar (PCA, Oxoid Ltd., UK) by the pour plate technique according to ISO 17410 (2019). The petri dishes were incubated at 6.5°C for 10 days. Data obtained from the bacterial counts are expressed in Log CFU/g.

**Table 1. Quality Index Method. Reproduce from: Ozyurt *et al.* (2009).**

| Quality parameters | Description | Demerit point |
|---|---|---|
| Skin appearance | Very bright | 0 |
| | Bright | 1 |
| | Mat | 2 |
| Blood on gills cover | None | 0 |
| | Same | 1 |
| | Much | 2 |
| Flesh texture | Hard | 0 |
| | Firm | 1 |
| | Soft | 2 |
| Belly texture | Firm | 0 |
| | Soft | 1 |
| | Burst | 2 |
| Odour | Fresh sea odour | 0 |
| | Neutral | 1 |
| | Slight off odour | 2 |
| | Strong off odour | 3 |
| Eyes appearance | Bright | 0 |
| | Somewhat lustreless | 1 |
| Eyes shape | Convex | 0 |
| | Flat | 1 |
| | Sunken | 2 |
| Gills odour | Characteristic red | 0 |
| | Somewhat pale, mat, brown | 1 |
| Gills colour | Fresh, seaweddy, metallic | 0 |
| | Neutral | 1 |
| | Slight off odour | 2 |
| | Strong off odour | 3 |
| Quality Index (Demerit points) | | 0-18 |

### Sensory analyses revised QIM scheme and shelf-life estimation

After validation of QIM scheme performed by Ozyurt *et al.* (2009), 70 samples were performed to validate a revised scheme and estimate shelf-life. More in details, revised QIM was carried out in three phases: a descriptive valuation of sensory analysis of Ozyurt *et al.* (2009) scheme focusing on correlation analysis of the descriptors with the QI, to assess their relevance. These sensory analyses were carried out with samples that were thawed at refrigeration temperatures for different, previously selected lengths of time. In order to validate the QIM scheme, first a correlation analysis of the descriptors with the QI, to assess their relevance, was done. Secondly, the scope of the objective of the QIM scheme was verified by means of a regression analysis. Shelf life determination was solved as a calibration problem in which its estimate and associated precision were obtained using inverse prediction methods based on the linear fit obtained in the validation phase of the QIM scheme

### Data analysis

Data obtained from the sensory evaluation of raw and cooked mullet were submitted to time-dependent linear regression analysis. The equation of best fit and correlation coefficient ($R^2$) of QI, Torry index, TMC and TPC against storage time in ice, were calculated using XL Stat (2001) software package.

## Results and discussion

### Sensory evaluation of raw

The QIM scheme developed by Ozyurt *et al*. (2009) lists a careful description of quality attributes in whole raw red mullet (*M. barbatus*) during storage in ice. However, the QIM scheme for *M. barbatus* used by the authors wasn't developed specifically for the red mullet but it is the result of the modification of the pre-existing scheme developed for herring (Nilsen and Esaiassen, 2005). The number of mullets of each batch was sufficient to describe the variability between individuals, as no significant differences were observed between batches during the analyses.

Initial changes in the following parameters were listed: skin brightness, the presence of blood in the operculum, flesh texture, belly texture, odor, the appearance and the shape of eyes, gills color and odour. During the storage in ice, the mullet showed gradual and consistent changes for all sensory evaluation parameters and all the tests carried out the demerit score, however, it never reached its maximum value (Zarr, 1999). Sensory rejection, in fact, occurred on day 10 of storage with a mean score of 13.83, consistent with what was observed by Ozyurt *et al*. (2009), even though with a day earlier than observed by the other authors (Nielsen *et al*., 2002; Ozyurt *et al*., 2009).

The values of the quality index of the various fish examined, in the different time intervals, recorded minimal fluctuations, probably due to variables specific to each lot such as the catch area, the fishing method and the manipulations suffered.

The flesh texture, the odor of skin and gills are the quality parameters most influenced by storage in ice. These parameters, in fact, compared to all the others presented a high correlation with the days of storage

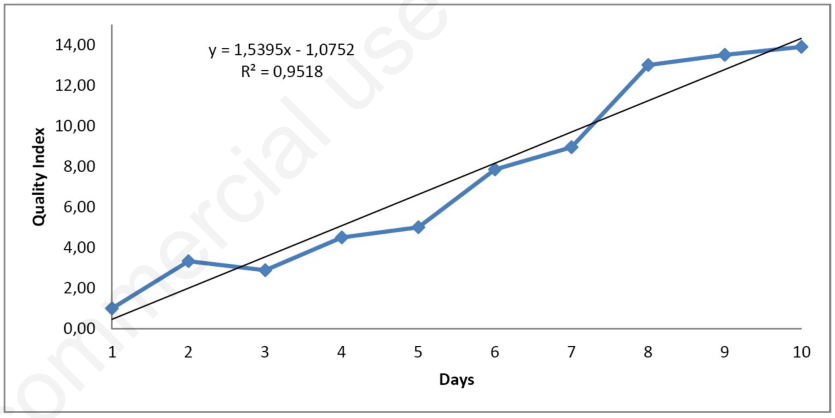


**Figure 1. Quality Index proposed by Özyurt *et al.*, (2009). Average values over each day of storage analysed against days in ice.**

**Table 2. Torry scheme for red mullet (modified by Alasalvar *et al.*, 2001).**

| Odour | Flavour | Texture (mouth feel) | Score |
|---|---|---|---|
| Initially weak sweetish odour | Watery, metallic, starchy, initially no sweetish but compact. It could develop a weak sweetish scent | Dray, crumbly with short tough fibres | 10 |
| Shellfish, seaweed | Sweet, meaty, green plant | Dray, crumbly with short fibrous succulent | 9 |
| Loss of odour, boiled milk, boiled potato | Sweet and characteristic flavours but reduced intensity | Dray, below succulent, fibrous, stick | 8 |
| Boiled potato | Neutral | Slight dry, below succulent, sticky, fibrous | 7 |
| Condensed milk, caramel | Insipid | Slight dry, below succulent, sticky, fibrous | 6 |
| Boiled milk | Slight sourness, trace of "off-flavours" | Below succulent, below fibrous | 5 |
| Lactic acid, stale | Slight bitterness, sour, "off-flavours" | Initial firm going softer with storage | 4 |
| Lower fatty acids (*e.g.* acetic acid or butyric acids) composed grass | Stronger bitter, slight sulphide, putrid | Initial firm going softer with storage | 3 |

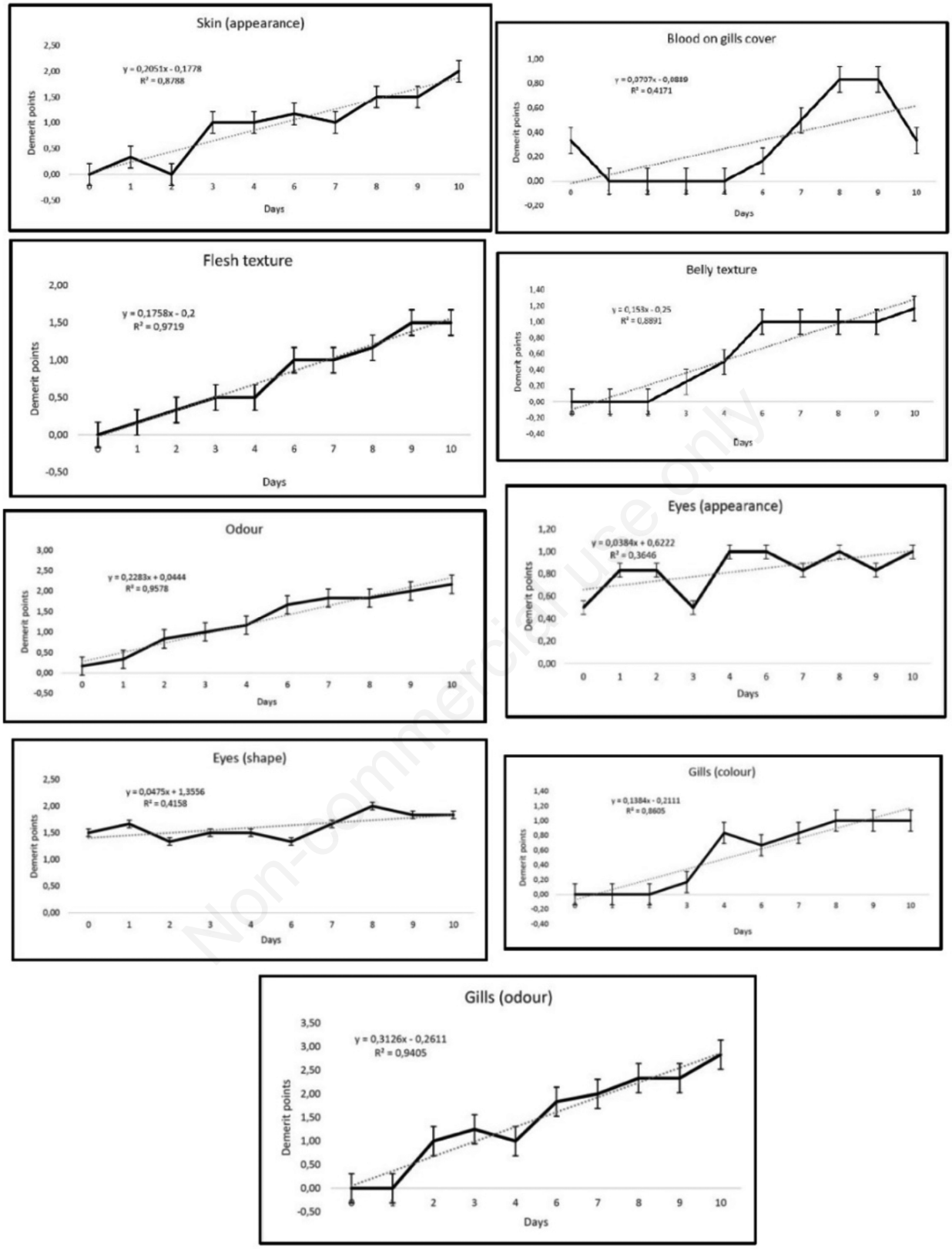




**Figure 2. Average scores of different parameters assessed with Quality Index Method scheme for red mullet.**

($R^2$=0.9719, 0.9578, and 0.9405, respectively). However, all the other parameters showed excellent correlation indices, higher than 0.8000, except for the parameters *blood on the operculum*, *eye (shape)* and *eye (aspect)* which presented $R^2$ values of 0.4171, 0.4158, and 0.3646, respectively (Figure 2).

Concerning the presence of blood on the operculum, this is a typical attribute of the *bluefish*, already taken into consideration even on the Regulation EC 2046/1996 (Council Regulation, 1996); an attribute that did not appear linearly in the mullet. In this regard, it should be remembered that the scheme based on the Quality Index Method proposed by Ozyurt *et al*. (2009) was a scheme applied to herring, notoriously a species belonging to the *bluefish* category (Table 1).

About the eye this showed, from the first day of analysis, a flat appearance and, in some sample, a certain degree of opacity. Regarding flat appearance it could be caused by breed related to orbital bone, which hide the eye convexity. During the days of storage, a haemorrhagic halo appeared at the corneal level that wasn't considered in the previous work (Ozyurt *et al.*, 2009) (Figure 3).

Following the processing of the average values of the individual parameters, it was decided to remove from the scheme those ones that showed a rather poor correlation with the days of storage and to modify the parameter e*ye's appearance* and *odour*. Therefore, a revised scheme with a final score of 15 was developed (Table 3).

The use of this scheme has highlighted a good correlation between the quality index and the days of storage in ice, with $R_2$ value equal to 0.9698 (Figure 4). Variations in sensory characteristics on the cooked muscle presented a temporal sequence well correlated with the days of storage (Figure 4). The predictability of QI was analysed using partial least-squares regression (PLS).

**Figure 3. Particular of eye and a typical haemorrhagic halo of mullet (8th day of storage).**

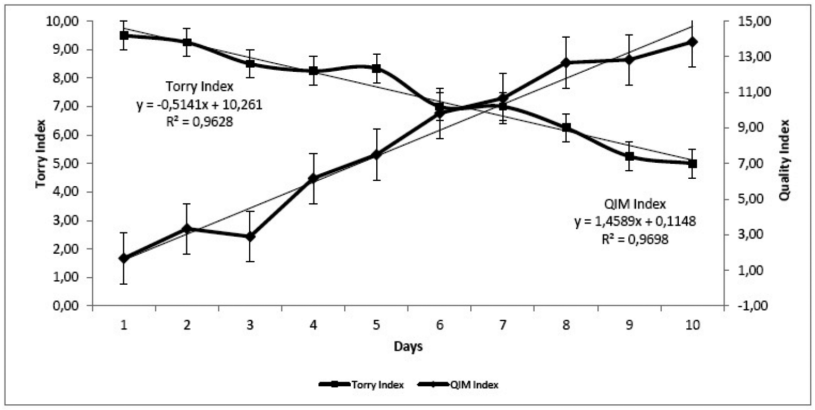


**Figure 4. Quality Index and Torry index of red mullet. Average values over each day of storage analysed against days in ice.**

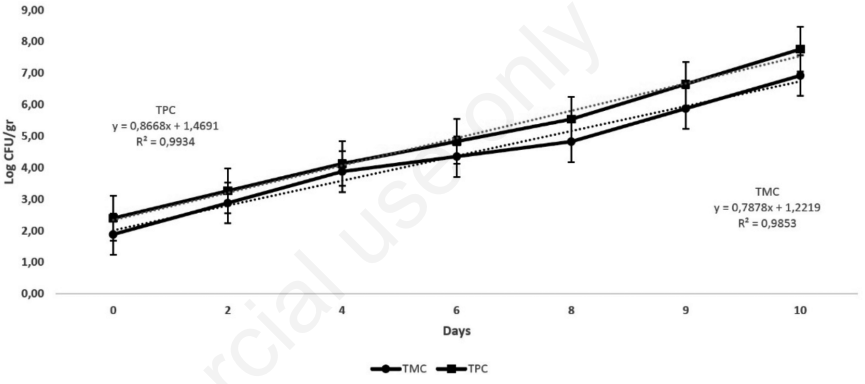


**Figure 5. Average scores of total mesophilic count and of total psychrophilic count in flesh of red mullet against days in ice.**

**Table 3. Modified Quality Index Method for red mullet.**

| Quality parameters | Description | Demerit point |
|---|---|---|
| Skin appearance | Very bright | 0 |
| | Bright | 1 |
| | Mat | 2 |
| Flesh texture | Hard | 0 |
| | Firm | 1 |
| | Soft | 2 |
| Belly texture | Firm | 0 |
| | Soft | 1 |
| | Burst | 2 |
| Odour | Fresh sea odour | 0 |
| | Neutral | 1 |
| | Slight off odour | 2 |
| | Strong off odour | 3 |
| Eyes appearance | Bright | 0 |
| | Somewhat lustreless | 1 |
| | Hemorragic halo | 2 |
| Gills odour | Characteristic red | 0 |
| | Somewhat pale, mat, brown | 1 |
| Gills colour | Fresh, seaweddy, metallic | 0 |
| | Neutral | 1 |
| | Slight off odour | 2 |
| | Strong off odour | 3 |
| Total demerit points | 0-15 | |

Observed and predicted values by linear regression were used to estimate the uncertainty (Standard Error of Estimate) of the prediction of the QI. Correlation between mean parameter values over time (in days) was then estimated for any parameter in each developing QIM and used to establish a modified QIM scheme.

### Sensory evaluation of cooked mullet

The characteristic fresh odors and flavors of the species were strong for the first 3-4 days, rapidly diminishing towards the 6th day, at which time the panellists perceived a *neutral* taste and odor.

Slight extraneous tastes/odours were evident in the sample on the ninth day of storage, remaining so until the tenth day, when the bitter flavors became evident.

Some authors hypothesize that, in subjects kept in contact with the covering ice, the leaching of the constituents using meltwater could occur. This phenomenon could enhance the general reduction of flavor during the early stages of storage, but it could also have the opposite effect, *i.e.*, slightly improve quality by reducing the concentration of undesirable flavours.

The limit of acceptability of mullet in ice was 10 days when the global sensory score reached the value of 5.5.

### Microbial counts

The flesh of healthy alive fish is sterile because the immune system prevents the bacterial growth on it; however, when the fish dies, the immune system collapses, and during storage, bacteria invade the flesh (Gram and Huss, 1996).

Trends of the microbial loads and the degree of correlation with the storage days are shown in Figure 5.

As it can be seen, the average values observed for the total psychrophilic count on the tenth day of storage were slightly higher than 7.00 log CFU/g, which is considered, precisely for the TPC, the maximum acceptability limit for marine and freshwater species, as established by the International Commission on Microbiological Specification for Food (ICMSF, 1986).

The average value of the TPC was always higher than the corresponding TMC; this observation underlines the original microflora of the mullet, characterized by bacteria naturally present in cold waters that are not affected by the cooling action of the covering ice and the storage temperatures.

The TMC and the TPC of the musculature remained rather low before 8 days (<5 logs CFU/g). In line with what has already been observed by other authors, it has been hypothesized that the sensory changes, which occurred during the first half of mullet conservation, could be mainly due to autolytic activity (Lougovois *et al*., 2003).

The TMC values observed were significantly lower than those observed by Ozyurt *et al.* (2009) and by Pournis *et al*., (2005) probably because the predominant flora, observed in the present work were mainly constituted by the psychrophilic microorganisms.

## Conclusions

QIM is now considered the most effective sensory method to evaluate the freshness of fish products. Its strengths are represented by practicality, simplicity, speed of execution, non-destructiveness, and its ability to estimate the residual shelf-life of the product.

According to some authors, with the QIM method, it is possible to arrive at the evaluation of the shelf-life with an error of 1-2 days, however acceptable, compared to the classic laboratory methods, which require chemical and microbiological tests, difficult to carry out routinely (Lougovois *et al.*, 2003).

The basic principle of QIM (its species-specificity) represents a weakness, since it limits the sensory evaluation procedure only to the species to which it is applied, thus it's not able to generalize the results (Bernardo *et al.*, 2020).

According to the QIM-EUROFISH project goals, the present study has been optimizing the QIM scheme of Ozyurt *et al.* (2009) that it has been the only study about *M. barbatus*.

It would therefore be desirable for experts in the sector to contribute in the development of new assessment schemes applicable to fish species not yet taken into consideration, as well as to carry out constant work of validating and updating the pre-existing schemes.

In this context, the QIM scheme for *M. barbatus* represents a serious contribution. This showed a clear correlation between QI and days of storage on ice. The final scheme developed by this investigation, evaluating 7 parameters, assigns a total of 15 demerit points. From the results obtained, it could be stated that the maximum storage time on ice for this species was reached in 9-10 days with error value of less than 24 hours. Data further confirmed by the Standard Prediction Error (ESP) which showed a value of 0.72 days.

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