Eur Food Res Technol (2017) 243:2225–2233
DOI 10.1007/s00217-017-2924-0

ORIGINAL PAPER

Computer vision system (CVS): a powerful non-destructive technique for the assessment of red mullet (*Mullus barbatus*) freshness

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Received: 16 January 2017 / Revised: 19 April 2017 / Accepted: 4 June 2017 / Published online: 17 June 2017

Abstract The evaluation of fish freshness can be performed using chemical, sensory and physical methods. Besides sensory methods, several instrumental techniques have been applied with the objective of replacing sensory assessment. The aim of this study was to set up and test objective physical methods mainly based on computer vision system (CVS) to assess red mullet (*Mullus barbatus*) freshness evolution during 10 days of storage, at two different storage temperatures (0 and 4 °C). To check the effectiveness of the purposed physical methods, CVS features (loss in the epidermis pigmentation, development of gill mucus and eye concavity index) and firmness have been compared with chemical trimethylamine content and sensory (QIM) attribute scores. As expected, fish degradation was faster at the higher temperature. Instrumental texture evaluation of fish by penetration test enabled to detect distinctive firmness changes due to onset and resolution of rigor mortis, and the successive tenderization phenomenon. Among CVS parameters, the epidermis pigmentation loss, and particularly the eye shape modification (eye concavity index) evidenced a high sensibility for the estimation of fresh red mullet quality loss, as a function of the two different storage conditions, and a good agreement with trimethylamine content and QIM response evolution.

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Keywords *Mullus barbatus* · Freshness · Computer vision system · Eye concavity index

Introduction

Fish freshness is a basic requirement for the quality of fish products and it is strictly bound to the appropriate storage conditions, in particular time and temperature after catch [1]. The optimal storage temperature for fresh fish is between −1 and 0 °C, while a temperature increase of 5 °C can lead to shelf-life reduction to more than a half [2].

More in general, endogenous biochemical changes in fish tissues after catch are strongly influenced by the holding temperature, the capture method and the previous condition of the live fish [1], representing the primary cause of freshness loss, while spoilage by microorganisms occurs later [3].

Because of the various factors contributing to this phenomenon, the precise estimation of fish shelf-life is difficult and complex [4, 5]. Actually it seems that a single parameter alone is never representative of the freshness depletion phenomena during storage of fish. Hence, a fast and reliable method able to monitor various parameters is needed.

Current methods for the evaluation of fish freshness include chemical, sensory and physical measurements and in some cases, the associated microbial growth.

Different chemical and biochemical parameters can be used for this purpose such as pH and ATP content, content of undesirable compounds such as products of lipid oxidation, trimethylamine (TMA-N) and total volatile basic nitrogen (TVB-N).

Nevertheless, chemical and microbiological methods, besides being time consuming and destructive, are not suited to the early stage of fish storage [4].

Texture is an important physical property of fish and its determination by empirical–imitative texture analyser instruments could provide a good indication of freshness evolution during storage [6]. Variation of textural parameters in fish can include an increase of toughness due to rigor mortis or frozen storage, or the occurrence of soft and mushy consistency due to autolytic degradation [7]. After rigor resolution, the tissues are subjected to relaxation and softening, while prolonging the refrigerated storage, both endogenous and microbial enzymatic activity, could be responsible for an ulterior firmness loss [8].

Because characteristic sensory changes occur in the appearance, odour, taste and texture of fish when deterioration takes place, sensory inspection, and for the industry the European Union scheme, has been currently adopted as method for quality and freshness assessment of raw fish [9]. Because it employs only general parameters, this scheme does differentiate between species. To take into account specific sensory parameters for each species, a suggested alternative sensory method is the quality index method (QIM) [9]. Originally developed by Bremmer [10], QIM is based on the observation of variations occurring during ice storage in some fish characteristics and it gives the possibility to estimate the remaining storage time in ice before the unacceptability level is reached [11]. Nevertheless, it remains a labour-intensive method that involves the use of a trained panel.

For this reason, the current trend in fish freshness evaluation is the replacement of sensory methods with non-invasive and fast instrumental methods that use low-cost equipment. The inspection of fish surface by image analysis provides a contactless and non-destructive method for monitoring the fish quality. The evaluation of chromatic and geometric elemental features (i.e. colour, size, shape and structure) of food products with the aim of quality assessment and control has been widely applied by the industry. Moreover, the significant improvement of the computer hardware and processing speed may open the possibility of using the whole image as input data, obtaining a more complete set of information related to quality [12].

The various applications of machine vision techniques for fish quality evaluation reviewed by Dowlati et al. [13] and by Hong et al. [14] include assessment of fish morphology, species detection, and some physical and chemical properties during fish processing and storage. Nevertheless, the application of image analysis for evaluation of fish freshness is still scarcely used [13]. Dowlati et al. [4] applied machine vision methods to monitor colour of eyes and gills of gilthead sea bream as an index of freshness during storage. However, results were not correlated to other analytical methods.

Since for the assessment of fish freshness, sensory methods are mainly based on visual parameters, the development of an objective method might start from the visual quality monitoring by a computer vision system [2]. Nevertheless, to set up a reliable objective method, it has to be consistent with sensory methods [15], not only measuring particular features within the image but also identifying the most suitable ones that can be correlated to QIM [15]. Di Natale [16] studied the construction of an artificial quality index by performing a calibration procedure of a set of instruments on the basis of the evaluations of a trained panel.

Red mullet (*Mullus Barbatus*) belongs to the *Mullidae* family and lives in the benthic zones of the Mediterranean and Black seas. Its proximate and fatty acid composition is reported in the literature [17]. Red mullet is considered of great commercial value in Europe and it is highly demanded on the market of Mediterranean countries, hence studies about the freshness decay of this fish are of interest for both retailers and consumers. Özyurt et al. [18] studied the shelf-life of red mullet during storage in ice in terms of sensory, microbiological and chemical changes. They found that the sensory acceptability limit was 11 days, with a maximum QIM total demerit score of about 14.

In this direction, the aim of this study was to set up and test objective physical methods based on computer vision system (CVS) and empirical–rheological texture analysis to assess fish freshness evolution during storage at two different temperatures (0 and 4 °C). To check the effectiveness of the purposed physical methods, results were compared with chemical (TMA-N) and sensory (QIM) evaluations.

Materials and methods

Fish samples

Sample preparation and storage conditions

Fresh red mullets (*M. barbatus*) were caught in the Adriatic Sea (Cesenatico, Italy) during autumn by a fishing vessel. After fishing, the fishes were placed in polystyrene boxes, covered with ice flakes and, after unloading, immediately carried to the laboratory of the Campus of Food Science. After about 60 min, in the laboratory, 74 ungutted fishes were individually inserted in open plastic pouches and placed in polystyrene boxes in two different conditions: 0 °C, samples covered with ice flakes (fish-to-ice ratio 2:1), replenishing melted ice daily; 4 °C, samples placed in polystyrene box without ice. Boxes were successively stored in a 4 °C refrigerated room up to 10 days.

Chemical trimethylamine (TMA-N) assessment

The TMA-N determination has been performed by ultraviolet (UV) spectrophotometry according to the AOAC official method [19], based on Dyer's method. Dyer [20] encompasses a liquid–liquid extraction of TMA-N with toluene and its subsequent reaction with picric acid reagent to form a yellow complex, the picrate. The latter, and consequently also the TMA-N concentration, was quantified by measuring the absorbance at 410 nm. Three fishes (for each sampling time and storage condition) were analysed in triplicate after QIM and texture assessment.

Sensory evaluation

Before texture assessment, samples (three fishes for each sampling time and storage condition) were taken for sensory evaluation at 0, 1, 3, 7 and 10 days of storage. A trained panel of six members evaluated the fish throughout the storage period on each sampling time, according to the quality index method (QIM) [18]. This sensory scale is based on the freshness quality grading system for herring developed by Nielsen and Hyldig [21].

The QIM involves specifying the characteristics of appropriate sensory attributes of the raw fish, assigning a demerit score ranging from 0 to 1, 2 or 3 depending on the different sensory attribute (Table 1). The scores for all characteristics are then summed to give an overall sensory score, the so-called quality index [11]. The scale gives zero score for absolutely fresh fish and increasing total demerit points during fish deterioration. The examined parameters were peculiars of the whole fish (appearance of skin; blood on gill cover; texture; texture of belly; odour), eyes (appearance and shape) and gills (colour and odour).

Table 1 QIM scheme for sensory evaluation of red mullet modified by Özyurt and other (2009)

Quality parameter	Description	Score
Whole fish		
Appearance of skin	Very bright	0
	Bright	1
	Mat	2
Blood on gill cover	None	0
	Some	1
	Much	2
Texture	Hard	0
	Firm	1
	Soft	2
Texture of belly	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
Shape	Convex	0
	Flat	1
	Sunken	2
Gills		
Colour	Characteristic red	0
	Somewhat pale, mat, brown	1
Odour	Fresh, seaweedy, metallic	0
	Neutral	1
	Some off odour	2
	Strong off odour	3

Total demerit points (0–18)

Texture assessment

For each sampling time, three fish at each temperature condition were taken and, after equilibration at 23 °C, submitted to texture analysis (one measurement for fish).

Flesh fish firmness was evaluated by performing a penetration test on the dorsal lateral line areas of the fish using a texture analyzer mod. TA-HDi500 (Stable Micro Systems, Surrey, UK) equipped with a 250-kg load cell. Among mechanical tests, penetration test has been chosen because it is the most suitable for fish and fish products' texture evaluation [8].

The selected area for texture measurement was chosen on the basis of preliminary experiments, performed on different parts of the fish, that showed that bony plates and the thoracic area have to be avoided to obtain consistent results.

The test was run with a metal probe of 6 mm diameter, a rate and depth of penetration of 1 mm s^{-1} and 5 mm, respectively. At the beginning of the test, the probe does not break the skin and an increasing compression force is measured. The flesh firmness (F_{max}, N) was evaluated as the peak registered at the point of skin rupture.

Visual quality assessment using computer vision system (CVS)

Image acquisition

Visual quality assessment was performed on the same ten fishes for all the period of analysis (ten fishes stored under ice and ten stored at 4 °C). Red mullet images were rapidly captured using the image acquisition system developed

by Rocculi et al. [22] with slight modifications. The samples were illuminated using two parallel lamps (with two fluorescent tubes by lamp, model TL-D deluxe, natural daylight, 18 W/965, Philips, NY, USA) with a colour temperature of 6500 K (D65, standard light source commonly used in food research) and a colour-rendering index (*R*a) close to 90%. The four fluorescent tubes (60 cm long) were situated 35 cm above the sample and at an angle of 45° with the sample. Additionally, light diffusers covering each lamp and electronic ballast assured a uniform illumination system. A colour digital camera (CDC), Canon PowerShot A70, was located vertically over the sample at a fixed distance. The angle between the camera lens and the lighting source axis was 45°. Lamps and CDC were inside a wooden box with internal walls that were painted black to avoid the light and reflection from the room. RGB images from the two sides of each fish were taken on the matte black background using the following camera settings: manual mode with the lens aperture at *f* of 4.5 and speed 1/125, no zoom, no flash, 2592 × 1944 pixel resolution of the CDC and storage in JPEG format. The camera was connected to the USB port of a PC provided with a Canon remote capture software (version 2.7.0) to visualize and acquire the digitalized images directly from the computer.

Image analysis and feature extraction

The CDC was positioned vertically above the sample at a variable distance (*d*) depending on the particular type of scanning. In this study, three different parameters were measured: loss in the pigmentation of the epidermis ($d = 23$ cm), development of gill mucus and eye concavity index ($d = 18.5$ cm).

The image analysis of sample pictures was performed with an advanced image analysis software (Image-Pro 6.2, media cybernetics, USA), using RGB or grey scale.

Loss in the pigmentation of the epidermis

The change in pigmentation of the epidermis (two sides for each fish) was observed on the fish surface. To evaluate the modification of the epidermis pigmentation, a chromatic model was created. Fish lateral images were evaluated in two steps: selection of total sample area (Fig. 1a) and of areas characterized by different pigmentation (Fig. 1b). For this aim, on the bases of the chromatic characteristics of all samples, a colour model composed of 10 RGB classes was built (Table 2). Ten colour

Table 2 RGB classes of the chromatic model for the evaluation of the changes of red mullet epidermis pigmentation

Class	Real colour	Colour channel		
		R	G	B
1	Pink	193–255	164–229	161–239
2	White	239–255	231–255	228–255
3	Grey	161–249	151–252	150–252
4	Orange/light brown	216–250	157–216	153–206
5	Blue/grey	118–217	201–229	197–224
6	Violaceous	204–216	154–164	204–218
7	Light yellow/light green	227–251	203–244	297–228
8	Brown	128–155	87–127	74–106
9	Dark green	149–200	107–116	95–150
10	Pink/very light green	227–252	213–239	217–244

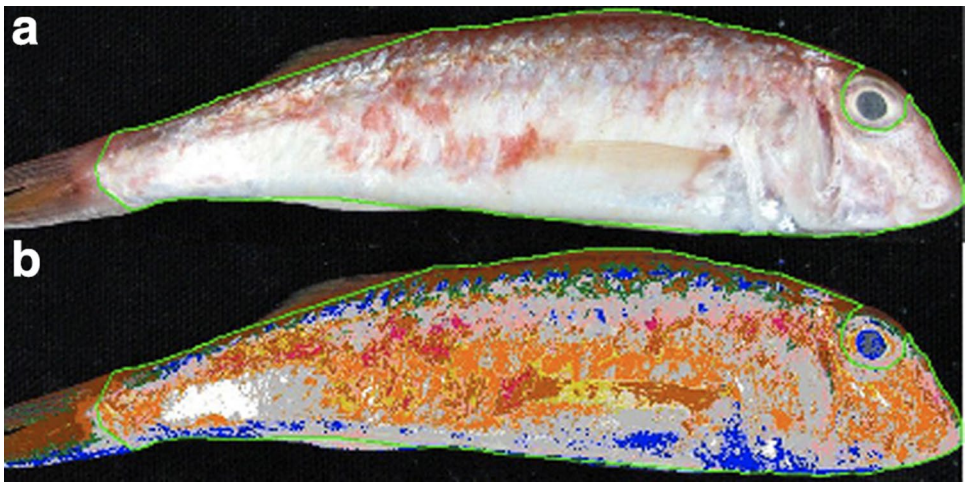

Fig. 1 Example of images of red mullet after selection of the area of interest (**a**) and application of the chromatic model (**b**)

classes were created to cover 100% of the general fish surface. The objectification of the classes has been performed on the basis of the colour change visually detectable by the operator, thus maintaining a strict relationship with the sensory methods used for the sensory evaluation. The same colour model was applied to all fish images. The software, examining all pixels in the image, calculates the area percentages of each single class (Fig. 1b).

The application of the chromatic model on the fish surface images, obtained at different storage time, permitted to monitor the modification of each single class during storage, at the two different temperatures investigated. The percentages of the classes with significantly decreasing trend were added up to obtain an average of the epidermis pigmentation loss (EPL, %), considering the initial value of the summed classes equal to 100%.

Development of gill mucus

For the determination of gill mucus (two sides for each fish), gill images were converted in grey-scale 8BPP. A grey achromatic model (two classes) was created to determine the percentage of the glossy area that can be ascribable to the presence of superficial mucus in the gill. The area was expressed in terms of % of the considered total area of interest.

Concavity eye index

The eye is one of the most sensitive and, therefore, perishable parts of the fish. It consists of a large proportion of liquid, the loss of which, during storage, leads to a change in its shape. In particular, during storage, the eye tends to sink into the eye socket. The concavity eye index (CEI) was obtained by comparing the distance created as a result of this phenomenon with that of fresh red mullet, as reported in Fig. 2 that shows an image of the eye of fresh (a) and stored at 4 °C for 10 days (b) red mullet. The CEI (%) was expressed in terms of increased distance compared to the initial one, considered as 100%.

Statistical analyses

Analyses of variance (ANOVA) and the test of mean comparisons according to Fisher's least significant difference (LSD) were applied, with a level of significance of 0.05. Data were also evaluated using Pearson's correlation analysis, with a level of significance of 0.001, among chemical, sensory and instrumental data. The statistical software used was STATISTICA (StatSoft, Tulsa, Oklahoma), version 8.0.

Results and discussion

Figure 3 shows the evolution of TMA-N content during storage on red mullet samples at the two different temperatures investigated.

Concentration of TMA-N in fish has been found to be closely related to organoleptic estimations [23], being an objective indicator for quality determination in fish samples. In fact, TMA-N generation is attributed to the

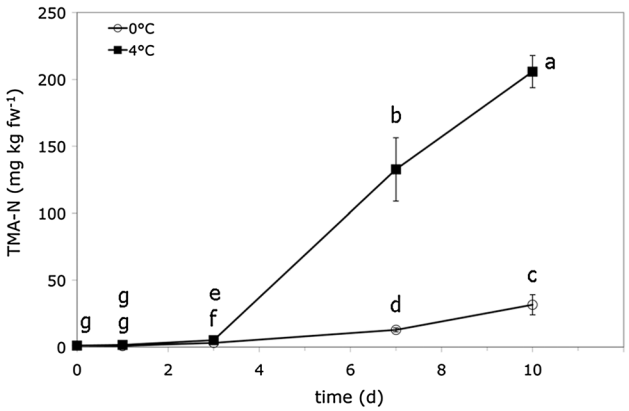

Fig. 3 Trimethylamine content (TMA-N, mg kg fw^{-1}) of red mullets stored at 0 and 4 °C for 10 days. *Error bars* represent standard deviation, $n = 9$

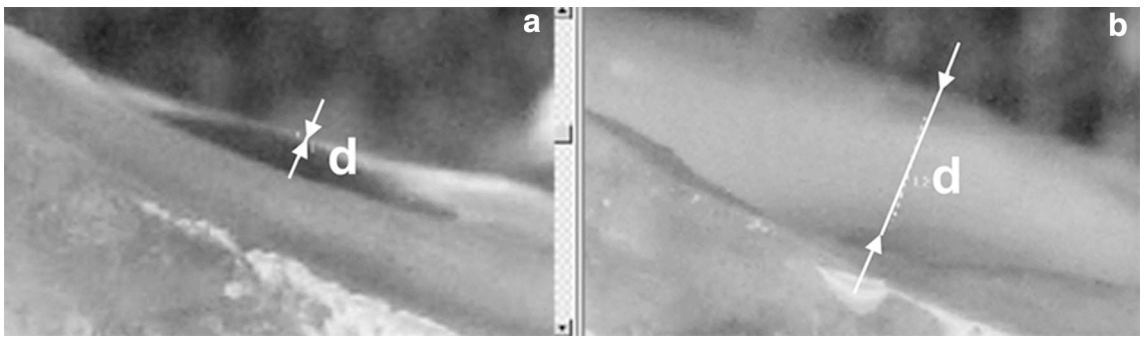

Fig. 2 Example of images of red mullet eye of fresh (**a**) and after 10 days of storage at 4 °C (**b**) fishes

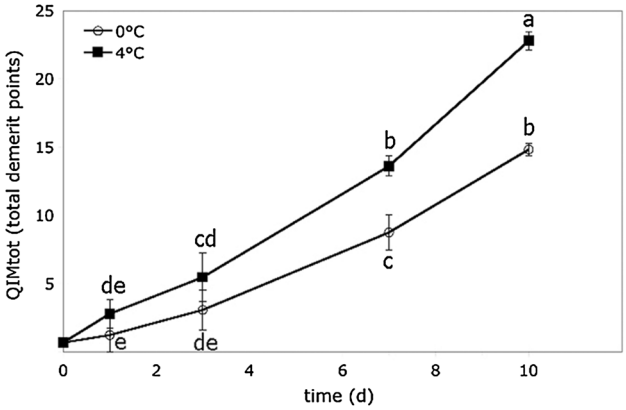

Fig. 4 Total demerit points (QIMtot) of red mullets stored at 0 and 4 °C for 10 days. *Error bars* represent standard deviation, $n = 18$

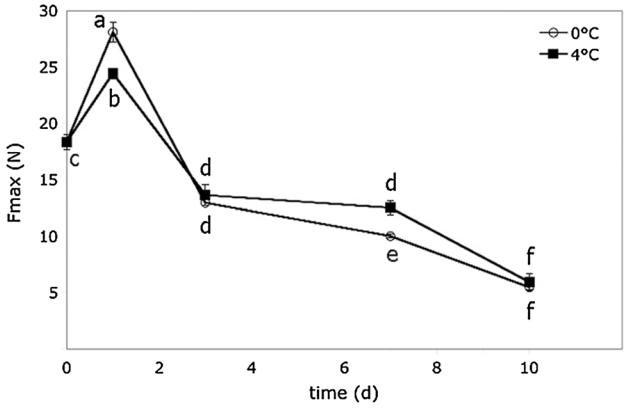

Fig. 5 Firmness (F_{max}) of red mullets stored at 0 and 4 °C for 10 days. *Error bars* represent standard deviation, $n = 3$

gradual conversion of trimethylamine oxide (TMA-O) by bacterial or enzymatic reduction. In particular, studies of TMA-N formation in stored fish after capture show an exponential increase in concentration, perhaps following a dwell period when the TMA-N does not increase [24].

In the present study, the formation of TMA-N starts dramatically after 3 days for samples stored at 4 °C and increases linearly reaching about 200 mg kg fw^{-1} at the end of the storage, while for 0 °C sample the increasing trend of TMA-N content was slower and less intense reaching values of about 25 mg kg fw^{-1} after 10 days. This slower increase at 0 °C may have been caused by a slower conversion of TMA-O into TMA-N, because at this temperature a specific enzymatic reaction, which principally leads to the formation of dimethylamine (DMA) and formaldehyde from TMA-O, takes place [7].

The total demerit points of red mullet stored in ice and at 4 °C are reported in Fig. 4. Initially, red mullet were characterized by a very bright appearance, a hard texture, bright and convex eyes and a fresh odour. As expected, demerit points increased at both temperatures during storage time, with a higher rate at 4 °C compared to storage in ice. Özyurt et al. [18] using the QIM method found a limit of acceptability for red mullet stored in ice corresponding to about 14 demerit points, and a relative storage life of 11 days. According to this finding, in our experimental conditions this value was reached after about 10 days at 0 °C and 7 days at 4 °C.

It is well known that there are several biochemical changes directly associated with the onset and the resolution of rigor mortis that can affect dramatically the texture of fish muscle. Immediately after death, the fish texture is soft and elastic; then the onset of rigor makes the fish texture harder, condition that usually lasts for a day or two [8].

After 1 day of storage, firmness (Fig. 5) increased for both 0 and 4 °C samples of about 50 and 25% compared to the fresh sample. The increase of firmness values was probably due to the incidence of rigor mortis [3], the possible explanation of the detected differences is that at 4 °C its occurrence (and the associated firmness increase) was faster and took place during the first 24 h of storage [1].

After rigor mortis, both samples showed very similar decreasing values until the end of the experiment, as a consequence of an opposing process to the stiffness occurring in rigor, called tenderization [25]. This tenderization affects the main structural proteins in the myofibrils and the extracellular matrix, as well as the proteins involved in the connections myofibril–myofibril and myofibril–sarcolemma. The extent of these changes and their effect on muscle texture depends on many factors, including species, the preslaughter conditions or methods of capture, management and post-mortem treatment, storage time and temperature [26].

According to previous findings [8], obtained results confirmed that the texture evaluation of whole fish using penetration test can detect distinctive firmness changes due to onset and resolution of rigor mortis, and the consequent tenderization phenomenon.

Results on the evaluation of epidermis pigment modifications during storage are reported in Fig. 6a. To our knowledge, just few papers have been published on instrumental colour measurements performed on the skin of whole fish during storage in ice, to monitor colour changes as a result of prolonged storage. Dowlati et al. [4] used computer vision system and image analysis to monitor colour of gilthead sea bream during storage. By applying artificial neural networks they correlated the evolution of the colour of different fish parts to storage time, concluding that changes in the fish eye could be used for fast and non-destructive evaluation of freshness. Nevertheless, to use image analysis for quality evaluation a correlation with other instrumental, chemical or sensorial analysis is needed.

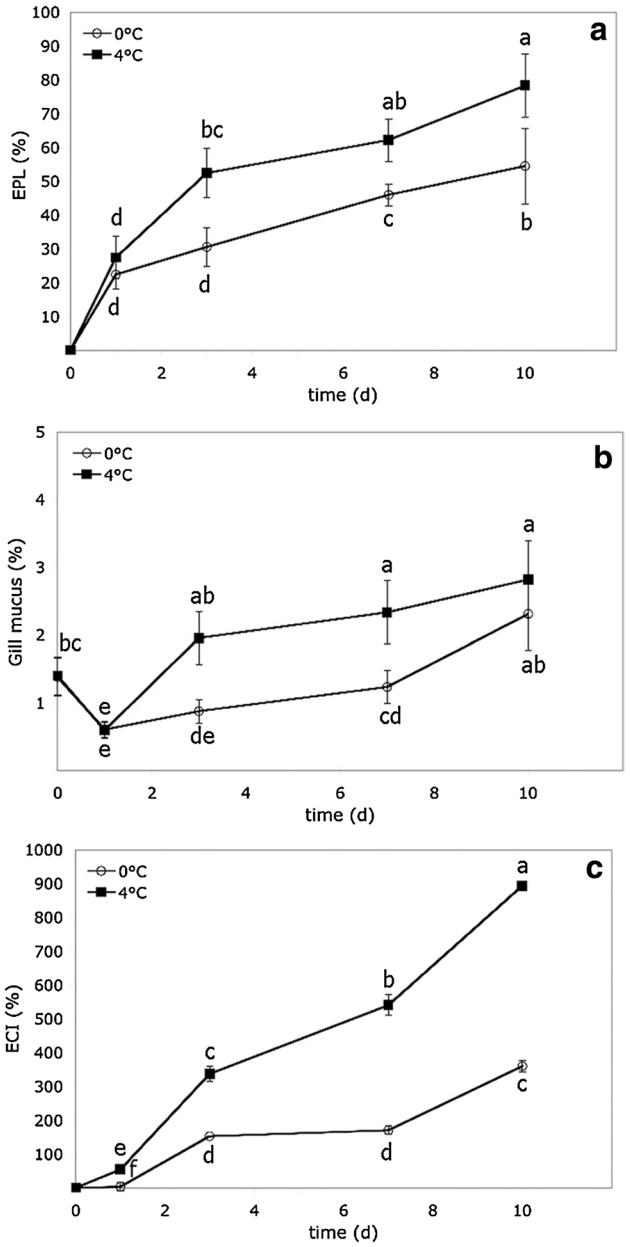

Fig. 6 Epidermis pigment loss (%) (**a**), gill mucus (%) (**b**) and eye concavity index (%) (**c**) of red mullets stored at 0 and 4 °C for 10 days. *Error bars* represent standard deviation, $n = 20$

A good correlation between changes in colour measured instrumentally and sensorial quality was found in squid during storage in ice [27] and in fresh and frozen cod [28], but so far no researches have been performed on red mullet.

After the first day of storage, the degradation of colour for both the samples was very similar, while from the third to the tenth day sample at 4 °C showed a faster and more intense colour loss.

The amount of gill mucus, evaluated in terms of detected glossy area, is reported in Fig. 6b. Both samples showed a slight decrease during the first day of storage, while successively they showed an increasing trend, which was faster and more intense for samples stored at 4 °C. This result was evident also from a visual examination. In our opinion, with this technique, it was possible to recognize as glossy area two different typologies of mucus, one peculiar of fresh fish, and the other produced during gill deterioration.

Data of the eye degradation expressed in terms of eye concavity index (%) are reported in Fig. 6c. Both samples showed an increasing trend that even this parameter was more pronounced for fish stored at 4 °C, in comparison with that stored under ice, in which the beginning of the eye collapse was delayed and started only after the first day of storage.

Di Natale [16], to define an artificial quality index, correlated the sensory QIM results with the response of a set of instruments able to capture the same physical and chemical parameters as described by the senses.

In our experiments, to highlight the existing relation among chemical, sensory and instrumental parameters, correlation analysis has been performed. Results reported in Table 3 showed that instrumental texture (F_{max}) correlated neither with chemical and sensory, nor with image analysis data. Among visual quality results evaluated using CVS, while gill mucus was not correlated with any of the investigated parameters, both the epidermal pigment loss (EPL), and particularly the eye concavity index (ECI) showed a strict positive correlation with QIM parameters, and the latter also with TMA-N. In addition, TMA-N results showed a high positive correlation also with QIM, confirming the close relation of this chemical index with organoleptic estimations, evidenced in previous studies [21].

Conclusions

Results of this study indicated that the development of physical methods for the evaluation of red mullet freshness, particularly image analysis performed by CVS, is very promising.

Instrumental texture evaluation of fish by penetration test enabled to detect distinctive firmness changes due to onset and resolution of rigor mortis, and the consequent tenderization phenomenon.

Among CVS parameters, the epidermis loss of colour, and particularly the eye shape modification (eye concavity index) evidenced a high sensitivity for the estimation of fresh red mullet quality loss, as a function of the two different storage conditions, and a good agreement with chemical and sensorial response.

In our experimental conditions, according to QIM results, the shelf-life of fresh red mullet was, respectively, 7 days at 4 °C and 10 days at 0 °C.

Table 3 Correlation coefficients among chemical (TMA-N), sensory (whole fish, gills, eyes and total QIM) and physical (F_{max}, EPL, gill mucus and ECI) parameters of red mullet

	Whole fish	Gills	Eyes	QIMtot	F_{max}	EPL	Gill mucus	ECI	TMA-N
Method	QIM	QIM	QIM	QIM	Texture	CVS	CVS	CVS	Chemical
Whole fish	–								
Gills	0.963*	–							
Eyes	0.983*	0.955*	–						
QIMtot	0.999*	0.974*	0.988*	–					
F_{max}	NS	NS	NS	NS	–				
EPL	NS	0.909*	0.919*	0.898*	NS	–			
Gill mucus	NS	NS	NS	NS	NS	NS	–		
ECI	0.953*	0.894*	0.963*	0.951*	NS	0.902*	NS	–	
TMA-N	0.903*	NS	0.894*	0.891*	NS	NS	NS	0.926*	–

* Statistically significant correlation at $p < 0.001$

This corresponded to a loss of characteristic epidermis colour of, respectively, 60 and 50% and an increase of the eye concavity index of five times at 4 °C and three times at 0 °C, compared to the fresh one. These values could hence be used to define the end of the shelf-life measured through CVS analysis. The main advantage of the proposed method is that it is rapid, non-destructive and well correlated to the chemical and sensorial results. Nevertheless, it is species specific and needs validation for different raw material conditions (e.g. species, season). For these reason, further researches are in due course in our lab, to improve the performances of the purposed physical methods, and to verify their suitability in a wider range of conditions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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