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Freshness represents a pivotal aspect in fish product for both security and quality. Its evaluation still represents the key factor driving the consumer’ choices. Fish appearance is affected by many different factors that demand the contribution of different disciplines to be understood: from the physical and optical properties to the slaughtering and post-slaughtering conditions. An innovative preservation system is represented by the
Passive Refrigeration PRS™ developed for the preservation and transport of perishable food products. Scientific methods for product freshness evaluation may be conveniently divided into two categories: sensorial and instrumental. In this study, an instrumental method of colour calibration and discrimination is proposed at pilot scale for automatic evaluation of gilthead seabream (Sparus aurata) freshness. We propose a non-destructive method based on the colorimetric imaging of the whole external body of seabreams to evaluate through multivariate partial least squares which approach the differences in the freshness preservation under four refrigeration modalities. The matrix of the independent variables is represented by RGB values for each pixel belonging to an extracted region of interest (129,633 values). The dependent variable is composed by two dummy variable corresponding to fresh (T0) or non-fresh (T2) individuals. T1 individuals were used as external test. The results quantified significant colorimetric differences between fresh and non-fresh fish. All fish used to create the model (T0 and T2) were correctly classified as fresh or non-fresh, while external test individuals (T1) were all classified as fresh. The proposed imaging method merges different image analysis techniques: (a) colorimetric calibration, (b) morphometric superimposition and (c) partial least square discriminant analysis modelling. This innovative and non-destructive approach allows the automatic assessment of fish freshness.

**Keywords**
Fish freshness assessment - Gilthead seabream - Colorimetric calibration - PLS - Warping - Passive refrigeration system

**Footnote information**

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Abstract Freshness represents a pivotal aspect in fish product for both security and quality. Its evaluation still represents the key factor driving the consumer’s choices. Fish appearance is affected by many different factors that demand the contribution of different disciplines to be understood: from the physical and optical properties to the slaughtering and post-slaughtering conditions. An innovative preservation system is represented by the Passive Refrigeration PRS™ developed for the preservation and transport of perishable food products. Scientific methods for product freshness evaluation may be conveniently divided into two categories: sensorial and instrumental. In this study, an instrumental method of colour calibration and discrimination is proposed at pilot scale for automatic evaluation of gilthead seabream (Sparus aurata) freshness. We propose a non-destructive method based on the colorimetric imaging of the whole external body of seabreams to evaluate through multivariate partial least squares which approach the differences in the freshness preservation under four refrigeration modalities. The matrix of the independent variables is represented by RGB values for each pixel belonging to an extracted region of interest (129,633 values). The dependent variable is composed by two dummy variable corresponding to fresh (T₀) or non-fresh (T₂) individuals. T₁ individuals were used as external test. The results quantified significant colorimetric differences between fresh and non-fresh fish. All fish used to create the model (T₀ and T₂) were correctly classified as fresh or non-fresh, while external test individuals (T₁) were all classified as fresh. The proposed imaging method merges different image analysis techniques: (a) colorimetric calibration, (b) morphometric superimposition and (c) partial least square discriminant analysis modelling. This innovative and non-destructive approach allows the automatic assessment of fish freshness.

Keywords Fish freshness assessment · Gilthead seabream · Colorimetric calibration · PLS · Warping · Passive refrigeration system

Introduction

Appearance is used throughout the production, storage, marketing and utilisation chain as the primary means of judging the quality of individual units of product. Fish appearance can be due to many different factors such as optical properties, physical form and health status, chemical composition and microbial load, method of slaughtering and preservation and the environmental conditions in which it has lived. The most important conservation factor is to chill fish with ice to about 0 °C to increase the thermal stability but this requires a huge amount of ice (1:1, Jeyasekaran et al. 2004) and produce drip loss, textural toughness, nutrient loss and decreases in protein extractability (Putro 1989). Lately, an innovative preservation system (Passive Refrigeration PRS™—NOMOS S.p.a. Olgiate Molgora, Italy) was developed for the preservation and transport of perishable products. The system guarantees perfect shelf life preservation through the maintenance of...
optimal temperature and relative humidity, without the use of power during operation.

As reported by Olafsdottir et al. (2004), fish freshness evaluation carried out by physical techniques is generally more rapid than with chemical ones, indeed optical and electrical measurements are almost instantaneous. Since the consumer is the ultimate judge of quality, most instrumental methods must be correlated with sensorial measures related to the sight, the touch or the odour perception (Menesatti et al. 2010; Quevedo et al. 2010). For whole fish the EU quality grading scheme (Howgate et al. 1992) is used as required by EU regulation (European Community 1996), but some initiatives have been taken to implement a new sensory method named Quality Index Method (QIM) to standardise sensory assessment for each species (Martinsdottir et al. 2004; Olafsdottir et al. 2004).

Generally, species-specific colour is a critical sensory characteristic of fish quality as it is used by consumers as an indicator of perceived quality and freshness. All sets of colour values show a fairly good linear relationship with both the QIM values and the values for appearance of skin (Olafsdottir et al. 2004). The functioning of modern colorimeters is comparable to the principle of colour perception used by the human eye (Li-Tsang et al. 2003).

In this scenario, this study aimed to test the ability of a novel, automated and non-destructive methodology of assessing the freshness of whole fishes, based on external colour appearance of samples preserved with a conventional system and an innovative passive refrigerator. Digital images of whole gilthead seabream (Sparus aurata Linnaeus, 1758) were taken soon after harvest, after four different refrigeration modalities and following three different periods of preservation were calibrated, with respect to a standard colour chart, with a Partial Least Squares (PLS) approach.

Materials and Methods

Samples

Thirty gilthead seabreams reared at the commercial farm Civitaltica s.r.l. (Civitavecchia, Italy) were used for the experiments. The fishes were split in four groups, following four refrigeration modalities: (a) stored accordig to traditional market techniques, in a polystyrene tray covered with a plastic seafood film, displayed into an industrial freezer at 2 °C for 2 days (Frz, seven fish); (b) stored in a polystyrene tray covered with a plastic seafood film with crushed ice placed above the film and placed outside for 1 day (7–12 °C), in order to simulate a market exposition, and then placed into an industrial refrigerator laying on the right side (0.6 m³) at 2 °C for 5 days (Out, seven fish); (c) stored in a polystyrene tray covered with a plastic seafood film, without ice, and placed into a PRSTM Passive Refrigeration System Thermopallet EI (1.93 m³; 1.21×0.81×1.95 m height) at 2 °C for 5 days (Frz, eight fish); (d) stored in a polystyrene tray covered with a plastic seafood film, displayed into an industrial freezer at −10 °C for 3 days and then thawed during placement into an industrial refrigerator (0.6 m³) at 2 °C for 2 days (Frz, seven fish).

Each fish group was analysed after 4 h post-mortem (T₀) and after 2 days (T₁), except for Fruz, and after 5 days (T₂). The fish used were sampled within the commercial size of gilthead seabreams (mean body weight=342.8±32.3 g).

Thermo-hygrometric data inside the PRS and the industrial refrigerator were acquired through automated acquisition instruments (every 5 min throughout the testing period), consisting of air temperature and relative humidity (RH) sensors integrated with a datalogger (H2 Testo AG. Lenzkirch-DE: precision 0.5 °C for temperature e, 1% for relative humidity). The temperature inside the conventional industrial refrigerator was measured also in contact with ice.

The weight loss of samples was measured for each refrigeration modality and conservation time above mentioned.

PRSTM Passive Refrigeration System

The system is composed of two units: (a) the container, internally hosting the products and built with walls filled with eutectic liquid solution of water and salt to obtain a specific ice fusion point temperature, and (b) the refrigeration unit, filled with ethylene glycol that cools down and sends back to the first unit through a closed circuit. Once the product unit is fully charged and the walls of the system are frozen, it can hold the temperature for several days or, inversely, it can be used attached to the charging unit, after setting the most appropriate temperature. In both cases, the products are stored with a high percentage of humidity and a very low ΔT.

Digital Image Acquisition and Processing

In order to measure the colour pattern, the camera was mounted on a tripod and images of single fish were acquired. For the acquisition, the samples were taken out from each refrigeration system for about 2 min. A high-resolution Nikon Coolpix P6000 camera (13.5 real MP) was used to acquire TIFF 8-bit images. Manual white balance control, exposure and metering methods were enabled. ISO sensibility was set to 100 to avoid any noise appearance. Colour calibration and validation were carried out using a GretagMacbeth ColorChecker 24 colour patch, as reference standard. Samples were...
illumination with four photographic low-consumption gas lamps with a power of 60 W, producing a light corresponding to 270 W of the traditional bulbs. Such lamps present a nominal illumination power of 3,800 lm, paired with a light temperature of 5,000 K (daylight) and an electronic converter that avoids the flickering effect.

Matlab (rel. 7.1, PLSToolbox Eigenvector rel. 4.0) was used to perform the image calibration based on PLS calibration (Costa et al. 2009a). RGB declared values of the ColorCheker (24 patch) were used as y-block. The x-block was represented by the mean RGB value of the same 24 patch.

Colorimetric Warping Analysis

After colour calibration, a total number of 18 landmarks were digitised (Fig. 1a) on the left side of each fish image, in order to allow the comparison of the entire body fish area. The first 13 landmarks were used to determine the region of interest (ROI) to be compared among samples. Following the landmarks configuration, the image RGB matrices were warped through a geometric morphometric procedure (Costa et al. 2009b; Menesatti et al. 2010). In this way, each pixel inside each ROI could be compared with the one in the same position of the other samples. For each individual, the three RGB values of the 43,211 pixels composing the ROI were decomposed in a single row (129,633 values).

Fig. 1 a Landmarks used for the warping procedure. Description: 1 snout tip; 2 position of the gold stripe on the profile; 3 curvilinear projection of the opercular plate on the profile; 4 insertion of anterior-most dorsal spine; 5 insertion of anteriormost soft dorsal ray; 6 insertion of the posteriormost soft ray; 7 and 9 dorsal and ventral insertion of the caudal fin; 8 posterior most caudal peduncle extremity; 10 and 11 posterior and anterior insertion of the anal fin; 12 insertion of the pelvic fin; 13 ventro-lateral insertion of the opercular plate; 14 centre of the eye; 15 begin of trunk lateral line; 16 vertical projection of the anteriormost soft dorsal ray on the lateral line; 17 and 18 upper (dorsal) and lower (ventral) insertion of the pectoral fin. b The two Sub-ROIs (region of interest; Sub-ROI1, Sub-ROI2) identified by the major pixel’s contribution to the PLSDA classification

Statistical Analyses

The matrix (60 × 129,633) representing the RGB colour values inside the ROI of each seabream at T0 and T2 was analysed with a Partial Least Square Discriminant Analysis (PLSDA; Sabatier et al. 2003; Costa et al. 2010). PLS is a soft modelling method for constructing predictive models with many and highly collinear factors. The technique looks for correlations among the 129,633 RGB values of each pixel (x-block); the y-block was composed by two dummy variables correspondent to fresh (T0) or non-fresh (T2) individuals. The x-block was pre-processed with an ‘autoscale’ procedure. The load of each pixel (x-block), in each latent vector (LV), was extracted (Costa et al. 2009b) in order to determine the pixel’s contribution to the classification (fresh vs non-fresh). Thirty individuals at T1 were used as external test.

Basing on the pixel’s contribution to the classification of PLSDA, two ROIs were identified (Sub-ROI1, Sub-ROI2), one below the dorsal fins and the other below the lateral line (Fig. 1b). The RGB mean values on these ROIs were extracted to statistically test the significance of differences with repeated measures MANOVA. Such comparison was carried out within Sub-ROI1 values and Sub-ROI2 values (not between them), respectively, at T0 and T2. A dendrogram based on the mean Euclidean distances, between the different refrigeration modalities and conservation times, based on the three RGB values decomposed in a single row (129,633 values), was built.

Results and Discussion

The PRSTM resulted as the best of the two tested refrigerator systems: as show in Fig. 2, the RH% and temperature trend lines of the two systems show totally different trends. The RH% industrial refrigerator oscillates between 83% and 100%, repeatedly during all the conservation time. Such a trend affects the product probably shortening visual freshness and more generally its organoleptic characteristics. Conversely, the RH% trend shown by the PRSTM does not show irregular peaks, but gradual reaching and keeping high humidity values (flat line, Fig. 2), so reducing the impact on the conserved products. The same pattern is observed when the temperature trends of the two systems are compared. A rapid and non-invasive technique able to monitor fish appearance, as image analysis combined with colour metrics presented in this work, could be very important for the industrial practices and to meet consumers’ preferences.

As reported in Table 1, the results obtained with the PLSDA model seem to confirm the feasibility of its application in food bioprocess for the automated identification of freshness status. In fact, it is possible to observe as the mean percentages...
of correct classification of the model, sensitivity and sensibility are equal to 100%. This is a promising result leading to future applications. All fish used to create the model (T0 and T2) were correctly classified as fresh or non-fresh, respectively, while external test individuals (T1) were all classified as fresh.

Figure 3 shows the loading values (contribution) to each LV that each pixel gives to the construction of the model (the white pixels correspond to higher loadings). The first LV, which expresses the main variance on both x- and y-blocks (9.9% and 29.4%, respectively), shows that the most important areas for the freshness discrimination are three: (a) the area below the dorsal fins (Sub-ROI1); (b) the central area below the lateral line (Sub-ROI2); and (c) the anterior region of the cephalic area. The first two areas are easily and quickly identifiable—thanks to the scarcity of organs and to a higher homogeneity of pigmentation pattern—then the third one was excluded from further analysis.

The results of this study shown as MANOVA quantified significant colorimetric differences for the Sub-ROI1 and Sub-ROI2, the two of the most informative areas extracted by the PLSDA model, between fresh (T0) and non-fresh fish (T2) and between the four different refrigeration modalities, in all the three RGB components (p<0.001). Consequently, T0 and T2 were correctly classified as fresh or non-fresh, respectively, while external test individuals (T1) were all classified as fresh.

### Table 1 Results of PLSDA modelling to highlight colorimetric differences between fresh and non-fresh seabream

| t1.2 | No. of classified elements | 60 |
| t1.3 | No. of units (y-block) | 2 |
| t1.4 | No. of LV | 4 |
| t1.5 | % Cumulated variance x-block | 28.29 |
| t1.6 | % Cumulated variance y-block | 49.43 |
| t1.7 | Mean specificity (%) | 100 |
| t1.8 | Mean sensitivity (%) | 100 |
| t1.9 | Mean classification error (%) | 0 |
| t1.10 | Mean RMSEC | 0.5028 |
| t1.11 | Random probability (%) | 50 |
| t1.12 | Mean% correlation classification model | 100 |
| t1.13 | Mean% correlation classification independent test (T1) as T0 | 100 as T0 |

See the text for further explanations

*LV* latent vector, *RMSEC* root mean square error of calibration
those two areas could be used for an instrumental colorimetric evaluation device.

The dendrogram in Fig. 4 shows the mean Euclidean distances between the different refrigeration modalities and conservation times. It is possible to observe that the lots closest to the fresh (indicated in the dendrogram as T0-ini) are those stored in PRSTM (indicated as T1-Prs and T2-Prs); then follow the lot T1-Out. All the other lots are very distant, including those conserved with the traditional method in a refrigerator with crushed ice (T1-Frg and T2-Frg). The most distant lots are T2-Frz and T2-Out. These results show very remarkable colour differences between fresh (T0) and non-fresh (T2) fish. It is the first time that the effect of passive refrigeration on fish quality is quantitatively measured, so showing on a sound and qualitative basis that the fresh seabream (independently from their experienced life history) has a lighter colour with respect to non-fresh. It was also demonstrated that samples conserved under the PRSTM show the greatest similarity to the overall coloration pattern of T0 fishes, also at the T2.

The samples weight loss from T0 to T2 showed values significantly lower ($p<0.005$) for Prs-preserved samples (0.20%) compared to Frg (0.67%) and Out (0.95%), lower but not significantly with the Frz (0.42%). Practically, the use of the PRSTM without ice showed a qualitative fish aspect better than the one preserved in traditional refrigerators, suggesting a higher economic margin.

From a technical point of view, the combination of calibration colour (Costa et al. 2009a) with warping (Costa et al. 2009b) (two advanced methods of image analysis) results is very promising for qualitative analysis of quality aspects, such as colour and shape. As reported by Olafsdottir et al. (2004), the European fish industry is still reluctant to implement methods other than sensory to monitor freshness and quality of fish products, although general consensus exists about the importance of various quality attributes and the need for methods to monitor quality.

**Conclusions**

The proposed imaging method brought out two main important issues: (1) a sound, qualitative, automated and non-destructive evaluation of fish freshness based on visual characteristics by merging different image analysis techniques, a three-dimensional colorimetric calibration, the morphometric superimposition and PLSDA modelling and (2) an innovative passive refrigeration system (PRSTM) is proposed for the best fish freshness conservation, at least in view of the overall coloration pattern.
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References


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