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This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Rocculi, P., Cevoli, C., Tappi, S., Genovese, J., Urbinati, E., Picone, G., et al. (2019). Freshness assessment of European hake (*Merluccius merluccius*) through the evaluation of eye chromatic and morphological characteristics. *FOOD RESEARCH INTERNATIONAL*, 115(January 2019), 234-240 [10.1016/j.foodres.2018.08.091].

Availability:

This version is available at: <https://hdl.handle.net/11585/654109> since: 2021-09-09

Published:

DOI: <http://doi.org/10.1016/j.foodres.2018.08.091>

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Accepted Manuscript

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PII: S0963-9969(18)30708-7
DOI: doi:[10.1016/j.foodres.2018.08.091](https://doi.org/10.1016/j.foodres.2018.08.091)
Reference: FRIN 7899
To appear in: *Food Research International*
Received date: 15 June 2018
Revised date: 23 July 2018
Accepted date: 27 August 2018

Please cite this article as: Pietro Rocculi, Chiara Cevoli, Silvia Tappi, Jessica Genovese, Eleonora Urbinati, Gianfranco Picone, Angelo Fabbri, Capozzi Francesco, Marco Dalla Rosa , Freshness assessment of European hake (*Merluccius merluccius*) through the evaluation of eye chromatic and morphological characteristics. *Food Research International* (2018), doi:[10.1016/j.foodres.2018.08.091](https://doi.org/10.1016/j.foodres.2018.08.091)

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Freshness assessment of European hake (*Merluccius merluccius*) through the evaluation of eye chromatic and morphological characteristics

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ABSTRACT

The most commonly used method for fish freshness determination is the sensory inspection; alternative sensory methods such as the Quality Index Method (QIM), based on the significant sensory parameters of one specific species, have been recently suggested. Considering that most of the sensory parameters are based on chromatic and morphological visual impression, the set-up of an objective method using computer vision techniques is very promising. The objective of this research was to characterize the changes in eye chromatic and morphological characteristics of European hake (*Merluccius merluccius*) during 13 days of storage on ice, using a tailored computer vision technique and a 3D scanner. Results obtained by multivariate statistical analysis of the colour spectra of eye images and by the eye concavity index using a 3D scanner permitted to estimate fish unacceptability after 7 days of storage, in agreement with results obtained by QIM sensory analysis. Moreover, ^1H -NMR was used to evaluate the production of trimethylamine (TMA) and the Ki index, confirming a good correlation with eye chromatic and morphological features. This preliminary study showed the high potentiality of the developed method as a non-destructive technique for raw fish freshness characterization / prediction, being a promising approach to create a robust portable instrument for the evaluation of fish freshness in real transport and marketing conditions.

Keywords: fish freshness, image analysis, 3D scanning, ^1H -NMR spectroscopy

1. INTRODUCTION

The quality of fish products, indispensably linked to the freshness of the raw material, is one of the most important attributes to define its market value.

Fresh fish are highly perishable products; their quality degradation starts immediately after fishing due to the occurrence of a series of autolytic processes that lead first to rigor mortis, and afterwards, to autolysis of proteins and fats, creating a favourable environment for bacteria to grow (García et al., 2017). The loss of freshness becomes increasingly evident with time. Its evaluation can be performed using chemical, sensory and physical methods. Because traditional chemical and microbiological determinations are destructive, time consuming, and not suited to describe the early stage of freshness degradation (Dowlati et al., 2013), nowadays the most commonly used method is the sensory inspection based on the valuation of appearance parameters such as skin, slime, eyes, gills, belly and odor, for whole fish stored in ice (Nollet and Toldra, 2010). Among sensory methods, the Quality Index Method (QIM), developed by Bremner (1985) is based on significant, well-defined characteristics of appearance, odour and texture attributes changing through storage time evaluated with demerit points and it is developed specifically for each fish species. However, the need for a trained panel represents an important drawback. For this reason, various attempts have been made to obtain objective methods for the assessment of fish freshness. Considering that most of the sensory parameters are based on visual impression, the set-up of a method using computer vision systems (CVS) is very promising. Dowlati et al. (2013) has used image processing to monitor eye and gills color changes during storage predicting fish freshness through artificial neural networks. However, correlations with other types of determination were missing. On the contrary, in a study on red mullet, Tappi et al. (2017) a good correlation between eye colour and shape evaluated by CVS and other traditional chemical and sensorial parameters has been found.

A 3D scanner is a device that can be used to describe the shape and the appearance of an object collecting distance information about surfaces within their fields of view (Uyar & Erdoğdu, 2009).

3D scanners can be used to construct digital 3D models for a wide variety of applications including food processing. Siripon, Tansakul, & Mittal (2007) obtained a 3D scan of a chicken body to be used for the simulation of the cooking process. Uyar & Erdoğdu (2009) evaluated the potential of 3D scanners for obtaining accurate digital images and geometrical description of complex and irregular shaped materials for food processing modelling.

During fish storage, the eye tends to sink into the eye socket. The eye concavity index (ECI) has been used as a parameter to describe loss of freshness using CVS (Tappi et al., 2017). However, the use of a 3D scanner could be a promising tool for improving the assessment of the changes of this geometric feature of fish during storage.

The objective of this research was to characterize the changes in eye chromatic and morphological characteristics of European hake (*Merluccius merluccius*) during 13 days of storage on ice, using a tailored computer vision technique and a 3D scanner. To validate the obtained data, results were compared with chemical parameters as trimethylamine (TMA) and Ki index obtained by ^1H -NMR spectroscopy (Ciampa, Picone, Laghi, Nikzad, & Capozzi; 2012) and sensory (QIM) evaluations.

2. MATERIALS AND METHODS

2.1. Fish samples preparation and storage conditions

Fresh Mediterranean Hake (*Merluccius merluccius*) were caught in the Adriatic Sea (Cesenatico, Italy) during autumn by a fishing vessel and immediately eviscerated. 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. In the laboratory, samples were individually inserted in open plastic pouches, and placed in polystyrene boxes covered with ice flakes (fish-to-ice ratio 2:1), replenishing melted ice daily. Boxes were successively stored in a 4 °C refrigerated room up to 13 days. In details, 15 fishes were used for non-destructive analysis (QIM, CVS and 3D scanner) while 3 fishes for each sampling time for chemical determinations at 0, 2, 3, 4, 5, 8, 11 and 13 days of storage.

The length of storage was chosen on the basis of literature studies (Orban et al., 2011) and intervals for analytical determinations were carried out after preliminary tests aimed at evaluating the kinetic of quality change.

2.2. Chemical parameters

Sample Preparation. Samples were prepared according to Picone et al. (2011): for each sample, three aliquots of 4 gr of samples were homogenized with 8 ml of 7% perchloric acid. The acid mixtures, transferred into 2 mL centrifuge tubes, were neutralized to pH 7.8 using 9 M KOH and then centrifuged at 14k rpm for 10 min at 4 °C in order to remove potassium perchlorate precipitate. 720 µl of supernatant was aliquoted and placed in Eppendorf microfuge tube adding 80 µl of 3-(trimethylsilyl)-propionic-2,2,3,3-d₄ acid sodium salt (TSP) and then centrifuged at 14k rpm for 10 min at 4 °C. 800 µl of the centrifuged sample were placed in a standard 5 mm NMR tube with a TSP final concentration of 10 mM and measurement were performed.

¹H-NMR measurements. All ¹H-NMR spectra were recorded at 300 K on a Bruker US+ Avance III spectrometer operating at 600 MHz, equipped with a BBI-z probe and a B-ACS 60 sampler for automation (Bruker BioSpin, Karlsruhe, Germany). Each spectrum was acquired using 32 K data points over a 7211.54 Hz spectral width and adding 256 transients. A recycle delay of 5 s and a 90° pulse of 11.4 µs were set up. Acquisition time (2.27 s) and recycle delay were adjusted to be 5 times longer than the T1 of the protons under investigation, which has been considered to be not longer than 1.4 s. Saturation of residual water signal was achieved by irradiating it during the recycle delay at δ equal to 4.703 ppm. For each sample 256 scans were collected into 32 K data points covering a 12 ppm spectral width and requiring 32 min of measurement time. The phase correction and baseline adjusted with TOPSPIN software version 3.0 (Bruker Biospin) and successively the spectra were calibrated taking the chemical shift of TSP signal to 0.000 ppm and integrals of the areas of the different diagnostic areas were calculated by using Chenomx NMR suit 7.7 software (<https://www.chenomx.com/>). For each sampling time, the analysis has been performed in triplicate (one determination for each fish).

2.3. Sensory evaluation

A trained panel of six members evaluated the fish throughout the storage period on each sampling time, according to the Quality Index Method (QIM) (Özyurt et al., 2009). This sensory scale is based on the freshness quality grading system for hake developed by Baixas-Nogueras et al. (2003). The QIM specifies 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 (Botta, 1995). The scale gives zero score for absolutely fresh fish and increasing total demerit points during fish deterioration. The examined parameters were peculiarities 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).

2.4. Visual quality assessment using computer vision system (CVS)

Image acquisition. Qualitative visual assessment was carried out analysing the same 15 fishes for all the period considered. Images of European hake were acquired placing the samples inside a black box and illuminated using four parallel lamps (mod. TL-D deluxe, natural daylight, 18W/965, Philips, NY, USA) with a colour temperature of 6500 K. The Colour Digital Camera

(CDC) (mod. D7000, Nikon, Shinjuku, Japan) used was equipped with a 105 mm lens (mod. AF-S micro, Nikkor) and located vertically over the sample at a fixed distance of 23 cm and at an angle with the lightning source of approximately 45° (Rocculi et al., 2007).

Standard capture conditions were used with following camera settings: manual mode with lens aperture at f of 16 and shutter speed $\frac{1}{2}$ s, no zoom, no flash, 4928×3264 pixels resolution of the CDC and storage in JPEG format. The camera was connected to the USB port of a PC provided with a Nikon Camera Control Pro software (version 2.13.0) to visualize and acquire the digitalized images directly from the computer.

Image analysis and features extraction. The image analysis of sample pictures was performed with ImageJ software (NIH, USA), and considering only the eye region of fishes. RGB images of eye pupils were converted into 8-bit grayscale, and the frequency of each brightness value (256 gray-levels) was plotted by histograms. Each histogram graph reported the percentage of pixels in the vertical axis and the number of gray levels in the horizontal axis. The gray level reporting the maximum pixels frequency has been used as the indicator of the chromatic modifications occurred during storage time.

2.5. Morphological features assessment using 3D scanning

The 3D eyes profile was evaluated by using the Next Engine 3D scanner (NextEngine, Inc. USA) on the same 15 fishes for all the period considered. This scanner is based on the optical acquisition of surfaces. The geometric accuracy of this 3D scanner was around ± 0.005 mm and the distance from the sample was of 16 cm. The fish sample was fixed on the turntable which is driven by the scanner. The scanning process was occurred in three stages: I) optical acquisition of the developed surfaces; II) conversion and assembly of the captured images during the scan; III) cleaning of any soft space element, and final smoothing of the 3D image (Scan Studio HD, Next Engine, Inc. USA). The scanning was carried out for 9 scanning sub-steps for samples in in both vertical and horizontal positions for a total scanning time of about 40 min. To reduce the transparency, the eyes were covered by a plastic wrap and a reflective powder was sprayed (**Figure 1**). MeshLab v1.3.2 (Visual Computing Lab –ISTI-CNR) software features were used for final surface refining operations and to measure the height of the edge of the pupil in the back of the eye.

2.6. Data analysis

For all evaluated parameters, Kolmogorov-Smirnov test were compiled to determine whether the data were distributed normally and Levene's test was used to test for homogeneity of variance. Significant differences (p-level <0.05) between the means at different storage times were explored by means of the analysis of variance (ANOVA with post-hoc Tukey HSD); Kruskal-Wallis test was used if significant differences emerged by the Levene test. Pearson's analysis, (p-level <0.05) was performed to evaluate the correlation between chemical and sensory data. Principal component analysis (PCA) was used as explorative technique to discriminate the samples as function of the storage time and to display the correlation between the parameters. All the statistical elaboration was made by Statistica 7 (StatSoft, INC, USA).

3. RESULTS AND DISCUSSION

3.1 Chemical parameters

Ciampa, Picone, Laghi, Nikzad, & Capozzi (2012) and Picone et al. (2011) showed the potentiality of ¹H-NMR for measuring simultaneously the concentration of metabolites related to fish freshness during storage. In the present study, through this technique, the components more commonly recognised as freshness indicators have been quantified. Trimethylamine oxide (TMAO) is a natural and nontoxic substance, found in almost all marine species, that is implicated in their osmoregulatory role. After death, TMAO content decrease due to gradual conversion into trimethylamine (TMA) by bacterial or enzymatic reduction. TMA is considered the main cause of the incidence of pungent and off-odours during refrigerated storage of fish products (Ólafsdóttir et al., 1997; Ocaño-Higuera et al., 2011), and being highly related to sensorial estimation, is often used as indicator of fish spoilage (Baixas-Nogueras, Bover-Cid, Veciana-Nogués, & Vidal-Carou, 2003).

The initial concentration of TMA-O (singlet at 3.238 ppm) was 71.1 mg/100g (**Figure 2**), similar to the amount found by Baixas-Nogueras et al. (2003) in fresh Mediterranean hake. During the first 5 days, the value remained fairly stable, then dropped significantly until reaching values around 19 mg/100g. TMA-N (singlet at 2.870 ppm) concentration followed a similar but opposite behaviour. The initial content of TMA-N was 0.47 mg/100g. Similar values have been observed by Baixas-Nogueras et al. (2003) and Orban et al. (2011) in hake species and according to Castell, Greenough, Rodgers, & MacFablane (1958) indicate an excellent quality grade. This value did not increase significantly until the 5th day and then rose significantly until reaching 25 mg/100g at the end of the storage.

The acceptability level for TMA is considered 5 mg/100g (Sikorski, Kolakowska, & Burt, 1990). In the present study, this limit was reached between the 5th and the 8th day of storage. After the fish death, autolysis and bacterial spoilage causes changes in the concentrations of adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP), adenosine-5'-monophosphate (AMP), and inosine-5'-monophosphate (IMP), which are turned into inosine (HxR) and hypoxanthine (Hx) (Ciampa et al., 2012).

The K index was first suggested by Saito (1959) as an objective index of fish freshness and it is calculated considering the concentration of ATP and its degradation components and it is defined as follow:

$$K (\%) = ([HxR] + [Hx])/([ATP] + [ADP] + [AMP] + [IMP] + [HxR] + [Hx]) \times 100$$

As the passage of ATP to IMP is fast, (Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E. & Toyama, 1984) modified K index with a new calculation (Ki) is defined as follow:

$$Ki (\%) = ([HxR] + [Hx])/([IMP] + [HxR] + [Hx]) \times 100$$

In the proton nuclear magnetic resonance spectra, the peaks used to calculate the Ki index are identified in the region between 8.16 and 8.60 ppm (IMP: singlet at 8.549, HxR: singlet at 8.301 and Hx: singlet at 8.160 ppm) (Ciampa et al., 2012). In the present study, an initial value of 18% was observed. This value increased fairly constantly throughout storage, reaching 94% (**Figure 3**). Moreover, according to Ciampa et al (2010), concentration and variation of free amino acids (FAAs) are also very sensitive to the changes occurring in fish muscle during storage.

Initial and final concentration (mg/100g) of the amino acids identified by ¹H-NMR are reported in **Table 2**.

Phenylalanine, tryptophan, methionine, isoleucine, leucin, valin and tyrosine are quite stable during storage. As other author suggested (Ruiz-Capillas et al., 2001), this indicate a dynamic balance between the production and he destruction of FAAs, associated with muscle enzymes.

On the other side, alanine, glycine and glutamate show a decreasing trend during storage indicating a higher rate of degradation compared to their production. This result differs from what observed by Ciampa et al. (2010) on bogue. Different fish are characterized by different enzymatic and microbial pools that may explain this discrepancy.

3.2. Sensory evaluation

Results of sensory evaluation, in terms of means of QIM demerit points, are shown in **Table 3**. The maximum sum of demerit points was 19. Quality descriptors were related to the visual appearance of whole fish, eyes and gills, in addition to the sum of total demerit points. As expected, demerit points increased as function of storage time, following a linear trend (R^2 from 0.943 to 0.981), as reported for other cod fishes (Cardenas Bonilla, Sveinsdottir, & Martinsdottir, 2007). In accordance to Baixas-Nogueras et al. (2003), the limit of acceptability for *Merlucciu merluccius* was set to 11.4 (0-19 demerit points), and in our experimental conditions this value was achieved between 7 and 8 days of storage.

3.3. Visual quality assessment using computer vision system (CVS)

Figure 4 shows the typical images of eyes colour changes during ice storage of fish. It is clear that eyes (pupil) tended to be white and cloudy (**Figure 4b**) over the period of storage. This phenomenon could be attributed to the chemical changes occurring in fish after death. The main chemical changes are related to lipolysis, lipid oxidation, and the interaction of the products of these processes with non-lipidic components, such as proteins (Dowlati et al., 2013). These occurrences could therefore play an important role in the determination of quality deterioration of fish with the extended storage. Reporting the pixels frequency (%) as function of the 256 brightness levels of eye pupils grayscale images (**Figure 4**), it is clear that distributions have a prevail tendency to shift from right-skewed to left-skewed, highlighting the variation of pupils from darker colours to the lighter ones.

The maximum pixels frequency of gray levels (%) over the storage time are shown in **Figure 5**. Mean values did not display significant differences within 5 days of storage; however, an exponential trend as function of storage time was determined ($R^2 = 0.942$). The chemical and physical changes of fish body are accompanied with the chemical and physical modifications in fish eyes, therefore a good evaluation of fish freshness can be obtained from eyes colour.

3.4. Morphological features assessment using 3D scanning

The eye concavity index (ECI) was determined by a 3D scanner, and it was reported in terms of pupils' height (mm) as shown in **Figure 6**. The eye consists of a large proportion of liquid, the loss of which, during storage, induces the collapse of the eye into the eye socket. Means values of the ECI linearly decreased passing from 0 to 8 days of storage ($R^2=0.954$) and being equal to zero for further storage days. The 8 days correspond to those established as freshness limit by means of QIM-tot.

3.5 Data correlations

Correlations between non-destructive parameters obtained by image analysis and scanner 3D and chemical (TMA, TMA-O and k-index) or sensorial analysis (QIM), are essential to understand if it is possible to monitor the evolution of fish freshness implementing only non-destructive methods (image analysis and 3D acquisitions). In this way, Tappi et al. (2017) reported that computer vision system (CVS) is a powerful non-destructive technique for the assessment of red mullet freshness. The Pearson's correlation matrix among all studied parameters is reported in **Table 4**. All the correlations were significant ($p > 0.05$). TMA and TMA-O results showed a high positive and negative correlation with QIM indexes, confirming the strong relation between chemical indexes and sensorial parameters, evidenced in previous studies (Baixas-Nogueras et al., 2003; Tappi et al., 2017). Visual quality results evaluated by CVS, reported strong correlations with both chemical (r from 0.824 to 0.977) and sensorial parameters (r from 0.908 to 0.982). Similar and robust results were obtained also considering the ECI.

Table 5 reports the correlation of FAAs with the other considered parameters. Only three amino acids were found to be correlated with the other indexes of fish freshness, namely alanine, glycine and glutamate. These were the FFAs that showed a decrease during storage and were highly correlated to the other considered chemical, physical and sensorial parameters.

PCA was developed considering all parameters evaluated in this study and the score plot is reported in figure 8. A clear separation over the storage time was observed. The samples were arranged along the PC1 (explained variance: 91.9%) from right to left, on the basis of the storage time (from 0 to 13 days). The loading plot of the variables (**Figure 9**) suggests that the discrimination is due to the contribution of all considered parameters. Loadings close to -1 or 1 indicate that the variable strongly influences the discrimination along the component, while loadings close to 0 indicate that the variable has a weak influence on the component. So, similar discrimination of the sample can be obtained considering just non-destructive parameters (visual quality and morphological index). Furthermore, considering that the variables that appear closer together on the chart are highly correlated, the correlation results showed in table 3 were confirmed.

CONCLUSIONS

Results of this research revealed a great correlation between chemical and sensory modification of fish occurring during ice storage with non-destructive analysis performed. In details, the changes of morphological aspects of eyes (ECI) and of their visual quality evaluated by CSV were highly correlated (r mean values of -0.89 and 0.95, respectively) with parameters evaluated by the official

sensory analysis (QIM), exhibiting the high potentiality of these techniques for the evaluation of hake freshness. Chemical parameters assessed showed a high correlation with sensory results, confirming a close relation between organoleptic and chemical modifications occurring during fish storage.

In our experimental conditions, during the ice storage of fish samples, in accordance to QIM evaluation, the total shelf life estimated for European hake was around 7 days.

On the basis of this results, it could be supposed a future implementation of non-destructive visual and morphological techniques on mobile system devices, which could allow the estimation of fish freshness in real time.

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Figure 1: Scanner 3D set up (a); eye covered by a plastic wrap and reflective powder (b).

Figure 2. Trimethylamine (TMA) and Trimethylamine oxide (TMA-O) of European hake for 13 days of storage. Error bars represent standard deviations. Same letters show no significant differences between mean values ($p>0.05$).

Figure 3. k-index of European hake for 13 days of storage. Error bars represent standard deviations. Same letters show no significant differences between mean values ($p>0.05$).

Figure 4. Typical images of eyes colour changes during storage at 0 (a) and 13 days (b); pixel frequency (%) as function of brightness levels of eye pupils grayscale images.

Figure 5. Gray levels that showed the maximum pixels frequency (GLMF) over 13 days of storage. Error bars represent standard deviations. Same letters show no significant differences between mean values ($p>0.05$).

Figure 6. 3D images of the pupil acquired by the scanner at 0 and 13 days of storage.

Figure 7. Concavity eye index (CEI) for 13 days of storage. Error bars represent standard deviations. Same letters show no significant differences between mean values ($p>0.05$).

Figure 8. PCA score plot of the samples as function of storage time.

Figure 9. Loading score plot of variables used for the PCA.

Table 1. QIM scheme for sensory evaluation of European hake by Baixas-Nogueras et al. (2003).

	Parameter	Descriptor	Demerit ponts	
General appearance	Surface	Bright, iridescent	0	
			Less bright, dull	1
			Tenuous rosy grey (dorsal zone)	2
			Tenuous yellowish rosy grey (dorsal zone)	3
	Flesh firmness	Firm, elastic	0	
			Firm, hard	1
			Elastic, flexible	2
			Soft	3
Eyes	Clarity (cornea)	Transparent, bright	0	
			Slightly opaque	1
			Opaque	2
	Pupil	Bright black and circular	0	
			Greyish black, less bright	1
			Greyish black, distorted	2
			Milky grey and distorted	3
	Shape	Slightly convex	0	
			Plane	1
			Slightly concave	2
Gills	Color	Bright red, none mucus	0	
			Dull red, clear mucus	1
			Brownish red, milky mucus	2
			Colorless, slats stick together	3
	Smell	Fresh, seaweedy (algea, sea)	0	
			Fresh, slightly seaweedy	1
			Slightly sweet, rancid	2
			Off odors (acid, metallic, rancid)	3
		Range of the total demerit points		(0 - 19)

Table 2. Concentration (mg/100g) of free aminoacids identified by ^1H -NMR.

Aminoacid	^1H (ppm)	Concentration (mg/100g)	
		C_i	C_f
Isoleucine	0.94	12.96 $\pm$ 4.58	14.01 $\pm$ 5.50
Leucine	0.96	21.57 $\pm$ 3.49	24.22 $\pm$ 7.20
Valine	1.00-1.05	20.40 $\pm$ 5.73	19.73 $\pm$ 2.94
Alanine	1.49	166.21 $\pm$ 56.73	50.62 $\pm$ 30.62
Methionine	2.14	16.90 $\pm$ 4.99	23.36 $\pm$ 12.98
Glycine	3.56	189.28 $\pm$ 74.93	29.58 $\pm$ 3.80
Glutamate	3.75	106.32 $\pm$ 45.07	35.41 $\pm$ 8.45
Tyrosine	6.88	16.77 $\pm$ 5.56	22.04 $\pm$ 6.23
Tryptophan	7.19	5.77 $\pm$ 2.41	6.76 $\pm$ 4.67
Phenylalanine	7.32	11.59 $\pm$ 6.24	16.45 $\pm$ 6.76

C_i : initial concentration; C_f : final concentration

Table 3. Sensory results over the storage time

Storage (time)	QIM-tot			QIM-WF			QIM-E			QIM-G		
0	1.2	±	0.3	0.0	±	0.2	0.7	±	0.2	0.0	±	0.2
2	2.0	±	0.3	0.8	±	0.2	0.8	±	0.3	1.0	±	0.3
3	4.2	±	1.0	0.8	±	0.3	1.3	±	0.2	2.2	±	0.2
4	5.2	±	0.9	0.8	±	0.2	1.7	±	0.3	3.0	±	0.3
5	7.5	±	1.8	1.0	±	0.5	3.2	±	0.2	3.2	±	0.2
8	12.8	±	0.4	3.0	±	0.2	4.2	±	0.3	3.8	±	0.3
11	14.7	±	1.3	3.7	±	0.5	5.0	±	0.3	5.8	±	0.3
13	18.7	±	0.3	5.5	±	0.3	7.0	±	0.0	6.7	±	0.0

Note: WF: whole fish, E:eyes, G:gills

Table 4. Correlation matrix among chemical (TMA, TMA-O), sensory (QIM) and visual (CSV) and morphological (ECI) parameters.

	TMA	TMAO	K-index	CVS	ECI	QIM-tot	QIM-WF	QIM-E	QIM-G
TMA	-								
TMAO	-0.893	-							
K-index	0.716	-0.831	-						
CVS	0.977	-0.948	0.824	-					
ECI	-0.618	0.837	-0.880	-0.752	-				
QIM-tot	0.860	-0.960	0.915	0.947	-0.917	-			
QIM-WF	0.930	-0.933	0.887	0.982	-0.821	0.971	-		
QIM-E	0.887	-0.984	0.907	0.960	-0.880	0.990	0.965	-	
QIM-G	0.839	-0.932	0.864	0.908	-0.929	0.963	0.925	0.953	-

Table 5. Correlation among FAAs concentration and physical, chemical and sensorial parameters.

Aminoacid	TMA	TMAO	K-index	CVS	3D	QIMtot	QIMW	QIME	QIMG
Alanine	-0.903*	0.915*	-0.891*	-0.929*	0.809*	-0.942*	-0.942*	-0.949*	-0.911*
Phenylalanine	0.365	-0.418	0.474	0.369	-0.261	0.351	0.402	0.400	0.236
Tryptophan	0.151	-0.165	0.547	0.187	-0.346	0.333	0.310	0.287	0.127
Glycine	-0.890*	0.889*	-0.835*	-0.906*	0.853*	-0.929*	-0.927*	-0.917*	-0.964*
Methionine	0.601	-0.696	0.776*	0.619	-0.727*	0.715*	0.706	0.710*	0.646
Isoleucine	-0.080	0.073	0.016	-0.087	0.277	-0.164	-0.084	-0.099	-0.304
Leucine	0.131	-0.140	0.170	0.128	0.162	0.031	0.113	0.110	-0.143
Valine	-0.254	0.236	-0.121	-0.256	0.403	-0.322	-0.264	-0.256	-0.457
Tyrosine	0.135	-0.170	0.402	0.145	-0.173	0.176	0.222	0.196	0.069
Glutamate	-0.873*	0.830*	-0.825*	-0.892*	0.776*	-0.914*	-0.912*	-0.891*	-0.857*

*: significant at $p < 0.05$

Highlights

- A non-destructive method for fish freshness assessment was developed
- Eye chromatic and morphological characteristics were useful indexes of fish freshness
- High correlation among chemical, sensorial and non-destructive results were observed
- Total shelf life estimated for European hake was around 7 days

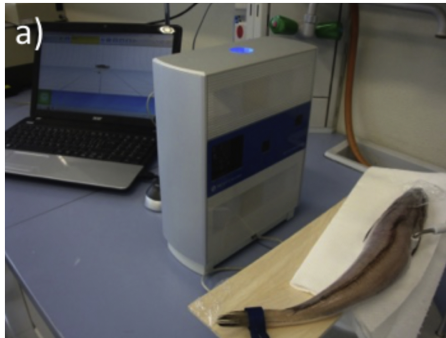


Figure 1

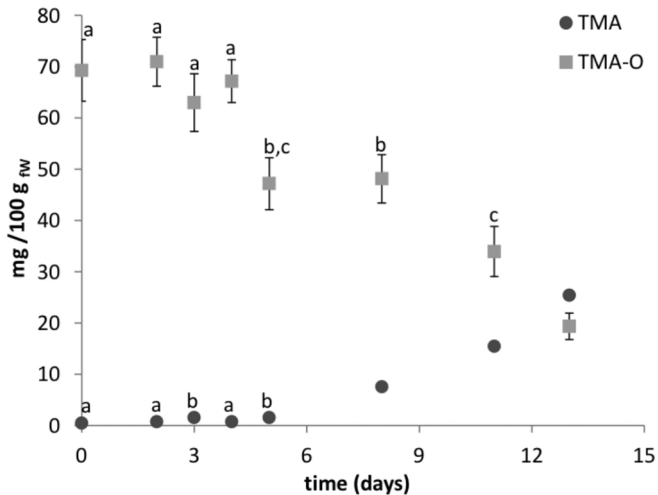


Figure 2

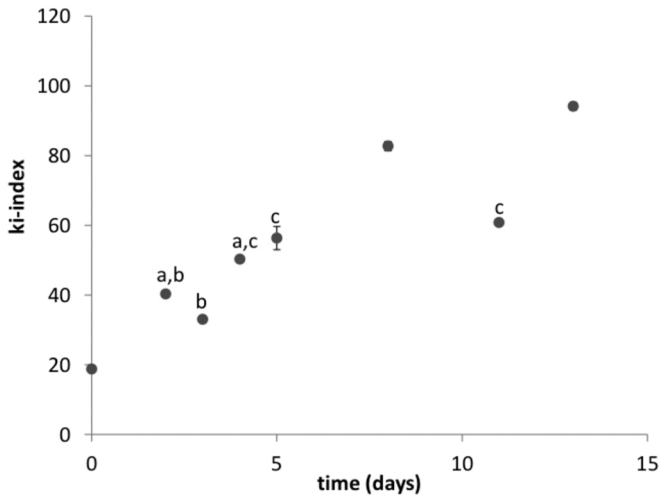


Figure 3

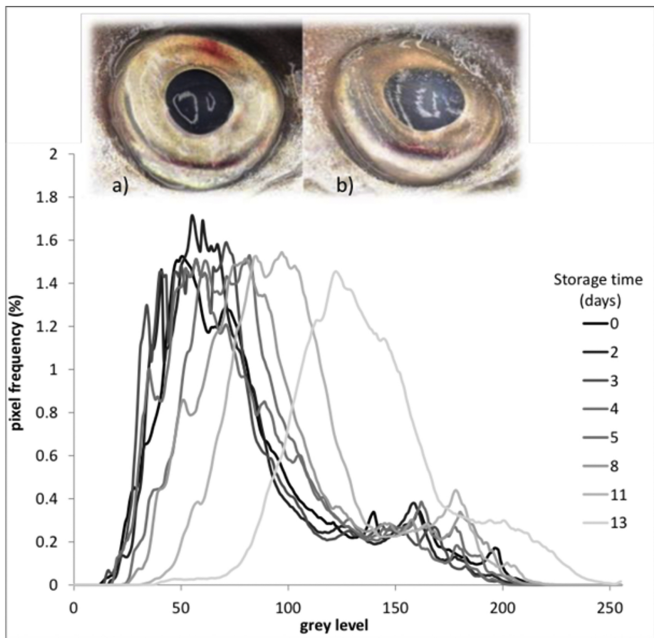


Figure 4

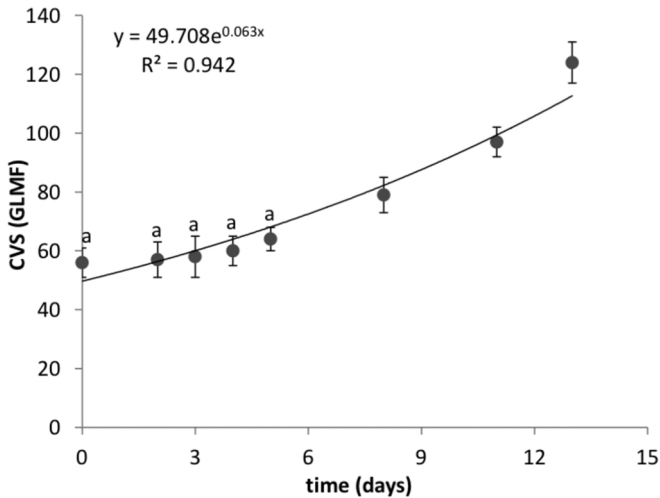


Figure 5



Figure 6

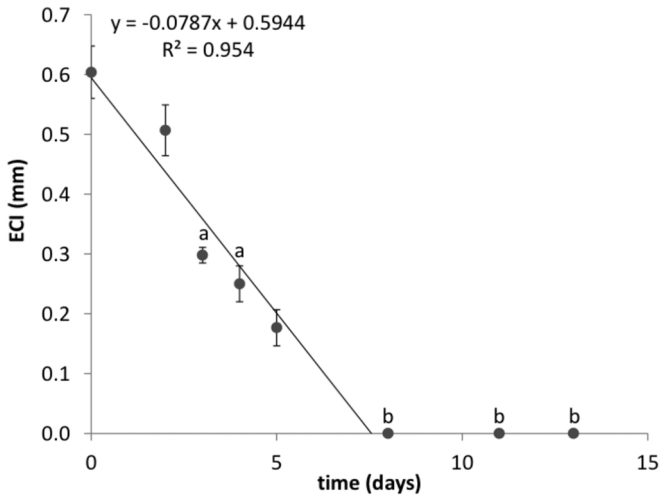


Figure 7

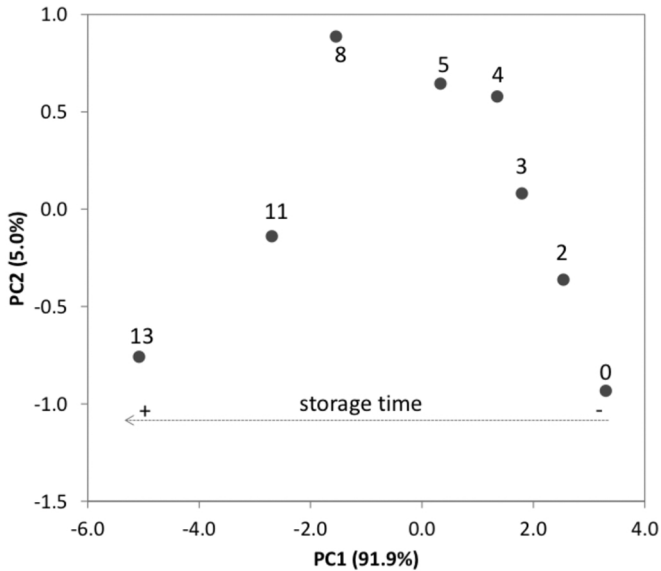


Figure 8

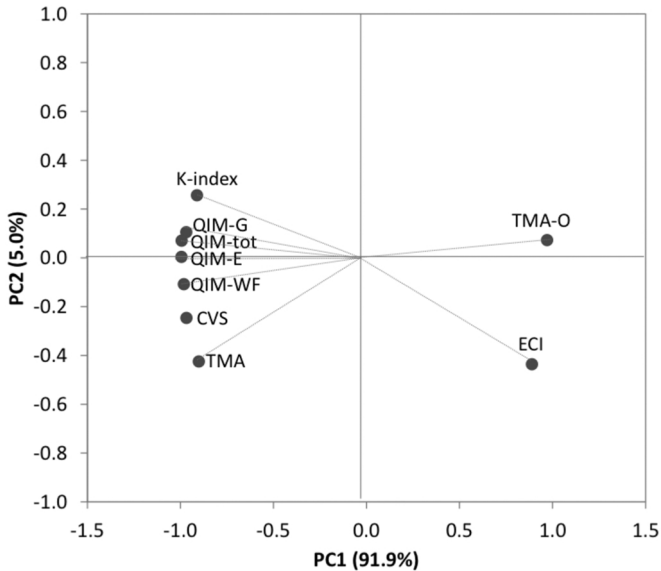


Figure 9