Smart sensor to predict retail fresh fish quality under ice storage

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Abstract

Fish wastage and market prices highly depend on accurate and reliable predictions of product shelf life and quality. The Quality Index Method (QIM) and EU grading criteria for whitefish (Council Regulation(EC) No 2406/96, 1996) are established sensory methods used in the market to monitor fish quality. Each assessment requires the consultation of a panel of trained experts. The indexes refer exclusively to the current state of the fish without any predictions about its evolution in the following days.

This work proposes the development of a smart quality sensor which enables to measure quality and to predict its progress through time. The sensor combines information of biochemical and microbial spoilage indexes with dynamic models to predict quality in terms of the QIM and EU grading criteria. Besides, the sensor can account for the variability inside the batch if spoilage indexes are measured in more than one fish sample.

The sensor is designed and tested to measure quality in fresh cod (*Gadus morhua*) under commercial ice storage conditions. Only two spoilage indexes, psychrotrophic counts and total volatile base-nitrogen content, were required to get accurate estimations of the two usual established sensory methods. The sensor is able to account for biological variability as shown with the validation and demonstration data sets. Moreover, new research and technologies are in course to make these measurements faster and non-destructive, what would allow having at hand a smart non-intrusive fish quality sensor.

Keywords: Smart sensor, retail fish quality, quality index method, predictive microbiology, core predictions, fish-to-fish variability

1. Introduction

Freshness is one of the most important attributes to define the market value of fish. Fresh fish are highly perishable, with shelf-lives ranging from some days to a few weeks. Immediately after fishing, a series of autolytic processes start to occur. These processes lead first to rigor mortis, and afterwards, to autolysis of proteins and fats, creating favorable conditions for bacteria to growth. The loss of quality becomes increasingly evident with time.

In the industry, quality is commonly assessed by sensory analyses relying on the visual inspection of fish by a panel of trained experts (Cheng et al., 2015).

The method consists of evaluating a list of sensory attribute separately and select the most repeated score. In the European Union, sensory assessment of fresh fish is defined by [Council Regulation\(EC\) No 2406/96 \(1996\)](#), which classifies fish quality into four categories (Extra, A, B and unfit or NA). The EU Standard Quality Method (from now on, SQM) is based on the state of skin, skin mucus, eyes, gills, and flesh. However, the regulation has been -and still is- a subject of considerable controversy, what has encouraged the emergence of the quality index method (QIM, [Luten & Martinsdottir \(1997\)](#)) as an alternative. The QIM scores depend on the fish species and are defined attending to several properties such as

skin color, odor, texture; eyes pupils and form; gills color, mucus, and odor; flesh color and viscera. The QIM defines 3-4 scores per quality parameter thus enabling a more refined characterization as compared to SQM.

Sensory indexes, however, refer exclusively to the current state of the fish without predicting shelf life or the loss of quality in the following days. Despite some attempts to estimating shelf life using sensory indexes (Bonilla et al., 2007; Heising et al., 2012) or spectroscopy (Sivertsen et al., 2011), most works (Dalgaard, 1995; Jørgensen et al., 1988; Koutsoumanis & Nychas, 2000; McMeekin et al., 1992; Taoukis et al., 1999) propose to correlate shelf life with a particular microbial load using the concept of predictive microbiology. These authors then suggest the use of microbial growth models to predict when this bacterial load is achieved and thus predict shelf life.

Whereas shelf life allows to plan ahead to avoid fish wastage, quality models are preferable as they can also estimate differences on sensory quality and can be used to fix market prices. However, the non-linear nature of fish quality characteristics hinders the modeling and prediction of fish quality as characterized by the established sensory indexes. First attempts to estimate quality proposed the use of non-established sensory indexes (Nuin et al., 2008; Tsironi et al., 2009). García et al. (2015) proposed a model that is able to correlate *Pseudomonas* and *Shewanella* growth with the SQM. However, these indexes were insufficient to predict QIM. Giuffrida et al. (2013), on the other hand, were able to predict the QIM, but at the expense of measuring six different microbiological indexes (skin, gills and flesh sulphide producers and non-producers).

The majority of quality and shelf life models in the literature focus on the most probable prediction. However, these indexes may vary significantly due, for example, to the use of different fishing gear or variations in season or ground. Even within the same batch variability may be substantial due to intrinsic fish-to-fish variability and differences in handling. It is, therefore, desirable to accompany most probable quality dynamics with the associated variability. García et al. (2015) and Koutsoumanis et al. (2002) explored that variability in the SQM and shelf life respectively.

Nevertheless, and for practical purposes, it is of

the highest interest to design a strategy to assess and predict fish quality together with its variability from a limited number of measurements. In this context, we propose the development of a smart fish quality sensor. Smart, here, refers to the capability of the sensor to assess the quality and, in addition, to predict its evolution through time and variability. The first step to develop the smart sensor is to define the minimum measurements that allow characterizing quality indexes. The second, is to define and calibrate the mathematical relationship (predictive model) that correlates those measurements with quality. The third step, is to validate the smart sensor.

In this work, the sensor was designed, calibrated and validated to assess quality of fresh cod (*Gadus morhua*) under commercial ice storage conditions exploring the relationship between psychrotrophic counts and total volatile base-nitrogen (TVB-N) content with established sensory indexes.

2. Materials and methods

The design of the smart sensor includes the definition of the purpose (output) based on the available measurements (inputs) as depicted in Figure 1.

This is a complex problem that requires the design of the three main parts:

- **Sensor observations or outputs** In this work we aim to take a step forward and to predict, not only shelf life, but the usual established quality indexes used nowadays in the market (QIM and QSM). The sensor output estimates those indexes at current time and predicts their evolution through time. Moreover, fish-to-fish variability inside the same fishing batch are studied to provide its impact on quality variability.
- **Sensor inputs or necessary measurements** The smart sensor will combine a number of “hardware” sensors and a mathematical model which is able to characterize the relationship between measurements, quality indexes and time. In this work we tested the possibility of using a minimum number of measurements as the inputs for the model. Psychrotrophic counts and TVB-N were finally selected based on previous findings, the most important of these are described in the following:

Figure 1: Smart sensor design.

Psychrotrophic counts Fish spoilage under cold storage has been correlated with the growth of some particular gram-negative psychrotrophic bacteria. Specifically, *Shewanella* spp. has been reported as the specific spoilage organism (SSO) of fresh air-stored chilled fish from cold marine waters. However, different SSOs were found when fish was caught in warmer waters or stored under a CO_2 -enriched atmosphere (Gram & Dalgaard, 2002). Recently, Chailou et al. (2015) found that spoilage bacteria mainly originated from the environment (water reservoirs) and that storage conditions exert strong selective pressure on the initial bacteria. Also, that seafood spoilage involves the growth of various bacterial communities, with specific groups of psychrotrophic bacteria as key components.

TVB-N Spoilage also occurs because of action of microbial metabolites of psychrotrophic bacteria responsible for off-flavors and off-odors (Cheng et al., 2015; Gram & Dalgaard, 2002; Vogel et al., 2005). Among those metabolites, trimethylamine, ammonia and, to a lower extent, dimethylamine are the most abundant. The total volatile base-nitrogen (TVB-N) index combines these amines into a unique indicator used to assess spoilage of fresh fish. In fact, the content of TVB-N is the only biochemical index presently included in EU regulations to discriminate fit from unfit fish in commerce or official inspection. Neither TVB-N nor trimethyl-

amine (TMA) content can be used as indexes of quality in the early stages of storage (Dalgaard, 2000; Howgate et al., 2010). However, some studies have found significant correlations between TVB-N and TMA and storage time (Baixas-Nogueras et al., 2001; Oehlenschläger, 1998; Ruiz-Capillas & Moral, 2001). Therefore indicating that the modeling of TVB-N or TMA dynamics could be used to predict shelf life.

- **Sensor engineering, input-output relationship or model** Finding the appropriate, usually non-linear, relationship between the inputs and the sensor outputs is a typically rather complex task (Saguy, 2016). We will follow an iterative procedure which combines experimental, statistical and numerical methods as illustrated in Fig. 2. This procedure consists of iterating between theory and data to select the best mathematical structure (model selection) and the set of unknown parameters (model calibration). Each pair of model structure and set of parameters are tested until the predictive capabilities of the sensor are considered satisfactory (sensor validation). Details are described in the sequel.

2.1. Experimental methods

2.1.1. Fish handling and storage conditions

Cod fish (*Gadus morhua*) was caught in the Central North Sea (FAO Major Fishing Area 27, Division IV b) by trawling. Once gutted, fish was ice-stored and then landed at Grenaa fishing harbor (Denmark), from where it was transported to Vigo (Spain) by road. About four days old fresh cod (1.5-2 kg / fish)

Figure 2: **Iterative procedure for sensor engineering** Sensor engineering is structured around three main steps: model selection and calibration and sensor validation. Model selection and calibration rely on a set of experimental data (subset 1) while sensor validation is approached by means of a different set of data (subset 2). Different steps in the sensor design call for different optimization and simulation-based methods.

were purchased from a local fish wholesaler on six different dates (batches) between March and May of 2015 (detailed in Table 1) in Vigo. Fish were transferred to the laboratory in expanded polystyrene boxes with ice and there stored in a cold chamber at $2 - 3^{\circ}\text{C}$. Between four and eight fish were removed from storage on sampling days for sensory, microbiological and biochemical analyses.

2.1.2. Sensory analysis

A panel of six trained assessors evaluated fish freshness using two sensory methods. On one hand, freshness was rated according to the grading criteria set out for whitefish in the [Council Regulation\(EC\) No 2406/96 \(1996\)](#), which is presently the Standard Quality Method (SQM) in the European Union. Ac-

cordingly, fish can be graded in four different categories of freshness, that is, extra (E), good (A), fair (B) and unfit (NA). On the other, freshness was also evaluated following the Quality Index Method (QIM) developed for fresh cod ([Luten & Martinsdotir, 1997](#)), in which a number of quality parameters are scored and all scorings added to give an overall value, the quality index. The QIM scheme for fresh cod used in the study is shown in Table A.3.

2.1.3. Microbiological analysis

A quantity of 25 ± 1 g of fish muscle was homogenized in 100 mL of peptone water for 30 s in a stomacher (ITUL Instruments, 2997/400, Spain). This quantity complies with the recommendations made by the International Commission on Microbiologi-

Batch	Day of purchase	Purpose	Analyses	Number of	
				samples	data
1	2/3/2015	Calibration	Psychrotrophs, TVB-N, QIM, SQM	16	64
2	13/3/2015	Calibration	Psychrotrophs, TVB-N, QIM, SQM	16	64
3	23/3/2015	Calibration	Psychrotrophs, TVB-N, QIM, SQM	12	48
4	11/5/2015	Calibration	Psychrotrophs, TVB-N, QIM, SQM	22	88
5	30/4/2015	Validation	Psychrotrophs, TVB-N, QIM, SQM	22	88
6	14/5/2015	Demonstration	Psychrotrophs and TVB-N at $t = 0$	1 ^a	2

Table 1: **Experimental design.** Each experiment corresponds to a batch. Different batches were purchased in different days and, therefore, correspond to different catching conditions. The number of samples indicates the number of fishes used per batch. The number of data indicates the total number of data used to compute the mean values and the corresponding standard deviations for each batch. Remark that a total of 264 data were used for calibration and 88 for validation.

^a For further validation of the sensor, QIM was also evaluated for 7 more cod samples after 5 days ($t = 5$). See Section 3.3 for details

cal Specifications for Foods (ICMSF, 1986). Homogenates were then ten-fold serially diluted in peptone water. Aliquots (0.1 mL) of adequate dilutions were spread on plate count agar (APHA ISO 4833, Panreac, Spain) and the number of psychrotrophic bacteria was counted after incubation at 17 °C for 3 – 5 days.

2.1.4. Total volatile base nitrogen

Total volatile base-nitrogen (TVB-N) content was determined as described by the method of Lücke & Geidel (1935), following modifications by Antonacopoulos (1960). Briefly, about 10 g of cod muscle were blended and transferred to a Kjeldahl distillation unit. A few drops of silicone antifoaming agent (Panreac, Spain) and 2 g of MgO (Panreac, Spain) were then added. About 75 mL of distillate were collected after steam distillation. Next, TVB-N content was determined by titration of the distillate with 0.1 N HCl using 5 mL of a 1 % (w/v) boric acid as indicator (pH 5.5). Results are expressed as mg TVB-N per 100 g of muscle.

2.2. Sensor engineering

2.2.1. Model selection and calibration

Model selection and calibration of the key sensory and spoilage indexes run in parallel. The different model candidates are first calibrated to compute the unknown parameter values that give the highest likelihood to the experimental data.

The calibration is formulated as an optimization problem where the objective is to compute the unknown parameter values that minimize the so-called log-likelihood function. For normal and independent distribution data, the log-likelihood function corresponds to:

$$J(\theta) = \sum_i^{nd} \frac{(\tilde{y}_{mi} - y_i(\theta))^2}{\sigma_{y,i}^2} \quad (1)$$

where i indicates the measurement point, being nd the total amount of data (sum of number of sampling times for the total number of experiments); $\tilde{y}_{mi}$ and $\sigma_{y,i}$ correspond, respectively, to the mean and standard deviation at measurement time i , whereas $y_i(\theta)$ refers to the corresponding sensor prediction for a given value of the parameters θ to be estimated. Note that, the available information on the nature of data noise and variability is being considered (Walter & Pronzato, 1997), i.e. more relevance is given to those data with less variability.

It should be stressed that, following good practices (Vilas et al., 2016), estimated parameter values are common to all experiments. This means that model parameters attain a unique value for all experiments (multi-experiment calibration), and less number of experimental data are required. Therefore these unique values, together with their associated uncertainty, may be used for the purpose of the smart sensor without any further calibrations. In other words, same parameters are being used for validation and demonstration.

The multi-experiment calibration allows us also to gather information even if the time from fishing to purchasing varies among batches, as it can be deducted from the initial bacterial load, and is unknown. In fact, in our experiments time and sampling times are relative variables with respect to the day of purchase (time=0). Note that, therefore, even if same relative sampling times are considered in two different experiments, they are actually different absolute sampling times whenever the initial condition of the experiments are different.

It should be stressed that the parameter values are not unique when considering fish-to-fish variability and, to a lesser extent, the error of the method. While solving problem in Eq. (1) gives us the most probable set of parameter values, different realizations of the experimental data within the experimental noise distribution can be used to determine the **parameter confidence intervals** (Balsa-Canto et al., 2010). To analyze the effect of the parameter confidence intervals on the sensor we compute **state confidence**.

Confidence intervals for both states and parameters are estimated using a Monte Carlo sampling approach. This technique generates various data realizations (500 in this work) following the statistical distributions of the experimental data. Each of these realizations is used to estimate the model parameters. All parameter values are collected in a matrix. To remove possible outliers, we select those parameters in the 0.95-0.05 interquartile range. The mean and the standard deviation for each parameter is used to express the parameter value and its confidence interval $\theta_i \pm \sigma_{\theta_i}$. The corresponding model predictions result in the state confidence. Note that for normal distributions this corresponds with a confidence interval of 68.27%.

Experimental data, model dynamics and state confidences are analyzed to select a good sensor design to be validated in the next step. Visually the sensor is considered good when

1. data lie inside the state confidence,
2. their mean value are close to the most probable estimated dynamics and
3. the state confidence is sufficiently narrow to distinguish the dynamics.

When several model candidates are available, the log-likelihood value is another measure of the sensor capacity to reproduce the experimental data.

2.2.2. Sensor validation

In this step, the model structure and the set of parameters are tested to check whether it is possible to predict the established sensory indexes from measurements of microbial and biochemical indexes when fish arrives to the market (t_0). These initial conditions encode information about previous factors affecting quality (such as catching ground and season, fishing gear and initial handling among others). For the measured initial conditions and the set of parameters found in the model calibration, sensor predictions are compared with a new set of experimental data (data for validation).

Confidence in the prediction due to fish-to-fish variability, commonly known as core predictions (García et al., 2015), is evaluated using a Monte Carlo sampling approach. Here we use the model to simulate a large number of solutions for different realizations of the parameter confidence intervals and the measured initial fish-to-fish variability. We compare the core predictions with the data for validation to check if the three conditions defined at the end of previous subsection 2.2.1 are satisfied.

2.3. Numerical methods

The solution of the parameter estimation requires the use of advanced numerical techniques. In this work, we made use of AMIGO2 (Advanced Model Identification using Global Optimization), a multi-platform toolbox implemented in Matlab which covers parameter estimation but also sensitivity analysis and experimental design (Balsa-Canto et al., 2016).

3. Results and Discussion

The smart sensor proposed in this work includes two spoilage indexes (psychrotrophic counts and TVB-N content) as functions of time, and defines their correlation with the established fish sensory methods, QIM and SQM. In the following sections, we will describe the selection and calibration of the different sub-models corresponding with the four indexes. Following, we compile the selected equations and the values of the estimated parameters to assess the predictive capabilities of the sensor in the validation step. At the end of the section we demonstrate the use of the sensor in practice.

3.1. Model Selection and calibration

3.1.1. Standard dynamics for psychrotrophic counts

Measurements of psychrotrophic counts after arriving to the market ($t = 0$) exhibited only exponential and stationary phases following the logistic model:

$$\frac{dm(t)}{dt} = \mu(1 - 10^{m(t)-m^*}), \quad (2)$$

with $m(t) = m(0)$ when $t = 0$ and where $m(t)$ and m^* refer, respectively, to the concentration and maximum concentration (concentration in the stationary phase) of the psychrotrophic population [CFU/g] in log-10 scale. In the equation t describes the time elapsed since the fish arrived to the market in days [d] and μ represents the growth velocity [$1/d$].

Note that model (2) is equivalent to the commonly used expression of Baranyi & Roberts (Baranyi & Roberts, 1994) without lag phase with the transformation $m = \log_{10}(CFUs/g)$ and being now μ the Baranyi's velocity divided by $\ln(10)$ and m^* the maximum concentration in log-10 scale. We should stress that logarithmic variables are dimensionless and calculations are more exact using this transformation. Bacterial concentrations follow a log-normal distribution (Busschaert et al., 2010), and therefore a normal distribution in the logarithmic scale, and we avoid dynamics over different ranges of magnitude what could derive in numerical errors.

The performance of the model was tested in experiments 1 to 4, regarded as subset 1 in Fig. 2. For this purpose we estimated the unknown model parameters which include the growth velocity (μ) and the maximum population (m^*). Note that simulation of Eq. (2) requires information about the initial bacterial population when the fish arrives to the market, i.e. a measure of $m(0)$. Many factors such as fishing gear, handling, or catching ground and season, affect such condition (Huss, 1995). A wrong choice for the initial condition may lead to over- or under-estimation of the growth curve. To avoid this situation, initial conditions for each experiment are estimated within the range computed from the experimental data statistics at $t = 0$ ($\bar{y}_0 \pm \sigma_{y_0}$).

Fig. 3 shows the sensor performance with the selected model. Continuous black lines indicate the most probable solution and gray lines define the state confidence (experiments 1-4). Results show that (1) data lie inside the state confidence, (2) their mean

values are close to the most probable estimated dynamics and (3) the state confidence is sufficiently narrow to distinguish psychrotrophic counts for different storage times. No other model candidates were tested based on these satisfactory results achieved.

3.1.2. A new model for TVB-N content as a function of microbial load

The most common model of TMA or TVB-N formation is usually modeled using an exponential model (Tsironi et al., 2009; Yao et al., 2011):

$$N(t) = N(0) \cdot e^{kt}, \quad (3)$$

where N stands for TVB-N content, k is the velocity of the reaction and t describes the time elapsed since the fish arrived to the market in days [d]. $N(0)$ is the TVB-N content at $t = 0$, i.e. when the fish arrives to the market

A critical drawback of conventional models of TVB-N content is their inability to reproduce the initial dwell and the exponential behavior (Heising et al., 2014b; Howgate et al., 2010). Besides, although some works claim that psychrotrophic bacteria produce TVB-N content, the role of bacterial counts is not considered in the TVB-N models (Gram & Dalgaard, 2002; Vogel et al., 2005). To include such effects we considered the following extension of the exponential model:

$$\frac{dN(t)}{dt} = k a(t) m(t) \quad (4a)$$

$$a(t) = \frac{a_0}{a_0 + (1 - a_0) \exp(-kt)} \quad (4b)$$

where the variable $m(t)$ describes the psychrotrophic bacteria growth of Eq. (2) and k is the production velocity [$1/d$]. Inspired by Baranyi's model (Baranyi & Roberts, 1994), we modified the velocity with a time-function $a(t)$ that simulates the dwell phase. The function $a(t)$ depends on the unknown parameter a_0 that goes from 0 to 1.

Fig. 4 shows how TVB-N content increased exponentially after a dwell of a few days, as observed previously by Howgate et al. (2010). The exponential model (3) cannot reproduce the dynamics of TVB-N content and, based on the maximum likelihood objective, it focus in reproducing the data with less associated variability, i.e. the first sampling times. As a result, the model (3) performs well only during

Figure 3: **Calibration for the selected model 2.** Experiments 1-4 corresponding with a total of 66 fishes purchased in 4 different days. Time here regards the number of days after purchase (relative time). Remark that, the time from fishing to purchasing varies among batches, as it can be deduced from the initial bacterial load. Therefore the multi-experiment parameter estimation allows to study an experimental design that it is, at least, as informative as using a single experiment with around 10 sampling times. Dots and bars represent, respectively, the mean and standard deviation of the experimental data ($\bar{y} \pm \sigma_y$) calculated using four to eight replicas, whereas continuous black lines indicate the most probable model solution and gray lines define the state confidences. Remark that psychrotrophic counts are in log-10 scale and only two model parameters, common to the 4 experiments, are estimated: the growth velocity and the maximum concentration.

the first storage days. Instead, the model with the dwell was able to follow the TVB-N content dynamics even for longer experiments (Fig. 4). The proposed model also performs better regarding the log-likelihood value in Eq. (1): the exponential model value ($1.28 \cdot 10^5$) is one order of magnitude larger than the proposed model ($4.26 \cdot 10^4$).

Fig. 5 shows the performance of the sensor when considering fish-to-fish variability, obtained by computing the state confidence. Again the model performs as required, being the only exception the last sampling time in experiment 4.

3.1.3. A correlation of Quality Index Method (QIM) with spoilage indexes

The models of QIM dynamics found in the literature were inadequate for the purpose of this work. Nuin et al. (2008) proposed a model independent of any spoilage indexes whereas the works by García et al. (2015) and Giuffrida et al. (2013) were developed for different fish species and considering only microbial counts. Similar formulations were tested using psychrotrophic counts and TVB-N content re-

sulting in inaccurate QIM predictions. In fact, Giuffrida et al. (2013) used a total of six microbial indexes (sulphide non-producers and producers in skin, gills, flesh) to reproduce the dynamics of QIM. García et al. (2015) used QIM estimates only as an intermediate variable to determine the SQM, which is a method with less scoring marks and therefore that requires less precision.

In this work, we propose a new neural network based model to estimate the scorings of the QIM inspired by the perceptron model with logistic activation. Note that a more mechanistic model would require the analysis of a large number of microbial and biochemical spoilage indexes as in Giuffrida et al. (2013). Although such model would be interesting for understanding the dynamics of quality, it would be of limited use in practice since it requires to measure a large number of microbial and biochemical indexes. Specifically, we explore the use of Artificial Neural Networks (ANN), recently considered to study different fish spoilage indexes (Liu et al., 2015; Wang et al., 2015). Multi-layer ANNs are universal approximators to any continuous function. In our case, the simplest

Figure 4: **Model calibration of TVB-N content using the exponential model (3) and the model we propose (4).** Dots and bars represent, respectively, the mean and standard deviation of the experimental data ($\tilde{y} \pm \sigma_y$) whereas continuous lines depict sensor dynamics.

Figure 5: **Model calibrations of TVB-N content (experiments 1-4) using model (4).** Dots and bars represent, respectively, the mean and standard deviation of the experimental data ($\tilde{y} \pm \sigma_y$) whereas continuous black lines indicate the most probable solution and gray lines define the state confidences.

single neuron ANN is enough to predict quality indexes. The relationship is given as:

$$QIM = \text{nint} \left(23 \frac{1}{1 + \exp(-\alpha m - \beta N - \gamma)} \right). \quad (5)$$

where m is the concentration of psychrotrophic population in log-10 scale and N stands for TVB-N content.

In this equation, the sigmoid depends on the spoilage indexes and three model parameters α, β and γ . This function goes from 0 for spoilage indexes equal to zero to the maximum QIM score for cod (23) when those spoilage indexes are large, provided the bounds for γ are properly selected. QIM takes only discrete values and hence the function is also rounded to the next integer value with the “nint” function. Fig. 6 shows the good performance of the model regarding maximum probability dynamics and state interval. Note that the staircase shape of the function is due to the rounding to the closest integer value in 5. On the contrary, the panel assessments of QIM (asterisks) were not rounded to show better the variability between different panelists and fish samples.

3.1.4. A correlation of the EU Standard Quality Method (SQM) with QIM

SQM estimates were obtained using the approach proposed in [García et al. \(2015\)](#). The idea was to correlate SQM and QIM making use of the available experimental data. Note, however, that some QIM scores may correspond to two different but consecutive SQM levels. To overcome this difficulty we define in-between zones where QIM scores correspond with any of the two SQM marks. These regions simulate the usual situation where the different panelists provide different SQM marks. In this way, we define seven SQM levels: extra (E), extra to good (E-A), good (A), good to fair (A-B), fair (B), fair to unfit (B-NA) and unfit (NA).

The empirical correlation between SQM and QIM

is described as:

$$SQM = \begin{cases} \text{E,} & \text{if } QIM \in [1, 3] \\ \text{E-A,} & \text{if } QIM \in (3, 5] \\ \text{A,} & \text{if } QIM \in (5, 10] \\ \text{A-B,} & \text{if } QIM \in (10, 13] \\ \text{B,} & \text{if } QIM \in (13, 14] \\ \text{B-NA,} & \text{if } QIM \in (14, 21] \\ \text{NA,} & \text{if } QIM \in (21, 23] \end{cases} \quad (6)$$

It should be stressed that no optimization is needed to find this correlation. Fig. 7 shows the estimates (black line) and state confidences (gray areas). The estimates can take more than one SQM value because of the definition of the in-between zones. In the figure, the state confidence region shows the possible SQM variability considering fish-to-fish variability. Note that, instead of showing all individual solutions, the plot presents the area where all possible solutions lie.

3.2. Sensor validation

Core predictions are calculated to test the predictive capabilities of the designed sensor, including mathematical structure, best parameters found and their confidence intervals. Eqs. (2), (4), (5) and (6) define the mathematical structure whereas the complete set of estimated model parameters, with their respective optimization bounds and confidence intervals is presented in Table 2.

θ	Units	θ_{min}	θ_{max}	$\tilde{\theta} \pm \sigma_{\theta}$
m^*	–	5	8.5	7.7 ± 0.28
μ	1/d	0.1	1	0.51 ± 0.060
k	1/d	0	10	0.94 ± 0.19
a_0	–	0	0.1	0.0036 ± 0.0073
α	–	0	2	0.95 ± 0.17
β	–	0	2	0.044 ± 0.031
γ	–	-100	10	-7.67 ± 0.62

Table 2: Bounds and estimations of the unknown parameters using experiments 1-4. In this procedure initial conditions for psychrotrophic counts and TVB-N content were also estimated considering the statistics of their respective measurements ($\tilde{y}_0 \pm \sigma_{y_0}$) for each experiment at time zero.

Fig. 8 depicts results of experiment 5, not used in the calibration step, and the corresponding sensor

Figure 6: **Calibrations (experiments 1-4) using model (5)**. Evaluations of each panel expert are depicted with asterisk whereas continuous black lines indicate the most probable solution and gray lines define the state confidences.

Figure 7: **Calibrations (experiments 1-4) using relationship in (6)**. Evaluations of each panel expert are with asterisks whereas continuous black lines indicate the most probable sensor solution and gray areas define the state confidences, i.e. the intervals of confidence considering fish-to-fish variability.

Figure 8: **Sensor validation against experimental data.** Dots represent the mean value of microbial and biochemical measurements. Asterisks represent the assessments of the expert panel. Continuous black lines indicate the sensor prediction while colored areas correspond to prediction confidences.

predictions for psychrotrophic counts, TVB-N content, QIM and SQM. The mean absolute residuals correspond to 0.14 (CFU/g in log-10 scale) for psychrotrophs; 7 (mg TVB-N/100 g) for TVB-N and 2 for QIM. In relative terms this means an average prediction error of 1.7% for psychrotrophs; 9% for TVB-N and 8.7% for QIM. Remarkably these relative errors are within the margins of the expected variability among samples.

The core predictions of psychrotrophic counts (first row in Fig. 8) show how confidence in the predictions increases during the exponential phase but tends to decrease during the stationary phase. This fact reveals that the main source of variability during the first days, encoded in the initial conditions, corresponds to the fish-to-fish variability inside the fishing batch.

On the other hand, the width of TVB-N core predictions tends to increase with time. This property is a common feature of exponential models: even for scenarios where the initial fish-to-fish variability is narrow, core predictions increase with time. Interestingly enough, as we will see later, this effect is not crucial to get accurate estimates of the sensory indexes.

First figure in last row of Fig. 8 shows the main results of this work: accurate estimates of QIM can be

predicted using the proposed smart sensor. Once the model and parameters are selected and calculated, dynamics of QIM for every new fishing batch are calculated based on the statistics of psychrotrophic counts and TVB-N content variability in the fishing batch at a given time (day 0 in our validation dataset). Maximum variability in the predictions usually corresponds to long storage times, being those QIM scores between 15 and 20 for experiment 5. We should stress, however, that the results reported by panelists are also subject to substantial variability.

Although the use of QIM has increased in the last years, SQM continues to be the quality method of reference in the industry. The correlation found in (6) gives us the relationship between both indexes for the data analyzed in cod under ice storage. As in the QIM, it is usual to observe discrepancies between different panelists. Our model considers these discrepancies by defining intermediate zones where the model can take two adjacent scores. Figure 8 illustrates this point showing how the most probable SQM estimates sometimes have several values for the same sampling time. In all cases, the most probable prediction coincides with the experimental data.

To further analyze the significance of the sensor predictions we run a chi-square goodness of fit test for the validation experiment. For the test we take the

following null-hypotheses H0: The data are consistent with the model predictions. Since the QIM and the QSM are categorical variables we run the test with the frequencies of each value as obtained by the model core predictions (expected, E_i) and the experiments (observed, O_i). The statistic reads as follows:

$$\chi^2 = \sum_{ki=1}^n \frac{(O_i - E_i)^2}{E_i}$$

The test statistic follows, approximately, a chi-square distribution with ν degrees of freedom which corresponds to 23 for QIM and 6 for QSM. Therefore, the resulting χ^2 value can be compared to the chi-square distribution to determine the goodness of fit.

For the case of QIM the mean χ^2 value over sampling times is 24.39. The corresponding p-value for 23 degrees of freedom is 0.38 which is higher than conventional criteria for statistical significance (.001 – .05). For the case of QSM the mean χ^2 value over sampling times is 0.47. The corresponding p value for 6 degrees of freedom is 0.99 which is higher than conventional criteria for statistical significance (.001 – .05).

Since the null hypothesis cannot be rejected in either case, the data are consistent with a specified model predictions.

3.3. Demonstration on the use of the smart sensor in practice

Once the smart sensor has been validated against experimental data, we will illustrate how the sensor would be used in real practice. We only need to initialize the model with measurements of psychrotrophic counts and TVB-N content in at least one fish sample to run the prediction. For a new experiment (number 6 in Table 1), we measure psychrotrophic counts and TVB-N content at $t = 0$, i.e. when the fish arrives to the market. This information is fed to the sensor as input and the sensor provides the result in Figure 9, for example using a code QR located on the fish batch. If the user is interested in the variability of the sensory indexes within the fishing batch, statistics of the fish-to-fish variability has to be measured or assumed attending to previous experience.

To test further the sensor, the QIM was evaluated on 7 cod samples 5 days after arriving to the

market. While the sensor predicted a QIM of 11, the resulting mean value of the panel was of 10.4 ([8.610.712.110.510.69.110.8]).

Remark that new research and technologies are in course to make these measurements faster and non-destructive, what would allow having at hand very reliable statistics of the initial conditions. In this respect, impedance methods (see for example Koutsoumanis & Nychas (2000)) can be used to determine microbial counts, whereas TVB-N content can be measured using non-destructive colorimetry sensor arrays (Huang et al., 2015) or using different types of ph-electrodes (Heising et al., 2014a; Bhadra et al., 2015).

4. Conclusions

This work proposed the combination of psychrotrophic counts and TVB-N content as a means to develop a smart sensor to assess and predict fish quality and its variability with a limited number of measurements.

First we established the mathematical relationship between measurements and desired smart sensor outputs, i.e. Quality Index Method (QIM) and EU Standard Quality Method (SQM). To do so we used a sequence of model selection, calibration and sensor validation.

We tested the performance of the smart sensor in the case of fresh cod (*Gadus morhua*) under commercial ice storage conditions. A rigorous iterative procedure was used to iteratively improve sensor predictive capabilities. The best candidate model was selected using the calibration data set and validated against a (new) validation data set.

The proposed methodology to obtain the smart sensor is general and it can be applied to any fish species. Of course parameter values -and maybe the perceptron model- need to be adapted for each species by means of data fitting.

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Figure 9: **Sensor output.** The colors represent the most probable prediction of the QIM model (5). The shelf life, depicted in the plot using the usual terminology “best before”, is based on the first “NA” estimation of the SQM model (6)

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Appendix A. Quality Index Method scheme for fresh cod

Quality parameter		Description	Scoring
Appearance	Skin	Bright, iridescent pigmentation	0
		Rather dull, becoming discoloured	1
		Dull	2
		In rigor	3
	Stiffness	Firm, elastic	0
		Soft	1
		Very soft	2
Eyes	Cornea	Clear	0
		Opalescent	1
		Milky	2
	Pupil	Black	0
		Opaque	1
		Grey	2
	Form	Convex	0
		Flat, slightly sunken	1
		Sunken, concave	2
Gills	Colour	Bright	0
		Less coloured, becoming discoloured	1
		Discoloured, brown spots	2
		Brown, discoloured	3
	Smell	Fresh, seaweedy, metallic	0
		Neutral, grassy, musty	1
		Yeast, bread, beer, sour milk	2
		Acetic acid, sulphuric, very sour	3
	Mucus	Clear	0
		Milky	1
		Milky, dark, opaque	2
Flesh, fillets	Colour	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
Blood	Colour	Red	0
		Dark red	1
		Brown	2

Table A.3: Quality Index Method (QIM) scheme for fresh cod [Luten & Martinsdottir \(1997\)](#). The scoring goes from 0 (best quality) to a maximum of 23

Appendix B. Measured quality indexes

	t	QIM						QSM					
Batch 1	0	0.50	1.00	0.00	1.00			E	E	E	E		
	2	3.50	6.40	2.40	6.40			A ⁺	A	E	A		
	4	9.42	11.00	12.83	13.20			A	B ⁺	B ⁺	B		
	7	17.00	18.83	17.00	16.80			B	B	B	B		
Batch 2	0	1.00	2.00	1.00	1.00			E	E	E	E		
	3	7.50	4.17	5.00	4.80			A	A	A	A		
	4	9.00	9.67	8.50	7.83			A	A	A	A		
	6	12.00	12.80	15.00	14.40			A	A	B	A		
Batch 3	0	1.80	2.00	1.40	2.00			E	E	E	E		
	2	6.83	6.50	8.50	3.80			A	A	A	A		
	4	5.80	8.80	11.20	9.80			A	A	A	A		
Batch 4	0	4.80	1.70	1.20	2.80			E	E	E	E		
	2	7.20	8.70	7.30	8.20	7.30	7.00	A	A	A	A	A	A
	4	10.30	11.30	12.30	13.30	14.30	15.30	B	B	A	A	A	B
	7	16.30	17.30	18.30	19.30	20.30	21.30	NA	B	B	B	B	B
Validation	0	3.60	3.20	3.60	2.80			E	E	E	E		
	4	12.20	6.50	11.30	10.50			A	A	A	A		
	6	12.30	14.50	20.00	17.00	19.50	21.30	18.80	B	NA	NA	NA	NA
	7	15.00	17.00	22.00	21.00	22.00	23.00	20.00	NA	NA	NA	NA	NA

Table B.4: Experimental data of QIM and SQM for all fish batches and sampling times (t)