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Research Article

Shelf-life Estimation of Mullet *(Mugil cephalus)* Fillets by Mathematical Models Based on Some Biochemical Parameters and Sensory Evaluation

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Abstract

This study aimed to estimate the shelf-life of mullet fillets stored in ice. Cadaverine, putrescine, total volatile base nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were evaluated together with sensory analyses. Empirical mathematical models were used to describe the data, and shelf-life was estimated by using acceptability limit and the model proposed for each parameter. The models proposed were all in agreement with the experimental data and can be safely used to estimate the shelf-life of mullet fillets. The lowest shelf-life was found as 5.2 days for the sensory analysis performed by trained panelist. Shelf-lives were determined as 6.5 and 7.5 days according to TBARS and PV, respectively. Lipid oxidation seemed to be the reason of the results since mullet is considered as a medium-fat fish. Highest shelf-life was calculated as 11.7 days according to cadaverine which is an indicator of late spoilage due to late formation. The results presented here revealed that predictive modeling can be used to describe the kinetic data for fish quality and further to estimate the shelf-life.

Keywords: *Predictive modeling, fish, shelf-life, TVB-N, TBARS, Peroxide value*

Kefal *(Mugil cephalus)* Filetolarının Bazı Biyokimyasal Parametrelere ve Duyusal Değerlendirmeye Dayalı Matematik Modellerle Raf Ömrü Tahmini

Öz

Bu çalışmada buzda depolanan kefal filetolarının raf ömrü tahmini amaçlanmıştır. Kadaverin, putresin, toplam uçucu bazik azot (TVB-N), peroksit değeri ve tiyobarbitürik asit (TBARS) değeri duyusal analiz sonuçları ile birlikte değerlendirilmiştir. Bu verileri tanımlamak için deneysel matematiksel modeller kullanılmış, her parametre için kabul edilebilirlik sınırı ve önerilen model kullanılarak raf ömrü tahmin edilmiştir. Önerilen modellerin tümü deneysel verilerle uyum gösterdiğinden, kefal filetolarının raf ömrünü değerlendirmek için güvenle kullanılabilmektedir. En düşük raf ömrü duyusal analiz için 5.2 gün olarak bulunmuştur, ancak eğitimli panelistler tarafından gerçekleştirilen duyusal analizler gerçek tüketici davranışını temsil etmeyebilir. Raf ömrü TBARS'a göre 6.5 gün, PV'ye göre ise 7.5 gün olarak hesaplanmıştır. Kefal orta yağlı balık olarak kabul edildiğinden, bu sonuçların nedeninin lipid oksidasyonu olduğu düşünülmektedir. En yüksek raf ömrü ise geç bozulmanın indikatörü olarak kullanılan kadaverine göre 11.7 gün olarak hesaplanmıştır. Burada sunulan sonuçlar, tahminsel modellemenin balık kalitesi için kinetik verileri tanımlamak ve ayrıca raf ömrünü tahmin etmek için kullanılabileceğini ortaya koymaktadır.

Anahtar sözcükler: *Tahminsel modelleme, balık, raf ömrü, TVB-N, TBARS, Peroksit değeri*

Introduction

Fish and other seafood products are considered one of the most important food commodities due to their delicacy with high nutritive value, hence their consumption has risen substantially over the past few decades. Therefore, it is very important to ensure the safety of edible fish for global fishing industry [1,2]. Fish production is estimated to reach about 179 million tons in 2018 globally and 156 million tons (about 88%) ended up in our plates whereas

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12% was used for non-food purposes. Live, fresh or chilled fish still represent the largest share of fish utilized for direct human consumption [3].

Striped mullet *(Mugil cephalus)* is an important and one of the most widely distributed seafood fish species around the world, occurring in the coastal waters of the tropical, subtropical and temperate zones of all seas. The fish has a good market in some countries, especially in the southern and eastern Mediterranean region. It is also consumed in many Asian countries. It is usually consumed fresh [4,5]. Fish and other seafood products are the most valuable nutrients, however, high levels of moisture, free amino acids, and unsaturated fatty acids make them extremely perishable foods [6]. These products are especially susceptible to chemical, enzymatic and microbiological spoilage during processing or storage [2]. Fresh and storage in ice are the common commercial practice for most fishes. Keeping fish in ice is one of the most efficient treatments for retarding spoilage [7].

The rate of spoilage during ice storage of fish depends on species, storage conditions, handling and processing from the catch to the consumer. Under improper conditions, fish are susceptible to changes in physicochemical properties and microbial spoilage. Therefore, maintaining and monitoring safety and freshness of fish are very important for consumers and food industry. There are many conventional techniques to point the state of freshness including sensory analysis, physical detection techniques, (bio)chemical and microbiological parameters such as total volatile basic nitrogen (TVB-N), thiobarbituric acid value (TBA), peroxide value (PV), biogenic amines and total viable counts (TVC). Most of these traditional techniques are time-consuming, expensive and tedious, requiring well trained operators [8-10].

Shelf-life determination of fresh fish is very important when assessing the quality. Unfortunately, because of many factors affecting freshness, the precise estimation of fish shelf-life is difficult and complex [11]. In shelf-life studies, (bio)chemical parameters and volatile compounds such as TVB-N can be monitored and modelled. In recent times, predictive modeling is a key component in quantitative risk assessment and management of seafood safety and quality.

Predictive models that are able to estimate quality aspects of fish and seafood products can be used as tools for quality management in the seafood industry [7]. Since a single parameter alone does not reflect the freshness during storage of fish, models resulting from the combination of several parameters (physical, microbiological, chemical, sensory attributes) could be more potent and comprehensive. Therefore, the main purpose of this study was to develop models in order to predict the shelf-life of striped mullet fillets stored in ice by using TVB-N, TBA, PV, biogenic amines (cadaverine and putrescine) and sensory analysis data.

Material and Methods

Data Set

All data used in this study were taken from a recent work published by Pilavtepe-Celik et al.[12] Experimental procedures (sample preparation, storage conditions, sensory evaluation, chemical analyses) were explained in detail by the authors. Briefly, biogenic amines (cadaverine and putrescine), total volatile base nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were measured during storage (up to 13 days) at 0°C. Sensory analyses were performed with 11 trained panelists during storage (up to 11 days) at 0°C.

Modeling

To describe cadaverine and putrescine over time, following model was proposed:

$$C(t) = \ln\{1 + \exp[k \cdot (t - t_c)]\} \tag{1}$$

where $C(t)$ is the concentration of cadavarine or putrescine at a time t (day), t_c is the time level at which cadavarine/putrescine concentration starts to accelerate and k is the rate (time^{-1}) at which cadavarine/putrescine concentration climbs as the time passes to a level well above t_c. Note that according to this model initially i.e., t = 0 cadavarine or putrescine concentration is zero. This model was originally proposed by Campanella and Peleg [13] as the secondary model in microbial inactivation.

The same model with an intercept (C_0) incorporated was used to describe total volatile base nitrogen (TVB-N):

$$C(t) = C_0 + \ln\{1 + \exp[k \cdot (t - t_c)]\} \tag{2}$$

where $C(t)$ is the value of TVB-N at a time t (day), C_0 is the initial (t = 0) TVB-N value, t_c is the time level at which TVB-N value starts to accelerate and k is the rate (time^{-1}) at which TVB-N value climbs as the time passes to a level well above t_c.

For peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) the following model was used:

$$C(t) = C_{max} \cdot \left(1 + \frac{t_{max} - t}{t_{max} - t_{rate}}\right) \cdot \left(\frac{t}{t_{max}}\right)^{\frac{t_{max}}{t_{max} - t_{rate}}} \tag{3}$$

where $C(t)$ is the value of PV or TBARS at a time t (day), C_{max} is the maximum PV/TBARS value attained at time t_{max} and t_{rate} is the time at which maximum rate is achieved.

Finally, sensory evaluation was described by using simple linear model viz.,

$$C(t) = k \cdot t \tag{4}$$

where $C(t)$ is the sensory score at a time t (day), k is the slope (time^{-1}) of the line which shows the rate of increase of the score.

Goodness-of-Fit Evaluation

Models were assessed by coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and root mean square error (RMSE) values. Higher R^2 and R^2_{adj}, and lower RMSE (compared to the *y* axis) values indicated good fit. SigmaPlot (Version 12.0, Chicago, IL) was used for linear and non-linear regression, and also to obtain the parameter values and goodness-of-fit indices.

Determination of Shelf-life

Putrescine and cadaverine are the biogenic amines commonly analyzed in seafood and their sum should be less than 20 mg/kg, or putrescine concentration should be less than 10 mg/kg [14,15]. Therefore, 10 mg/kg was used as the limit of consumption for cadaverine and putrescine to estimate shelf-life. One of the most common indicators of fish spoilage is TVB-N. Fish, according to TVB-N value, can be considered as spoiled above 35 mg/100 g fish [16,17] and this value was used as the limit value to calculate shelf-life. Upper limits of acceptability for PV and TBARS values were set to 1 mmole CPO/kg fish and 6.5 mg MDA_{eq}/kg fish, respectively for shelf-life prediction. Finally, for sensory evaluation rejection point was set to 5 out of 15 for overall quality parameter to estimate shelf-life of mullet fillets [12].

The shelf-life of fish samples were obtained as the intersection point of the model fits and the acceptability limits given above for each analysis. Therefore, shelf-life could be calculated by using the numerical values of the model parameters in the model equation and acceptability limit values. Moreover, intersections of the 95% confidence bands (upper and lower) and the acceptability limits were used as the 95% confidence interval of the shelf-life.

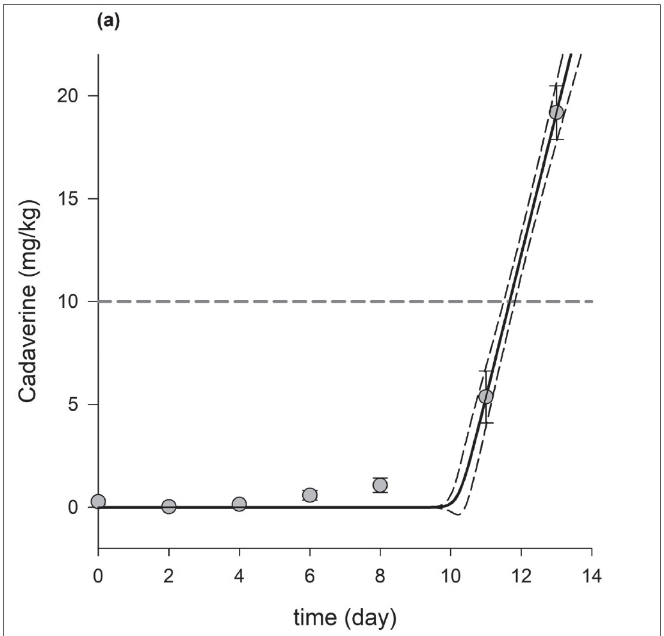
Results

Model Fits

Figure 1 shows the fit of Eq.(1) to cadaverine and putrescine, and *Fig. 2* shows the fit of Eq.(2) to TVB-N. The fit of Eq.(3) to PV and TBARS is shown in *Fig. 3* while the fit of Eq.(4) for sensory data is shown in *Fig. 4*. Confidence bands (95%) of each fit (black dashed lines) is also shown together with the maximum allowance of each analysis (gray dashed line) during the storage. *Table 1* and *Table 2* list the model parameters and goodness-of-fit indices. Fitted curves went near the data *(Fig. 1, 2, 3* and *4)*, and higher (and closer to 1.0) R^2, R^2_{adj} (≥0.9190) and lower RMSE (≤1.0064) values were obtained *(Table 1* and *Table 2)* indicating that models proposed were in good agreement with the experimental data. Moreover, parameter uncertainties (standard errors) were very low compared to parameter values so that all parameters were statistically significant ($P<0.05$).

Shelf-life Estimation

By using the parameter values of the models given in *Table 1* and *Table 2* and the maximum allowance level of each analysis, shelf-life of the mullet fillets was calculated. Furthermore, by using the 95% confidence bands of each fit 95% confidence intervals of shelf-life were also estimated. These are given in *Table 3*. The lowest shelf-



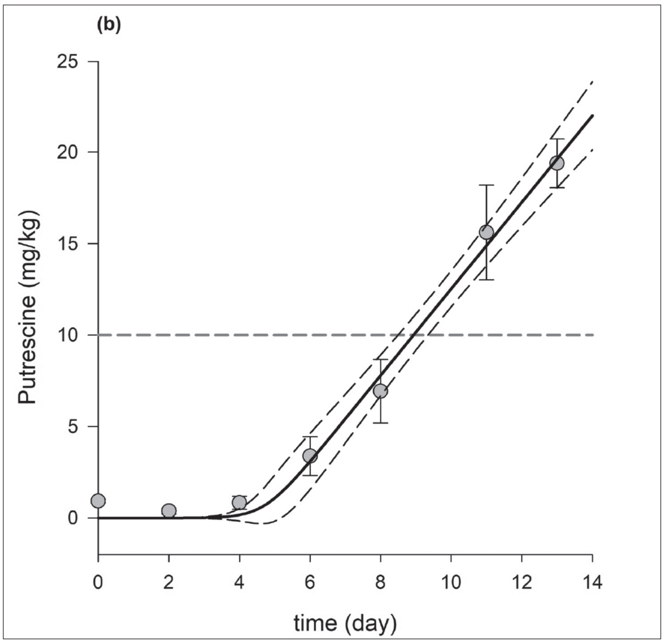


Fig 1. Change of cadaverine (a) and putrescine (b) in mullet fillets at 0°C with respect to time. Gray circles are the experimental data points; error bars represent standard deviation of three independent replications. Solid black line is the model fit [Eq. (1)], dashed black lines are the 95% confidence bands and dashed gray line is the limit for the shelf-life. Original data are from Pilavtepe-Celik et al.[12]

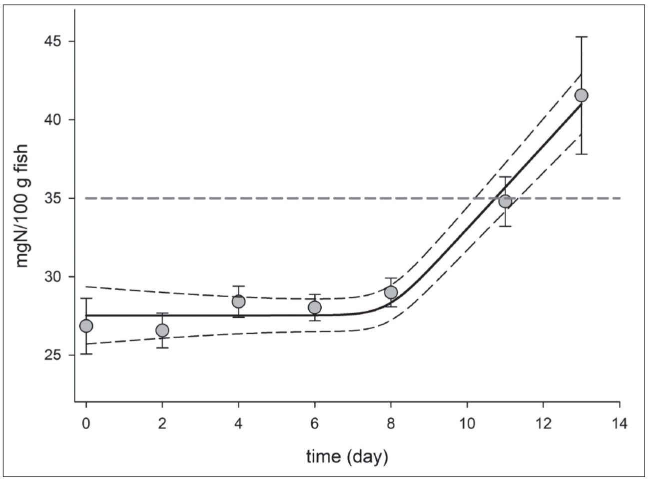


Fig 2. Change of TVB-N in mullet fillets at 0°C with respect to time. Gray circles are the experimental data points; error bars represent standard deviation of three independent replications. Solid black line is the model fit [Eq.(2)], dashed black lines are the 95% confidence bands and dashed gray line is the limit for the shelf-life. Original data are from Pilavtepe-Celik et al.[12]

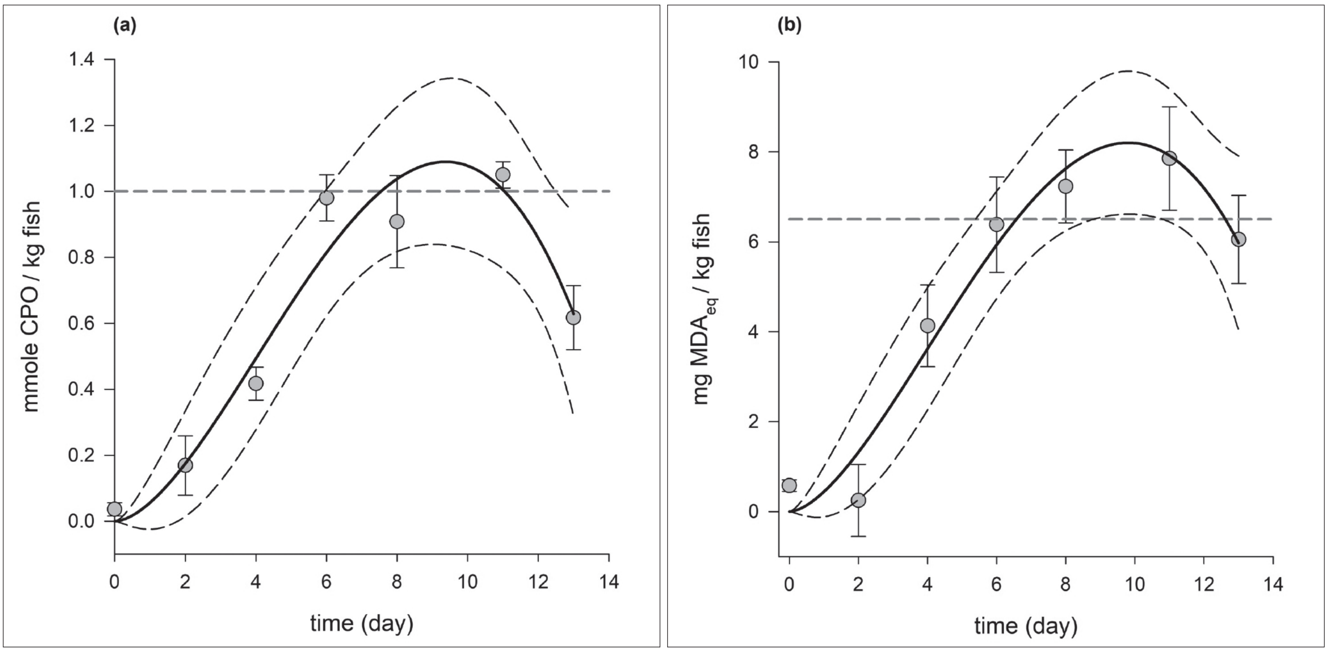


Fig 3. Change of PV (a) and TBARS (b) in mullet fillets at 0°C with respect to time. Gray circles are the experimental data points; error bars represent standard deviation of three independent replications. Solid black line is the model fit [Eq.(3)], dashed black lines are the 95% confidence bands and dashed gray line is the limit for the shelf-life. Original data are from Pilavtepe-Celik et al.[12]

life was determined in sensory analyses as 5.2 days with 95% confidence interval of 4.4 to 6.4 days followed by TBARS as 6.5 days with 95% confidence interval of 5.4 to 8.8 days. Highest one was obtained in cadaverine as 11.7 days (11.5 to 11.8 days) followed by TVB-N as 10.7 days (10.2 to 11.3 days) *(Table 3)*. Note that upper limit of PV cannot be calculated because the acceptability limit never intersected with the lower 95% band *(Fig. 3-a)*.

Discussion

Shelf-life of fish during storage is mainly dependent on storage temperature. Since the storage temperature (0°C) was lower than the refrigeration temperature (4°C) in this study, it may be expected to have longer shelf-life. However, the minimum shelf-life was estimated as low as 5.2 days: the lowest shelf-life was determined from the modeling of sensory analysis (5.2 days with 95% confidence intervals of 4.4-6.4 days) and this was followed by TBARS (6.5 days) and PV (7.5 days) *(Table 3)*. Some studies showed that the filleted fish has shorter shelf-life than whole fish since the filleting procedure causes cross-contamination and exposure of fish lipids to atmospheric oxygen leading to enhanced enzymatic activity and accelerated oxidation[18,19]. Average fat content of mullet fillets was found as about 5%, indicating medium-fat fish. Hence, quick deterioration or lipid oxidation was the main reason of low shelf-life even at 0°C. Both PV and TBARS increased and then decreased during the shelf-life *(Fig. 3)*. Decomposition of hydroperoxide and malonaldehyde to other lipid oxidation products caused the decline in both PV and TBARS [12]. The results of PV and TBARS were in agreement with earlier reports [20,21].

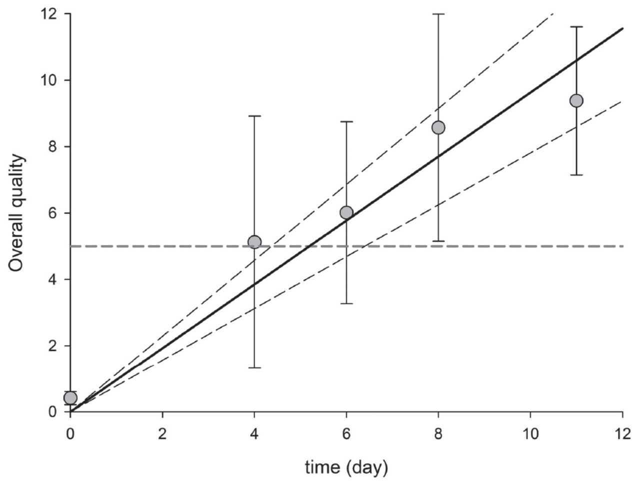


Fig 4. Sensory evaluation of mullet fillets at 0°C with respect to time. Gray circles are the score points; error bars represent standard deviation of the scores from 11 panelists. Solid black line is the model fit [Eq.(3)], dashed black lines are the 95% confidence bands and dashed gray line is the limit for the shelf-life. Original data are from Pilavtepe-Celik et al.[12]

Table 1. *Model equations with respect to analysis (criteria) and model parameters±standard errors obtained by regression together with the goodness-of-fit indices*

Criteria	Eq.	C_0 (mg N/100 g fish)	k (day^{-1})	t_c (day)	R^2	R^2_{adj}	RMSE
Cadaverine	1	–*	6.9±0.4	10.2±0.1	0.9946	0.9936	0.5642
Putriscine	1	–	2.4±0.1	4.7±0.3	0.9921	0.9906	0.7509
TVB-N	2	27.5±0.5	2.6±0.4	7.9±0.7	0.9921	0.9906	0.7509
Sensory	4	–	0.96±0.06	–	0.9190	0.9190	1.0064

** Not a model parameter for the given equation*

Table 2. *Model equations with respect to analysis (criteria) and model parameters±standard errors obtained by regression together with the goodness-of-fit indices*

Criteria	Eq.	C_{max*}	t_{max} (day)	t_{rate} (day)	R^2	R^2_{adj}	RMSE
PV	3	1.1±0.09	9.4±0.4	4.0±0.9	0.9453	0.9180	0.1157
TBARS	3	8.2±0.6	9.8±0.3	4.0±0.9	0.9636	0.9453	0.7272

** Unit of this parameter is mmole CPO/kg fish for PV and mg MDA_{eq}/kg fish for TBARS*

Table 3. *Estimated shelf-life and their calculated 95% confidence intervals of Mullet fillets stored at 0°C*

Criteria	Estimated Shelf-life (day)	95% Interval of the Estimated Shelf-life
Cadaverine	11.7	11.5-11.8
Putriscine	8.9	8.5-9.4
PV	7.5	6.0-ND*
Sensory	5.2	4.4-6.4
TBARS	6.5	5.4-8.8
TVB-N	10.7	10.2-11.3

* Not determined

Subzero temperatures may be used to increase the shelf-life of mullets. Choubert et al.[22] measured TBARS in packed rainbow trout stored at -20°C during a period of 18 months and observed a significant increase in TBARS at the end of first month, but not other changes occurred during the next 5 months. These results revealed that lipid oxidation could be controlled by preservative action of subzero temperatures [23]. The concentration of TBARS in good quality frozen and chilled fish or in fish stored on ice is typically between 5 and 8 mg MDA/kg whereas the levels of 8 mg MDA/kg are generally regarded as the limit of acceptability for most species [24]. In this study, the limit of acceptability was set to 6.5 mg MDA/kg [12]. The duration of shelf-life would have been much higher if the acceptability limit of TBARS had been set to 8 mg MDA/kg.

Not only the overall quality but also flesh color, skin, texture, gaping and odor were also scored during the sensory evaluation [12]. Mathematical modeling, on the other hand, were only applied to overall quality parameter since it was the sole parameter that was used to identify the acceptance and/or rejection point of fish fillets. Sensory analyses were performed with trained panelists as explained above. People in such panels can be considered as "human tools" and are not typically representative for the reaction of consumers since they are trained and selected on their capacity to detect certain changes [25]. Therefore, it was not surprising that the shelf-life based on sensory evaluation was low (5.2 days). Moreover, the uncertainties of the scores were relatively high *(Fig. 4)* revealing the human subjective inspection [12]. On the other hand, with respect to cadaverine, putrescine and TVB-N much longer shelf-life (8.9 to 11.7 days) were determined compared to PV, TBARS and sensory evaluation *(Table 3)*. Cadaverine and putrescine are good indicators of late spoilage due to late formation [12] hence longer shelf-life were estimated according to these biogenic amines.

It should be noted that according to Eq.(1), cadaverine and putrescine values were assumed to be zero initially (t = 0); however, they were not. Nevertheless, this had no consequence and the goodness-of-fit of the model since initially very low (close to zero) values were observed and this was also true for Eq.(3) to estimate PV and TBARS values. It should also be noted that models used in this study were ad hoc or empirical models and had no mechanistic background. In other words, they could all be considered as product oriented yet successful to describe the data since higher R^2 and R^2_{adj}, and lower RMSE values were obtained *(Table 1* and *Table 2)*. Furthermore, empirical models are useful for predicting the shelf-life of fish products [26,27]. It may still be possible to use alternative models with similar goodness-of-fit, but the aim of this study was not to make a comparison between the models.

Predictive modeling can be used to estimate shelf-life of fish products and especially microbial growth models were used for this purpose [28,29]. However, to the best of our knowledge, there are not many modeling studies on sensory and (bio)chemical parameters such as cadaverine, putrescine, PV, TBARS and TVB-N. A notable exception is the work of Calanche et al.[7] where shelf life of sea bream stored in ice were evaluated by using predictive models.

We demonstrated the modeling of mullet fillets with respect to some (bio)chemical parameters and also sensory evaluation to estimate the shelf-life in this study. The procedure presented here can be used for any type of fish with ad hoc models. Of course, more modeling studies based on kinetic mathematical models can be carried out by combining the microbial spoilage with physical and chemical analyses.

Availability of Data and Materials

The authors declare that data supporting the findings of this study are available upon request.

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Competing Interest

The authors declare that they have no competing interests.

Author Contributions

Authors contributed equally to this work (design of the study, literature search, data analysis, writing, reviewing) and approved the final manuscript.

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