# Predictive models for evaluation of mesophilic and psychrophilic bacterial loads in muscles of fresh ice-stored silver pomfret by impediometric technique

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## Abstract

Current microbial methodologies to determine fish quality are laborious and have long time required to obtain results. The impediometric technique as a rapid sensitive method was used to determine the correlation between impedance detection times (IDTs) and conventional reference psychrophilic and mesophilic plate counts of fish in order to develop models for predicting the microbial quality and determining fish shelf-life. The changes in sensorial factores, psychrophilic and mesophilic bacterial loads of ice stored fresh silver pomfret (*Pampus argenteus*) were measured by two different methods including conventional reference plating techniques and also impediometric monitoring method at 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27 days of storage. The primary psychrophilic (3.44 ± 0.69 logCFU/g) and mesophilic (3.64 ± 1.08 logCFU/g) bacterial loads increased to more than acceptable limit (6 logCFU/g) on days 12 and 21, respectively. The calibration curves for the two methods and their equations were designed with linear regression models. IDTs were highly correlated with psychrophilic (r=-0.9614) and mesophilic (r=-0.9547) bacterial loads. This study suggests that impediometric technique can be used as a rapid and reliable method to accurate estimation of silver pomfret bacterial loads and determine its shelf-life as seafood. According to results, the sensorial data were correlated with psychrophic bacterial load. The shelf-life of ice stored silver pomfret determined 9-12 days based on sensorial data and psychrophic bacterial load.

**Keywords:** Psychrophilic, Mesophilic, Bacterial Load, Silver Pomfret *(Pampus argenteus)*, Storage time, Impedance

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## Introduction

Increasing demand for seafood products and consumer awareness of food quality has called for better quality seafood products. However, the extremely perishable nature of seafood products makes this difficult (Metcalfe and Marshall, 2004). Deterioration of fish, either marine or freshwater, occurs mainly as a result of enzymatic and microbial activities, which lead to loss of quality and spoilage (Arashisar et al., 2004). Fish freshness is the most important and fundamental single criterion for judging the quality of fish and fishery products (Rodriguez-Jerez et al., 2000). The loss of quality of fish depends on many factors, including the fish species, handling and storage conditions (Venugopal and Shahidi, 1996). Time passed after catch and the temperature ‘‘history” of fish is considered to be the key factor determining the final quality characteristics of a fish product (Olafsdottire et al., 2004).

The silver pomfret *(Pampus argenteus)*, is a member of the Stromateidae family and is widely distributed throughout the Indo-West Pacific from the Persian Gulf to Indonesia, Japan, West and Southwest of Korea and Eastern parts of China (Fischer and Bianchi, 1984). Silver pomfret is one of the most commercially important fish in the Northern Persian Gulf and its stock is shared by Iran, Iraq and Kuwait. They can grow up to 4-6 kg . However, due to overfishing, specimens weighing less than 1 kg (2 lb) are more commonly seen (Al-Hussaini, 2003). This fish is prized in the Indo-Pacific region for its taste and due to the high market demand and high value; commercial fisheries in the Persian Gulf usually target this species (Amrollahi et al., 2011).

The quality of silver pomfret like other fishes is sensitive to changes in microbial load during storage. The silver pomfret fish are usually ice stored after fishing till marketing and the variation in bacterial load is one of the major causes of its quality deterioration and putrefaction.

Besides the offer of this fish to consumers in the most domestic markets in various regions of Iran and mainly in Khuzestan province, it usually exports to some other neighbor countries like Iraq, Kuwait etc. So the main aim of this study was to investigate the effect of icing on growth pattern of mesophilic and psychrophilic bacterial loads and the quality deterioration of silver pomfret during storage in ice.

Since traditional microbiological methods for bacterial counts are laborious and have long time required to obtain results, the growth models have to be combined with rapid methods for microbial enumeration. Among the microbiological methods which can be used to determine bacterial counts within a short period of time, the impedance is the most promising (Koutsoumanis et al., 1999). Impedance measurement is based on the principle that during bacterial growth, metabolic processes produce electrically measurable changes in the growth medium due to the metabolism of high molecular weight nutrients into smaller charged ionic components that increase the electrical conductivity of the medium. Variation in electrical conductivity is proportional to the change in the number of bacteria and therefore

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bacterial growth can be measured. Impedance measurement has been used for quality control in food industries and especially in identification, enumeration (Flint and Brooks, 2001; Fontana et al., 2002; Dupont et al., 2004; Yang and Bashir, 2008) of indicator microorganisms and estimation of antimicrobial activity (Gerolimatou et al., 2004). A combination of mathematical models and impedance technique could provide information about the remaining shelf-life within less than 24 hours and significantly improve distribution and marketing in the fish industry. Impedance is the resistance to flow of alternating current through a conducting material (e.g. growth medium). It is a complex entity composed of a combination of a conductive element and capacitive element. Therefore, in monitoring microbial growth, conductance measurements can be indicative of changes taking place in the bulk solution, while capacitance measurement can be associated with changes in close proximity to the electrodes (Koutsoumanis and Nychas, 2000).

Also another objective of the present study was to establish and apply a systematic, standard experimental procedure based on microbiological studies, mathematical modeling and studies on impedance technique in order to develop a microbial model for rapid predictions of shelf-life of silver pomfret *(P. argenteus)*, a species of great importance in Iran and Middle East. To our knowledge, there are no studies in the bibliography investigating the use of the impedance measurement method in silver pomfret fish.

Also this study was conducted to compare impedance-splitting method as impediometric technique and conventional reference plate count methods to evaluate microbial quality of silver pomfret fish in north-west of Persian Gulf from the aspect of mesophilic and psychrophilic bacteria. Impedance-splitting method and conventional reference method were used and the results compared to obtain a rapid and sensitive equation as a method for evaluation of bacterial load in this seafood.

## Materials and methods

In total, 30 fresh silver pomfret fish, (average weight and length, 685±74 g and 242±27 mm, respectively) were collected from Abadan landing (48°35' E - 30°10' N) .

The fish samples were delivered to the food hygiene laboratory in Shahid Chamran University of Ahvaz in less than 3 h. Three fish were sampled immediately (day zero), and the rest were covered with ice and stored up to 27 days with monitoring its ice content. The ice/fish (3:1, w/w) ratio was maintained constant throughout the experiment and the melted ice was replaced daily, as required. The box was provided with an outlet for water drainage. After 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27 days, three randomly chosen fish were removed from the ice and analyzed in triplicate (Rezaei et al., 2007).

The evaluation of mesophilic and psychrophilic bacterial loads after fishing were considered as indicator factor for the freshness estimation of fish meat by using two different methods including conventional reference plate count and impedance-splitting techniques in order to find a reliable equation between two methods and develop a faster technique

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for evaluation of microbial load of silver pomfret fish meat.

Samplings for microbial test were performed. In order to this matter, the fishes were skinned aseptically and sampling was taken from the flesh of the anterior-dorsal region of each fish. Then 10 g of fish flesh were transferred aseptically to 90 mL sterile physiological saline (0.85% NaCl) and homogenized. Serial dilutions of each homogenate were carried out with the same diluents (1:10, by vol.) up to 10-6. (Vanderzant and Splittstoesser, 1992)

For conventional psychrophilic bacterial enumeration, 0.1 mL samples of serial dilutions were spread on the surface of dry media (standard plate count agar). Psychrophilic bacteria were counted, after incubation for 48 hour at 21ºC. For the conventional mesophilic viable count, a 1.0 mL sample was inoculated into 10 mL of molten (45ºC) standard plate count agar. After setting, a 10 mL overlay of molten medium was added, and then incubated for 48 hour at 37ºC (Vanderzant and Splittstoesser, 1992; Metcalfe and Marshall, 2004). All above mentioned microbial cultures were conducted in triplicates. Data were obtained from the mean ± SD of three randomly different fish which were analyzed for each sampling time. Sampling and cultures were continued over a 27 day storage period. Then the microbiological data were transformed into a logarithm of the number of colony forming units per gram (logCFU/g).

All samples were also analyzed using the impediometric monitoring technique (Bactrac 4300 microbial analyzer) by inoculation 1.0 ml of 10-1 from each diluted samples into two separate impedance glass tubes (cells) containing 9 ml 001A nutrient broth media (BiMedia Sy-Lab company) for mesophilic and psychrophilic bacterial loads. By means of an electrode system, mounted in the system's glass tubes, impedance values can be registered over time during a pre-selected incubation period. Each incubator contains a microprocessor controlled electric unit for measurement and temperature control. This system has the ability to evaluate simultaneously changes of the growth media impedance (M-value) and changes of the electrode impedance (E-value). In this study, measurable results were obtained by the M-value only. In our present study the changes of electrical resistance were measured each 10 minutes in 001A nutrient broth media (M-value) which used in this method up to 24 hours for mesophilic and 48 hours for psychrophilic bacterial loads at 37°C and 21ºC respectively. Reverse to the electrical resistance, the amount of electrical conductance were calculated by the software of instrument.

In impedance method, the concentration of ions generated by the bacteria reach a magnitude in which a measurable increase in conductivity can be detected. The bacterial level associated with this change in conductance is called the bacterial threshold level (Fig. 1). This time is called the detection time. The detection times were defined as the point in hours where the electrical resistance threshold level changes equal to 10 micro siemens. In impedance measurement, the detection time (the time at which impedance change reaches a particular threshold) is recorded. "Impedance Detection Time" (IDT)

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is usually inversely proportional to the log number of bacteria in the sample and therefore, bacterial counts can be calculated or estimated by measurement of the IDT. Finally after termination the incubation periods (24 and 48 h ) at 37°C and 21ºC for mesophilic and psychrophilic bacterial loads respectively, the printouts of the impedance patterns and the IDTs expressed in hours for each test cell or glass tube were produced. (ISIRI No.7726, 2004; Metcalfe and Marshall, 2004).

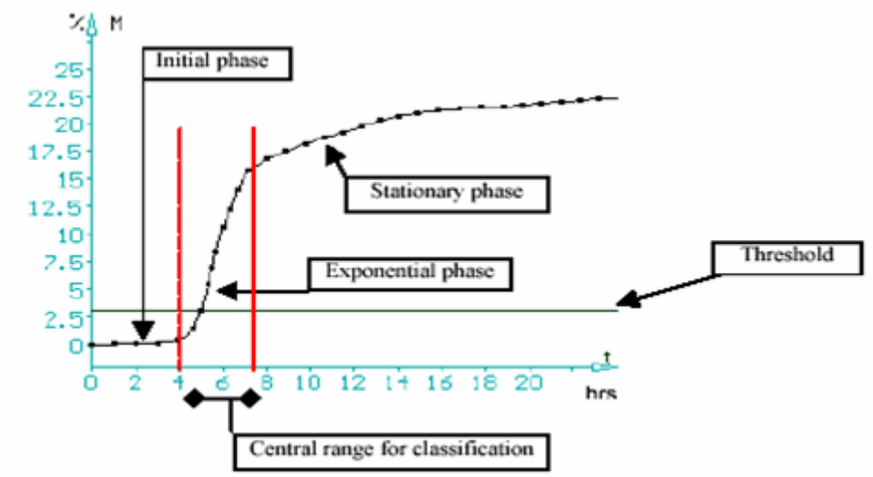



**Figure 1: Typical impedance signal**

Initially, a calibration curve must be adjusted to establish the correlation between total bacterial populations (logCFU/g) of a specific type of food like fish flesh with IDT. The calibration curve is designed by measuring the IDTs of adequate number of samples of known bacterial population, determined by a conventional reference plate count method (ISIRI No.7726, 2004). So in present study, the IDTs (hours) resulting from changes in the capacitance measurements were obtained in triplicate at both 21ºC and 37°C and then compared with the initial psychrophilic and mesophilic bacterial loads obtained by conventional reference plate count methods. In this way, for each fish sample, two pairs of data were obtained for psychrophilic and mesophilic bacteria: “the bacterial population (logCFU/g) and the corresponding IDT (in hours)”. A total of 60 data pairs were subjected to linear regression analysis to adjust two regression lines or calibration curves for psychrophilic and mesophilic bacterial loads respectively.

For sensory analysis, triplicate samples were taken at regular intervals before performing microbiological analysis. Sensory analysis was assessed according to the guidelines presented in Table 1 (Lin and Morrissey, 1994; Koutsoumanis et al., 2002; Rezaei et al., 2008). Four categories were ranked; 0= excellent; 1= good; 2= acceptable; >2 = reject. Sensory assessment included the evaluation of the following parameters: texture, general appearance, gill odor, gill appearance and eyes. Each assessment was

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carried out by a minimum of four trained persons.

**Table 1: Descriptive sensory evaluation definitions and descriptors**

| Texture | General appearance | Gill odor | Gill appearance | Eyes | Score |
|---|---|---|---|---|---|
| Flesh is firm and resilient and springs back immediately when released | Good overall appearance, skin lustrous and shiny, no fading | Characteristic of species, fresh | Bright red, little mucus | Clear, bright convex eyes | 0 |
| Reasonably firm some loss of resiliency, thumb indentation slowly fills out | Good overall appearance, very slight bleaching of skin | Neutral. Total absence of odor characteristic odor no longer detectable but off-odors have not developed | Red, some mucus | Slightly sunken or some what dull | 1 |
| Moderately firm, thumb indentations may remain in flesh | Some loss of metallic luster, some bleaching | Slight to moderate sour odor | Pinkish red to brownish, some mucus | Dull and/or cloudy | 2 |
| Excessively soft flesh | Bloom gone from skin, color faded and bleached | Very sour, strong, or putrid | Brown, may be covered with mucus | Very dull, sunken and cloudy | 3 |

Source: Lin and Morrissey (1994)

Data were subjected to one-way analysis of variance (ANOVA). The least significant difference (LSD) was used for differences between means. Linear regression was calculated to determine correlation of IDTs with mesophilic and psychrophilic bacterial counts. Significance of differences was defined at $p<.05$. All statistical tests were done using the statistical software for Windows SPSS, version 16.0.

## Results

Changes in bacterial counts of psychrophilic bacteria, total mesophilic count; in whole silver pomfret during ice storage is shown in Table 2 and Figure 2. All of these parameters increased significantly ($p<.05$) during storage period. Psychrophiles comprised the main bacterial load. From an initial count of 3.44 ± 0.69 logCFU/g (day 0), with a stepwise trend, they finally reached critical point 6.18 ± 0.40 logCFU/g on the day 12 and finally reached to 8.13 ± 0.20

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logCFU/g on the day 27. The primary total mesophilic count (3.64±1.08 logCFU/g), reached critical point 6.34 ± 0.96 log CFU/g on the day 21 and finally attained to a maximum level of 7.49 ± 0.52 logCFU/g at the end of the storage period.

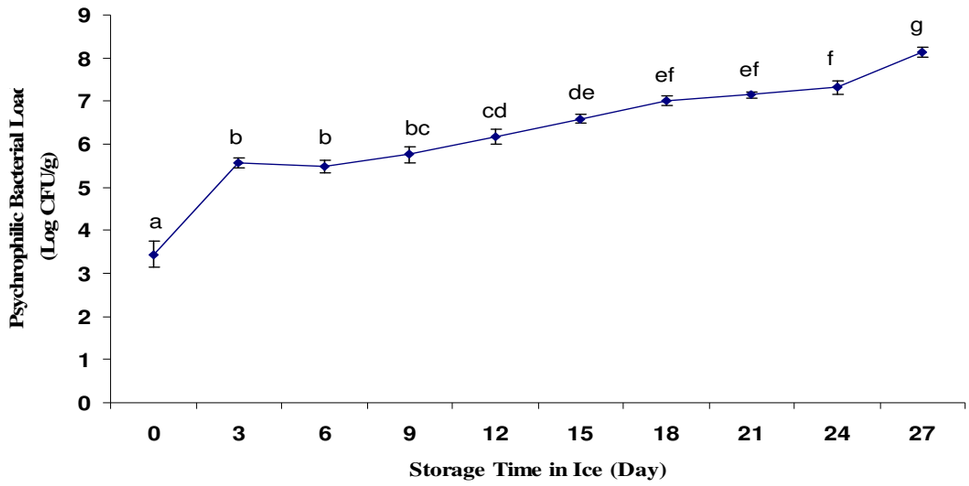
**Table 2: Mesophilic and psychrophilic bacterial loads in meat of ice stored silver pomfret fish**

| Day | Mesophilic Bacterial Load (Mean ± SD) Log CFU/g | Psychrophilic Bacterial Load (Mean ± SD) Log CFU/g |
|---|---|---|
| 0 | 3.64 ± 1.08a | 3.44 ± 0.69a |
| 3 | 4.07 ± 0.30ab | 5.57 ± 0.28b |
| 6 | 4.61 ± 0.66b | 5.48 ± 0.30b |
| 9 | 4.52 ± 0.20ab | 5.67 ± 0.41bc |
| 12 | 4.69 ± 0.40b | 6.18 ± 0.40cd |
| 15 | 4.31 ± 0.24ab | 6.58 ± 0.24de |
| 18 | 4.50 ± 0.09ab | 7.02 ± 0.19ef |
| 21 | 6.34 ± 0.96c | 7.15 ± 0.12ef |
| 24 | 6.21 ± 0.54c | 7.32 ± 0.29f |
| 27 | 7.49 ± 0.52d | 8.13 ± 0.20g |

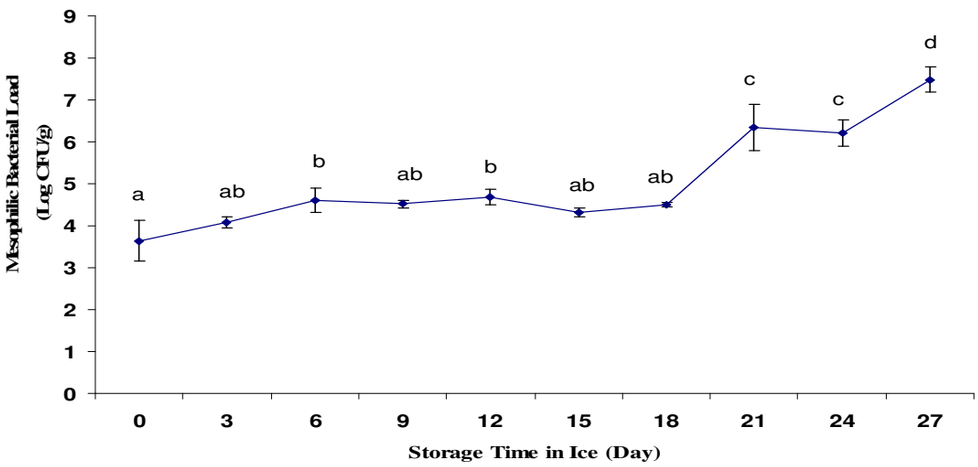
Different parameters below values, means significant difference

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(A)




(B)

**Figure 2: Changes in Psychrophylic (A) and mesophilic (B) bacterial loads in meat of ice stored silver pomfret**

Different parameters in figure, means significant difference between values

According to the results obtained from both methods the calibration curves for the two methods and their equations were obtained using Excel software (Figures 3 and 4) and the regression models were represented mathematically by the equations. The obtained correlation coefficients "r" and dispersion values "Syx" for mesophilic and psychrophilic bacterial loads were "r = -0.9547, Syx = 0.355" and "r = -0.9614, Syx = 0.358", respectively.

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As shown in the Figures 3 and 4, the equations of linear regression or calibration curves between two methods for psychrophilic and mesophilic bacterial loads were Y1= -0.2705 X + 10.43 and Y2= -0.5364 X + 10.269 with coefficient of determinations ($R^2$) equal to 0.9243 and 0.9115, respectively. The parameters "Y1" and "Y2" in the above mentioned equations, means psychrophilic and mesophilic bacterial loads (logCFU/g) respectively and also "X" is the IDT (hours) which obtained from impedance or electrical resistance technique. Indeed the calibration curves of the present study indicate that the more bacterial loads (logCFU/g) increased, the more detection times decreased.

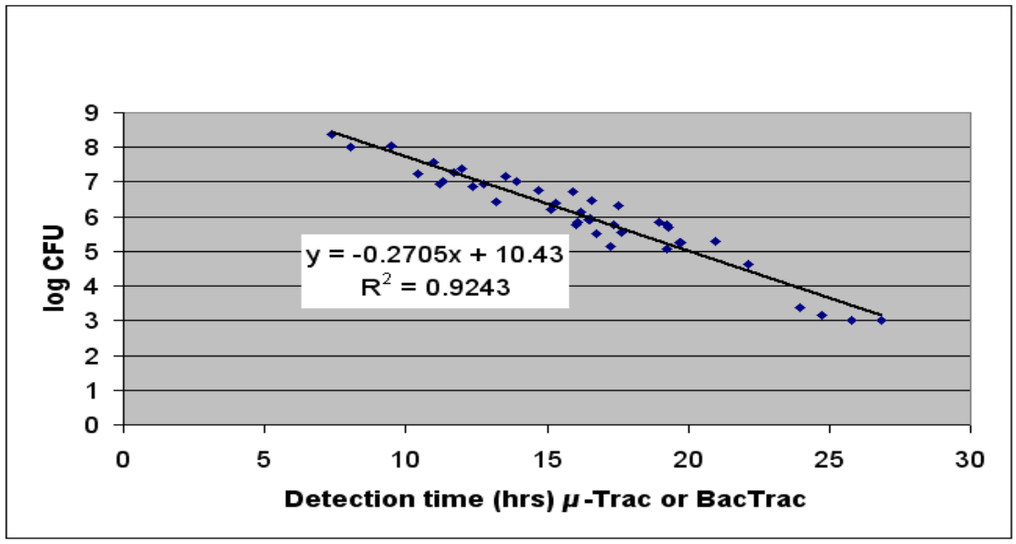



**Figure 3: Calibration curve of the psychrophilic bacterial load (logCFU/g) by reference surface plate count method and IDT (Hours)**

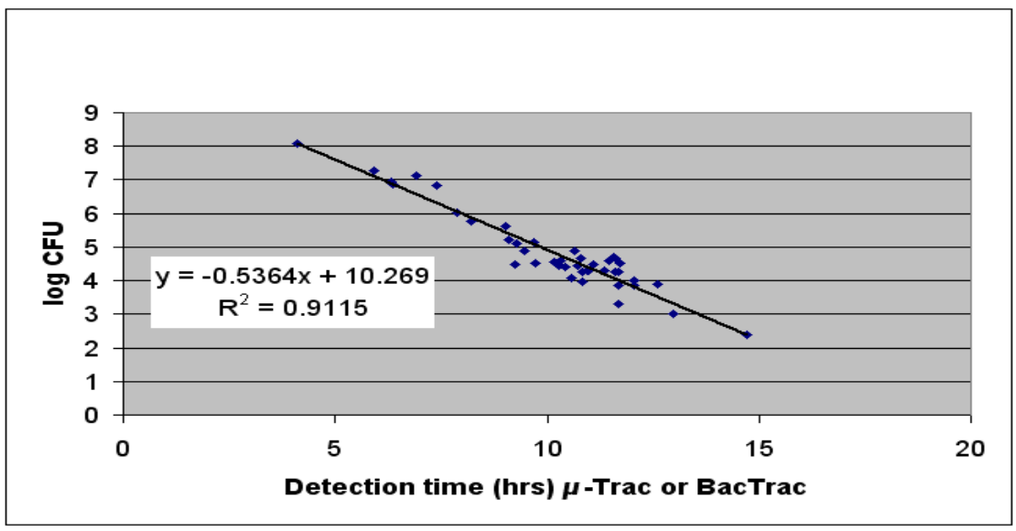



**Figure 4: Calibration curve of the mesophilic bacterial load (logCFU/g) by reference pour plate count method and IDT (Hours)**

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Table 3 shows the results of the sensory analysis of the silver pomfret fish stored in ice after icing for various time intervals. According to the results of the sensory analysis, the silver pomfret samples maintained excellent to good quality up to day 3 of storage. After this time, quality decreased, and by day 12 the samples was no longer acceptable. The main aspect related to quality loss was the gill odor. The silver pomfret samples being rejected on day 12. In this day, the only gill odor was the limiting factor of acceptability, but in the day 15, all the sensory factors including texture, eyes, gill odor and appearance except general appearance were the limiting factors of acceptability. In the 18th day and the days after that, all the studied sensory factors were being in the rejected limit. These results indicated that sensory analysis of silver pomfret correlated well with microbiological analysis and especially with psychrophilic microbial load.

**Table 3: sensory acceptability of silver pomfret fish stored in ice**

| Sensory factors | Storage days in ice | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | **27** |
| **Texture** | 0 | 0 | 0.67 | 1.33 | 1.67 | 2.33 | 2.67 | 3.00 | 3.00 | 3.00 |
| **General appearance** | 0 | 0 | 0.33 | 1.00 | 1.33 | 2.00 | 2.33 | 3.00 | 3.00 | 3.00 |
| **Gill appearance** | 0 | 0.33 | 0.67 | 1.33 | 2.00 | 2.33 | 2.67 | 3.00 | 3.00 | 3.00 |
| **Gill odor** | 0 | 0.67 | 1.00 | 1.67 | 2.33 | 2.67 | 3.00 | 3.00 | 3.00 | 3.00 |
| **Eyes color** | 0 | 0.33 | 0.67 | 1.00 | 1.67 | 2.33 | 2.67 | 3.00 | 3.00 | 3.00 |

Scoring was: 0 = Excellent; 1 = Good; 2 = Acceptable; >2 = Reject

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## Discussion

In present study, the mesophilic and psychrophilic bacteria in the flesh of ice stored fresh silver pomfret fish were increased significantly during the storage time and reached to 6.34 ± 0.96 logCFU/ g and 6.18 ± 0.40 logCFU/g on the 21th and 12th day, respectively. If 6 logCFU/g is considered the microbial limit of acceptability in fish (Antoine et al., 2002; Ozogul et al., 2005; Scherer et al., 2006; Mol et al., 2007), the shelf-life of silver pomfret fish stored in ice is approximately 18-21 and 9-12 days according to mesophilic and psychrophilic bacterial loads, respectively. Also based on sensorial data (Table 3), the silver pomfret fish were acceptable until the 12th day of storage period which is correlated with psychrophilic bacterial load. It seems that the cause of difference between the mentioned bacterial shelf-lives could be the influence of cold temperature and slow growing of mesophile bacteria in comparing to psychrophiles in cold (icing) storage conditions. This is in agreement with the results of Mol et al. (2007) on shelf-life estimation in ice stored fresh Anchovy, horse mackerel, cod and rainbow trout. They

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were revealed that psychrophilic microorganisms give better results than mesophilic bacteria for the shelf-life estimation of chilled fish (Mol et al., 2007). According to above mentioned shelf-lives, the psychrophilic bacterial load comprised the main bacterial load in the shelf-life determination of silver pomfret fish. Similar results reported for rainbow trout *(Oncorhynchus mykiss)* stored in ice (Rezaei et al., 2007) and also sea bass *(Dichentrachus labrax)* (Koutsoumanis et al., 2002). These researchers are indicated that the sensory acceptability shelf-life was correlated with psychrophilic bacterial load. It appeared from the results of our present study too, that due to the psychrophilic nature of the bacterial flora of fresh silver pomfret fish, the bacterial counts determined at 21°C were about 1.5 logCFU/g more than the bacterial counts determined at 37°C in the 12th day of storage period. Therefore a temperature of about 21°C is advised for the microbial analysis and determining the shelf-life acceptability in fresh silver pomfret fish.

It should be taken into consideration that based on all studied sensorial factors (Table 3); the silver pomfret were not acceptable in the 18th day of storage period and the fish samples shown major signs of spoilage, such as putrefaction, gill odor and loss body texture and appearance. Despite this, the mesophilic bacterial load on the day 18 was lower than the critical limit of 6 logCFU/g for acceptability (equal to 4.50 ± 0.09 logCFU/g). According to these obtained results, it seems that the digestive enzyme interactions may have accelerated the muscle degradation, especially in latter stages of storage (Ozogul and Ozogul, 2004). Furthermore, this is in agreement with other findings which suggest predominance of enzymatic (autolytic) degradation in comparing to microbial putrefaction (Vaz-Pires et al., 2008). In attention to these ideas, the autolysis or the activity of endogenous enzymes could be the main responsible for the change in sensory attributes. Anyway, in our present study the shelf-life of ice stored silver pomfret fish determined 9-12 days based on both correlated sensorial data and psychrophilic bacterial load.

The impedance method has not been extensively applied in the food products, mainly due to the fact that an efficient correlation and calibration curve must be first determined for every type of food. (Batrinou et al., 2005). The ideal and significant correlation coefficient (r) and the dispersion value (Syx) in the impedance calibration curves must be between -0.85 to -1.0, and less than 0.5, respectively. In the other word, the correlation coefficient more than -0.85 means indicative of a high correlation between the impedance technique and the reference method (Yang and Bashir, 2008). In our present study, the obtained correlation coefficients (r) and dispersion values (Syx) for mesophilic and psychrophilic bacterial loads were in the significant limits and equal to "r = -0.9547, Syx = 0.355" and "r = -0.9614, Syx = 0.358", respectively. Therefore, the obtained calibration curves are acceptable as the standard curve for silver pomfret fish microbial loads. The obtained coefficient of determinations ($R^2$) of regression equations equal to 0.9115 and 0.9243 respectively, indicates an accurate estimation of mesophilic and psychrophilic bacterial loads by the

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impedance technique. The correlation coefficients were high which means that conventional methods were in good agreement with the electrical impedance method. Finally, as a result of high correlation obtained between methods in our present study in the detection of psychrophilic and mesophilic bacterial loads, the impedance technique is considered as a suitable alternative in the quantitative detection of microbial loads in silver pomfret fish. The regression lines equations and their parameters confirm that IDTs is inversely proportional to the logCFU/g of studied bacterial loads in silver pomfret fish. These calibration curves can be used to measure mesophilic and psychrophilic bacterial loads from IDTs. Total mesophilic and psychrophilic bacterial count are obtained by calculating the antilogarithm of the "$Y_1$" and "$Y_2$" estimation in the obtained equations. Such findings are also confirmed by other researches on the basis of the mathematical regression equations about impedance technique and its application in food sciences. For example, similar research have been previously carried out in determine the microbiological quality of raw shrimp *(Penaeus setiferus)* by Metcalfe and Marshall (2004). They reported that the impedance technique was highly correlated with aerobic plate count (r = -0.91) and psychrophilic plate count (r = -0.89). These results are also in accordance with those of Batrinou et al. (2005), who have also reported that the impedance technique was highly correlated with standard pour plate method (r = -0.95). Other researches has shown the IDTs were correlated with conventional pseudomonad count as a major psychrophilic spoilage organism on sea bass *(Dichentrachus labrax)* (r = -0.96) (Koutsoumanis et al., 2002) and gilt-head seabream (*Sparus aurata*) (r = -0.95) (Koutsoumanis et al., 2000).

Also in present study the minimum and maximum time needed to obtain impedance results (IDTs) were 4.11 and 14.71 hours for mesophilic and 7.35 and 26.83 hours for psychrophilic bacterial loads respectively, which are more less than 24 and 48 hours spending time for provide the results of mesophilic and psychrophilic bacterial loads in conventional reference plating methods. In other meaning, in comparing with the conventional microbiological tests, the impediometric method gave results in one-second to one-sixth of the time. Similar results are reported on gilt-head seabream *(Sparus aurata)* by impedance technique in one-fourth of the time. (Koutsoumanis and Nychas, 2000). According to the obtained regression equations of these models, IDTs for silver pomfret fish with maximum microbial limit of acceptability equal to 6 logCFU/g can be detected in about 8 and 15 hours for mesophilic and psychrophilic bacterial loads, respectively. Indeed the result of the present study reveals that in case of the mesophilic and psychrophilic bacterial loads more than acceptable limit of 6 logCFU/g, the IDTs can be detected in more less than 8 and 15 hours respectively, in compared to the 24 and 48 hours required for the conventional reference plating methods. These results are in agreement with other finding which reported that, the IDTs for microbial loads of shrimp were attained in less than 9 h (Metcalfe and Marshall, 2004). Therefore, this method offers the advantage of a rapid microbial screening of

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silver pomfret fish to reveal as soon as possible, if the microbial criteria are not in the range of acceptable limit. These results are similar to the research of Koutsoumanis and Nychas (2000) on gilt-head seabream *(Sparus aurata)*, Batrinou et al. (2005) on microbial population of chocolate mix and also Salvat et al. (1997) on pseudomonads in poultry meat which a satisfactory agreement was reported between IDT and pseudomonads count (logCFU/g) (Koutsoumanis and Nychas, 2000; Salvat et al., 1997). Impedance instruments have been used for the determination of microbial count in different food products like milk, ice-cream, water, meat products etc. (Koutsoumanis and Nychas, 2000). These results suggest that impedance technique could be used as a fast and reliable method to evaluate the microbial load of fresh silver pomfret fish and determine its shelf-life as seafood.

The above mentioned results from present study and other researchers indicate about the impedance method as a successful predictive model for microbial load in foods. The microbial growth models usually can be used to predict the effect of various time–temperature combinations on fish shelf-life in a production and distribution chain. However, the use of a model which accurately predicts growth over a range of conditions may often lead to misleading shelf-life predictions. This can be explained by the fact that depending on intrinsic and extrinsic factors, each product has its own specific spoilage microflora and as a consequence, a microbial growth model is applicable within a limited range defined by the origin of the species, the type of product and the storage conditions. Thus, in order to achieve accurate predictions of shelf-life, it is essential to choose and apply a microbial model based on the spoilage process of the product (Koutsoumanis and Nychas, 2000). This matter is exactly the same and in agreement with our research methodology and idea about silver pomfret fish which we kept fresh and stored up to 27 days under controlled condition (icing) to monitoring their spoilage process and developing the mathematical predictive model for psychrophilic and mesophilic bacterial loads based on impedance–splitting method.

As a general conclusion impedance measurement which is more rapid, automated and less laborious which tedious dilutions are not needed, could be used as an alternative method for the early detection of microbial levels in fresh silver pomfret. Finally, it should be pointed out that impedance measurement is closely dependent on the fish species and meat composition of the fish being tested and the obtained calibration curves for silver pomfret should not be used for other fish species. As a consequence, variations in muscle ingredients of another fish species may result in differences in the pattern of impedance change and therefore a readjustment of the calibration curve should be performed for each type of fish.

In conclusion, this study revealed that based on psychrophilic bacterial load and sensorial data, the shelf-life of silver pomfret stored in ice is 9-12 days and the impedance-splitting method as a faster new technique can be used instead of reference conventional plating methods for determining microbial quality of silver pomfret fish in less time than conventional reference methods. Therefore a

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temperature of about 21°C is preferred and advised for the quick screening of samples by impedance electrical measurements.

The present study shows a systematic experimental procedure to be followed for chilled silver pomfret fish shelf life modeling in order to achieve an effective tool for quality monitoring and prediction. However, further studies on the kinetic behavior of spoilage should verify the developed models under dynamic storage conditions.

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