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Using grey and regression prediction models to estimate the aerobic plate bacteria counts on frozen squid rings (*Loligo Vulgaris* Lamarck, 1798) during the thawing process

Die Grau- und Regressionsvorhersagemodelle zur Schätzung der aeroben Plattenbakterien von gefrorenen Tintenfischringen (Loligo vulgaris Lamarck, 1798) beim Auftauen

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Summary

In this study, grey and regression models were developed to estimate the aerobic plate bacteria development on frozen squid rings during the thawing process. The regression model was found to be suitable for predicting the microbiological growth on frozen squid rings during the thawing process for different temperatures and times (R^2_{adj} = 0.918). This model allowed predictions to be made based on time, temperature and treatment factors. The other model developed for the data in this study was grey modeling, which is also used to predict the total aerobic bacteria growth on frozen squid rings during the thawing process. The GM (1,1) model allowed modeling that could only be based on time. Therefore, a comparison of the groups was not possible with the GM (1,1) model, compared to the regression modeling technique. However, the grey model appeared to be a good alternative when there was little data and time-dependent measurements were made. The proposed modeling techniques are be useful for predicting the total aerobic bacteria growth on frozen squid rings during the thawing process because they are economical and timesaving.

Keywords: frozen, thawing process, squid rings, predicting, regression model, GM (1,1)

Introduction

Many important techniques have been used in the estimation of microbial development in the culture environment, such as the prediction of potential damage of plant pathogens in agriculture, the determination of inhibitory effects of compounds, the disease and development rates of microbes etc. The measurement of bacterial development in solid environments has often been carried out by trained technicians using time-consuming techniques. The conversion of microbial areas into digital images with developed functional models have been shown to be significantly faster than manual measurement methods, with minimal data used and more comparable results (Ancin-Murguzur et al., 2018). In addition, predictive models can be easily implemented with accurate constrained data, for separate and biological interpretation (Akkermans et al., 2017).

Mathematical methods have often been used to predict microbial development in food products (Teleken et al., 2008). Understanding the character of microbial development could be of considerable importance in scientific research, in the food industry and in many other areas such as public health and agriculture. Many methods have been developed to characterize the process of microbial growth. It has been preferred to sample transfer in online and automated methods because the development of these methods facilitated the monitoring of simple, safe and effective microbial development (Zhang et al., 2019).

Food safety and quality can be affected by the presence of pathogenic and spoilage microorganisms that cause food products to deteriorate during their shelf life. Mathematical models have been developed in the field of microbiology for the prediction of microbial changes in foods (Impe et al., 2005). Estimated models have been used in microbial risk determination for pathogenic microorganisms and shelf life studies in fishery products (Bolivar et al., 2018).

Microorganisms can play a natural role in various ecosystems. The characterization of the microorganisms, understanding the microbial function and organization and the interactions between them, can be a very important step. Therefore, in recent years, prediction methods have been frequently used for the studies of microbial interaction (Li et al., 2016).

Analysis of published literature has shown that many studies have been conducted to determine the factors affecting the development of bacteria using a response surface method (Buchanan and Phillips, 1990; Dengremont and Membre, 1995; Oscar, 1999; Shih et al., 2008; Dikshit and Tallapragada, 2014; Zhang et al., 2016). There have also been numerous modeling studies that have been conducted to predict the development of various types of microorganisms in foods under different storage and packaging conditions (McMeekin and Olley, 1992; Ross, Dalgaard and Tienungoon, 2001; Impe et al., 2005; Huang, 2013; Costa et al., 2016; Lytou et al., 2016; Guillard et al., 2016; Bolivar et al., 2018; Juneja et al., 2019; Costa et al., 2019).

In this study, two predicting techniques (regression modeling and grey modeling) were used. Although regression modeling is an old and widely used method, grey modeling is a newer modeling technique. Grey prediction models, proposed by Julong Deng in 1982, are a methodology that focuses on the study of problems involving a low level of information and small samples using many forecasting applications (Huang et al., 1997). The basic assumptions in this approach are that the data used are positive and the time intervals are fixed (Deng, 1989). Although there are various types of grey models, the GM (1,1) model is widely used because of its computational efficiency (Lin et al., 2012, Wang et al., 2017, Ma and Liu, 2017, Deng, 2002, Hsu and Wen, 1998, Akay and Atak, 2007, Lu et al.,2009). GM (1,1) known as the 'grey model first order one variable', is the simplest model with a first order differential equation and one variable. To use the GM (1,1) model, the minimum number of inputs required is four (Deng, 1989). Using the accumulating generation operator (AGO), a new set of data was generated from the raw data. To obtain the predicted system values, the differential equation was solved. Finally, to find the predicted values for the original data, the inverse accumulating generation operator (IAGO) was applied in the study.

In light of the above studies *showing* that statistical methods have provided many advantages to predict the microbial growth in determining the quality and the shelf-life of fishery products. The aim of this study was to develop new models for predicting the total aerobic bacteria growth on frozen squid rings during the thawing process at different temperatures and for different times.

Material and Methods

Materials

In this study, frozen squid rings (*Loligo Vulgaris* Lamarck, 1798), which were stored at –18 ˚C for 1 month, were used as the test material. They were purchased from a market and brought to the Ege University Fisheries Faculty in a cooler box in approximately 40 minutes. As soon as the frozen squid rings were brought into the laboratory of the Fisheries Faculty, they were divided into two groups. The first group was the frozen+ thawed squid (Group A) and the second group was the frozen+ boiled+ thawed (Group B). For the second group the frozen squid rings were put into hot water at 60 ˚C for 2 minutes to apply a boiling process before being removed from the hot water. After that, each of these groups (Group A and B) were thawed at different temperatures (4 °C, 10 °C, 15 °C and 25 °C) over different times (0, 2, 4, 6 and 8 hours). The groups of frozen squid rings thawed at different temperatures and times are given in Table 1. For each group 10 squid rings were placed onto each styropor plate. Three styropor plates were used for each microbiological analysis. A total of 30 squid rings were used from each group. An aerobic plate count (APC) analysis was done in triplicate for each group. The microbiological results were given as the mean value from three styropor plates for each group.

Microbiological methods

Squid rings (10 g) were taken aseptically according to microbiological rules. Afterwards, 10 g of the squid rings were placed into 90 ml of 0.1 % peptone water (Merck, 1.07228.0500), and then a stomacher (IUL, Barcelona, Spain) was used for 2 minutes for homogenization. A 1 ml amount of the homogeneous liquid was put into 9 ml of peptone water (Merck, 1.07228.0500). Further decimal dilutions were then prepared from this homogenization. To determine the aerobic plate count (APC) of the squid rings, 1 ml of inoculum from each dilution was taken and placed into separate petri dishes.

Plate count agar (PCA) (Merck, 1.05463.0500) was used as the medium for the APC. Thereafter, the PCA medium was poured onto each inoculum using the Pour Plate Method. After inoculation, the petri dishes were

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incubated at 30 °C for 24 to 48 hours for APC measurements according to the method described by Harrigan and McCance (1976). After the incubation period, the colonies on the petri dishes were counted and converted into log cfu/g units. The results for the APC of squid rings were given as the mean value of three microbiological analyses.

Statistical methods

All statistical analyses were performed using SPSS Version 25.0 software. One-way analysis of variance (ANOVA) (Montgomery and Runger, 2003) was performed to determine whether or not there was a significant difference between the group means. The differences between the two groups were checked with post hoc Tukey tests. The homogeneity of variance for the dependent variables was checked using the Levene test. In addition, the Kolmogorov-Smirnov test was used for the goodness of fit to a normal distribution. In cases where the assumption of normality could not be achieved, the differences between the group means were checked by the nonparametric Kruskal-Wallis test (for more than two groups) and the Mann-Whitney test (for two groups) test (Gamgam and Altunkaynak, 2013).

To determine the factors affecting the bacterial growth measured by the APC regression analysis was applied. The relationships between dependent and independent variables of the mathematical model were determined by using regression analysis (Montgomery and Runger, 2003). For checking the adequacy of the final model, the goodness of fit errors from a normal distribution were tested using the Kolmogorov-Smirnov test.

Different from regression modeling, the grey prediction models are used for time series prediction with small samples and low levels of information (Hamzaçebi and Karakurt, 2015). The GM (1,1) model, which was one of the most commonly used grey prediction models, was used in this study. A detailed modeling procedure for GM (1,1) was outlined in Badhrudeen et al. (2016), see figure 1.

Results and Discussion

Microbiological results

Squid *can be rapidly spoiled* just like other fish species and seafoods (Kumar et al., 2015). Shafieipour and Sami (2015) reported that one of the best and most common ways of preserving seafood for long periods of time is freezing. However, the freeze thawing process can influence the quality and shelf-life of seafoods.

The effect of different thawing methods (in air at ambient temperature, in water, in a refrigerator and in a microwave oven) on the quality of eels was studied by Ersoy et al., (2008). In this study the authors reported that the lowest total aerobic bacteria were determined in the water-thawed eels. Therefore, the authors declared that water thawing was the most suitable method for handling frozen eels (Ersoy et al., 2008). Genç et al., (2015) reported in another study that Meagre fillets were thawed in the air at ambient temperature (+16 ºC for 3.5 h). In this study, the total mesophilic aerob bacteria count of the air-thawed fish was found to be 4.62 log CFU/g at +16 ºC after 3.5 h. In addition, thawing was indicated to be a very important process for the quality of frozen fish and seafoods, otherwise these products could be spoiled in a short time because of the growth of bacteria.

Owing to the importance of the thawing process, in this study, frozen squid rings were thawed in the air at different

Consider a raw data series of equal time intervals as follows. $X^{(0)} = \left(x^{(0)}(1), x^{(0)}(2), \dots, x^{(0)}(n)\right), n \geq 4.$

Applying the AGO to this raw series, the new set of inputs were:

$X^{(1)} = (x^{(1)}(1), x^{(1)}(2), \dots, x^{(1)}(n)).$

In here, the AGO could be expressed as $x^{(1)}(k) = \sum_{i=1}^{k} x^{(0)}(i)$, $k = 1,2,\dots,n$. Then the grey difference equation of GM (1,1) was as follows:

$$x^{(0)}(k) + az^{(1)}(k) = b \quad , k = 1,2,\dots,n$$

where, $x^{(0)}(k)$ was called the grey derivative, a the development coefficient and b the grey input.

Here, $z^{(1)}(k) = 0.5x^{(1)}(k) + 0.5x^{(1)}(k-1)$. The coefficients a and b could be obtained using the least square method as $\left[\hat{a},\ \hat{b}\right]^T = (B^T B)^{-1} B^T Y$, where

$$Y = \begin{bmatrix} x^{(0)}(2) \\ x^{(0)}(3) \\ \vdots \\ x^{(0)}(n) \end{bmatrix}, \qquad B = \begin{bmatrix} -z^{(1)}(2) & 1 \\ -z^{(1)}(3) & 1 \\ \vdots & \vdots \\ -z^{(1)}(n) & 1 \end{bmatrix}, \qquad \hat{r} = \begin{bmatrix} \hat{a} \\ \hat{b} \end{bmatrix}$$

Here the B matrix is called data matrix.

The whitening differential equation for GM (1,1) was:

$$\frac{dx^{(1)}(k)}{dk} + ax^{(1)}(k) = b \qquad (1)$$

Equation (1) was solved to find $\hat{x}^{(1)}(k)$ as follows:

$$\hat{x}^{(1)}(k+1) = \left[x^{(0)}(1) - \hat{b}/\hat{a}\right]e^{-ak} + \hat{b}/\hat{a}. \qquad (2)$$

The IAGO was then applied to (2) to obtain the restored series value $\hat{x}^{(0)}(k)$.

$$\hat{x}^{(0)}(k+1) = \hat{x}^{(1)}(k+1) - \hat{x}^{(1)}(k) \qquad (3)$$

where $\hat{x}^{(0)}(1) = x^{(0)}(1)$.

Then substituting (2) in (3) we obtion:

$$\hat{x}^{(0)}(k+1) = \left[x^{(0)}(1) - \frac{\hat{b}}{\hat{a}}\right](1 - e^{\hat{a}})e^{-\hat{a}(k)} \quad , \quad k = 1,2,\dots,n,n+1,\dots$$

$\hat{x}^{(1)}(k)$ denotes the predicted values using the smoothed data using the AGO function and $\hat{x}^{(0)}(k)$ denotes the predicted value for the actual raw data.

FIGURE 1:

temperatures (4, 10, 15 and 25 °C) and over different times (0, 2, 4, 6 and 8 hours). The aerobic plate count (APC) of frozen+ thawed squid ring reached 3.27 log CFU/g after thawing at 4 °C for 8 hours (A5), whilst the APC of frozen+ thawed squid ring increased to 6.70 log CFU/g after thawing for 8 hours at 25 °C (A20). *Pseudomonas aeruginosa,* which was sensitive to the heat treatment, was thawed at 65 °C for 5 seconds per log (90 %) reduction (Spinks et al., 2006).

TABLE 1: The groups of frozen squid rings groups thawed at different temperatures and times.

Groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	4 °C					10 °C					15 °C					25 °C				
Group A	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h
Group B	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h

Group A: frozen + thawed, Group B: frozen + boiled + thawed, h: hour

In this present study the heat treatment applied to the frozen squid rings decreased the APC of the groups (B1, B6, B11 and B16) to <1 log CFU/g at the beginning of the thawing process. In addition to this, the APC of frozen+ boiled squid (B2) was also inhibited <1 log CFU/g after thawing for 2 hours at 4 °C. However, it was felt that all the types of microorganisms may not have been destroyed by the applied heat treatment because some heat-resistant bacteria could have survived and begun to grow during the thawing process. In fact the APC of, Group B3 began to grow (0.96 log CFU/g) after the thawing process had been carried out for 4 hours at 4 °C. At the end of the thawing process over 8 hours at 4 °C, the APC of frozen thawed+ boiled squid rings reached 2.55 log CFU/g (B5), while the APC of frozen +boiled + thawed squid rings reached 4.98 log CFU/g (B20) after the thawing process over 8 hours at 25 °C.

In one study, a heated water treatment was reported to reduce the pathogenic bacteria on foods by 5 log CFU/g (Kharel et al., 2018). Furthermore, Abel et al., (2019) reported, that increasing heat treatment could decrease the bacterial loads of foods. In this present study, the APC of squid rings was determined to exhibit lower values in all the boiled samples (B groups) compared to the other groups (A groups). These results were found to correlate well with the above studies regarding the effects of heat treatment on decreasing the bacterial loads of boiled samples (B groups). Ragnarsson and Vidarsson, (2017) also reported in their study that the thawing process was an important factor in determining the final quality of the product. Therefore, the results of this study showed that the temperature of the thawing process had an important effect on accelerating or decelerating the growth of spoilage microorganisms and also the shelf-life of fishery products.

The samples thawed at 25°C were determined to have higher bacterial loads (A17, A18, A19, A20, B17, B18, B19 and B20) than the other samples thawed at temperatures 4, 10 and 15°C. The consumption limit for the total APC of fresh and frozen fishery products is indicated to be 7.0 log CFU/g according to the ICMSF (1986). Given this microbiological limit, none of the groups of squid rings exceeded the limit of consumption after the thawing process at different temperatures (4, 10, 15 and 25°C) and over different times (0, 2, 4, 6 and 8 hour).

In one study, thawed hake *(Merluccius capensis)* and plaice *(Pleuronectes platessa)* were stored at different temperatures (0 °C and 10 °C). These fish species were found to have exceeded the limit for bacterial counts (10^7 cfu/g) within 2 to 3 days at 10 °C and 7 to 8 days at 0 °C (Zotta et al., 2019). In another study Parlapani et al. (2018), reported that Cephalopods were such perishable fishery products that they could deteriorate much faster than fish species due to microbiological spoilage. The shelf-life of thawed cuttlefish *(Sepia officinalis)* stored at 2 °C was determined to be 4 days by sensory evaluation, while the aerobic plate count of thawed cuttlefish had reached 6.6 logs CFU/g by that day. The results of this present study were in accordance with the above studies indicating that increasing temperatures gave rise to the development of bacteria in samples.

Statistical results

When the frozen+thawed squid rings were checked for goodness of fit to a normal distribution with the Kolmogorov-Smirnov test it was observed that all groups except those at 4 °C conformed to a normal distribution. It was concluded that data for the frozen thawed squid rings were normally distributed (at 4 °C with a *p-value* = 0.012; at 10 °C with a *p-value* = 0.101; at 15 °C with a *p-value* = 0.200; at 25 °C with a *p-value* = 0.200). The homogeneity of variances of these groups was checked with the Levene Test. Since $p>0.05$ and with a *p-value* = 0.144. The variances were found to be homogeneous. Since these two assumptions were valid, a one-way analysis of variance (ANOVA) was performed to determine if there was a significant difference between group means according to temperatures. Since $p<0.05$ and with a *p-value* = 0.022. We concluded that there was a significant difference between group means according to temperatures. The differences between the group means were checked with the post hoc Tukey tests and found that there was a notable difference between the groups at 4 °C and 25 °C (with a *p-value* = 0.014).

Similar analyses were conducted for frozen+boiled+thawed squid rings. The goodness of fit to a normal distribution was checked with the Kolmogorov-Smirnov test and it was concluded that data for the frozen+ boiled+ thawed squid rings were not normally distributed (at 4 °C and a *p-value* = 0.001; at 10 °C and a *p-value* = 0.000; at 15 °C and a *p-value* = 0.003; at 25 °C and a *p-value* = 0.047). The homogeneity of variances of these groups was checked with the Levene Test. Since $p>0.05$ and with a *p-value* = 0.777 the variances were found to be homogeneous. Since the normality assumption was not valid, the differences between the group means were checked by the nonparametric Kruskal-Wallis test. Since $p<0.05$ with a *p-value* = 0.006 we concluded that there was a remarkable difference between group means according to temperatures. The differences between the groups means were checked with the Mann Whitney test, and it was found that there were significant differences between the groups 4 °C and 10 °C, 4 °C and 15 °C, and 4 °C and 25 °C (for 4 °C and 10 °C with a *p-value* = 0.056; for 4 °C and 15 °C with a *p-value* = 0.019; for 4 °C and 25 °C with a *p-value* = 0.001; for 10 °C and 15 °C with a *p-value* = 0.345; for 10 °C and 25 °C with a *p-value* = 0.081; for 15 °C and 25 °C with a *p-value* = 0.233).

Comparisons of all results are given in Table 2. For this reason, the Mann Whitney results were also obtained for the frozen+thawed squid rings. According to this, there were significant differences between the groups 4 °C and 10 °C, 4 °C and 15 °C, 4 °C and 25 °C, 10 °C and 25 °C (for

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4 °C and 10 °C with a *p-value* = 0.045; for 4 °C and 15 °C with a *p-value* = 0.037; for 4 °C and 25 °C with a *p-value* = 0.002; for 10 °C and 15 °C with a *p-value* = 0.567; for 10 °C and 25 °C with a *p-value* = 0.045; for 15 °C and 25 °C with a *p-value* = 0.106).

For frozen+ thawed squid rings, the differences between the group means according to time were checked with the Mann Whitney test, and it was found that the differences between all groups except for the 2 h and 4 h, 4 h and 6 h, and 6 h and 8 h were significant (for 0 h and 2 h, with a *p-value* = 0.000; for 0 h and 4 h, with a *p-value* = 0.000; 0 h and 6 h, with a *p-value* = 0.000; for 0 h and 8 h, with a *p-value* = 0.000; 2 h and 4 h, with a *p-value* = 0.078; 2 h and 6 h, with a *p-value* = 0.000; 2 h and 8 h, with a *p-value* = 0.000; 4 h and 6 h, with a *p-value* = 0.128; 4 h and 8 h, with a *p-value* = 0.001; 6 h and 8 h, with a *p-value* = 0.068.

TABLE 2: Differences between the group means of aerobic plate counts (APC) log CFU/g of frozen squid rings during the thawing process according to temperatures and time.

Thawing Time	Thawing temperatures			
	4 °C	10 °C	15 °C	25 °C
Frozen+thawed (0h)	(A1) <1[a]	(A6) <1[a]	(A11) <1[a]	(A16) <1[a]
Frozen+thawed (2h)	(A2)1.37±1.191[b]	(A7) 2.50±0.112[b]	(A12)2.65±0.032[b]	(A17)3.39±0.112[b]
Frozen+thawed (4h)	(A3)2.36±0.151[b]	(A8) 3.15±0.222[b]	(A13)3.25±0.042[b]	(A18)4.57±0.042[b]
Frozen+thawed (6h)	(A4)2.72±0.041[b]	(A9) 3.52±0.162[b]	(A14)4.48±0.042[b]	(A19)5.84±0.032[b]
Frozen+thawed (8h)	(A5)3.27±0.031[b]	(A10)4.62±0.062[b]	(A15)5.85±0.032[b]	(A20)6.70±0.172[b]
Frozen+thawed+boiled (0h)	(B1) <1[c]	(B6) <1[c]	(B11) <1[c]	(B16) <1[c]
Frozen+thawed+boiled (2h)	(B2) <1[c]	(B7) 0.48±0.834[d]	(B12)1.40±0.204[d]	(B17)2.58±0.034[d]
Frozen+thawed+boiled (4h)	(B3)0.96±0.933[d]	(B8) 2.60±0.064[e]	(B13)2.65±0.074[e]	(B18)2.91±0.014[e]
Frozen+thawed+boiled (6h)	(B4)2.27±0.043[e]	(B9) 2.81±0.034[f]	(B14)2.97±0.034[f]	(B19)4.01±0.034[f]
Frozen+thawed+boiled (8h)	(B5)2.55±0.053[e]	(B10)2.94±0.014[f]	(B15)3.07±0.044[f]	(B20)4.98±0.064[f]

The results were shown as X ± SD. n=3; The results were given as the mean value of three experiments. The letters in the same row show statistical difference) between the groups according to temperatures and the letters in the same column show the difference). between the groups during the storage period.

Similarly, for frozen + boiled + thawed squid rings, the differences between the group means according to time were checked with the Mann Whitney test, and it was found that the differences between all groups except for 6h and 8h were significant (for 0 h and 2 h, with a *p-value* = 0.014; for 0 h and 4 h, with a *p-value* = 0.000; 0 h and 6 h, with a *p-value* = 0.000; for 0 h and 8 h, with a *p-value* = 0.000; 2 h and 4 h, with a *p-value* = 0.006; 2 h and 6 h, with a *p-value* = 0.000; 2 h and 8 h, with a *p-value* = 0.000; 4 h and 6 h, with a *p-value* = 0.039; 4 h and 8 h, with a *p-value* = 0.005; 6 h and 8 h, with a *p-value* = 0.242).

In addition, for frozen + thawed and frozen + boiled + thawed squid rings, the differences between the group means of total aerobic bacteria counts were compared according to temperatures. The differences between group means (for two groups) were checked with the Mann-Whitney test. The differences between all group means were found to be significant at a 10 % significant level (for 4 °C, with a *p-value* = 0.071; for 10 °C, with a *p-value* = 0.049; for 15 °C, with a *p-value* = 0.046; for 25 °C, with a *p-value* = 0.061).

In the frozen + thawed and frozen + boiled + thawed squid rings, the differences between the group means of the total aerobic bacteria counts were compared according to time. According to the Mann-Whitney test, the differences between all group means were found to be significant except for 0 h (for 0 h, with a *p-value* = 1.000; for 2 h, with a *p-value* = 0.004; for 4 h, with a *p-value* = 0.012; for 6 h, with a *p-value* = 0.039; for 8 h, with a *p-value* = 0.001).

Regression Modeling

To determine the factors affecting the bacterial growth in frozen+thawed squid rings stored at different temperatures, the APC (aerobic plate count) was chosen as the dependent variable. The independent (explanatory) variables were determined to be storage times (0, 2, 4, 6 and 8 hours), different temperatures (4 °C, 10 °C, 15 °C and 25 °C) and treatments (frozen+thawed squid ring and frozen+boiled+thawed squid ring). Treatment variables were qualitative, while the other variables were quantitative variables. Storage times, temperatures and treatment variables were selected at certain levels depending on expert opinion, and a fixed effect model was used. The mathematical model related to these variables was determined by using regression modelling technique (Montgomery and Runger, 2003). Qualitative variables were expressed as dummy variables in the regression models, and defined as below. The frozen+ thawed treatment was taken as a reference category (Carrascosa et al., 2014) (see Fig. 2, F and B).

In order to the relationship aerobic plate count (APC) dependent variable and the independent variables of storage times (0, 2, 4, 6 and 8 hours), different temperatures (4, 10, 15 and 25 °C), and different treatments (frozen+thawed and frozen+boiled+thawed), scatter plots were drawn from the measurements.

The relationships between the dependent variable APC and the independent variables (temperature, time and treatment) are shown in Figure 3.

With the help of Figure 3, various regression models were tested to try and match the relationships between the variables. The most appropriate linear fixed-effect model for the estimation of APC was found to be (see Fig. 2, logAPC).

In this equation, *TE* and TI were used for the temperature and time, respectively. Also, *fbt* was used as an abbreviation for frozen+boiled+thawed and *ft* was used as an abbreviation for frozen+ thawed. *TIxTE* showed the interaction effect between time and temperature factors. According to the estimation equation, the APC value was affected by temperature factors both linearly and quadratically. Except for the constant term, all of the coefficients of this model were significant with a *p-value*=0.000 and R^2_{adj}=0.918. The goodness of fit errors of this model compared to a normal distribution were tested with the Kolmogorov-Smirnov test with a *p-value*=0.099.

$$F = \begin{cases} 1, & \text{if treatment frozen + thawed is used} \\ 0, & \text{if treatment frozen + thawed is not used} \end{cases}$$

$$B = \begin{cases} 1, & \text{if treatment frozen + boiled + thawed is used} \\ 0, & \text{if treatment frozen + boiled + thawed is not used} \end{cases}$$

$$\widehat{logAPC} = 0.144 + 0.034TE + 0.677TI - 0.044\mathrm{TI}^2 - 1.051(fbt - ft) + 0.014TIxTE$$

FIGURE 2:

For example, using this estimation equation, the aerobic plate bacteria count of frozen+thawed squid ring was estimated to be 1.57 at the end of 2 hours at a temperature of 4 °C, while the total aerobic plate bacteria count of frozen+boiled+thawed squid ring was estimated to be 0.52 at the end of 2 hours at a temperature of 4°C. Using this estimation equation, the estimation values for all observation results were obtained and are given in Table 3.

TABLE 3: The observed and estimated values of the aerobic plate counts (APC) log CFU/g of frozen squid rings during the thawing process at different temperatures and times according to the regression modeling.

	Frozen+Thawed Squid Rings				Frozen+Boiled+Thawed Squid Rings			
	4 °C	10 °C	15 °C	25 °C	4 °C	10 °C	15 °C	25 °C
t=0	0.0000 (0.2787)	0.0000 (0.4813)	0.0000 (0.6502)	0.0000 (0.9878)	0.0000 (–0.7721)	0.0000 (–0.5695)	0.0000 (–0.4007)	0.0000 (–0.0630)
t=2	1.3700 (1.5695)	2.4967 (1.9431)	2.6467 (2.2545)	3.3867 (2.8773)	0.0000 (0.5186)	0.4800 (0.8923)	1.3967 (1.2037)	2.5833 (1.8264)
t=4	2.3567 (2.5044)	3.1500 (3.0491)	3.2533 (3.5030)	4.5733 (4.4109)	0.9633 (1.4535)	2.6033 (1.9983)	2,6533 (2.4522)	2,9100 (3.3600)
t=6	2.7167 (3.083)	3.5167 (3.7992)	4.4767 (4.3957)	5.8400 (5.5886)	2.2733 (2.0326)	2.8133 (2.7484)	2.9700 (3.3449)	4,0067 (4.5378)
t=8	3.2733 (3.3067)	4.6200 (4.1935)	5.8467 (4.9325)	6.6967 (6.4106)	2.5533 (2.2559)	2.9467 (3.1427)	3.0733 (3.8817)	4.9767 (5.3597)

The values in this table show the values observed at the end of the experiment and the values in brackets show the predicted values. Therefore, the closeness of the actual and predicted values shows the efficiency of the estimation equation obtained.

The observed and estimated values of the aerobic plate bacteria counts of frozen+thawed and frozen+boiled+thawed squid rings depending on both temperature and time are given in the above table. Values in parentheses were estimation values and other values were observed values. The changes in the total aerobic plate counts of squid rings when the temperatures were increased can be clearly seen from the values in Table 3.

The estimated values of the total number of aerobic plate count for frozen+thawed and frozen+boiled+thawed squid rings depending on both temperature (0 °C and 30 °C) and time were obtained using the regression modeling and are given in Table 4. If frozen squid rings were thawed at 30 °C, the estimated value of the aerobic plate count of frozen+thawed squid rings according to regression modeling reached 7.12 log CFU/g, which was the spoiled microbiological value after 8 hours according to (ICMSF, 1986). However, if squid rings were thawed at 0 °C, the estimated aerobic plate bacteria count of squid rings could have increased to 2.74 log CFU/g (t=8). After 8 hours of the thawing process at 30 °C, the estimated total aerobic bacteria count of squid rings was determined to be approximately 4.5 log higher than the estimated total aerobic bacteria count of squid rings thawed at 0 °C. This regression model was found to be very suitable in predicting the microbiological growth of squid rings during

TABLE 4: The estimated values of aerobic plate counts (APC) log CFU/g of frozen squid rings during the thawing process at 0 ° and 30 °C according to the regression model.

	Frozen+Thawed Squid Rings		Frozen+Boiled+Thawed Squid Rings	
	0 °C	30 °C	0 °C	30 °C
t=0	0.144	1.164	–0.907	0.113
t=2	1.322	3.182	0.271	2.131
t=4	2.148	4.848	1.097	3.797
t=6	2.622	6.162	1.571	5.111
t=8	2.744	7.124	1.693	6.073

The estimated values of APC obtained from the regression model for 0°C and 30°C that were not experimentally observed, are given.

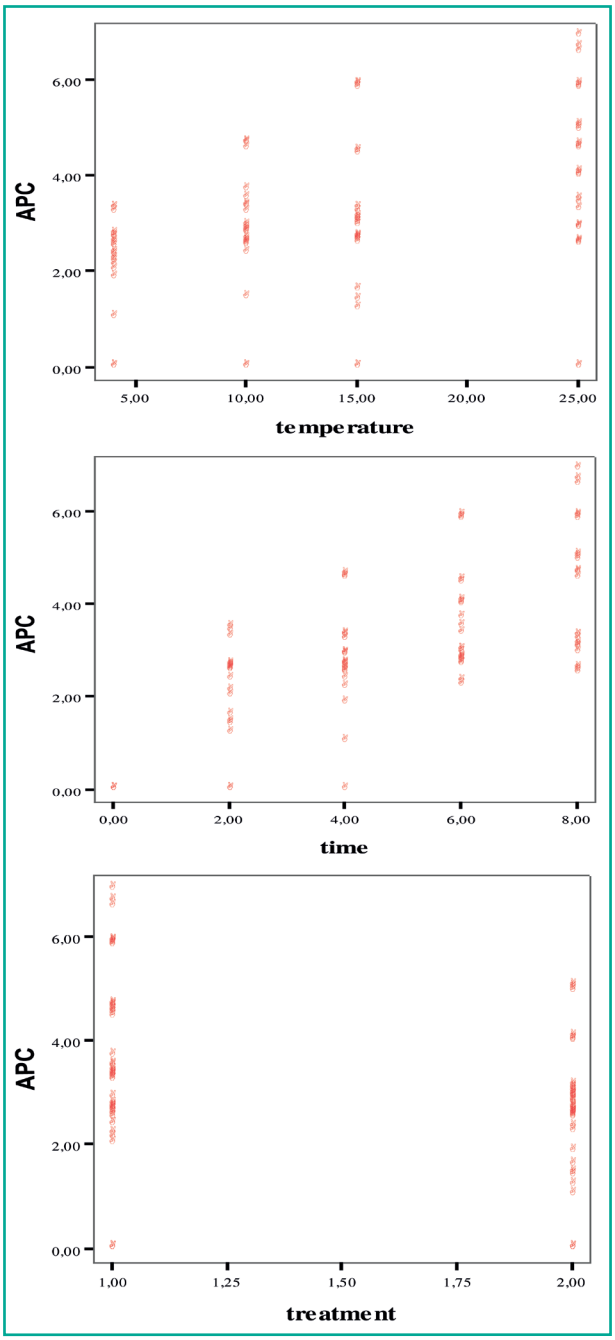

FIGURE 3: *Scatter plots of the dependent variable (APC) and independent variables (temperature, time and treatment).*

APC values also increased as the values of temperature and time variables increased. The APC values differed for the two treatment level of the variable (frozen+thawed and frozen+boiled+thawed).

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the thawing process for different temperatures and times. Additionally, this regression model has the advantage of being able to compare groups with bacterial growth according to the temperature and time.

Grey Modeling

Using five volume data for frozen+ thawed squid ring at 25°C, the steps mentioned in the statistical methods section are illustrated as follows (see Fig. 4).

For illustration, calculations were made for a subgroup within this study. In a similar way, using the procedure outlined above, the grey model prediction results can be obtained for the other subgroups given in Table 3.

Moreover, there were different subgroups in terms of temperature and treatment in the observed data set. Different GM (1,1) models should be proposed for each of these subgroups according to the method. While calculating the different subgroups, we encountered different grey difference equations and different whitening equations. Therefore, a single model could not be created as it could be in regression modeling. For this reason, performance measures related to the errors obtained from the models for only one subgroup were calculated and are given in Table 6.

To quantify the prediction errors for both models, the performance measures Root Mean Square Error (RMSE), Mean Absolute Error (MAE) and Mean Squared Error (MSE) were used. These measures were calculated as follows (see Fig. 5).

These performance measures were obtained for the values given in Table 5 and the results of the performance measures are given in Table 6.

The performance measures given in Table 6 were based on the differences between the actual values and the estimated values, i. e. errors. Therefore, the model with lower errors should give more accurate estimates. The values of the three performance criteria (MSE or mean square error, RMSE or root mean square error and MAE or mean absolute error) were lower for the grey model. Therefore, the grey model appeared to be a good alternative when there was little data and time-dependent measurements were made.

In one study the authors reported that the temperature-related microorganism development models of Baranyi, Huang, modified Gompertz and logistic models were very consistent with the stated data on microorganism development R^2 values 0.90 to 0.99. It was also reported that all four models fitted very well with the modified Ratkowsky square-root model (Juneja et al., 2019). In another study, the authors declared that the model was successful

Step 1: There were three observations at 25°C for each time period. The raw data set was obtained by taking the mean of the three observations in each time period:

$$X^{(0)}(k) = (0,\quad 3.39,\quad 4.57,\quad 5.84,\quad 6.70)$$

Step 2: Applying the Accumulating Generating Operation (AGO) to the raw data, the following cumulative values were obtained:

$$X^{(1)}(k) = (0,\ 3.39,\ 7.96,\ 13.8,\ 20.5)$$

Step 3: Finding the mean values of two adjacent cumulative values in $X^{(1)}(k)$ produced $Z^{(1)}(k+1) = (1.695,\ 5.675,\ 10.88,\ 17.15)$.

Step 4: Using the $[\hat{a},\ \hat{b}]^T = (B^TB)^{-1}B^TY$ equation, the coefficients a and b were found to be $\begin{bmatrix}\hat{a}\\ \hat{b}\end{bmatrix} = \begin{bmatrix}-0.214\\ 3.234\end{bmatrix}$. Here,

$$Y = \begin{bmatrix}3.39\\ 4.57\\ 5.84\\ 6.7\end{bmatrix}, \qquad B = \begin{bmatrix}-1.695 & 1\\ -5.675 & 1\\ -10.88 & \vdots\\ -17.15 & 1\end{bmatrix}.$$

Step 5: Using the following:

$$\hat{x}^{(0)}(k+1) = \left[x^{(0)}(1) - \frac{\hat{b}}{\hat{a}}\right](1 - e^{\hat{a}})e^{-\hat{a}(k)} \quad , \quad k = 1, 2, \dots, n, n+1, \dots$$

with the initial condition $\hat{x}^{(0)}(1) = x^{(0)}(1)$, predicted values for the raw data were obtained and are given below:

$$\hat{x}^{(0)}(k) = [0 \quad 3.61 \quad 4.47 \quad 5.53 \quad 6.85]$$

FIGURE 4:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}|y_i - \hat{y}_i|^2}{n}}, \quad MAE = \frac{\sum_{i=1}^{n}|(y_i - \hat{y}_i)|}{n}, \quad MSE = \frac{\sum_{i=1}^{n}|(y_i - \hat{y}_i)|^2}{n}.$$

where n is the number of time intervals, y_i denotes the observed values obtained from the experiment, and $\hat{y}_i$ denotes the predicted value obtained from the models, for $i = 1,2,3, \dots, n$, respectively.

FIGURE 5:

TABLE 5: The observed and predicted values of the aerobic plate bacteria counts (APC) log CFU/g of squid rings thawed at 25 °C during the thawing process at different times according to the regression and grey models.

	Observed values 25 °C	Predicted value from the regression model 25 °C	Predicted value from the Grey model 25 °C
t=0	0.00	0.99	0.00
t=2	3.39	2.88	3.61
t=4	4.57	4.41	4.47
t=6	5.84	5.59	5.53
t=8	6.70	6.41	6.85

The values observed for the APE values in frozen+thawed squid rings stored at 25 °C depending on the time and also showed the estimated values obtained from both the regression and the grey model.

TABLE 6: Performance measures for the regression and grey model for the values given in Table 5.

	Regression model	Grey model
MAE	0.440	0.156
MSE	0.282	0.035
RMSE	0.531	0.188

The values observed for the APE values in frozen+thawed squid rings stored at 25 °C depending on the time and also showed the estimated values obtained from both the regression and the grey model.

in estimating the microbial safety of packaged fresh food products under O_2, CO_2 and N_2 modified atmospheric conditions and their effects on microbial development without defining any parameters (Guillard et al., 2016). In another study, the use of microbial models to evaluate the effects of UV-C light and trans-cinnamaldehyde on the microbial load of grape juice by using Weibull, modified Gompertz and logistic models were studied. Ochoa-Velasco et al., (2018) The three model estimations used in this study showed that trans-cinnamaldehyde was more effective than UV-C light in stopping microbial development. Our study was found to be well correlated with the above studies, which were used for estimating the microbial development.

Conclusion

A regression model that predicted the total aerobic bacteria growth of frozen squid rings during the thawing process at different times and temperatures was developed. This newly developed mathematical model that was dependent on different temperatures and times can be applied to the thawing process for frozen squid rings. This predictive model will not just be of great interest in exploring bacterial interaction for different temperatures and times, but also for estimating the bacterial growth according to temperature and time changes during the thawing process.

Grey modeling was also developed for the data of this study to predict the total aerobic bacteria growth of squid rings during the thawing process at different temperatures and times. A comparison of the groups was not possible with the grey modeling, while it was with regression modeling. However, the grey model appeared to be a good alternative when there was little data and time-dependent measurements were made. As a result the regression modeling in this study was seen to be more suitable than the grey modeling because it enabled comparison between all groups.

The two developed mathematical models in this study can be used for estimating the total aerobic bacteria growth of frozen squid rings during the thawing process because of they are economical and save time in comparison to microbiological analyses. The aerobic bacteria counts of frozen squid rings during the thawing process can be estimated by using these developed regression and grey models without doing the microbiological analysis. It would be advisable to do more studies on predicting the different growth of microorganisms on different aquatic species by using different statistical prediction models.

Conflict of interest

The authors have declared no conflicts of interest for this article.

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