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## The effects of Artichoke (*Cynara scolymus* L.) Leaf Extract on the microbial and chemical quality of Sardine (*Sardina pilchardus* Walbaum, 1792) fish patties and shelf life modeling

*Die Auswirkungen von Artischockenblattextrakt (Cynara scolymus L.) auf die mikrobielle und chemische Qualität von Sardinenfrikadellen (Sardina pilchardus Walbaum, 1792) und Haltbarkeitsmodelle*

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### Summary

In this study, the influence of artichoke leaf extract on the microbiological (total mesophilic aerobic bacteria, total coliform count, total psychrophilic bacteria, and the total yeasts and molds) and chemical (pH, TVB-N and TBA) quality of sardine fish patties were investigated during cold storage period at +4 °C. The microbiological and chemical effects of artichoke leaf extract on fish patties were analyzed with a nonlinear regression model. From the measured values, R<sup>2</sup> values of the models were between 0.845 and 0.958 – close to 1 in terms of model fit. The chemical properties of the samples had pH values between 4.75 to 6.89, TVB-N values of 17.80 to 45.15 mg/100 g and TBA values of 1.02 to 2.55 mgMDA/kg. In conclusion, it was observed that fish patties with 1.5 % added artichoke leaf extracts were found to be of acceptable quality when stored at 4 °C and contributed to increased shelf life.

**Keywords:** Shelf life, fish patties, artichoke, quality, nonlinear regression

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## Introduction

One of the most important food groups addressing the issue of healthy nutrition is fish products, and it is a leading food group. Products derived from aquaculture, especially fish and fish meat, occupy an important place due to both the healthy and perishable characteristics of other foods of animal origin. In order to benefit more from fish, they should be consumed in a short period after being caught or they should be kept in appropriate conditions so that the best quality can be delivered to consumers. Fish meat is processed in different ways to supply consumers. Processed fish, especially, offers different flavors and alternatives for hotels and restaurants and has become a sought after product in such places. One of these products is fish patties (Öksüztepe, Emir Çoban and Güran, 2010).

Fish patties are a fish product obtained by adding spices after the fish meat is cleaned, boiled and minced. Gökoğlu (1994) and Akkuş, Varlık, Erkan, and Mol (2004) stated that fish patties retained their quality up to 8 days but were spoiled after 10 days of storage at  $4 \pm 1$  °C. Therefore, alternatives techniques need to be developed to increase shelf life and to preserve the nutritional qualities of processed aquatic products such as fish patties. Plant extracts and organic acids are known as natural alternatives to ensure the microbiological safety of foodstuffs and to increase shelf life (Koos, 1992; Cubina, 1995).

Artichoke (*Cynara scolymus* L.) is a vegetable that is widely consumed in the Mediterranean diet. It belongs to the *Asteraceae* family, grows to between 50 and 150 cm tall and has purple flowers (Ergezer, Kaya and Şimşek, 2018). It is rich in phenolic acids such as caffeic acid, mono- and di-caffeoylquinic acid (i.e. Synarin) and chlorogenic acid. These characteristics of these compounds are increasing bile, decreasing cholesterol and protecting the liver, as well as anti-carcinogenic and antioxidant properties (Pierluigi and Piergiorgio, 2000; Schutz, Kammerer, Carle and Scieber, 2004). In published literature, artichoke leaf extracts have also been reported to have antimicrobial properties. Moreover, it has also been reported that the antioxidant structure of artichoke might prevent liver damage. With its peppery-like taste and water solubility capacity of its dried leaves, the use of artichoke in medicine is gradually increasing (Liorach, Espin, Tomas-Barbera and Ferreres, 2002; Speroni et al. 2003). Artichoke extract has been used as a medicine for hundreds of years, but its use as an antimicrobial is very rare. The hydroalcoholic extracts obtained from artichoke (*Cynara scolymus*) have been shown to have a significant inhibitory effect when tested against strains of *Listeria innocua* and *Bacillus cereus* (Ionescu et al., 2013).

Mathematical models offer analyses that yield results in shorter times when compared to time-consuming and expensive quality control analyses, as well as being economically beneficial. Another important characteristic of the models is the use and development of known factor parameters in determining food characteristics (Wijtzes, De Wit, HuisInVeld, Van Riet, and Zwietering, 1995). By using microorganism density and distribution, the methods used in modeling allow strategies to be developed. The aim of using mathematical models is to determine the concentration of microorganisms, which threaten human health, and to estimate when this risk might occur (Soboleva, Pleasants and Roux, 2000; Keyvan and Özdemir, 2010). The mathematical models used in food production aim to estimate the changes in quality of food occurring over the course of time and thus prolong the shelf life of foods. One

of these models, the microbial inactivation model, is used in various phases of meat product manufacturing such as mincing, cooling, packaging and distribution. Its purpose is to determine the shelf lives of meat and milk products, in controlling the special starter cultures used in fermentation, in ensuring the environmental factors required for development of microorganisms, in optimizing different processes for production, and in determining the effect of food additives on the target microorganisms (Oğuzhan and Yangilar 2013).

The outer leaves of the artichoke can be used as a natural additive due to their rich phenolic content, which has antimicrobial and antioxidant effects (Lattanzio, Kroon, Linsalata and Cardinali, 2009). However, the use of artichoke by-products in food matrices has very rarely been reported on before. This study aimed to contribute to the literature by determining the chemical and microbiological properties of sardine fish (*Sardina pilchardus* Walbaum, 1792) with added artichoke leaf extract during its storage time. This was done by using nonlinear regression models to assess the effect of artichoke leaf extracts on the microbiological quality parameters during the storage period. The microbial shelf life was modeled by choosing the mathematical model that best estimates the microbial change.

## Material and Methods

### Material

The sardine fish (*Sardina pilchardus* Walbaum, 1792) that were used in this study were procured from a local company in İzmir, Turkey. The fish were immediately brought to the Aquaculture Processing Technology Laboratory of İzmir Katip Çelebi University. The filleted sardine meat was kept in polystyrene plates at 4°C. For each test repetition, approximately 10 to 11 kg of sardine fish was used, and each fish weighed approximately 20 grams. The artichoke leaves that were used in this research were collected from an artichoke farm from a district of İzmir in Turkey, and then brought to the same laboratory as the fish samples.

### Extraction of the artichoke leaf

The artichoke leaves were rinsed twice using municipal water and then filtered in order to remove the dirt and agricultural pesticides on the leaves. The rinsed artichoke leaves were dried in an oven at 50°C during 4 hours. The dried samples were shaken up using a blender. Twenty grams of the dried sample was put in an erlenmeyer flask with 100 ml of solvent (80% organic alcohol), and the mixture was put into a shaking water bath at 55°C for 6 hours. At the end of 6 hours, the samples were taken out of the water bath and then filtered into bottles using Whatman no.1 filter paper. This procedure was repeated three times. A rotary evaporator was used for removing the solvent from the filtered solutions. Following this step, the water remaining within the extracts was freeze-dried. The pulverized samples were kept at -20°C before being used (Keyrouz et al., 2011).

### Preparation of the fish patties

Sardine fish were used to prepare the patties. The fish were cleaned by removing the heads and organs. Following the boning and washing procedure, the fish meat was ground up. Some of the fish patties were prepared using artichoke leaf extract (ALE) in addition to the standard ingredients

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(1% breadcrumbs, 1% onion powder, 1% garlic powder, 10% boiled rice, 8.1% egg, 0.9% salt, 0.2% black pepper, 0.2% cumin, 0.2% allspice and 0.1% red pepper flakes), while the remaining portions were prepared only with spices and ground meat. The patties prepared at predetermined concentrations were kneaded, and the artichoke leaf extracts were added at concentrations of 0.5%, 1%, 1.5%, and 2%. The patties without extract were used as a control group. The kneaded patties were formed into 50 gram pieces and kept in a polystyrene plate by covering with stretch film and kept at 4°C. These samples kept at 4°C, were examined for chemical and microbiological aspects on day 0, 2, 4, 8, 10, 12, 14, 16, 18, and 20 of storage.

### Chemical analyses

The chemical changes occurring during storage of all the fish patties samples were investigated using pH, Thiobarbituric Acid Analysis (TBA) and Total Volatile Basic Nitrogen (TVB-N) analysis. Ten gram samples was homogenized in 100 mL of distilled water and the mixture was filtered. The pH value was measured using a digital pH meter (Ohaus Starter 3000, Parsippany, NJ 07054, USA) (Guran et al. 2015). The TBA and TVB-N analyses were performed as described by Kılınç, Çaklı and Tolasa (2008).

### Microbiological analyses

The total mesophilic aerobic bacteria, the total psychrophilic bacteria, the total coliform counts and the total yeast and molds, were determined for microbiological analysis. For this purpose, a 10 gram patty sample was put into a 90 mL sterilized Maximum Recovery Diluent solution under aseptic conditions, and the mixture was then homogenized. By using a 1/10 dilution rate, the homogenate was diluted to a dilution level of  $10^6$ . By taking 0.1 mL from the appropriate dilutions, the homogenate was cultivated in petri boxes containing the medium. A Plate Count Agar (PCA, Merck) was used at 30°C for 24 to 48 hours to determine the total mesophilic aerobic bacteria; at 6.5°C for 10 days for the total psychrophilic bacteria; Potato Dextrose Agar (PDA, Merck) was used at 30°C for 4 to 5 days for the total yeast and mold counts, and Fluorocult Violet Red Bile Agar (FVRBA, Merck) was used at 37°C for 24 hours incubation for coliform bacteria counts. At the end of the incubation, the colonies that developed in the media were counted in order to calculate the bacteria (FDA, 1998).

### Establishment of the nonlinear regression models

In this study, the total mesophilic aerobic bacteria, total coliform bacteria, total number of yeast-molds and total number of psychrophilic bacteria, and some chemical properties, were determined for fish patty samples supplemented with ALE. The data obtained were used as effective parameters for the preparation of the mathematical models. Generally, in the nonlinear regression method, the second-order polynomial equation is used for modeling. Such equations are also known as quadratic equations. The regression model (Eq. 1), based on plant extract concentration ( $B_k$ ) and storage time ( $t_d$ ) is shown below:

$$M = a_0 + a_1 B_k + a_2 t_d + a_3 B_k^2 + a_4 t_d^2 + a_5 B_k t_d \quad (\text{Eq. 1})$$

The coefficients of  $a$ , in this equation, were determined using Windows SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). Regression coefficients for the mathematical models used were calculated, and these coefficients were substituted into equation (1) to obtain mathe-

matical models for the pH, TBA, TVB-N values and total mesophilic bacteria, total coliform bacteria, total yeast and mold, and total psychrophilic bacteria counts. Experimental and theoretical values were compared using the mathematical models. In addition, the analysis of variance (ANOVA) method was applied to the mathematical models values obtained from the chemical and microbiological analysis, and regression and determination coefficient ( $R^2$ ) values were determined for the regression models. These values indicated the model's constant, quadratic and statistical effects on the chemical and microbiological values of the product terms, and they were used to evaluate their model conformity (Kalkan, 2016).

Response surface graphs of the measured values were created using Surface Response Method software from MATLAB. The effects of the processing parameters on the microbiological properties of the product can be observed more clearly with the help of the graphs generated.

## Results and Discussion

### Chemical properties of the samples

The pH, TVB-N and TBA results obtained from performing chemical storage analyses are presented in Table 1, and the response surface graphs of the measured chemical values are shown in Figure 1. As seen in Table 1, the pH values decreased from the beginning to the end of storage and then reached a minimum on day 20. When compared to the control group, a decrease was observed in the pH level of the group with added artichoke leaf extract. The minimum pH value was found to be 4.74 on day 20 in samples with 0.5% added artichoke leaf extract. The pH value increases due to autolysis and bacterial activities because of rigor mortis immediately after death. The pH value of fresh fish meat varies between 6.00 to 6.50 and increases depending on the storage period. Looking at the microbial results, an expected increase in pH occurred during storage due to alkaline bacterial metabolites. The consumption limit for fish meat in terms of pH value is between 6.80 to 7.00 (Galdos et al. 2002). In this study, the pH value of fish patties ranged from 4.74 to 6.89 and changes were observed during storage. However, it is known that the pH value alone is not enough to decide on suitability for consumption and results must be supported by sensory, chemical and microbiological analyses. Kılınççeker (2014) determined that pH values of fish patty coatings with added sage tea and stinging nettle to be 6.65, 6.65, 6.62, 6.63 and 6.63, respectively. Similar results were obtained in this study, and it was determined that the pH value changed during the storage period; however, it did not exceed the consumption limit value of 7.00.

Given the TVB-N values obtained, it was determined that minimum values were observed at the beginning, while the maximum values were obtained on day 8 of storage. Compared to the control group, it was found that the maximum level was 38.80 on day 20 of storage in samples containing 1% artichoke leaf extract. The TVB-N value is used to determine the degree of degradation of protein and product quality in marine and freshwater fish. According to TVB-N classification value for aquatic products, 25 mg/100 g TVB-N is "very good"; 30 mg/100 g TVB-N is "good", 35 mg/100 g TVB-N is "marketable" and >35 mg/100 g TVB-N is "unsuitable/spoiled" (Gökoğlu, Özden and Erkan, 1998). Experimental groups containing 0.5% and 1% ALE were deemed to be spoiled on day 8 of

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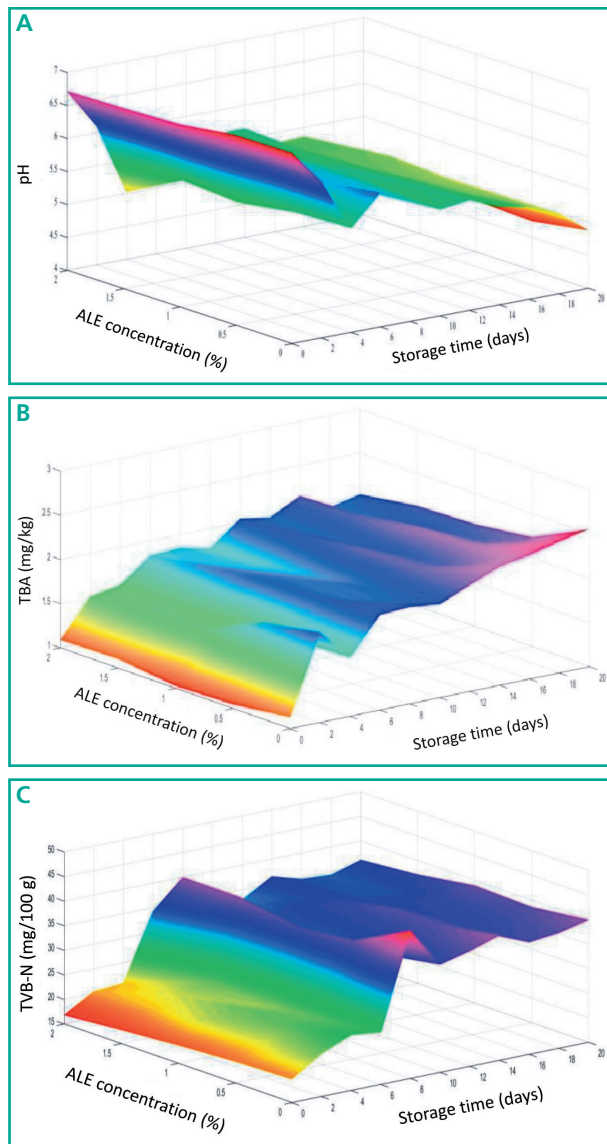


FIGURE 1: Response surface graphs of the chemical values of the samples (A: pH; B: TBA values (mg/kg); C: TVB-N values (mg/100g)).

storage; other groups containing 1.5% and 2% were deemed to be spoiled on days 12 and 14 of storage because they exceeded 35 mg/100 g TVB-N. TVB-N values indicated that the shelf life increased as the ALE used in patty production increased. The shelf life of the experimental group, which contained 2% ALE, was found to be 6 days longer than the control group. The TVB-N values of the experimental groups containing 1% and 1.5% ALE were found to be similar, and they were deemed to be corrupted with 35 mg/100 g TVB-N by day 12. Mahmoud et al. (2004) reported that the TVB-N value of carp fish fillets stored at 5°C by immersion in thymol and carvacrol reached 30 mg/100 g by day 12. Erkan et al. (2011) researched the effect of added essential oils on the shelf life of sea bass fillets. TVB-N values were determined to be 28.14 and 31.17 mg/100 g in laurel and thyme essential oils by day 13 of storage. Although the essential oils and their application methods were different from the studies carried out here, similar results were obtained.

For the TBA values, the minimum value was obtained on day 0 of storage. The maximum level in the control group was found to be 2.55 on day 20 of storage, while the maximum value of other groups with added artichoke leaf extract was determined to be on day 16 of storage. The TBA value is one of the most important criteria for the deterioration of fish meat because of the occurrence of fat oxidation. The consumption limit for the TBA content was reported to be 7 to 8 mgMDA/kg and rancidity started when the fish meat exceeded 4 mgMDA/kg (Can, Yalçın and Arslan, 2011). In this study, the TBA value of fish patty samples stored at 4°C for 20 days did not exceed 8 mgMDA/kg, which is the limiting value for consumption. TBA values were increased in all groups during storage. While the TBA value of the control group doubled the initial value, the increase in the other groups with the addition of the ALE extract remained lower. TBA levels were significantly lower in the experimental group containing 2% artichoke extract compared to the other groups between day 14 and 18 ( $p \leq 0.05$ ). In a similar study, sheep patty samples with different extracts were stored at 4°C for 13 days. During the storage period, TBA values for extract-supplemented patties were lower than the control group. In addition, the most effective antioxidant compound was repor-

TABLE 1: pH, TVB-N and TBA values of samples during storage.

ALE Concentrations (%)	Storage time (Days)										
	0	2	4	6	8	10	12	14	16	18	20
<b>pH</b>											
Control	6.89±0.03 <sup>KG</sup>	6.35±0.31 <sup>F</sup>	5.59±0.25 <sup>CD</sup>	6.04±0.07 <sup>EF</sup>	5.81±0.12 <sup>DE</sup>	5.62±0.15 <sup>CD</sup>	5.67±0.10 <sup>gCD</sup>	5.49±0.09 <sup>BCD</sup>	5.40±0.04 <sup>cBC</sup>	5.21±0.04 <sup>ab</sup>	4.88±0.01 <sup>BA</sup>
0.50	6.88±0.07 <sup>KG</sup>	6.34±0.27 <sup>F</sup>	5.55±0.10 <sup>CD</sup>	5.92±0.04 <sup>E</sup>	5.89±0.10 <sup>E</sup>	5.55±0.12 <sup>CD</sup>	5.67±0.27 <sup>DE</sup>	5.46±0.02 <sup>cBC</sup>	5.29±0.02 <sup>cBC</sup>	5.20±0.08 <sup>ab</sup>	4.74±0.02 <sup>BA</sup>
1	6.77±0.01 <sup>KG</sup>	6.20±0.09 <sup>F</sup>	5.42±0.22 <sup>BCD</sup>	5.92±0.04 <sup>EF</sup>	5.85±0.16 <sup>HE</sup>	5.72±0.14 <sup>DE</sup>	5.60±0.32 <sup>ICDE</sup>	5.42±0.03 <sup>cBC</sup>	5.26±0.07 <sup>cBC</sup>	5.19±0.04 <sup>ab</sup>	4.77±0.02 <sup>BA</sup>
1.50	6.73±0.00 <sup>KG</sup>	6.17±0.11 <sup>F</sup>	5.48±0.07 <sup>BC</sup>	5.92±0.19 <sup>EF</sup>	5.81±0.19 <sup>DE</sup>	5.50±0.10 <sup>BC</sup>	5.59±0.23 <sup>gCD</sup>	5.33±0.00 <sup>bc</sup>	5.29±0.08 <sup>ab</sup>	5.25±0.04 <sup>ab</sup>	4.87±0.07 <sup>BA</sup>
2	6.71±0.02 <sup>KE</sup>	6.10±0.02 <sup>D</sup>	5.03±0.71 <sup>BA</sup>	5.79±0.08 <sup>DE</sup>	5.80±0.12 <sup>DE</sup>	5.65±0.32 <sup>gCDE</sup>	5.64±0.02 <sup>ICDE</sup>	5.33±0.02 <sup>ab</sup>	5.35±0.01 <sup>bc</sup>	5.08±0.10 <sup>cABC</sup>	4.75±0.04 <sup>BA</sup>
<b>TVB-N (mg/100 g)</b>											
Control	19.80±1.13 <sup>3A</sup>	23.80±1.69 <sup>3A</sup>	26.40±6.22 <sup>4A</sup>	25.70±0.98 <sup>3A</sup>	45.15±2.47 <sup>7C</sup>	37.00±2.82 <sup>6B</sup>	38.50±0.98 <sup>6BC</sup>	40.50±0.70 <sup>6BC</sup>	37.85±5.86 <sup>6BC</sup>	38.50±0.00 <sup>6BC</sup>	39.90±2.82 <sup>6BC</sup>
0.50	19.40±1.97 <sup>3A</sup>	22.00±2.82 <sup>3AB</sup>	24.40±2.26 <sup>3B</sup>	24.30±0.70 <sup>3B</sup>	39.20±0.00 <sup>6C</sup>	35.00±1.97 <sup>6C</sup>	37.10±2.96 <sup>6C</sup>	38.55±2.05 <sup>6C</sup>	36.75±0.49 <sup>6C</sup>	37.45±1.48 <sup>6C</sup>	38.50±2.96 <sup>6C</sup>
1	18.70±2.40 <sup>3A</sup>	22.40±3.95 <sup>3A</sup>	23.80±9.89 <sup>4A</sup>	22.90±1.83 <sup>3A</sup>	38.15±3.46 <sup>6B</sup>	34.30±0.98 <sup>6B</sup>	36.40±8.20 <sup>6B</sup>	38.50±0.00 <sup>6B</sup>	36.05±2.47 <sup>6B</sup>	37.10±1.97 <sup>6B</sup>	38.80±0.49 <sup>6B</sup>
1.50	17.80±7.07 <sup>3A</sup>	20.80±0.28 <sup>3A</sup>	22.20±3.95 <sup>3A</sup>	23.95±1.48 <sup>4A</sup>	39.90±0.98 <sup>6B</sup>	34.65±2.47 <sup>6B</sup>	35.70±0.00 <sup>6B</sup>	36.05±4.45 <sup>6B</sup>	35.00±1.97 <sup>6B</sup>	34.65±3.46 <sup>6B</sup>	37.50±0.98 <sup>6B</sup>
2	16.80±1.69 <sup>3A</sup>	20.40±7.35 <sup>3A</sup>	20.80±1.69 <sup>3A</sup>	34.30±0.00 <sup>6B</sup>	39.90±0.98 <sup>6C</sup>	31.50±2.96 <sup>6B</sup>	32.90±0.00 <sup>6B</sup>	36.40±2.96 <sup>6B</sup>	34.65±2.47 <sup>6B</sup>	34.30±3.95 <sup>6B</sup>	36.05±1.48 <sup>6B</sup>
<b>TBA (mg/kg)</b>											
Control	1.13±0.14 <sup>3A</sup>	2.02±0.12 <sup>3BC</sup>	1.66±0.15 <sup>3B</sup>	2.07±0.04 <sup>3BCD</sup>	2.10±0.03 <sup>3BCD</sup>	2.05±0.10 <sup>3BCD</sup>	2.20±0.11 <sup>3CDE</sup>	2.34±0.15 <sup>3CDE</sup>	2.40±0.06 <sup>3CDE</sup>	2.48±0.04 <sup>3DE</sup>	2.55±0.50 <sup>3E</sup>
0.50	1.04±0.15 <sup>3A</sup>	1.90±0.14 <sup>3CB</sup>	1.64±0.03 <sup>3B</sup>	2.03±0.10 <sup>3DE</sup>	1.95±0.12 <sup>3DE</sup>	2.01±0.08 <sup>3DC</sup>	2.19±0.05 <sup>3DE</sup>	2.12±0.08 <sup>3DE</sup>	2.29±0.05 <sup>3E</sup>	2.17±0.19 <sup>3DE</sup>	2.23±0.13 <sup>3DE</sup>
1	1.02±0.05 <sup>3A</sup>	1.61±0.26 <sup>3BC</sup>	1.51±0.01 <sup>3B</sup>	2.01±0.19 <sup>3DE</sup>	1.95±0.02 <sup>3DE</sup>	1.89±0.13 <sup>3CD</sup>	2.19±0.21 <sup>3DE</sup>	2.04±0.08 <sup>3DE</sup>	2.27±0.04 <sup>3E</sup>	2.07±0.02 <sup>3DE</sup>	2.15±0.08 <sup>3DE</sup>
1.50	1.10±0.16 <sup>3A</sup>	1.61±0.07 <sup>3B</sup>	1.58±0.10 <sup>3B</sup>	1.94±0.01 <sup>3BCD</sup>	1.86±0.37 <sup>3BCD</sup>	1.81±0.13 <sup>3BC</sup>	2.09±0.12 <sup>3CD</sup>	1.99±0.01 <sup>3CD</sup>	2.18±0.05 <sup>3D</sup>	2.00±0.02 <sup>3CD</sup>	2.13±0.14 <sup>3CD</sup>
2	1.09±0.24 <sup>3A</sup>	1.50±0.15 <sup>3B</sup>	1.56±0.23 <sup>3BC</sup>	1.82±0.29 <sup>3BCD</sup>	1.85±0.07 <sup>3BCD</sup>	1.76±0.02 <sup>3BCD</sup>	2.04±0.28 <sup>3D</sup>	1.98±0.15 <sup>3CD</sup>	2.15±0.01 <sup>3D</sup>	1.95±0.10 <sup>3CD</sup>	2.03±0.07 <sup>3D</sup>

<sup>a-k</sup> values in the row with different letters are significantly different ( $p \leq 0.05$ ); <sup>A-E</sup> values in the column with different letters are significantly different ( $p \leq 0.05$ ); values are means ± standard deviations

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ted to be ginseng (İbrahim, Abou-Arab and Abu Salem, 2011). Banerjee et al. (2012), Pereira et al. (2011), Kanatt, Chander and Sharma (2010) and Naveena, Sen, Vaithiyathan, Babji and Kondaiah (2008) reported that natural antioxidants of different qualities prevent the lipid oxidation of meat and meat products.

### Microbiological properties of the samples

The changes in microbiological quality during the storage are presented in Table 2, and response surface graphs of the measured microbial values are shown in Figure 2. As seen in Table 2, the TMAB for sardine fish patties was determined to be 7.52 logcfu/g with 2% ALE at the end of the storage period. The total viable count is an important criterion for quality evaluation (Kaba, Yücel, Çorapçı, Özer and Eryasar, 2012). These results indicate that the ingredients of fish patties, such as parsley and other ingredients, could contribute high amounts of bacteria since these ingredients are not sterilized (Guran, Öksüztepe, Coban and İncili, 2015). The TMAB count increased with an increased storage time for all group samples ( $p \leq 0.05$ ). The maximum number of psychrophilic bacteria was found to be 9.06 logcfu/g in the control group on day 18 of storage, while the maximum level was found to be 8.09 logcfu/g on day 20 of storage with the addition of 2% ALE. In contrast to the other microbiological parameters, the total number of coliform bacteria reached a minimum level on day 4 of storage, while the maximum values were obtained on days 18 and 20 of storage. The control group and groups with added ALE were compared with respect to the total number of coliform bacteria. It was found that the maximum level was found to be 6.44 logcfu/g in the control group on day 20, while the maximum level of samples with 2% added ALE was found to be 5.38 logcfu/g on day 20 of storage.

In regard to the total number of yeasts and molds, the minimum values were observed on day 0 of storage for all of the groups, while the maximum values were observed on day 20. Comparing the control group and the groups with added ALE, no difference was observed in terms of the total number of yeast and molds. It was determined

that the maximum level in the control group was found to be 8.78 logcfu/g on day 20 of storage, while the maximum level of samples with added 2% ALE was determined to be 8.29 logcfu/g on day 20 of storage. Given all these results, it can be stated that ALE had an antimicrobial effect on the bacteria due to four caffeoylquinic acid derivatives, chlorogenic acid, cynarin, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid and decreased them, while its inactivation effect on the yeast and molds was not statistically significant ( $p \leq 0.05$ ).

### Evaluation of the nonlinear regression models

A non-linear regression method and second-order polynomial equation was used in the mathematical modeling of the effects on the general microflora and chemical composition of ALE extracts at different concentrations (0.5, 1, 1.5 and 2%) added to fish patties. The regression and coefficient of determination ( $R^2$ ) were determined according to Eq. (1) during the storage. For ALE extracts, the regression coefficients and  $R^2$  values of the mathematical models of the measured units are shown in Table 3.

When these coefficients were substituted into Eq. (1), the calculated values for the mathematical models are as follows:

$$M_{TMAB} = 7.103 - 3.630.B_k + 0.094.t_d + 0.123.B_k^2 - 0.003.t_d^2 + 0.440.B_k.t_d^d$$

$$M_{TC} = 5.431 - 3.755.B_k + 0.041.t_d + 0.236.B_k^2 - 0.001.t_d^2 + 0.582.B_k.t_d^d$$

TABLE 3: Regression coefficients and  $R^2$  values of models.

Measured values	Regression coefficients					$R^2$	
	a	$a_1$	$a_2$	$a_3$	$a_4$		$a_5$
TMAB	7,103	-3,630	0,094	0,123	-0,003	0,440	0,860
TC	5,431	-3,755	0,041	0,236	-0,001	0,582	0,887
TYM	6,538	-4,297	0,072	0,141	-0,002	0,576	0,845
TPB	7,008	-2,664	0,177	0,365	-0,006	0,222	0,946
pH	6,273	-1,937	0,068	0,020	0,001	0,327	0,941
TBA	1,545	-0,572	0,093	0,072	-0,003	0,159	0,951
TVB-N	22,966	-11,784	1,924	1,144	-0,060	0,260	0,958

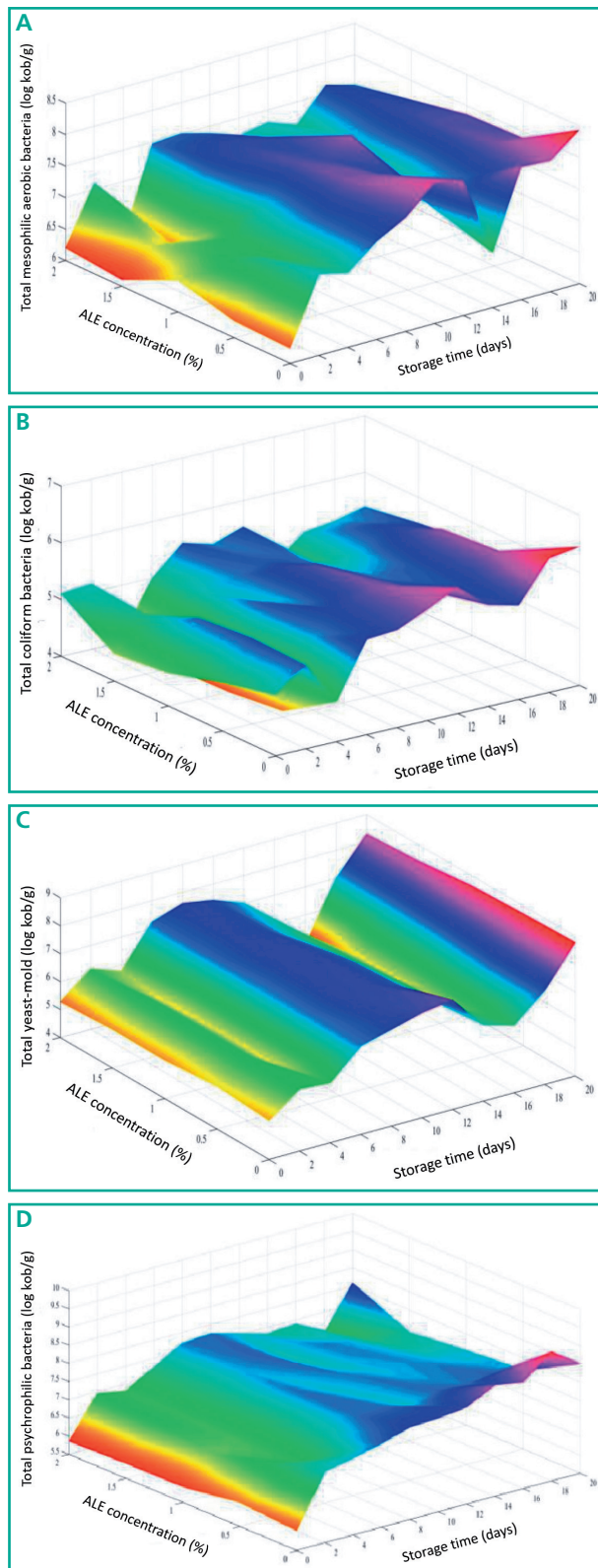
\*TPB: Total psychrophilic bacteria; TC: Total coliform bacteria; TYM: Total yeast and mold; TMAB: Total mesophilic aerobic bacteria

TABLE 2: The microbiological quality of samples during the storage period (log kob/g).

ALE Concentrations (%)		Storage time (Days)										
		0	2	4	6	8	10	12	14	16	18	20
TPB	Control	6.04±0.69 <sup>aA</sup>	7.45±0.59 <sup>aAB</sup>	7.61±0.26 <sup>aB</sup>	7.80±0.07 <sup>aAB</sup>	8.04±0.68 <sup>aAB</sup>	8.07±0.96 <sup>aAB</sup>	8.17±1.17 <sup>aAB</sup>	8.56±1.51 <sup>B</sup>	8.43±1.35 <sup>hB</sup>	9.06±0.76 <sup>hB</sup>	8.51±0.37 <sup>B</sup>
	0.50	6.15±0.72 <sup>aA</sup>	7.04±0.32 <sup>bB</sup>	6.86±0.02 <sup>bAB</sup>	7.40±0.07 <sup>bB</sup>	7.72±0.01 <sup>hB</sup>	7.74±0.62 <sup>B</sup>	7.55±0.52 <sup>hB</sup>	7.77±0.28 <sup>B</sup>	7.71±0.33 <sup>hB</sup>	7.78±0.12 <sup>B</sup>	7.67±0.19 <sup>B</sup>
	1	5.92±0.14 <sup>aA</sup>	6.66±0.31 <sup>bb</sup>	6.73±0.05 <sup>bB</sup>	7.24±0.31 <sup>bBC</sup>	7.73±0.24 <sup>hC</sup>	7.55±0.26 <sup>hC</sup>	7.71±0.00 <sup>C</sup>	7.58±0.58 <sup>hC</sup>	7.67±0.03 <sup>C</sup>	7.56±0.12 <sup>hC</sup>	7.55±0.36 <sup>C</sup>
	1.50	5.91±0.11 <sup>aA</sup>	6.93±0.14 <sup>bBC</sup>	6.79±0.00 <sup>hB</sup>	7.26±0.22 <sup>bCD</sup>	7.62±0.22 <sup>D</sup>	7.79±0.14 <sup>hD</sup>	7.45±0.48 <sup>hCD</sup>	7.71±0.13 <sup>D</sup>	7.58±0.26 <sup>hD</sup>	7.30±0.00 <sup>hCD</sup>	7.38±0.55 <sup>hCD</sup>
	2	5.86±0.05 <sup>aA</sup>	6.94±0.29 <sup>hABC</sup>	6.81±0.05 <sup>hAB</sup>	7.25±0.05 <sup>hBC</sup>	7.70±0.00 <sup>hBC</sup>	7.76±0.30 <sup>hBC</sup>	7.54±0.26 <sup>hBC</sup>	7.32±0.27 <sup>hBC</sup>	7.38±0.11 <sup>hBC</sup>	6.88±1.25 <sup>aABC</sup>	8.09±0.92 <sup>hC</sup>
TC	Control	5.08±0.09 <sup>aAB</sup>	5.48±0.24 <sup>hABC</sup>	4.71±0.33 <sup>aA</sup>	5.69±0.08 <sup>aABC</sup>	5.72±0.16 <sup>hABC</sup>	5.97±0.94 <sup>hBC</sup>	6.23±0.55 <sup>C</sup>	5.79±0.45 <sup>hBC</sup>	5.66±0.26 <sup>hABC</sup>	6.37±0.06 <sup>hC</sup>	6.44±0.57 <sup>hC</sup>
	0.50	4.86±0.25 <sup>hAB</sup>	5.52±0.28 <sup>hBC</sup>	4.10±0.09 <sup>aA</sup>	5.30±0.31 <sup>hCB</sup>	5.65±0.21 <sup>hBC</sup>	5.95±0.00 <sup>C</sup>	5.82±0.18 <sup>hBC</sup>	5.64±0.08 <sup>hBC</sup>	5.53±1.01 <sup>hBC</sup>	6.05±0.60 <sup>hC</sup>	5.74±0.38 <sup>hBC</sup>
	1	4.58±0.25 <sup>hAB</sup>	5.44±0.12 <sup>hAB</sup>	4.00±0.00 <sup>aA</sup>	5.47±0.51 <sup>hAB</sup>	5.24±0.23 <sup>hAB</sup>	5.87±0.04 <sup>hAB</sup>	5.45±0.63 <sup>hAB</sup>	5.38±1.95 <sup>hAB</sup>	5.46±1.08 <sup>hAB</sup>	5.99±0.03 <sup>hB</sup>	5.65±0.91 <sup>hAB</sup>
	1.50	4.48±0.31 <sup>hAB</sup>	5.05±0.26 <sup>hAB</sup>	3.87±0.02 <sup>aA</sup>	5.17±0.08 <sup>hB</sup>	5.36±0.51 <sup>hB</sup>	5.47±0.00 <sup>B</sup>	5.42±0.17 <sup>hB</sup>	5.11±1.57 <sup>hB</sup>	5.22±0.18 <sup>hB</sup>	5.61±0.06 <sup>hB</sup>	5.53±0.08 <sup>B</sup>
	2	5.19±0.14 <sup>hAB</sup>	5.15±0.47 <sup>hB</sup>	4.06±0.30 <sup>aA</sup>	5.00±0.00 <sup>hAB</sup>	5.49±0.27 <sup>hB</sup>	5.34±0.06 <sup>hB</sup>	5.53±0.39 <sup>hB</sup>	4.73±1.04 <sup>hAB</sup>	5.18±0.17 <sup>hB</sup>	5.25±0.72 <sup>hB</sup>	5.38±0.12 <sup>B</sup>
TYM	Control	5.39±0.08 <sup>aA</sup>	6.23±0.25 <sup>hABC</sup>	6.15±1.03 <sup>hAB</sup>	7.16±0.05 <sup>hBCD</sup>	7.46±0.03 <sup>hCDE</sup>	7.76±0.38 <sup>hCDE</sup>	7.92±1.48 <sup>hDE</sup>	6.73±0.37 <sup>hABCD</sup>	6.42±0.06 <sup>hABC</sup>	7.36±0.24 <sup>hBCD</sup>	8.78±0.03 <sup>hE</sup>
	0.50	5.53±0.16 <sup>aA</sup>	6.31±0.13 <sup>hAB</sup>	5.59±0.03 <sup>hA</sup>	7.15±0.23 <sup>hBC</sup>	7.61±0.12 <sup>hC</sup>	7.68±0.02 <sup>hC</sup>	7.32±0.21 <sup>hBC</sup>	6.52±0.29 <sup>hAB</sup>	5.87±1.37 <sup>hA</sup>	7.35±0.17 <sup>hBC</sup>	8.73±0.06 <sup>hD</sup>
	1	5.36±0.45 <sup>aA</sup>	6.29±0.00 <sup>hAB</sup>	5.62±0.19 <sup>hA</sup>	7.13±0.11 <sup>hBC</sup>	7.60±0.18 <sup>hCD</sup>	7.64±0.44 <sup>hD</sup>	7.21±0.67 <sup>hBC</sup>	6.44±0.19 <sup>hAB</sup>	5.65±1.18 <sup>hA</sup>	7.12±0.08 <sup>hBC</sup>	8.65±0.11 <sup>hD</sup>
	1.50	5.37±0.09 <sup>aA</sup>	6.29±0.07 <sup>hB</sup>	5.56±0.14 <sup>hA</sup>	7.22±0.07 <sup>hCD</sup>	7.56±0.10 <sup>hD</sup>	7.44±0.12 <sup>hCD</sup>	6.85±0.10 <sup>hBC</sup>	6.27±0.10 <sup>hB</sup>	5.57±0.84 <sup>hA</sup>	7.00±0.13 <sup>hCD</sup>	8.42±0.04 <sup>hE</sup>
	2	5.26±0.18 <sup>aA</sup>	6.15±0.02 <sup>hB</sup>	5.76±0.04 <sup>hB</sup>	7.24±0.03 <sup>hCD</sup>	7.60±0.12 <sup>hD</sup>	7.42±0.07 <sup>hD</sup>	7.01±0.58 <sup>hC</sup>	6.09±0.07 <sup>hB</sup>	4.93±0.08 <sup>hA</sup>	7.00±0.03 <sup>hC</sup>	8.29±0.07 <sup>hE</sup>
TMAB	Control	6.24±0.41 <sup>aA</sup>	7.29±0.08 <sup>hBC</sup>	7.18±0.26 <sup>hBC</sup>	7.56±0.03 <sup>hBCD</sup>	7.81±0.03 <sup>hCDE</sup>	8.22±0.35 <sup>hDE</sup>	8.15±0.25 <sup>hDE</sup>	6.87±0.04 <sup>hAB</sup>	8.16±0.05 <sup>hDE</sup>	8.07±0.44 <sup>hDE</sup>	8.43±0.61 <sup>hE</sup>
	0.50	6.26±0.38 <sup>aA</sup>	7.15±0.23 <sup>hBC</sup>	6.93±0.13 <sup>hAB</sup>	7.51±0.27 <sup>hBC</sup>	7.97±0.09 <sup>hC</sup>	7.96±1.07 <sup>C</sup>	7.60±0.18 <sup>hBC</sup>	7.23±0.08 <sup>hBC</sup>	7.51±0.00 <sup>hBC</sup>	7.96±0.13 <sup>hC</sup>	7.89±0.04 <sup>hC</sup>
	1	6.00±0.12 <sup>aA</sup>	6.79±0.63 <sup>hAB</sup>	6.92±0.10 <sup>hAB</sup>	7.31±0.24 <sup>hBC</sup>	7.89±0.09 <sup>hC</sup>	7.79±0.02 <sup>hC</sup>	8.04±0.24 <sup>hC</sup>	6.98±0.58 <sup>hAB</sup>	7.49±0.21 <sup>hBC</sup>	7.86±0.19 <sup>hC</sup>	7.86±0.02 <sup>hC</sup>
	1.50	6.10±0.71 <sup>aA</sup>	6.53±0.73 <sup>hAB</sup>	6.33±0.59 <sup>hA</sup>	7.67±0.10 <sup>hBC</sup>	7.76±0.17 <sup>hC</sup>	7.73±0.19 <sup>hC</sup>	7.53±0.08 <sup>hC</sup>	6.96±0.01 <sup>hABC</sup>	7.14±0.24 <sup>hBC</sup>	7.74±0.06 <sup>hC</sup>	7.69±0.20 <sup>hC</sup>
	2	6.19±0.28 <sup>hB</sup>	7.10±0.15 <sup>hC</sup>	6.09±0.33 <sup>hA</sup>	7.47±0.30 <sup>hC</sup>	7.51±0.58 <sup>hC</sup>	7.38±0.55 <sup>hC</sup>	7.22±0.15 <sup>hC</sup>	7.30±0.14 <sup>hC</sup>	6.97±0.23 <sup>hC</sup>	7.64±0.38 <sup>hC</sup>	7.52±0.37 <sup>hC</sup>

<sup>a-k</sup> values in the row with different letters are significantly different ( $p \leq 0.05$ ); <sup>A-E</sup> values in the column with different letters are significantly different ( $p \leq 0.05$ ); values are means ± standard deviations; TPB: Total psychrophilic bacteria; TC: Total coliform bacteria; TYM: Total yeast and mold; TMAB: Total mesophilic aerobic bacteria

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**FIGURE 2:** Response surface graphs of the microbial values of the samples (A: Total mesophilic aerobic bacteria; B: Total coliform bacteria; C: Total yeast-molds; D: Total psychrophilic bacteria).

$$\begin{aligned}
 M_{TYM} &= 6.538 - 4.297.B_k + 0.072.t_d + 0.141.B_k^2 - 0.002.t_d^2 + 0.576.B_k.t_d \\
 M_{TP} &= 7.008 - 2.664.B_k + 0.177.t_d + 0.365.B_k^2 - 0.006.t_d^2 + 0.222.B_k.t_d \\
 M_{pH} &= 6.273 - 1.937.B_k + 0.068.t_d + 0.020.B_k^2 + 0.001.t_d^2 + 0.327.B_k.t_d \\
 M_{TBA} &= 1.545 - 0.572.B_k + 0.093.t_d + 0.072.B_k^2 - 0.003.t_d^2 + 0.159.B_k.t_d \\
 M_{TVB-N} &= 22.966 - 11.784.B_k + 1.924.t_d + 1.144.B_k^2 - 0.060.t_d^2 + 0.260.B_k.t_d
 \end{aligned}$$

For a comparison of model fit, the experimental values obtained using different concentrations of ALE extracts and the predicted values using the mathematical models are shown in Table 4. The expression of the growth of microorganisms in foods by models is very important as these models allow estimation without experimentation for similar situations. The simplest way is to compare the determinant coefficients ( $R^2$ ), although there are some disagreements about which experimental data represents the model better (Kalkan, 2016).

In this study, the nonlinear regression model was used to determine the mathematical models showing the effects of artichoke extracts of different concentrations on the microbial flora during the storage period for fish patties, and  $R^2$  values were used to compare the model fit. In the models, an  $R^2$  value of 0.860 was determined for total mesophilic aerobic bacteria (TMAB), 0.887 for total coliform bacteria, 0.845 for total yeast-molds, 0.946 for total psychrophilic bacteria, 0.941 for pH values, 0.951 for TBA and 0.958 for TVB-N. The  $R^2$  values of the nonlinear regression models are close to 1, indicating that model suitability has been achieved.

Although there are many studies carried out to determine the shelf lives of processed aquaculture products under various conditions, there are no models explaining the quality changes in sardine fish and processed products – which are obtained from fish – during the storage period. In the modeling study by Alfora, Hernandez, Marc and Pin (2013), which is one of these studies, the estimation of bacterial development occurring in fish products at various temperatures and under various storage conditions (modified atmosphere and normal conditions) was performed, and the models were put into practice in a Visual Basic add-on called “Fishmap”. Churchill, Fernandez-Piquer, Shane and Tamplin (2016), established models for the microbiological and sensorial characteristics of salmon fish during storage at between 0 to 15°C using DMfit curve-fitting software V.2.1, and the  $R^2$ -values calculated for each of the models were compared. It was seen that the  $R^2$  values varied between 0.50 and 0.99. Similarly, Yang, Liu, Chen, Lv and Luo (2018) predicted quality changes in Pacific white shrimp (*Litopenaeus vannamei*) during storage at different temperatures (273, 276, 279, 282 and 285 K), changes in sensory assessment (SA) quality, total aerobic counts (TAC), total volatile basic nitrogen (TVB-N), and the  $K$ -value. Researchers stated that the radical basis function-neural network model was most effective for predicting the quality changes of the Pacific white shrimp during storage over the researched temperature range.

## Conclusions

The chemical and microbiological properties of patties changed with the use of ALE extracts. A significant increase in quality and shelf life was achieved depending on the extract concentration. It was seen that a 1.5% ALE extract has the potential to increase shelf life and be used as a food additive because of its contribution to quality parameters. According to the results obtained from this study, the chemical and microbiological properties were examined after 20 days’ storage when various concentrations of artichoke leaf extract were added into sardine fish patties. Accordingly, the decreases in pH levels of sardine fish patties during the storage period were attributed to the increase in the lactic acid level due to microorganism

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development. Thus, at the end of the storage period, the levels of acidity and decrease in pH were determined not to have exceeded the edible limits for fish patties. From the aspect of TVB-N values, the samples were seen to show irregular changes. However, the samples were found to be within the edible limits in terms of TVB-N. The samples also showed irregular changes in terms of TBA values, but these values were also observed to be within edible

limits. Considering the microbiological changes during the storage period, which were similar to the control groups, the samples with added artichoke extracts at various concentrations were observed to exceed the microbiological limits for edibility at the end of the storage period. Because the comparisons in terms of R<sup>2</sup> values ranged from 0.860 to 0.958 were close to 1 for a nonlinear regression model, it indicated a good model fit.

**TABLE 4:** Predicted and experimental values of microbial and chemical quality indicators.

Storage time (Days)	Concentration (%)	TMAB		TC		TYM		TPB		pH		TBA		TVB-N	
		E	P	E	P	E	P	E	P	E	P	E	P	E	P
0	0	6.24	7.10	5.08	5.43	5.40	6.54	6.05	7.01	6.90	6.27	1.13	1.55	19.80	22.97
	0.5	6.27	6.70	4.87	5.03	5.54	6.02	6.16	6.45	6.89	6.44	1.05	1.36	19.40	19.88
	1	6.51	6.46	4.59	4.58	5.37	5.47	5.93	6.03	6.77	6.57	1.03	1.21	18.70	17.19
	1.5	6.11	5.97	4.48	4.24	5.38	5.06	5.91	5.80	6.74	6.72	1.11	1.11	17.80	14.81
	2	6.20	5.79	5.10	4.80	5.27	4.58	5.86	5.74	6.72	6.87	1.09	1.04	16.80	12.71
2	0	7.29	7.28	5.48	5.51	6.24	6.67	7.46	7.34	6.36	6.14	2.02	1.72	23.80	26.57
	0.5	7.16	7.07	5.52	5.30	6.32	6.38	7.05	6.88	6.35	6.22	1.91	1.60	22.00	23.83
	1	6.79	6.76	5.45	5.16	6.30	6.15	6.67	6.52	6.21	6.25	1.62	1.48	22.40	21.76
	1.5	6.54	6.43	5.06	4.82	6.29	5.98	6.94	6.47	6.17	6.31	1.61	1.41	20.80	19.58
	2	7.10	6.76	5.15	4.94	6.15	5.73	6.95	6.56	6.10	6.34	1.51	1.34	20.40	18.19
4	0	7.19	7.43	4.72	5.58	6.16	6.79	7.62	7.62	5.59	6.02	1.67	1.87	26.40	29.70
	0.5	6.94	7.17	4.11	4.96	5.59	6.29	6.87	7.14	5.56	5.96	1.64	1.73	24.40	27.27
	1	6.93	6.97	4.00	4.39	5.62	5.88	6.73	6.82	5.42	5.87	1.51	1.61	23.80	25.25
	1.5	6.33	6.44	3.87	3.86	5.56	5.47	6.80	6.71	5.48	5.84	1.59	1.55	22.20	23.26
	2	6.09	6.02	4.06	3.74	5.77	5.41	6.82	6.78	5.04	5.52	1.57	1.51	20.80	21.53
6	0	7.56	7.56	5.69	5.64	7.17	6.90	7.80	7.85	6.05	5.90	2.08	2.00	25.70	32.35
	0.5	7.52	7.43	5.31	5.37	7.16	6.85	7.40	7.43	5.93	5.91	2.03	1.89	24.30	29.90
	1	7.32	7.27	5.48	5.31	7.14	6.85	7.24	7.16	5.93	5.92	2.02	1.82	22.90	27.66
	1.5	7.68	7.46	5.17	5.05	7.23	7.02	7.26	7.10	5.92	5.94	1.94	1.76	23.95	26.59
	2	7.48	7.37	5.00	4.90	7.25	7.21	7.25	7.21	5.79	5.89	1.83	1.72	34.30	31.19
8	0	7.81	7.66	5.73	5.70	7.47	6.99	8.05	8.04	5.82	5.79	2.10	2.10	45.15	34.52
	0.5	7.98	7.63	5.66	5.52	7.62	7.07	7.73	7.66	5.90	5.79	1.96	1.98	39.20	34.01
	1	7.89	7.63	5.25	5.23	7.61	7.21	7.74	7.46	5.86	5.79	1.96	1.91	38.15	33.80
	1.5	7.77	7.62	5.36	5.27	7.56	7.39	7.62	7.4	5.81	5.78	1.86	1.84	39.90	34.98
	2	7.51	7.50	5.50	5.53	7.61	7.72	7.7	7.59	5.81	5.80	1.85	1.83	39.90	36.27
10	0	8.23	7.74	5.97	5.74	7.77	7.06	8.08	8.18	5.62	5.69	2.06	2.18	37.00	36.21
	0.5	7.96	7.71	5.95	5.65	7.68	7.16	7.74	7.80	5.55	5.64	2.01	2.07	35.00	35.15
	1	7.80	7.67	5.87	5.64	7.65	7.31	7.55	7.56	5.72	5.65	1.90	1.98	34.30	34.48
	1.5	7.74	7.68	5.48	5.42	7.45	7.36	7.8	7.60	5.51	5.54	1.82	1.91	34.65	34.62
	2	7.39	7.48	5.35	5.4	7.42	7.58	7.76	7.76	5.65	5.59	1.77	1.88	31.5	33.59
12	0	8.16	7.80	6.24	5.78	7.92	7.11	8.17	8.27	5.68	5.60	2.21	2.23	38.5	37.41
	0.5	7.61	7.69	5.83	5.66	7.33	7.11	7.55	7.87	5.68	5.57	2.19	2.14	37.1	36.63
	1	8.05	7.83	5.45	5.43	7.22	7.12	7.71	7.68	5.60	5.52	2.19	2.08	36.4	36.24
	1.5	7.54	7.61	5.42	5.41	6.85	6.91	7.45	7.57	5.60	5.49	2.09	2.03	35.7	36.24
	2	7.22	7.38	5.53	5.65	7.02	7.17	7.55	7.75	5.64	5.50	2.04	2.02	32.9	35.53
14	0	6.87	7.83	5.80	5.81	6.73	7.15	8.57	8.31	5.49	5.52	2.35	2.26	40.5	38.14
	0.5	7.24	7.64	5.64	5.63	6.53	6.92	7.77	7.93	5.47	5.45	2.12	2.16	38.55	37.55
	1	6.98	7.40	5.39	5.43	6.45	6.71	7.59	7.70	5.43	5.38	2.05	2.08	38.5	37.51
	1.5	6.97	7.26	5.12	5.18	6.28	6.45	7.72	7.71	5.34	5.28	2.00	2.04	36.05	37.10
	2	7.31	7.50	4.74	4.76	6.09	6.14	7.32	7.69	5.33	5.21	1.98	2.03	36.40	38.08
16	0	8.13	7.84	5.67	5.83	6.43	7.18	8.44	8.30	5.40	5.44	2.40	2.27	37.85	38.39
	0.5	7.51	7.71	5.53	5.62	5.88	6.76	7.72	7.92	5.3	5.34	2.30	2.18	36.75	37.56
	1	7.35	7.57	5.47	5.50	5.65	6.28	7.68	7.71	5.27	5.25	2.28	2.13	36.05	37.12
	1.5	7.32	7.50	5.23	5.30	5.58	5.87	7.59	7.66	5.29	5.18	2.18	2.09	35.00	36.94
	2	7.14	7.35	5.19	5.31	4.94	4.84	7.38	7.71	5.35	5.15	2.16	2.10	34.65	37.42
18	0	8.08	7.82	6.37	5.85	7.37	7.19	9.06	8.25	5.21	5.37	2.49	2.25	38.50	38.16
	0.5	7.96	7.79	6.05	5.79	7.35	7.19	7.79	7.87	5.20	5.26	2.18	2.15	37.45	37.42
	1	7.86	7.77	6.00	5.82	7.12	7.13	7.57	7.63	5.20	5.16	2.07	2.08	37.10	37.16
	1.5	7.75	7.77	5.62	5.65	7.01	7.11	7.30	7.51	5.25	5.09	2.01	2.03	34.65	36.57
	2	7.65	7.79	5.26	5.4	7.01	7.23	6.89	7.44	5.09	4.91	1.96	2.01	34.30	37.00
20	0	8.44	7.78	6.45	5.85	8.79	7.18	8.51	8.15	4.88	5.31	2.56	2.21	39.90	37.45
	0.5	7.90	7.74	5.75	5.71	8.73	7.58	7.68	7.76	4.74	5.12	2.23	2.11	38.50	36.85
	1	7.87	7.74	5.65	5.62	8.65	8.01	7.56	7.53	4.78	4.96	2.16	2.05	38.85	36.91
	1.5	7.69	7.69	5.54	5.59	8.42	8.33	7.39	7.43	4.87	4.84	2.13	2.02	37.10	36.81
	2	7.52	7.63	5.39	5.56	8.30	8.70	8.10	7.88	4.76	4.63	2.03	1.99	36.05	37.20

E: Experimental; P: Predicted; \*TPB: Total psychrophilic bacteria; TC: Total coliform bacteria; TYM: Total yeast and mold; TMAB: Total mesophilic aerobic bacteriaTotal coliform bacteria; TYM: Total yeast and mold; TMAB: Total mesophilic aerobic bacteria

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## Conflict of interest

There is no conflict of interest between the authors.

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