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Modeling the Microbial Shelf-Life of Chicken Mince Added Rosemary Extract

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HIGHLIGHTS

- The microbiological properties of minced chicken changed with the use of rosemary extract.
- A significant increase in shelf-life and quality was achieved depending on the extract concentration.
- The use of 1% rosemary extract had a positive effect on the quality parameters.
- Storage day and rosemary (Rosmarinus officinalis) extract supplementation dependent microbial values in minced chicken meat can be modelled with values close to 1.

Abstract: Using the non-linear regression model, the present study aims to develop sample mathematical models for the microbial flora by utilization of the antimicrobial effects of rosemary (*Rosmarinus officinalis*) extracts at different concentrations (0.5, 1, 1.5, and 2%) on the raw chicken mince. For this purpose, 5 experimental groups were established for each plant extract. The samples (100 g each) treated with plant extract at different concentrations were vacuum-packaged under aseptic conditions. The packaged samples were kept in refrigerator (4ºC). The microbiological analyses of (total mesophilic aerobic bacteria, total coliform group bacteria, *S. aureus,* total yeast-mold, and total psychrophilic bacteria) were performed on 0^{th}, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, and 10^{th} days of storage. When compared to the control group the treatment with RE resulted in a decrease in the microbial numbers by 2.5 log units for TMAB number, by 3.5 log units for *S. aureus* number, by 3,5 log units for TMY number and by 1.5 log units for TP bacteria on the final day of storage. In establishing the model, the plant extract and storage period were used as variable parameters, whereas the shelf-life was used as output parameter. The changes in shelf-life of raw chicken minces by storage period and extract concentration were modeled, the compliance of obtained mathematical models was tested using Variance Analysis Method (ANOVA) and regression and determination coefficients (R^2) were determined. After determining their compliance of models based on R^2 values, the estimated values and real values were compared. As a result of study, it was determined that R^2 values of raw chicken mince models by total mesophilic aerobic bacteria, *S. aureus*, total coliform bacteria, total yeast/mold, and total psychrophilic bacteria during the storage period were found to range between 0.743 and 0.978 and the models representing the microbial change were found to have a high level of compliance.

Keywords: antimicrobial; chicken mince; rosemary extract; nonlinear regression.

INTRODUCTION

Because of its specific sensorial characteristics and the idea that white meat is healthier than red meat, the consumption of white meat has significantly increased in recent years in many countries and it is a popular food worldwide [1,2]. Chicken meat contains proteins, vitamins, and minerals that are necessary to maintain the health of humans [3]. Besides its high nutritional value, raw chicken meat's water activity (0.98-0.99) and pH value are also suitable for the growth of microorganisms. pH value of chicken breast ranges between 5.7 and 5.9 and that of chicken drumstick ranges between 6.4 and 6.7. Even though the skin acts as a physical barrier against microorganisms, it also hosts many microorganisms [4]. Thus, the microbiological quality and safety of chicken meat are very important for all producers, sellers, and consumers.

The poultry products are contaminated by various microorganisms, especially bacteria, during production, transportation, storage, marketing, and preparation for consumption. Although the core parts of meats obtained from healthy animals are sterile, the outer parts might be contaminated by the pathogen microorganisms depending on the hygiene of cutting. Since the intestinal content, skin, and furs of birds contain a high amount of microorganisms, microbial contamination during cutting is a significant problem. Since taken to the cutting line, the chicken might be exposed to direct or indirect contamination. The chicken carcass might be contaminated unless the required measures are taken during slaughtering, hair soaking, plucking, opening up, removal of visceral organs, cooling, partitioning, and packaging procedures. Moreover, the contamination might originate from personnel, water, and equipment. Since poultry slaughterhouses aim to have as many slaughters in a unit period as possible and there are many contamination points (hair soaking, plucking, removal of visceral organs, and cooling), cross-contamination is inevitable [5,6]. Besides them, the storage conditions during cooling and partitioning the carcass, packaging, and transferring to the customers significantly affect the product quality. Any problem in these steps might easily cause spoilage of chicken meats.

The poultry industry pays attention to the methods aiming to enhance the safety, quality, and shelf-life of poultry products, which are very vulnerable to spoilage [2]. For this purpose, researchers and the poultry industry make effort to develop new methods in order to minimize the microbial growth and improve the microbiological quality of meat, including chicken meat [7].

Nowadays, the consumers' concerns regarding the adverse effects of chemical preservatives resulted in the use of natural preservatives in the foods and the natural preservatives became more popular [7; 8]. Javadian and coauthors [9] stated that, synthetic food additives have possible toxic properties to human health and environment, and they can exhibit carcinogenic effects in living organisms. Because of these reasons, The microbial activity of plant extracts and oils, among the natural preservatives, lays the foundation of many practices including alternative medicine and natural therapies, protecting the raw and processed foods, and use of medications. Some of the oils have proven in vitro activity when used based on their known antimicrobial characteristics. Some of the studies focused on a specific oil or microorganism [10].

The antimicrobial activity level of essential oils depends not on the amount of the main component but the combination and ratios of different components [8; 11]. In many studies, the effects of the essential oils of plants such as marjoram, sage, turmeric, rosemary, thyme, basil, coconut, savory, mace, clove, and fennel were examined. Those studies were the ones, in which the plants and other essential oils were used together with the storage methods in order to improve the sensorial quality of meats or meat products or prolong their shelf-life [12]. But it should be known that plant extracts generally present some formulation problems, such as long-term instability, low bioavailability, and a significant burst release. Therefore, some reseachers suggest that encapsulation method for their productionas a convenient method for that purpose [13].

Belonging to the Lamiaceae family having different areas of use, *Rosmarinus officinalis* (rosemary) is a very old plant and is very common in the Mediterranean region [14]. Rosemary oil is used as spice in foods since it contains antibacterial, antifungal, and antioxidant chemical components. *Rosmarinus officinalis L*. is very rich in phenolic compounds. The phenolic compounds are obtained from its extracts and volatile oils [15]. Terpenoids, rosmanol, carnosol, ursolic acid, epirosmanol, carnosic acid, rosmaridiphenol, rosmarol, isorosmanol, rosmariquinon are among the identified secondary metabolites of *Rosmarinus officinalis.* The rosemary extract showed antimicrobial activity against *Listeria monocytogenes, Vibrio parahaemolyticus, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus typhi, Bacillus subtilis Escherichia coli, Staphylococcus intermedius, Staphylococcus aureus*, and other bacterial species [14, 16].

Predictive modeling of bacterial growth and inactivation is one of the important research subjects among food biologists. The estimation-based models might provide an idea about estimating the shelf-life of foods, isolating the critical points in the production and distribution process, and how the environmental factors affect the behavior of pathogenic or disruptive bacteria. The predictive microbiology allows estimating the potential growth of specific microorganisms under various conditions. The models used in predictive microbiology have been developed in the experimental studies generally carried out under laboratory conditions. These models can be then adapted to the foods [17; 18]. The mathematical models used in food production aims to estimate the changes in food quality in the course of time and thus prolong the shelf-life of foods. Being one of these models, the microbial inactivation model is used in various phases of the production of meat products such as mincing, chilling, packaging, and distribution [19].

In the present study, the microbiological characteristics of chicken minces during the storage period were determined by adding rosemary extract (RE) at different concentrations and the nonlinear regression models were used in assessing the effect of rosemary extracts on the microbiological quality parameters. The microbial shelf-life was modeled by using the mathematical model that most accurately estimates the microbial change.

MATERIAL AND METHODS

Material

The chicken meats were obtained from local stores in Giresun province. The chicken meats bought from those stores were the fillets separated from bone, fat, and nerves. The samples taken to the laboratory under refrigerated conditions were processed using mincer machine and the chicken minces were divided into 100 g packages and taken to analyses under aseptic conditions. The rosemary *(Rosmarinus officinalis)* extract (RE) was produced using water extraction method and bought from Awe Cembre (İstanbul-Turkey) in 50 ml glass bottles.

Sample preparation and microbiological analyses

RE added chicken mince samples were prepared according to Külcü and coauthors [20]. Chicken mince samples were divided into 5 equal portions (100 g each) under aseptic conditions and by using sterile gloves and 5 experimental groups were established for each plant extract used in the present study. 1st group was control group but 2nd group was submerged into 0.5% plant extract solution, 3rd group into 1% plant extract solution, 4th group into 1.5% plant extract solution, and 5th group into 2% plant extract solution for 1 minute. Then, they were vacuum-packaged under aseptic conditions and stored at 4 ºC until microbiological analyses. The microbiological analyses were performed on the 0th, 1st, 3rd, 5th, 7th, and 10th days of storage. In sterile stomacher bags, 10 g of chicken minces with RE was put and then 90 mL Maximum Recovery Diluent (Merck 1.12535) was added. Violet Red Bile Agar (Merck, 1.01406), Baird–Parker agar (Oxoid CM0275), Potato Dextrose Agar (Merck, 1.10130), and Plate Count Agar (Merck, 1.05463) were used in determining coliform group bacteria, *S. aureus,* total yeast/mold, total mesophilic aerobic bacteria (TMAB), and total psychrophilic bacteria (TPAB) numbers, respectively [21]. The study was carried out with 90 samples in 3 replications.

Development of nonlinear regression models

In the present study, the total mesophilic bacteria, coliform bacteria, *S. aureus,* total yeast/mold, and total psychrophilic bacteria numbers in chicken minces were determined at different RE concentrations and in different storage periods. These data were used as effective parameters in preparing the mathematical models.

While modeling in the non-linear regression method, the second-order polynomial equations are used in general. Such equations are also known as quadratic equations. For obtaining the mathematical model, the regression model depending on the RE concentrations (B_k) and storage period (td);

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

In this Equation 1, “a” coefficients were determined using “Windows SPPS 20.0 software” statistical package program (SPSS Inc., Chicago, IL, USA). The regression coefficients of the mathematical models of measured units were calculated and, by placing these coefficients in Equation (1), the mathematical models of total mesophilic bacteria, total coliform bacteria, *S. aureus*, total yeast/mold, and total psychrophilic bacteria were obtained. Using the experimental values and resultant mathematical models, the estimated

analysis results were compared. Moreover, the variance analysis (ANOVA) was performed for the mathematical models obtained from the total mesophilic bacteria, total coliform bacteria, *S. aureus,* total yeast/mold, and total psychrophilic bacteria values and the regression and determination coefficient (R^2) values of the regression models were determined. These values represent the statistical effects of model's constant, quadratic, and multiplication terms on the total mesophilic bacteria, total coliform bacteria, *S. aureus,* total yeast/mold, and total psychrophilic bacteria values and they were used in rating the compliance of models [22].

With response surface method, the response surface diagrams of measured values (total mesophilic bacteria, total coliform bacteria, *S. aureus*, total yeast/mold, and total psychrophilic bacteria) were created using MATLAB. By making use of the diagrams, the effects of parameters affecting the procedure on the microbiological parameters could be more clearly observed [19].

Statistical Analyses

The microbiological analysis results of chicken minces added with RE were evaluated using "Windows SPSS 20.0 software" statistical package program (SPSS Inc., Chicago, IL, USA) according to randomized blocks plan [23]. One-way analysis of variance (ANOVA) was used to compare differences in significance between trials, and Duncan's multiple comparison test was used to compare differences between groups ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effects of rosemary (*Rosmarinus officinalis)* extracts on the microbial growth in raw chicken minces

The changes in total mesophilic bacteria, total coliform bacteria, *S. aureus*, total yeast/mold, and total psychrophilic bacteria (TMAB) values of raw chicken minces treated with different concentrations of RE (0.5, 1, 1.5, and 2%) during the storage period (0^{th}, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, and 10^{th} days) under refrigerator conditions are presented in Table 1. The response surface diagrams of microbial changes in raw chicken minces during the storage period because of different rosemary extract concentrations are presented in Figure 1. As seen in Table 1, the microbial characteristics of chicken minces treated with rosemary extracts were better when compared to the control group ($p \leq 0.05$). It was determined that, when compared to the control group, all the microbiological values were found to be statistically significant as the extract concentration increased. As known, it is accepted that the meat and meat products containing high number of bacteria are not produced and/or stored under hygienic conditions. TMAB value of chicken minces containing 1% RE was found to be 7.20 log CFU/g. This finding indicates that treatment with RE resulted in a decrease in TMAB number by 2.5 logarithmic units. According to Raw Poultry Meat and Processed Meat Mixtures Communiqué of Turkish Food Codex, the upper limit for TMAB is 5.0×10^6 CFU/g in 3 of every 5 samples [24]. As a result of RE treatment, the number of TMAB decreased when compared to the control group but the results were found to be high according to the values set in the communiqué. As knowing, the nonnutrient secondary metabolites of rosemary such as the phenolic diterpenes, carnosol, carnosic acid, methyl carnosate, rosmanol, and epirosmanol, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids, have already been reported to possess diverse biological activities, including antioxidant and antimicrobial activity by many researchers [25]. Similar to the present study, Rižnar and coauthors [26] stored the vacuum-packaged chicken frankfurter treated with rosemary extract at 4°C and they found 3 log units decrease in TMAB numbers on the 33^{rd} day when compared to the control group. Ntzimani and coauthors [27] examined the antimicrobial effects of EDTA, lysozyme, rosemary extract, and thyme oil combinations in vacuum-packaged and cooked chicken meat during storage at 4°C and they reported 1-3 log units of decrease in TMAB value on 10^{th} day when compared to the control group. Examining the samples in terms of *S. aureus* number, they reported that no *S.aureus* was detected on 0^{th} and 1^{st} days of storage with treatment using 1.5-2% RE. It was determined that, in proportion to the increasing concentrations of RE, in comparison to the control group ($p \leq 0.05$). With 2% RE concentration, the number of *S. aureus* decreased by 3.5 log units on the final day of storage when compared to the control group. In a study carried out by Coşkun [28], similar to the present study, the author reported that the number of *S. aureus* in Tekirdağ meatballs treated with rosemary and then thyme extracts was lower on the last day of storage when compared to the other samples. Harmankaya & Vatansever [29] carried out minimum effective doses of rosemary (*Rosmarinus officinalis*) and clove (*Syzygium aromaticum*) volatile oils on food pathogens, poultry meat decontamination, and prolonging the shelf-life. Authors were reported that although 1 unit or more decreases were obtained at the beginning in

the groups in which rosemary and clove oils were used together, the difference was closed towards the end of the process and the values closer to the control group were achieved. Moreover, although the clove and rosemary oils had effect on the chicken meat microflora, they were not sufficient for prolonging the shelf-life. Examining the total coliform bacteria numbers in the samples, similar to the other microbiological values, a decrease by approx. 2.5 log units was achieved with 2% RE concentration on the last day of storage in comparison to the control group. It is known that the number of total coliform group bacteria including the enterobacteria is a hygiene indicator. In a previous study, 0.2% RE was added into ostrich mince and it was reported that no statistically significant change occurred in the number of enterobacteria on the 9th day of storage at 3°C [30]. In another study, the meatballs prepared using turkey meat mince were treated with 1% RE and then stored at +4°C and it was reported that the rosemary extract caused a decrease in the number of enterobacteria by 1-2 log units when compared to the control group [31]. Giatrokau and coauthors [32] reported that the number of enterobacteria in chicken products decreases when stored using thymol. Examining the experimental samples in terms of the total number of yeast/mold, it was determined that RE was effective on these microorganisms and the change in concentration yielded statistically significant results ($p \leq 0.05$).

Table 1. The microbiological quality of samples during the storage period (log kob/g)

	RE (%)	Storage time (Days)					
		0	1	3	5	7	10
TMAB	Control	3.49±0.08^{aC}	4.40±0.21^{bA}	6.23±0.77^{cA}	8.03±0.53^{dA}	8.52±0.43^{dC}	9.73±0.62^{eC}
	0.5	3.83±0.02^{aE}	3.77±0.20^{aA}	6.47±0.03^{bB}	8.13±0.58^{cA}	8.25±0.14^{cBC}	9.08±0.16^{dBC}
	1	3.68±0.03^{aD}	3.45±0.99^{aA}	6.65±0.09^{bC}	7.80±0.02^{bcA}	7.95±0.55^{bcBC}	8.72±0.75^{cB}
	1.5	2.17±0.01^{aA}	3.80±0.33^{bA}	6.21±0.10^{cA}	7.35±0.00^{eA}	7.19±0.09^{dA}	7.81±0.05^{fA}
	2	2.39±0.05^{aB}	3.53±0.05^{bA}	6.21±0.04^{cA}	7.61±0.28^{eA}	7.70±0.02^{eAB}	7.20±0.00^{dA}
S. aureus	Control	1.14±0.82^{aBC}	2.62±0.65^{bC}	3.88±0.44^{cB}	5.39±0.46^{dC}	7.10±0.03^{eE}	8.86±0.39^{fE}
	0.5	1.07±0.41^{aB}	2.03±0.09^{bB}	4.62±0.18^{cC}	5.28±0.18^{dC}	6.64±0.18^{eD}	7.95±0.23^{fD}
	1	1.57±0.01^{aC}	2.00±0.14^{aB}	3.46±0.20^{bB}	4.61±0.47^{cB}	5.81±0.05^{dC}	7.27±0.13^{eC}
	1.5	ND^{aA}	ND^{aA}	1.16±0.22^{bA}	2.44±0.40^{cA}	4.76±0.22^{dB}	6.47±0.01^{eB}
	2	ND^{aA}	ND^{aA}	1.41±0.21^{bA}	2.57±0.05^{cA}	3.13±0.13^{dA}	5.47±0.04^{eA}
TC	Control	2.40±1.05^{aA}	3.20±0.17^{aA}	5.21±0.25^{bA}	6.85±0.25^{cA}	8.58±0.42^{dC}	9.70±0.13^{eC}
	0.5	3.60±0.44^{aB}	3.75±0.12^{aC}	6.24±0.00^{bB}	7.61±0.13^{cC}	8.19±0.05^{dC}	8.11±0.13^{cdB}
	1	3.48±0.31^{aB}	3.70±0.32^{aBC}	6.11±0.01^{bB}	7.61±0.04^{cC}	7.67±0.33^{cB}	8.11±0.05^{cB}
	1.5	1.87±0.02^{aA}	3.40±0.14^{bABC}	6.01±0.06^{cB}	7.26±0.11^{dB}	7.10±0.04^{dA}	7.38±0.20^{dA}
	2	1.96±0.03^{aA}	3.30±0.01^{bAB}	6.10±0.26^{cB}	7.39±0.18^{deBC}	7.66±0.05^{eB}	7.13±0.01^{dA}
Mold-Yeast	Control	5.50±0.93^{aC}	6.81±0.20^{bC}	6.22±0.12^{bA}	8.35±0.07^{cC}	9.17±0.05^{dA}	9.39±0.03^{dB}
	0.5	5.66±0.03^{aC}	5.39±0.02^{aB}	6.62±0.31^{bAB}	8.20±0.30^{cBC}	8.34±0.30^{cB}	9.18±0.12^{dB}
	1	5.63±0.11^{aC}	5.44±0.03^{aB}	7.14±0.08^{bC}	7.82±0.21^{bcAB}	8.39±0.06^{cdB}	9.05±0.75^{dB}
	1.5	2.74±0.06^{aB}	5.28±0.28^{bAB}	6.72±0.19^{cBC}	7.84±0.06^{eAB}	7.38±0.04^{dA}	7.67±0.12^{dA}
	2	1.78±0.26^{aA}	4.93±0.24^{bA}	6.59±0.08^{cAB}	7.72±0.03^{eA}	7.12±0.15^{dA}	7.42±0.09^{deA}
TP	Control	5.10±0.10^{aA}	5.87±0.32^{bC}	6.43±0.46^{bC}	7.27±0.13^{cC}	8.37±0.53^{dCD}	8.67±0.23^{dC}
	0.5	5.69±0.04^{aC}	5.51±0.03^{aB}	6.36±0.17^{bBC}	6.45±0.26^{bB}	8.93±0.07^{dD}	8.56±0.00^{cC}
	1	5.71±0.00^{aC}	5.64±0.02^{aB}	5.93±0.04^{bB}	6.66±0.02^{cBC}	8.03±0.02^{dBC}	8.67±0.19^{eC}
	1.5	5.26±0.04^{abB}	4.67±0.13^{aA}	5.16±0.34^{abA}	5.45±0.50^{bA}	7.39±0.02^{cAB}	7.81±0.21^{cB}
	2	5.32±0.00^{abB}	4.59±0.02^{aA}	4.98±0.02^{abA}	5.72±0.15^{cA}	7.21±0.63^{dA}	7.06±0.42^{dA}

a–d values in the row with different letters are significantly different ($p \leq 0.05$); A–E values in the column with different letters are significantly different ($p \leq 0.05$); values are means ± standard deviations; TP: Total psychrophilic bacteria; TC: Total coliform bacteria; TMAB: Total mesophilic aerobic bacteria.

In studies carried out in cultivation environments, it was determined that essential oil, water, and extract of rosemary showed antifungal effect on molds at different rates [33]. In a study carried out by using different essential oils and packaging methods, it was reported that essential oils had no effect but the packaging conditions were effective on the number of yeast/mold [34]. Since the psychrophilic microorganisms can reproduce at temperatures between 0 and 10°C, they are important for the fresh and/or processed foods stored at these temperatures [35]. According to the Communiqué on Microbiological Criteria for Poultry Meat, the number of psychrophilic bacteria shall not exceed 10^5 CFU/g [36]. Given the number of psychrophilic bacteria detected in the experimental meatball samples, it was found that a decrease by 1.5 log units was

observed in the number of psychrophilic bacteria in the samples treated with 2% RE on the final day of storage in comparison to the control group. As can be seen in Table 1, the number of psychrophilic bacteria exceeded beyond the limits, which was set in the Communiqué, since the 5th day. Liu and coauthors [37] stored the chicken frankfurters added with 500, 1000, and 1500 ppm rosemary extract at 4°C. On the 14th day of storage, the number of psychrophilic bacteria was found to be 6 log CFU/g in the control sample, whereas the values in groups added with 500, 1000, and 1500 ppm rosemary extract were found to be 5.5-6.0, 5.5, and 5.0-5.5 log CFU/g, respectively. In their study, the increase in rosemary extract concentration was effective in the decrease in psychrophilic bacteria number. This finding is in corroboration with the present study. Consequentially, our study results showed that the increase of the antimicrobial activity depended on the formulation and the delivery systems, as well as on the microorganism's class.

Evaluating the mathematical models

Nonlinear regression model and second-order polynomial equation were used in modeling the effects of different RE concentrations (0.5, 1, 1.5, and 2%) on the general microflora of chicken minces. The regression and determination coefficients (R^2) during the storage were determined using Equation 1. The regression coefficients and R^2 values of all the mathematical models of measured units are presented in Table 2 for all the rosemary extract concentrations. Putting these coefficients in Equation (1), the mathematical models for total number of mesophilic aerobic bacteria (M_{TMAB}), number of *S. aureus* ($M_{S.aureus}$), total number of coliform group bacteria (M_{TKB}), total number of yeasts/molds (M_{TMK}), and total number of psychrophilic bacteria (M_{TPB}) are as follows;

$$M_{TMAB} = 3.706 - 1.216.B_k + 1.402.t_d + 0.353.B_k^2 - 0.076.t_d^2 - 0.081.B_k.t_d \quad (2)$$

$$M_{S.\ aureus} = 2{,}643 - 1.836.B_k + 0.751.t_d + 0.103.B_k^2 - 0.005.t_d^2 - 0.058.B_k.t_d \quad (3)$$

$$M_{TKB} = 3{,}618 - 1.170.B_k + 1.350.t_d + 0.225.B_k^2 - 0.087.t_d^2 + 0.021.B_k.t_d \quad (4)$$

$$M_{TMK} = 5.196 - 0.296.B_k + 0.949.t_d - 0.358.B_k^2 - 0.061.t_d^2 + 0.049.B_k.t_d \quad (5)$$

$$M_{TPB} = 5.966 - 0.106.B_k + 0.124.t_d - 0.332.B_k^2 + 0.021.t_d^2 - 0.039.B_k.t_d \quad (6)$$

Expressing the inactivation of microorganisms in foods is very important since these models allow estimating similar situations without an experiment. The simplest way to determine which model represent the experimental data best is, although controversial, is to compare the determination coefficients (R^2). However, in case that the numbers of parameters are not the same, different methods (F test) are used [22]. The nonlinear regression model was used in determining the mathematical models of the effects of different concentrations of plant extracts on the microbial flora of raw chicken minces during the storage period and the model compliance was analyzed comparing R^2 values. It was determined that R^2 values ranged between 0.952 and 0.978 for the total mesophilic aerobic bacteria model, between 0.961 and 0.989 for the model of *S. aureus,* between 0.955 and 0.971 for the total coliform group bacteria, between 0.869 and 0.967 for the model of total yeasts/molds, and between 0.743 and 0.971 for the model of total psychrophilic bacteria. High R^2 values (closer to 1) of nonlinear regression models indicate the model compliance.

Table 2. 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	3.706	-1.216	1.402	0.353	-0.076	-0.081	0.952
S.aureus	2.643	-1.836	0.751	0.103	-0.005	-0.058	0.961
TC	3.618	-1.170	1.350	0.225	-0.087	0.021	0.968
Yeast and Mold	5.196	-0.296	0.949	-0.358	-0.061	0.049	0.869
TP	5.966	0.106	0.124	-0.332	0.021	-0.039	0.743

*TP: Total psychrophilic bacteria; TC: Total coliform bacteria; TMAB: Total mesophilic aerobic bacteria

Although many studies were carried out in order to reveal the shelf-lives of chicken minces under various conditions, the models explaining the microbiological quality changes (*Escherichia coli, Listeria innocua,* Salmonella spp., *Campylobacter jejuni, etc.*) during the storage are related more with the pathogen bacteria. The models used in studies are the linear, Gompertz, Weibull Ratkowsky, Baranyi, and logistic models [19]. Similar to the present study, in their study, Külcü and coauthors [20] treated the chicken minces with garlic

extract and modeled the microbiological parameters by using the polynomial surface fitting (PSF) method and artificial neural networks (ANN) modeling method. As a result of their study, it was determined that the garlic extract has modelable effects for inhibiting the microbial growth in minced raw chicken meat. The compliance of models ranged between 97% and 99%. Although ANN yielded 1-1.5% better compliance, it was recommended to use both models together because of the disadvantages of ANN. In a different study carried out by Ozturk and coauthors [19], the effects of artichoke leaf extract on the microbiological (total mesophilic aerobic bacteria, total coliform bacteria, total psychrophilic bacteria, and total yeasts/molds) and chemical (pH, TVB-N, and TBA) quality of sardine meatballs were examined during storage at +4°C. The microbiological and chemical effects of artichoke leaf extract on fish meatballs were analyzed using a nonlinear regression model. The R^2 values of measured parameters ranged between 0.845 and 0.958 and the values closer to 1 indicate the model compliance. Another similar study, Menezes and coauthors [38] found that the average R^2 value for the control samples (± standard deviation) was 0.979 (± 0.014) and for samples with 0.4% OEO (oregano essential oil) was 0.941 (± 0.035) for Baranyi and Roberts model. In same study, for modified Gompertz, the average R^2 value for the control samples was 0.975 (± 0.027) and for samples with 0.4% OEO was 0.950 (± 0.055). According to the researchers, these R^2 values are acceptable once the microbial concentrations are from natural microbiota of solid food, which can lead to changes in scores. The values of accuracy factor found were close to 1, indicating that the observed response is as close as the predicted response as similar our present study.

In comparing the model compliances, the values estimated using mathematical models and experimental models obtained using different rosemary extract concentrations are presented in Table 3. In Table 3, it can be seen that, thanks to the model compliances obtained, the experimental data and the estimated data were closer to each other.

Table 3. Predicted and experimental values of microbial quality parameters (log kob/g)

		TMAB		*S. aureus*		TC		Yeast and Mold		TP	
Storage time (days)	**Concentration (%)**	**E**	**P**	**E**	**P**	**E**	**P**	**E**	**P**	**E**	**P**
0	**0.5**	3.83	3.19	1.07	1.75	3.60	3.09	5.66	4.96	5.69	5.94
0	**1**	3.68	2.84	1.57	0.91	3.48	2.67	5.63	4.54	7.71	5.74
0	**1.5**	2.17	2.68	0.00	0.12	1.87	2.37	2.74	3.95	5.26	5.38
0	**2**	2.39	2.69	0.00	-0.62	1.96	2.18	1.78	3.17	5.32	4.85
1	**0.5**	3.77	4.47	2.03	2.47	3.75	4.36	5.39	5.87	5.51	6.06
1	**1**	3.45	4.09	2.00	1.60	3.70	3.96	5.44	5.48	5.64	5.85
1	**1.5**	3.80	3.88	0.00	0.78	3.40	3.66	5.28	4.91	4.67	5.47
1	**2**	3.53	3.85	0.00	0.01	3.30	3.48	4.93	4.16	4.60	4.92
3	**0.5**	6.47	6.59	4.62	3.87	6.24	6.39	6.62	7.33	6.36	6.44
3	**1**	6.65	6.12	3.46	2.94	6.11	6.00	7.14	6.99	5.93	6.18
3	**1.5**	6.21	5.83	1.16	2.07	6.01	5.73	6.72	6.47	5.16	5.77
3	**2**	6.21	5.72	1.41	1.24	6.10	5.57	6.59	5.77	4.98	5.18
5	**0.5**	8.13	8.09	5.28	5.23	7.61	7.71	8.20	8.31	6.45	6.98
5	**1**	7.80	7.55	4.61	4.25	7.61	7.35	7.82	8.01	6.66	6.69
5	**1.5**	7.35	7.18	2.44	3.31	7.26	7.10	7.84	7.54	5.45	6.23
5	**2**	7.61	6.98	2.57	2.43	7.39	6.96	7.72	6.89	5.72	5.61
7	**0.5**	8.25	8.99	6.64	6.55	8.19	8.34	8.34	8.79	8.93	7.70
7	**1**	7.95	8.36	5.81	5.51	7.67	7.99	8.39	8.55	8.03	7.37
7	**1.5**	7.18	7.91	4.76	4.51	7.10	7.76	7.38	8.13	7.39	6.87
7	**2**	7.70	7.64	3.13	3.57	7.66	7.64	7.12	7.53	7.21	6.21
10	**0.5**	9.80	9.20	7.95	8.45	8.11	7.97	9.18	8.61	8.56	9.09
10	**1**	8.72	8.45	7.27	7.32	8.11	7.65	9.05	8.44	8.67	8.70
10	**1.5**	7.81	7.88	6.47	6.24	7.38	7.45	7.67	8.09	7.81	8.14
10	**2**	7.20	7.48	5.47	5.21	7.14	7.37	7.42	7.57	7.06	7.42

E: Experimental; P: Predicted; *TP: Total psychrophilic bacteria; TC: Total coliform bacteria; TMAB: Total mesophilic aerobic bacteria

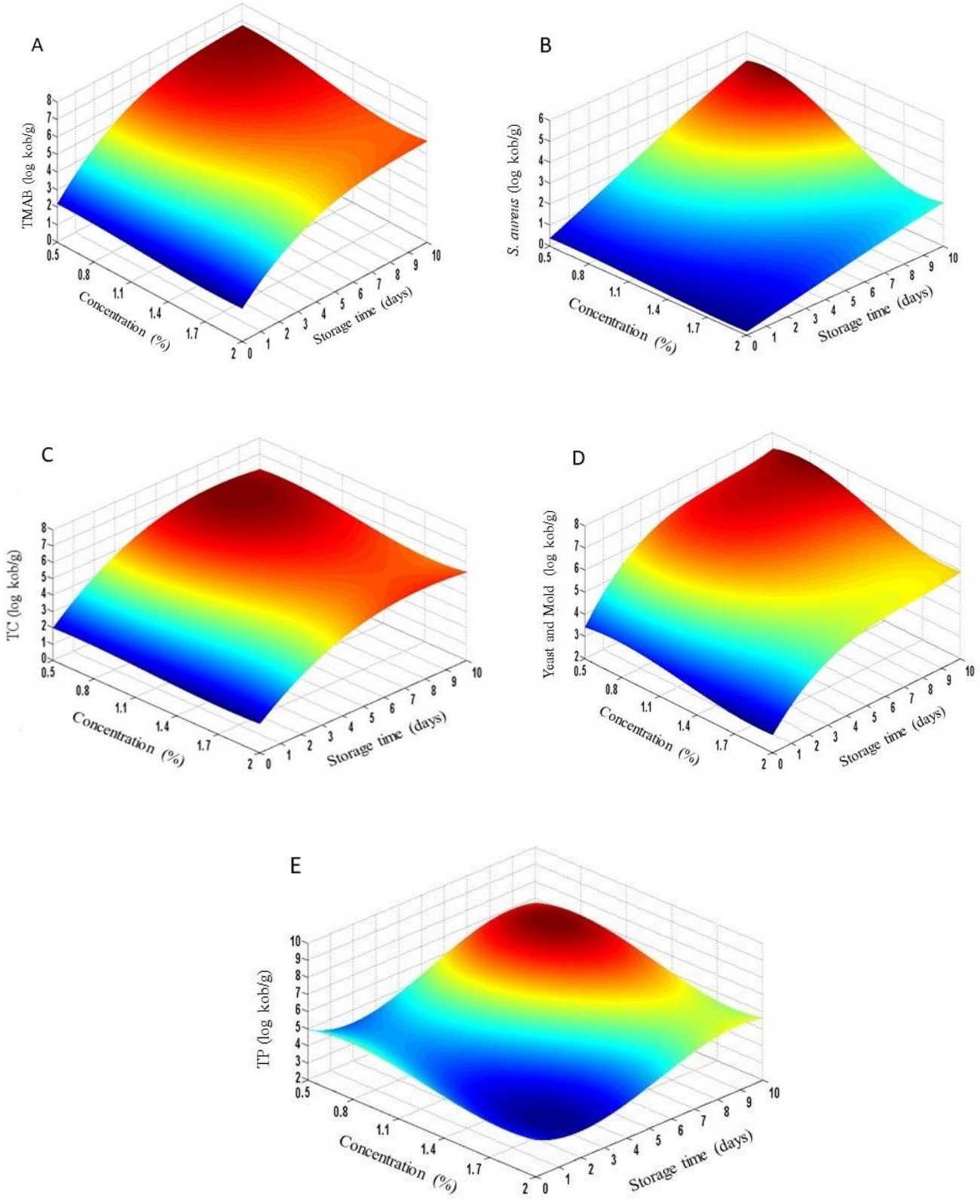

Figure 1. Response surface graphs of the microbial values of the samples; A, Total mesophilic aerobic bacteria, B, *S. aureus*, C, Total coliform bacteria, D, Total Yeast and Mold, E, Total psychrophilic bacteria.

CONCLUSION

In conclusion, it was found that the microbiological properties of chicken minces changed with the use of rosemary extracts. A significant increase in shelf-life was achieved depending on the extract concentration. It was determined that 1% RE extract concentration has potential for use as food additive, as well as

prolonging the shelf-life, thanks to its contribution to the quality parameters. Given the microbiological changes during the storage period, it was determined that the samples added with rosemary extract at different concentrations, similar to the control group, exceeded the microbiological edibility limit at the end of the storage period. Since R^2 values of nonlinear regression models were between 0.743 and 0.978 and close to 1, a good model compliance could be achieved.

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