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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Development of prediction models to estimate the total number of mesophilic aerobic and lactic acid bacteria of squid rings that were cooked before marinating

Marinasyon öncesinde pişirme işlemi uygulanan kalamar halkalarının toplam mezofilik aerobik ve laktik asit bakteri sayılarını tahmin etmek için modellerin geliştirilmesi

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Abstract: This study was conducted in order to develop different statistical models for estimating the bacterial count of squid rings marinated with lemon juice and mineral water after cooking. The marination ratios and times were as follows: (10:90; 90:10; 50:50; 100:100/100 g squid ring) and (1, 3, 6, 12, 24, 48 and 72 h), respectively. The effects of marination ratios and times on the microbiological and sensory changes of the cooked squid rings were observed at 4°C. Pathogenic bacteria (*Vibrio* spp., *Staphylococcus aureus* and *Escherichia coli*) were not found in the cooked (C) and cooked marinated (CM) squid rings in the present study. The TMC (total mesophilic aerobic bacteria counts) of all groups were determined as consumable at 72 h, whereas the TMC of C and CM samples (C7, CM7, CM14, CM21, CM28) increased to 5.92, 5.83, 5.71, 5.57 and 5.42 log cfu/g, respectively. Regression models were created to estimate the TMC and lactic acid bacteria count (LBC) of cooked squid rings during the marination process at 4°C to determine the increasing rates of bacterial growth of samples. As a result of this study; when compared with Model I and Model II; both of them can be preferred for predicting the TMC of C and CM samples. The variability in the TMC of C and CM squid samples was obtained as 93% in Model I, whereas the variability in the TMC of these samples was observed as 91% in Model II. So, these two models performed well, and they can be used for predicting the TMC of C and CM samples. Additionally, Model III was also developed for estimating the prediction value of LBC of cooked squid samples during the marination process at 4°C. This model was also determined very good performance (86%) to estimate the predicting values of LBC and it can be very essential together used with Model I or Model II for marinated fishery products to estimate the real shelf-life.

Keywords: Marination, prediction models, cooked squid ring, bacteria counts, estimation

Öz: Bu çalışma, pişirme sonrasında limon ve maden suyu ile marine edilen kalamar halkalarının bakteri sayılarını tahmin etmede farklı istatistiksel modeller geliştirmek amacıyla yapılmıştır. Marinasyon oranları (10:90; 90:10; 50:50; 100:100/100 g kalamar halkası) ve süreleri (1, 3, 6, 12, 24, 48 ve 72 saat) sırasıyla bu şekildedir. Marinasyon oranlarının ve sürelerinin pişmiş kalamar halkalarının 4°C'de depolama aşamasında mikrobiyolojik ve duyusal değişimleri üzerindeki etkileri gözlenmiştir. Bu çalışma sonucunda pişirilmiş marine edilmiş (CM) ve pişirilmiş kalamar halkalarında (C) patojenik olan *Vibrio* spp., *Staphylococcus aureus* ve *Escherichia coli* türü patojenik bakterilere saptanmamıştır. C ve CM örneklerinin (C7, CM7, CM 14, CM21, CM28) toplam mezofil aerobik bakteri sayısı (TMC) 72. saatte sırasıyla 5.92, 5.83, 5.71, 5.57 ve 5.42 log kob/g'a yükselmiştir. Marinasyon işlemi esnasında örneklerin artan bakteri gelişim oranlarının belirlenmesi için; 4°C'de marine işlemi sırasında pişirilmiş kalamar halkalarının TMC ve laktik asit bakteri sayılarının (LBC) tahmini için regresyon modelleri geliştirilmiştir. Bu çalışmanın sonucunda; Model I ve Model II karşılaştırıldığında, C ve CM örneklerinin her ikisi içinde iki modelde TMC sayılarını tahmin etmek için tercih edilebilir olduğu görülmüştür. TMC'deki değişkenliğin %93'ü Model I ile %91'i Model II ile açıklanabilmektedir. Bu nedenle bu iki modelinde C ve ve hem CM örneklerinde TMC sayılarını tahmin etmede çok iyi performans gösterdiği ve kullanılabileceği belirlenmiştir. Buna ek olarak, pişirilmiş kalamar örneklerinin 4°C'de marinasyon işlemi esnasında LBC sayılarını tahmin etmek için Model III geliştirilmiştir. Bu modelinde LBC sayılarının tahmini değerlerinin belirlenmesi için çok iyi performans gösterdiği ve Model III'ün marine edilen su ürünleri için gerçek raf ömrünü tayin etmede Model I veya Model II ile birlikte kullanıldığında çok önemli olabileceği belirlenmiştir.

Anahtar kelimeler: Marinasyon, tahmin modelleri, pişmiş kalamar halkası, bakteri sayısı, tahmin

INTRODUCTION

In traditional microbiological methods, the quality and safety of foods have been studied by microbiologists using challenge tests, which are only relevant to the characteristics of food products tested and the conditions of the foods. In addition, these tests are also very slow and very expensive (Roberts, 1995). Nevertheless, predictive microbiology models

propose an alternative to the traditional microbiological evaluations of food quality and food safety. In this concept, the effect of distribution, processing, and storage conditions on the microbial growth of foods can be expressed by mathematical models (McMeekin et al., 1992). Most predictions can be generated by these models that the results observed in the challenge studies. In certain conditions; the predictions show the product to be safe or fail. Otherwise, the growth rate of microorganisms predicted from the models is faster, or the predicted time to the growth of microorganisms is shorter than the time that occurred in the food products (Hyytia et al., 1999). In addition to this, predictive modeling provides not only for determining the shelf-life of food products during storage, but also gives rise to predicting the shelf-life of foods during distribution and retail sales (McMeekin and Ross, 1996).

Shelf-life has a significant concept and important property of today's food products. The safety or quality of food products, if unchecked, can be hazardous and limited the shelf-lives of many food products (Man, 2004). Statistical forecasting models can be used to estimate the shelf-life of food products, and for hygienic assessment of food processing conditions that are normal or not according to the characteristics of storage and distribution of food products. It can also help determine distribution times so that the shelf-life of food products becomes adequate (Dalgaard, 2002). Additionally, predictive modeling provides to estimate the remaining shelf-life of foods accurately (McMeekin and Ross, 1996). In both cases, the shelf-life prediction models may contribute to reducing the losses of food products due to spoilage. In addition, shelf-life prediction is used in research and also education, where the applications of mathematical shelf-life models of food products (Dalgaard, 2002).

Predictive modeling such as the Weibull hazard method also is used for the determination of the shelf-life of many products (Keklik et al., 2017). This model is used especially for predicting the true shelf-life of refrigerated foods (Fu and Labuza, 1993). The growth of surviving bacteria after the processing of fishery products during storage time can be predicted by a modified Gompertz model and a polynomial expression (Tomac et al., 2013). Furthermore, Baranyi and Roberts equation can be used for determining the growth rates of microorganisms are modeled as the function of time. Arrhenius Equation the rapid and reliable method; can be also used for predicting the shelf-life of fishery products (Li et al., 2019). Additionally, Grey and Regression prediction models can be also used for estimating the aerobic bacteria counts on frozen squid rings (*Loligo vulgaris* Lamarck, 1798) during the thawing process (Kilinc et al., 2021). Effective applications are necessary for modeling the temperature dependency and the shelf-life of fishery products. This prediction model would be made by establishing a time correlation among measured sensory, microbial, chemical and biochemical changes for the conditions of interest (Taoukis et al., 1999). The use of predictive mathematical models helps to reduce the requirements of process modifications, storage trials, product reformulations, and challenge tests, which are labor-intensive, expensive, and time-consuming (Blackburn, 2000).

There have been made many studies about the predictive modeling for the shelf-life control of chilled fishery products under storage terms and predictive quality changes of fishery products (Dalgaard et al., 1997; Taoukis et al., 1999; Hyytia et al., 1999; Bulat et al., 2020; Kilinc et al., 2021; Wang et al., 2022). In these studies, models predicting the growth of spoilage organisms in fresh and processed fishery products have been conducted. Squid is considered a very healthy fishery product owing to it has high nutritional composition. Nevertheless, it can be easily spoiled by microbial contamination (Xuan et al., 2017). So, it is very important to extend the shelf-life of squid with the processing technologies.

Therefore, the purpose of this study was firstly to investigate the effects of the different marinating time and the ratios of the different marination solutions on the microbiological, sensory and pH changes of cooked squid rings. Secondly, another aim was to create prediction models to estimate the TMC and LBC of cooked squid rings during marination process as well as predicting the shelf-life of samples in the refrigerated storage.

MATERIAL AND METHODS

Material and the preparation of marinade formulations

In this study, frozen squid rings (*Loligo vulgaris* Lamarck, 1798) were taken from the seafood processing factory, which was located in Izmir province of Türkiye. The length and weight of the squid rings were approximately 5-10 cm and 10-15 g, respectively. Frozen squid samples were transported from the factory to the laboratory of Fish Processing Technology of Ege University Fisheries Faculty in approximately 20-30 minutes with a cold chain. Squid rings were washed with tap water before the first treatments. After that, the heat treatment was applied to the squid rings at 50˚C for 5 minutes in a water bath. Cooked squid rings were divided into groups. Some of which were marinated by using the different solutions at different times.

The groups of C and CM samples are shown in Table 1 and 2. The group of frozen squid rings (F), from groups C1 to C7 indicated as the groups of cooked squid rings, which were stored at 4°C. Subsequently, cooked squid samples were marinated by using the different ratios of lemon juice and mineral water with the same ratios of sugar and carbonate indicated as the group CM. The components of the marinating solution (lemon juice, mineral water, sugar, carbonate) were determined as a result of sensory evaluations. The pH value of the lemon juice used in the marination was determined as 2.3±0.03. The ratio of (marinade solution: squid ring) was (1:1) from the groups CM1 to CM21, whereas the ratio of (marinade solution: squid ring) was (2:1) from the groups CM22 to CM28. Total groups of the squid rings were stored at 4ºC up to the experiment times. The temperature of the marinating solution applied for each group is 4ºC.

Table 1. The groups of C and CM squid rings

Frozen squid rings (F) / Cooked (C)		1 h C1	3 h C2	6 h C3	12 h C4	24 h C5	48 h C6	72 h C7
Cooked Marinated (CM)	100 g squid ring +10 ml lemon juice + 90 ml mineral water+0.5 g sugar+0.5 g carbonate	CM1	CM2	CM3	CM4	CM5	CM6	CM7
	100 g squid ring +90 ml lemon juice + 10 ml mineral water+0.5 g sugar+0.5 g carbonate	CM8	CM9	CM10	CM11	CM12	CM13	CM14
	100 g squid ring+50 ml lemon juice + 50 ml mineral water+0.5 g sugar+0.5 g carbonate	CM15	CM16	CM17	CM18	CM19	CM20	CM21
	100 g squid ring+100 ml lemon juice + 100 ml mineral water+0.5 g sugar+0.5 g carbonate	CM22	CM23	CM24	CM25	CM26	CM27	CM28

Frozen squid rings: F; Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28

Table 2. The prediction groups of cooked squid rings during predictive marination process for determining the estimation values of bacteria counts according to the regression models

Frozen squid rings (F) / Cooked (C)		75 h C8	80 h C9	85 h C10	90 h C11	100 h C12	120 h C13	150 h C14
Cooked Marinated (CM)	100 g squid ring +10 ml lemon juice + 90 ml mineral water+0.5 g sugar+0.5 g carbonate	CM29	CM30	CM31	CM32	CM33	CM34	CM35
	100 g squid ring +90 ml lemon juice + 10 ml mineral water+0.5 g sugar+0.5 g carbonate	CM36	CM37	CM38	CM39	CM40	CM41	CM42
	100 g squid ring+50 ml lemon juice + 50 ml mineral water+0.5 g sugar+0.5 g carbonate	CM43	CM44	CM45	CM46	CM47	CM48	CM49
	100 g squid ring+100 ml lemon juice + 100 ml mineral water+0.5 g sugar+0.5 g carbonate	CM50	CM51	CM52	CM53	CM54	CM55	CM56

Cooked; C8-C14, Cooked marinated squid rings; CM29-CM56

The methods of the analysis

pH analysis

Cooked (C) and cooked marinated (CM) squid rings (10 g) were calculated for pH analysis and then samples were put into the 10 ml of the distilled water to be dissolved in it. The pH values of squid ring groups were performed by using a pH meter (HANNA-HI 2211 model, Leighton Buzzard, England). For the determination of pH values of samples, analysis were performed in triplicate for each group (C and CM) according to the method (Bongiorno et al., 2018).

Sensory analysis

The sensory analysis had a hedonic scale (changed from 9; extremely liked to 1; extremely disliked) was used the determine the characteristics of color, texture, odor, and general acceptability of C and CM (Tomac et al., 2017). Randomly chosen 3 pieces of the groups (approximately 10-15 g of each) were served to each panelist to evaluate the sensory characteristics of the groups. A total of ten panelists, whose very experience in evaluating sensory characteristics of fish products in Ege University Fisheries Faculty, were joined to the sensory analysis for giving a numerical score to C and CM squid samples.

Microbiological analysis

For microbiological analysis of C and CM squid rings, firstly the dilutions were prepared according to the aseptic conditions. The sample of 10 g of was calculated and then put into 90 ml of 0.1% peptone water (Merck, 1.07228.0500, Darmstadt, Germany). Secondly, other decimal dilutions were prepared from the first dilution. After that, squid rings were homogenized by using a stomacher (IUL, Barcelona, Spain) for 60s.

For total psychrotrophic bacteria counts (TPC) and TMC, the Plate Count Agar (PCA, Merck, 1.05463.0500) was used as a medium for the pour plate method (Harrigan and McCance, 1976). After the inoculation stage, the inoculated petri dishes were incubated at 30˚C for 24-48 h in the incubator (EN500, Nevü, Ankara, Türkiye) for determining the TMC of the samples. For determining the TPC of squid samples, this time inoculated petri dishes were incubated in the refrigerator and were adjusted at 7˚C for 10 days (Harrigan and McCance, 1976).

Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, 1.16000.0500) was used as the medium for determining the yeast and mold counts (YMC) by using The Pour plate method. Accordingly, the inoculated petri dishes were incubated at 25°C for 3-5 days for enumerating the YMC (Anonymous, 2000) and Baird Parker Agar (BPA, Merck 1.05406) with egg yolk tellurite emulsion was used the determine the *Staphylococcus aureus* count (SAC). According to the spread plate method, 0.1 ml of inoculum was taken and inoculated onto the BPA. After the inoculation, the inoculum was spread onto this medium. After the inoculated petri dishes were incubated at 37°C'de for 30 h for SAC in an incubator (EN500, Nevü, Ankara, Türkiye) according to the method (Mossel and Moreno, 1985). The spread plate method was also used for determining the *Vibrio* bacteria count (VBC). 0.1 ml of inoculum was taken and then inoculated onto the TCBS Agar (Triosulfate Citrate Bile Sucrose). Then, inoculation was the TCBS Agar inoculated petri dishes were incubated at 20°C for 3-5 days for VBC (Serratore et al., 1999).

The medium of MRS Agar (De Man, Rogosa Sharpe, Merck, 1.10660.0500) was used for determining the LBC by using the double agar layer method and was incubated for 2-3 days at 30°C (Baumgart et al., 1986). In addition, the double agar layer method with the Violet Red Bile Agar (VRB Agar, Merck, 1.10275.0500) was used for determining the Enterobacteriaceae counts (EBC) for C and CM squid samples and were incubated at 30°C for 25h (Harrigan and McCance, 1976). For determining the coliform bacteria counts (CBC) of C and CM squid rings, the double agar layer method was used, and the Violet red bile agar (VRB agar, Merck, 1.10275.0500) was used as the medium. Subsequently, the inoculated petri dishes were incubated at 30°C for 24 h for determining the CBC of C and CM squid rings.

For determining the fecal coliform bacteria count (FCB) of C and CM squid rings, the Brilliant Green Lactose Broth (BGLB) was used as the medium. Afterward, the inoculated tubes were incubated at 44-45°C for 48 h according to the method of ICMSF (1986). For defining the *E. coli* (EC) of squid samples, the Eosin Methylene Blue Lactose Sucrose Agar (EMB, Merck, 1.01347.0500) was used as the medium. In this method, the EMB Agar spread by using the spread plate method was inoculated for *E. coli* at 37°C for 24 h (Mossel ve Moreno, 1985). On each analysis day in triplicate, were done for all microbiological analysis. On each experiment day, three repetitions were performed from each C and CM squid sample.

Statistical methods

Statistical Package Program for the Social Sciences Version 25.0 software (IBM SPSS Statistics 25) was used to determine the statistical changes between the groups according to the marination times of cooked squid rings during the same period and in the same groups. The differences in the results of all analysis were evaluated statistically. The ANOVA test (One-way analysis of variance) was carried out for examining the significant difference ($p>0.05$) according to the method by Montgomery and Runger (2003). In this ANOVA test, the normality assumption was checked using the Kolmogorov-Smirnov test, while the homogeneity assumption was checked using the Levene test. Kruskal-Wallis nonparametric test was used to determine the significant difference ($p>0.05$) between the groups according to sensory analysis. Moreover, the non-parametric Friedman S test and two dependent sample sign tests were also carried out using the method (Gamgam and Altunkaynak, 2017). Regression models were also used estimate the values of TMC and LBC of C squid rings with respect to the method (Montgomery and Runger, 2003).

In this study, various regression models were created to determine the factors affecting the TMC values in C and CM squid rings. In these regression models, TMC was the dependent variable, lemon juice (LJ), mineral water (MW), and marination time (T) were the independent variables. In the present study, the lemon juice and mineral water variables were 4 levels (10, 90, 50, 100 ml), whereas the T variable was 7 levels (1, 3, 6, 12, 24, 48, 72 h). The model equation containing the quadratic effect of independent variables and interaction terms with appropriate factor levels is given below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

In this equation, Y dependent variable (TMC), X_i's independent variables (lemon juice, mineral water, and T), β_0 constant terms, β_i's linear effects of the independent variables, β_{ii}'s were the quadratic effects of the independent variables, and β_{ij}'s were the interaction effects of independent variables on the dependent variable.

Mean Absolute Error (MAE), Mean Square Error (MSE), and Mean Square Error (RMSE) performance measurements are used to measure the prediction errors of the models. These measures are calculated as follows:

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

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} |y_i - \hat{y}_i|^2}{n}},$$

where n is the sample size, y_i denotes the actual values, and $\hat{y}_i$ denotes the predicted value obtained from the models for i=1,2,3,…,n, respectively (Sharma, 2019).

RESULTS

pH changes and the microbiological of all C and CM sample groups during refrigerated storage are shown in Table 3.

Table 3. Changes in microbial load and pH values of C and CM squid rings during refrigerated storage

Groups	TPC (log cfu/g)	TMC (log cfu/g)	LBC (log cfu/g)	YMC (log cfu/g)	Pathogenic bacteria	pH
F	<1^{A}	<1^{A}	<1^{A}	<1^{A}	Negative	7.06±0.02^{A}
C1	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	7.54±0.10^{B1}
C2	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	7.58±0.18^{C1}
C3	<1^{A1}	2.44±0.00^{B2}	<1^{A1}	<1^{A1}	Negative	7.65±0.02^{D1}
C4	<1^{A1}	2.78±0.03^{C2}	<1^{A1}	<1^{A1}	Negative	7.66±0.01^{D1}
C5	<1^{A1}	3.54±0.09^{D1}	<1^{A1}	<1^{A1}	Negative	7.65±0.07^{D1}
C6	<1^{A1}	4.57±0.68^{E1}	<1^{A1}	<1^{A1}	Negative	7.66±0.01^{D1}
C7	1.51±0.04^{B2}	5.92±0.07^{F1}	<1^{A1}	<1^{A1}	Negative	7.70±0.13^{E1}
CM1	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.90±0.06^{A2}
CM2	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.75±0.15^{B2}
CM3	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.87±0.09^{C2}
CM4	<1^{A1}	1.54±0.04^{B3}	<1^{A1}	<1^{A1}	Negative	6.54±0.03^{D2}
CM5	<1^{A1}	1.85±0.07^{C2}	<1^{A1}	<1^{A1}	Negative	6.17±0.16^{E2}
CM6	1.51±0.03^{B2}	3.41±0.55^{D2}	1.69±0.10 ^{B2}	<1^{A1}	Negative	6.45±0.07^{F2}
CM7	1.56±0.04 ^{C3}	5.83±0.05^{E2}	2.15±0.26^{C2}	1.51±0.03^{B2}	Negative	6.78±0.04^{G2}
CM8	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.83±0.02^{A3}
CM9	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.43±0.08^{B3}
CM10	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.82±0.05^{C3}
CM11	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.97±0.06^{D3}
CM12	<1^{A1}	1.61±0.05^{B3}	1.52±0.04^{B2}	<1^{A1}	Negative	3.67±0.01^{E3}
CM13	<1^{A1}	4.93±0.25^{C3}	3.03±0.24^{C3}	<1^{A1}	Negative	3.59±0.04^{F3}
CM14	1.49±0.01^{B2}	5.71±0.17^{D3}	3.67±0.05^{D3}	<1^{A1}	Negative	3.66±0.03^{G3}
CM15	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.11±0.03^{A4}
CM16	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.25±0.12^{B4}
CM17	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.92±0.05^{C4}
CM18	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.32±0.04^{D4}
CM19	<1^{A1}	1.54±0.05^{B4}	1.51±0,05 ^{B2}	<1^{A1}	Negative	3.84±0.08^{E4}
CM20	<1^{A1}	5.05±0.23^{C4}	2.76±0,29 ^{C4}	<1^{A1}	Negative	3.52±0.02^{F4}
CM21	<1 ^{A1}	5.57±0.03^{D4}	3.49±0,04^{D4}	<1^{A1}	Negative	3.92±0.03^{C4}
CM22	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	4.00±0.06^{A5}
CM23	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.78±0.01^{B5}
CM24	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.53±0.04^{C5}
CM25	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.56±0,01^{C5}
CM26	<1^{A1}	1.62±0.05^{B5}	1.59±0.03^{B3}	<1^{A1}	Negative	3.32±0.04^{D5}
CM27	<1^{A1}	4.46±0.05^{C5}	2.38±0.04^{C5}	<1^{A1}	Negative	3.61±0.01^{E5}
CM28	<1^{A1}	5.42±0.05^{D5}	3.45±0.08^{D4}	<1^{A1}	Negative	3.52±0.03^{C5}

Frozen squid rings: F; Cooked squid rings: C1-C8; Cooked marinated squid rings; CM9-CM36; TPC: Total Psychrotrophic bacteria count; TMC: Total mesophilic bacteria count; LBC: Lactic acid bacteria count; YMC: Yeast-Mould Count, Negative: Pathogenic bacteria (*S. aureus, Vibrio spp., E. coli*) were not detected. n=3; The microbiological and pH results were presented as the average value of the three analysis. The results are given as mean value (X) ± Standard deviation (SD). The different capital letters A-G in the same column indicate the difference ($p<0.05$) according to the marinating time in the same groups. The different numbers $^{1-5}$ in the same column indicate the difference ($p<0.05$) between the groups in the same marination time.

In the present study, the LBC of CM squid rings reached to 2.15, 3.67, 3.49, and 3.45 log cfu/g at the end of 72 h of marinating, of the groups CM7, CM14, CM21, and CM28, respectively. *Staphylococcus aureus, Vibrio* spp. and *Escherichia coli* were absent in the C and CM samples. The pH values of cooked squid rings increased from 7.54 (C1) to 7.70 (C7) at the end of the storage at 4°C. The marinating process has reduced the pH values of the cooked squid rings. Additionally, the higher the lemon juice concentration used in the marination process, the more caused the decrease in the pH values of cooked squid rings. At the end of the 72 h of marination process, the pH values of cooked squid rings decreased to 6.78, 3.66, 3.92, and 3.52 for the groups CM7, CM14, CM21, and CM28, respectively.

The sensory data of the C and CM squid rings are given in Table 4. In the study, both cooking and marination treatments caused to increase in the shelf-life of squid rings. In addition to this, TMC and LBC of all groups were increased during the marinating process. However, all the groups were determined as consumable in terms of microbiological as well as sensory evaluations. All groups remained below the TMC upper limit for processed fishery products (6 log cfu/g) set by the ICMSF (1986). Therefore, regression models were developed for estimating the TMC and LBC of cooked squid rings during

marination at 4°C. According to these created prediction models, the bacterial counts of cooked squid rings can be easily estimated during the marination process in the different solutions and marination times. In the light of the similar studies given, three models have been created in this study as follows: Model I and Model II were developed for predicting the values of TMC for C and CM squid rings, whereas Model III was developed for estimating the prediction value of LBC of cooked squid samples during marination process.

Table 4. Sensory changes of C and CM squid rings during refrigerated storage

Groups	Colour	Odour	Texture	General Acceptability
F	8.5±1.08^{A}	8.3±0.95^{A}	7.0±0.67^{A}	8.3±0.67^{A}
C1	8.1±1.19^{B1}	7.6±0.97^{B1}	8.2±0.79^{B1}	8.2±0.79^{B1}
C2	8.0±1.15^{B1}	7.5±0.85^{BC1}	8.2±0.79^{B1}	8.2±0.79^{B1}
C3	7.7±0.82^{C1}	7.4±0.70^{C1}	7.3±0.82^{C1}	7.3±0.82^{C1}
C4	6.8±0.79^{D1}	6.1±0.87^{D1}	6.7±0.67^{D1}	6.5±0.53^{D1}
C5	6.1±0.74^{E1}	5.3±0.67^{E1}	6.2±0.63^{E1}	5.8±0.63^{E1}
C6	5.5±0.97^{F1}	4.5±0.53^{F1}	6.1±0.74^{EF1}	4.9±0.74^{F1}
C7	4.9±0.74^{G1}	4.3±0.67^{G1}	6.0±0.82^{F1}	4.4±0.52^{G1}
CM1	7.6±0.52^{A2}	7.5±0.53^{A1}	7.3±0.48^{A2}	7.5±0.53^{A2}
CM2	7.3±0.48^{B2}	7.0±0.67^{B2}	6.8±0.42^{B2}	7.1±0.74^{B2}
CM3	6.8±0.63^{C2}	6.4±0.52^{C2}	5.8±0.63^{C2}	6.8±0.79^{C2}
CM4	6.4±0.52^{D2}	5.3±0.67^{D2}	5.5±0.53^{D2}	6.0±0.82^{D2}
CM5	6.1±0.32^{E2}	4.9±0.57^{E2}	4.8±0.63^{E1}	5.1±0.88^{E2}
CM6	5.6±0.52^{F2}	4.5±0.53^{F1}	4.5±0.53^{F2}	4.8±0.79^{F1}
CM7	5.4±0.52^{G2}	4.1±0.57^{G2}	4.1±0.57^{G2}	4.5±0.53^{G}
CM8	8.4±0.52^{A3}	8.4±0.52^{A2}	8.1±0.88^{A1}	7.8±0.63^{A3}
CM9	8.3±0.48^{A3}	8.3±0.48^{A3}	7.8±0.79^{B3}	7.4±0.52^{B3}
CM10	7.7±0.67^{B3}	7.6±0.51^{B3}	7.2±0.79^{C1}	7.1±0.74^{C3}
CM11	6.7±0.48^{C3}	7.2±0.42^{C3}	6.2±0.63^{D3}	5.8±0.42^{D3}
CM12	5.9±0.57^{D3}	6.1±0.57^{D3}	5.2±0.63^{E2}	5.3±0.48^{E3}
CM13	5.4±0.52^{E3}	5.1±0.74^{E2}	4.4±0.52^{F3}	4.7±0.48^{F1}
CM14	4.5±0.53^{F3}	4.4±0.52^{F1}	4.1±0.57^{G2}	4.3±0.48^{G1}
CM15	8.5±0.53^{A3}	8.5±0.53^{A2}	7.3±0.48^{A2}	8.0±0.82^{A4}
CM16	7.7±0.67^{B4}	8.4±0.52^{A3}	6.9±0.32^{B4}	7.6±0.70^{B4}
CM17	7.0±0.82^{C4}	7.9±0.32^{B4}	6.2±0.42^{C3}	7.3±0.48^{C4}
CM18	6.5±0.71^{D4}	7.5±0.53^{C4}	5.6±0.52^{D4}	6.1±0.74^{D4}
CM19	5.1±0.74^{E4}	6.1±0.74^{D4}	5.1±2.57^{E2}	5.4±0.84^{E3}
CM20	4.5±0.53^{F4}	4.6±0.52^{E3}	4.4±0.70^{F3}	4.9±0.87^{F1}
CM21	4.4±0.52^{F4}	4.2±0.63^{F2}	4.1±0.57^{G2}	4.4±0.52^{G1}
CM22	7.8±0.79^{A5}	8.0±0.82^{A3}	7.9±0.74^{A3}	8.0±0.82^{A4}
CM23	7.4±0.52^{B2}	7.5±0.53^{B4}	7.5±0.75^{B5}	7.5±0.71^{B4}
CM24	7.6±0.70^{C3}	6.5±0.85^{C5}	7.1±0.74^{C4}	7.0±0.67^{C3}
CM25	6.9±0.74^{D5}	5.8±0.63^{D5}	6.1±0.57^{D5}	6.0±0.82^{D2}
CM26	6.6±0.70^{E5}	5.3±0.48^{E5}	5.5±0.53^{E3}	5.6±0.52^{E4}
CM27	6.0±0.67^{F5}	4.9±0.57^{F4}	4.6±0.70^{F4}	4.7±0.48^{F1}
CM28	5.5±1.08^{G5}	4.7±0.48^{G3}	4.3±0.48^{G3}	4.4±0.52^{G1}

Frozen squid rings: F; Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28; TPC: Total Psychrotrophic bacteria count; TMC: Total mesophilic bacteria count; LBC: Lactic acid bacteria count; YMC: Yeast-Mould Count, n=3; The microbiological and pH results were presented as the average value of the three analysis. The results are shown as mean value (X) ± standard deviation (SD). The different capital letters A-G in the same column indicate the difference ($p<0.05$) according to the marinating time in the same groups. Dec Decimals 1-5 in the same column indicate the difference ($p<0.05$) between groups during the same storage period.

First of all, correlations between dependent variable TMC and independent variables time, LJ, MW were investigated. The correlation between TMC and time was obtained as 0.945 (it is significant at the 0.01 level), and the correlation between LJ and MW was obtained as 0.22 (it is significant at the 0.05 level). In order to estimate the model given by equation (1), the result model equation obtained by using the stepwise method was given below:

Model I: $\hat{Y} = 0{,}660 + 0{,}1046T - 0{,}0004T^2 - 0{,}0146LJ - 0{,}0099MW + 0{,}0001LJxMW + 0{,}0001TxLJ$

All coefficients of Model I was significant (p-value <0.05). According to the results, TMC was significantly (p–value <0.01) affected by both the linear (T, positively) and quadratic (T^2, negatively) effects of the T. We could say that the TMC was significantly (p–value <0.01) affected linearly (negatively) both lemon juice and mineral water. Moreover, the interaction of the lemon juice and the T affected the TMC positively and significantly (p–value <0.015), and the interaction of the mineral water and the lemon juice affected the TMC positively and significantly (p–value <0.01), the adjusted r^2 value of this model was calculated as 0.931. With the estimation model obtained, 93% of the variability in the TMC could be explained. In other words, there was a strong and significant effect of the quadratic effect of T and the rates of marinating on the TMC values of CM squid samples.

The basic principle of regression analysis is that the model is simple. Therefore, another estimation equation, which is simpler than the estimation equation given by Model I, but whose $AdjR^2$ value (0.913) is close to Model I, is obtained and is given below:

Model II: $\hat{Y} = 0{,}46354 + 0{,}0833T - 0{,}0060LJ - 0{,}0045MW$

Although Model II included only linear effects of independent variables, it explained 91% of the variability in the dependent variable. This value (%91) was determined very close to the value in Model I (%93). In order to make a comparison, for different values of independent variables, the actual (observed) values of TMC and the estimated values obtained from Model I and Model II are given in Table 5. In this table, the estimated values obtained from Model I and Model II according to different times and formulations (for different values of LJ and MW) are given in Table 5. According to the results, the TMC value for $LJ = 0$ and $MW = 0$ reached the unacceptable limit for consumption earlier than the other content values.

Table 5. The actual (observed) values of TMC (log cfu/g) and the estimated values of TMC (log cfu/g) of samples obtained from Model I and Model II

Time	Groups	LJ	MW	The observed TMC(log	The estimated TMC (log cfu/g) from Model I	The estimated TMC
1 h	C1	0	0	0	0.7638	0.5468
	CM1	10	90	0	-0.1719	0.0797
	CM8	90	10	0	-0.5403	-0.0365
	CM15	50	50	0	-0.1856	0.0216
	CM22	100	100	0	-0.6019	-0.5037
3 h	C2	0	0	0	0,9697	0.7134
	CM2	10	90	0	0,0368	0.2462
	CM9	90	10	0	-0.3094	0.1300
	CM16	50	50	0	0.0343	0.1881
	CM23	100	100	0	-0.3682	-0.3371
6 h	C3	0	0	2.44	1.2726	0.9632
	CM3	10	90	0	0.3438	0.4960
	CM10	90	10	0	0.0310	0.3798
	CM17	50	50	0	0.3580	0.4379
	CM24	100	100	0	-0.0237	-0.0873
12 h	C4	0	0	2.78	1.8567	1.4628
	CM4	10	90	1.54	0.9363	0.9957
	CM11	90	10	0	0.6900	0.8795
	CM18	50	50	0	0.9837	0.9376
	CM25	100	100	0	0.6436	0.4123
24 h	C5	0	0	3.54	2.9382	2.4621
	CM5	10	90	1.85	2.0344	1.9949
	CM12	90	10	1.61	1.9214	1.8787
	CM19	50	50	1.54	2.1485	1.9368
	CM26	100	100	1.62	1.8917	1.4116
48 h	C6	0	0	4.57	4.7547	4.4606
	CM6	10	90	3.41	3.8842	3.9935
	CM13	90	10	4.93	4.0377	3.8773
	CM20	50	50	5.05	4.1315	3.9354
	CM27	100	100	4.46	4.0413	3.4101
72 h	C7	0	0	5.92	6.1090	6.4592
	CM7	10	90	5.83	5.2719	5.9920
	CM14	90	10	5.71	5.6918	5.8758
	CM21	50	50	5.57	5.6524	5.9339
	CM28	100	100	5.42	5.7287	5.4087

Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28; LJ: Limon juice; MW: Mineral water; TMC: Total mesophilic bacteria count.

The performance measures are based on the differences between the actual values and the predicted values, that is, errors. Therefore, the model with lower errors should give more accurate estimates (Table 6).

Table 6. The estimation errors of the models

	Model I	Model II
MAE	0.4632	0.4965
MSE	0.3139	0.4044
RMSE	0.5603	0.6359

Mean Squared Error (MSE), Mean Absolute Error (MAE), and Root-Mean Square Error (RMSE)

According to this table (Table 6), both models were determined to be performed well in terms of being used for prediction. Moreover, TMC is only not a good indicator for determining the shelf-life of fermented food products such as marinated fishery products. This bacteria count should be considered with the LBC, which consists of the main microbial flora of fermented food products (Unluturk and Turantas, 2003). For this reason, Model III was developed for estimating the LBC of cooked marinated squid rings. The adjusted r^2 value of this model was calculated as 0.864.

Model III: $\hat{Y} = -0{,}1631 + 0{,}0004TLJ + 0{,}0154T + 0{,}00008TMW$

The estimated values of TMC (log cfu/g) of samples from Model I and Model II are given in Table 7. In addition to this, the estimated values of LBC (log cfu/g) of samples from Model III are also in given Table 7.

Table 7. The estimated values of TMC (log cfu/g) of samples from Model I, Model II and the estimated values of LBC (log cfu/g) of samples from Model III

Time	Groups	LJ	MW	$\hat{Y}_{MI}$	$\hat{Y}_{MII}$	$\hat{Y}_{MIII}$
75h	C8	0	0	6.2550	6.7110	0.9919
	CM29	10	90	5.3830	6.2460	1.8319
	CM36	90	10	5.6070	6.1260	3.7519
	CM43	50	50	5.6550	6.1860	2.7919
	CM50	100	100	5.5550	5.6610	4.5919
80h	C9	0	0	6.4680	7.1275	1.0689
	CM30	10	90	5.6010	6.6625	1.9649
	CM37	90	10	5.8650	6.5425	4.0129
	CM44	50	50	5.8930	6.6025	2.9889
	CM51	100	100	5.8180	6.0775	4.9089
85h	C10	0	0	6.6610	7.5440	1.1459
	CM31	10	90	5.7990	7.0790	2.0979
	CM38	90	10	6.1030	6.9590	4.2739
	CM45	50	50	6.1110	7.0190	3.1859
	CM52	100	100	6.0610	6.4940	5.2259
90h	C11	0	0	6.8340	7.9605	1.2229
	CM32	10	90	5.9770	7.4955	2.2309
	CM39	90	10	6.3210	7.3755	4.5349
	CM46	50	50	6.3090	7.4355	3.3829
	CM53	100	100	6.2840	6.9105	5.5429
100h	C12	0	0	7.1200	8.79354	1.3769
	CM33	10	90	6.2730	8.32854	2.4969
	CM40	90	10	6.6970	8.20854	5.0569
	CM47	50	50	6.6450	8.26854	3.7769
	CM54	100	100	6.6700	7.74354	6.1769
120h	C13	0	0	7.4520	10.45954	1.6849
	CM34	10	90	6.6250	9.99454	3.0289
	CM41	90	10	7.2090	9.87454	6.1009
	CM48	50	50	7.0770	9.93454	4.5649
	CM55	100	100	7.2020	9.40954	7.4449
150h	C14	0	0	7.3500	12.95854	2.1469
	CM35	10	90	6.5530	12.49354	3.8269
	CM42	90	10	7.3770	12.37354	7.6669
	CM49	50	50	7.1250	12.43354	5.7469
	CM56	100	100	7.4000	11.90854	9.3469

The predictive groups of cooked; C37-C40, Cooked marinated squid rings; CM41-CM56 LJ: Limon juice; MW: Mineral water; TMC: Total mesophilicbacteriacount;$\hat{Y}_{MI}$:The estimated TMC (logcfu/g) fromModel I; $\hat{Y}_{MII}$: The estimated TMC (log cfu/g) from Model II; $\hat{Y}_{MIII}$: The estimated LBC (log cfu/g) from Model III.

After 120 h of the predictive marination process, the predictive value of TMC of cooked squid rings increased to 6.62, 7.20, 7.07, and 7.20 log cfu/g, respectively for the groups CM34, CM41, CM48, and CM55 according to Model I.

Additionally, at that time the predictive value of LBC of samples increased to 3.02, 6.10, 4.56, and 7.44 log cfu/g, respectively for the groups CM34, CM41, CM48, and CM55 according to the Model III.

DISCUSSION

Squid meat is known to be sensitive to heat treatment due to its unique structure, which gives traditional squid products a harsh and harsh taste (Hu et al., 2014). This special structure of squid meat directly affects the acceptance of consumers (Jun-Hui et al., 2020). Therefore, this study, it was aimed to soften the squid rings by cooking and marinating them. Reported that cooking at lower temperatures (55°C) significantly decreased cooking loss, physicochemical and textural attributes of squid meat, when compared with cooking at a higher temperature of 77°C. In addition, it is noted that cooking temperature of 55°C, squid meat has both sufficient juiciness and a pleasant texture (Schmidt et al., 2021). The maximum softening level was reported and softening is achieved by incubating squid mantle rings at 45°C and pH 2 (Collignan and Montet, 1998).

Many studies have been conducted about the processing technologies that are applied to squid for decreasing the TMC. The TMC of squid treated with slightly acidic electrolyzed water ice significantly inhibited this value up to 1.46±0.10 log cfu/g, and also observed relatively slow microbial production during storage (Xuan et al., 2017). In a reported study that high-pressure processing at 300 MPa after 20 minutes reduced the number of TMC in squid by 1.26 log cfu/g (Gou et al., 2010). In addition to this, gamma irradiation was also reported by Tomac et al. (2017), reducing bacterial loads of highly perishable squid rings and resulting in significantly extended shelf-life under cooling conditions. Reported in another study that heat treatment was lethal to some types of microorganisms in seafood products (Zavadlav et al., 2020). In addition to this, each type of microorganism had its own special heat tolerance.

In the present study, the TMC of cooked squid rings began to increase after 6h stored at 4°C. The growth of most of the bacteria species could be inhibited by the heat and marination treatments. However, acid tolerance lactic acid bacteria began to grow in acidic conditions in CM squid products. The numbers of all C and CM sample groups of microorganisms were determined under the detectable limit at the beginning of the marination process (Table 3). However, all the groups of microorganisms began to increase during the marination process at 4°C. The number of TMC began to increase in CM4 after 12 h of the marination process, whereas the TMC of groups CM12, CM19, and CM26 increased after 24 h of the marination process. The population of TMC in groups C7, CM7, CM14, CM21, and CM28 significantly increased up to 5.92, 5.83, 5.71, 5.57, and 5.42 log cfu/g, respectively, then 72 h of the refrigerated storage. However, according to the ICMSF (1986), all groups C and CM did not exceed the microbiological consumption limit specified as 6.0 log cfu/g for processed aquaculture after 72 h of storage at 4°C.

Andrighetto et al. (2009) reported that LBC was found to be high in the Italian seafood salads discussed in this study and increased the cfu/g values by 8.0 log at the end of the shelf-life of the seafood salad. The results of the study correlated well with the literature studies provided (Andrighetto et al., 2009), stating that the increase in the marinating conditions of lactic acid bacteria is in accordance with the increased storage time. However, pathogenic bacteria *S. aureus, Vibrio spp.* and *E.coli* were not found in the C and CM squid rings in the present study.

The authors reported that longer cooking times could be caused to increase the pH values of cooked squid mantle during refrigerated storage (Stanley and Hultin, 1982). In the present study; after marinating process the decreasing of pH values of cooked squid samples are confirmed by these studies (Kilinc and Çaklı, 2004; Szymczak et al., 2020).

All the groups of C and CM squid rings were still not exceeded the limit of consumption after 72 h of storage at 4°C (Tomac et al., 2017). At that time the general acceptability results of the groups C7, CM7, CM14, CM21, and CM28 were determined as 4.4, 4.5, 4.3, 4.4, and 4.4, respectively. In one report, the panelists noted that the most important sensory quality of cuttlefish is the texture, which is the primary quality factor, and their most common complaint is a rubbery texture or stiffness. However, the longest cooking time (32 minutes) produced squid with the best sensory quality (Stanley and Hultin, 1982). In addition to this, taking into consideration of the processing technologies, they could be induced an increase in the shelf-lives of fishery products (Keklik et al., 2017). In another report, the higher pressurization was reported to be caused to extend the longest shelf-life of squid samples (Paarup et al., 2002). These results of all the studies given were found to be very similar to the findings of this study that cooking and marinating caused an increase in the shelf-life of squid rings. Andrighetto et al. (2009) reported that LAB was highly present in the Italian marinated seafood salads and it increased to a value of 8.0 log cfu/g at the end of the shelf-life of the seafood salad. In the present study, the predictive LBC of the group CM56 during the marination process stored at 4°C exceeded this value after 150 h according to the predictive regression model III.

CONCLUSION

As a result, three models were created for estimating the TMC and LBC of C and CM squid rings during marination at 4ºC. For marinated fishery products; Model III was also developed for estimating the value of LBC of samples. Therefore, Model III can be very essential together used with Model I or Model II because of observing the increasing LAB of

cooked squid rings during marination. These created models can be preferred not only for estimating bacterial counts of fishery products during marination process but also for deciding the shelf-life of marinated and fermented fishery products.

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AUTHOR CONTRIBUTIONS

All authors contributed equally.

CONFLICTS OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS APPROVAL

There are no ethical issues with the publication of this manuscript.

DATA AVAILABILITY

The authors confirm that the data that supports the findings of this study are available within the article.

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