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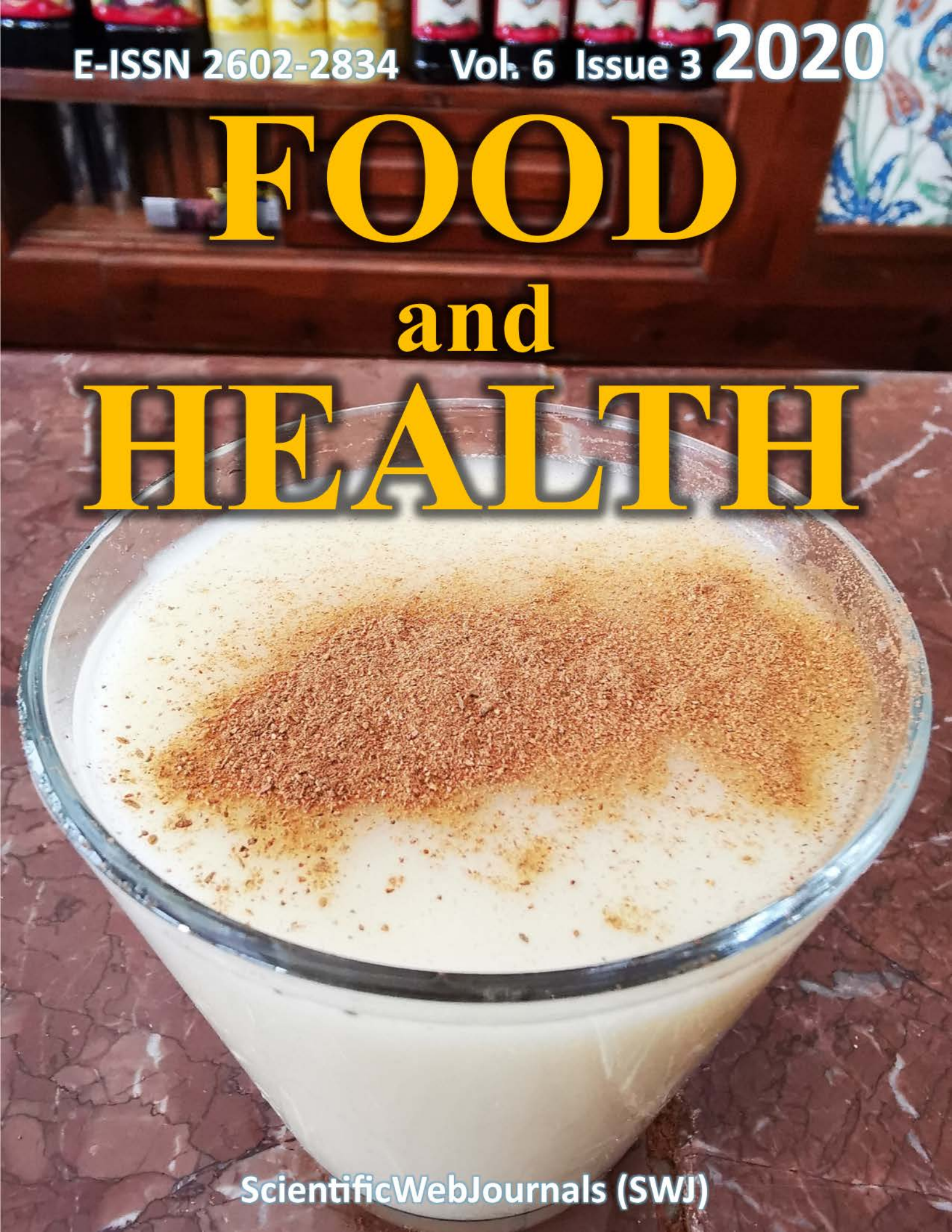
FOOD and HEALTH



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Aims and Scope

FOOD and HEALTH

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"Food and Health" journal will publish peer-reviewed (double blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their team of experts provides editorial excellence, fast publication processes and high visibility for your paper.

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Editor in Chief: Prof. Nuray ERKAN
Address: Istanbul University, Faculty of Aquatic Sciences, Department of Seafood Processing Technology, Ordu Cad. No: 8, 34134 Fatih/Istanbul, Türkiye
E-mail: nurerkan@istanbul.edu.tr

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Production of functional Turkish noodle (Erişte) supplementary probiotic and determining of some quality properties

Selin Kalkan, Mustafa Remzi Otağ, Elif İlkay Köksal, Nursena Şevval Bozkurt

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Giresun University, Faculty of Engineering, Department of Food Engineering, 28200, Giresun, Turkey

ORCID IDs of the authors:
S.K. 0000-0002-4142-3152
M.R.O. 0000-0001-5450-1546
E.İ.K. 0000-0003-0663-4939
N.Ş.B. 0000-0002-0457-3629

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Correspondence: Selin KALKAN
E-mail: selin.kalkan@giresun.edu.tr

ABSTRACT

In this study, it was aimed to production Erişte samples which have functional properties by the addition of *Bacillus clausii* and determined some quality properties. For this purpose, *B. clausii* was microencapsulated by using extrusion method and added up to 4% in dough. Dried vegetables were pulverized and then added to the noodles at a rate of 5 to 15%, in order to increase the sensory properties of the noodle samples. In addition, microbiological characteristics and survivor *B. clausii* for probiotic samples of all noodles samples were analyzed.

It was determined that the number of *B. clausii* was 9.99-11.06 log CFU/g in noodle dough on average; between the values of 9.35 – 10.11 log CFU/g after shaping the noodles; after cooking the noodles samples, it was determined that they ranged between 5.02 - 5.10 log CFU/g values. *B. clausii* values were sufficient to maintain the resistance of probiotic microorganisms in the gastrointestinal system.The enriched products by probiotic are in the nature of functional new product in accordance with the purpose of the study.

Keywords: Turkish noodle, Probiotic, *B. clasuii*, Quality, Functional product

Introduction

Humanity is providing the nutrients necessary for survival and social functioning from the vegetable and animal origin foodstuffs. Healthy nourishment can be achieved by taking these two groups of food materials in sufficient and balanced amounts (Martín et al., 2015). In Turkey, as in many parts of the world, many daily calories from grains and grain products are provided. Pasta products are highly preferred due to its low price, easy preparation, the wide variety of uses, flavor characteristics, and long shelf life (Bergman et al., 1994). Noodles are a primary food in much of the world, especially East Asia. Erişte (Noodles) is a food product originating from China and promoted to the whole world through the silk road. The prepared mix can be directly consumed after kneading, drying, boiling and cooking (Gunathilake and Abeyrathne, 2008). The quality of Erişte is assessed as a combination of their appearance, texture, and cooking properties (Zhang et al., 2015). It is the main goal of food production to provide safe and nutritious food that will ensure the healthy and happy life for people (Siró et al., 2008). In recent years, the changing of the nutritional characteristics of consumers, the increase in expectations of life expectancy of consumers, the development of healthy nutrition awareness, and the increase in obesity and other health problems have become effective at increasing of consumption of "functional food product" (Granato et al., 2010). The use of probiotic microorganisms to gain functional properties of foods has become more intense in recent years with the increase in demand for prebiotic products. Probiotics are live microbial sources that have a beneficial effect on human health by modifying the natural gut microbiota positively. Probiotic starter culture is a single or mixed culture that creates the mentioned effects (Parvez et al., 2006). The minimum number of probiotic bacteria in the product is 10^6-10^8 CFU / ml in order for probiotic bacteria to have a beneficial effect (Schrezenmeier and De Vrese 2001). Although it is important for probiotic bacteria to maintain the number of live cells in food, these bacteria have lost their vitality during the shelf life of the product for whatever reasons. Therefore, various strategies for increasing the viability and continuity of probiotic bacteria in products are constituted the focus of recent studies (Vinderola et al., 2000). Also, various techniques are applied to maintain and support the viability of probiotic cultures in product development (Ross et al., 2002). One of these techniques is microencapsulation. Microencapsulation (ME) can be defined as packaging by using protein or carbohydrate-based coating material of solid, liquid or gaseous food components, enzymes, microorganisms, cells and other substances (Gouin, 2004; Madene et al., 2006). Various techniques are used to form capsules in the ME process. Among these methods, spray drying, emulsion and extrusion methods are mostly used in probiotic foods (Iyer and Kailasapathy, 2005; Chen and Chen, 2007). *Bacillus* spp. which known as probiotic is used in nutritional supplements for humans, incentives to grow for the animals, and growth regulators or resistance to diseases for aquatic products. There are probiotic *Bacillus spp*. products that are produced and sold in the United States (USA), European and Asian countries. Although the Bacillus species have been used for about 50 years under the name of a medical support product, the scientific studies on this subject have been around for the last 15 years. Bacillus species that are studied the most are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus laterosporus*. Bacillus species are mostly safe, but, strains that produce enterotoxin and/or emetic toxin and contain an infectious antibiotic resistance agent may cause problems (Sorokulova et al., 2008; Cutting, 2011). It is known that *Bacillus clausii* has a direct effect by releasing antimicrobial substances against gram-positive pathogenic bacteria such as *Staphylococcus aureus*, *Enterococcus faecium*, and *Clostridium difficile*. *B. clausii* spores, which are taken orally, pass through the stomach-gall bladder due to its high resistance to chemical and physical influences, reaching intestinally intact. Here, it shows a number of beneficial effects aimed at regrowing under appropriate conditions and restoring the physiological balance of the flora. Thus, *B. clausii* helps to regulate the digestive tract and support the immune system. *B. clausii* can synthesize antimicrobial agents like bacteriocins that restrict the growth of various pathogenic bacteria. It also affects the immune system by stimulating the growth of antibodies (Cutting, 2011).

Scientific studies on this subject have been conducted over the last decade, although there are probiotics containing spore-forming bacteria such as *Bacillus* and commercially available products and so there are still some unexplained things about this subject such as safety of uses. For this reason, detailed studies must be done so that *Bacillus* species can be used as potential probiotic products for human consumption. The aim of this study was to investigate the potential use of *B. clausii* strains known to be probiotic in the production of Erişte among the most consumed foods. For this purpose, the chemical, physical, microbiological and sensory properties of Erişte samples produced have been determined. Thus, it is aimed to increase the production potential of probiotic Erişte which is beneficial to consumer health by protecting or improving the intestinal microbial balance of individuals and to develop a functional new product.

Materials and Methods

Microencapsulation of Bacillus clausii

Bacillus clausii T strain (Enterogermina; Sanofi, Italy) was used as a probiotic culture in the study. Alginate microcapsules were obtained using a modified encapsulation method of Kalkan et al. (2018) ; Chen et al. (2007). Accordingly, the *B. clasuii* culture concentrates, which was grown in 20 ml (10^9 CFU/ml) was slowly added and immobilized to a solution of 80 ml sterile alginate containing 2.5% sodium alginate for about 10 min using a magnetic stirrer. In this way, the bacteria were dispersed into the alginate solution. The obtained alginate bacterial suspension provides the alginate to form a spherical bead in the gel 500 ml 0.2M $CaCl_2$ solution was extruded by dropwise transfer of the distance to 30 cm with sterile 21 G syringe. When the droplets were dripped into the $CaCl_2$ solution, the cross-ionic binding takes place and the cells were trapped in the 3D mesh structure and the alginate encapsulation (microcapsules) was performed (Karthikeyan et al., 2014). The resulting calcium alginate microcapsules were additionally mixed in $CaCl_2$ solution for 30 min in a magnetic stirrer to provide a more rigid and robust form of the capsules and then the capsules were washed in sterile pure water (Chen et al., 2007). Finally, filtration was performed using Whatmann filter paper No.4 to obtain microcapsules in $CaCl_2$ solution. Microencapsulated probiotic culture was shown in Figure 1.

Figure 1. Microencapsulated probiotic *B. clausii* culture

Production of Erişte

All materials used in Erişte samples production were supplied from local grocery stores in Giresun. Erişte samples were prepared according to the method reported by Demir et al. (2010). For preparation control group (plain samples; without vegetable powder and microencapsulated *B. clausii*), 300 g wheat flour, 1 g salt, 2 pieces whole eggs, 30 ml milk and 50 ml water were mixed in mixer (Kiwi kmx 3633, Turkey) for 10 min. Then the Erişte dough was divided into four pieces (Control (A), Probiotic-Control (B), Control-added dried vegetable powder (C) and Probiotic-added vegetable powder (D), covered with cloth and rested for 30 min at room temperature. At the end of this period, the Erişte dough pieces were sheeted with a rolling pin. Then, the dough was sheeted at a thickness of about 2 mm and cut with a noodle machine (Maracato Ampia 180, Italy). Drying was made in a drying cabinet (Nüve FN 120, Turkey) at 50°C for 18 h. *B. clausii* was microencapsulated by using extrusion method and used in Probiotic Erişte production as 4% rate. For the production of vegetable Erişte, spinach, carrots, tomatoes and red berry vegetables, thoroughly cleaned and washed, are dried at 40°C (Nüve FN 120, Turkey) and powdered by grinding (Waring Blender 7011HS, Osaka Chemical Co. Ltd., Japan) with 60 mesh particul size. After Erişte production, all groups were stored at room temperature to be used in analysis by applying vacuum packaging.

Chemical analysis

Chemical components of Erişte samples were determined using Standard Methods (International Association for Cereal Science and Technology-ICC, 1992), for the determination of moisture, ash and protein. Water activity and acidity values of Erişte samples were determined according to Li et al. (2011) and Lu et al. (2003)

Color

Colors of Erişte samples were evaluated by measuring the L^* (whiteness/darkness), a^* (redness /greenness) and b^* (yellowness / blueness) parameters using Minolta CR-410 (Minolta Chroma, Osaka, Japan) (Ugarčić-Hardi et al., 2007).

Cooking Quality

Cooking quality parameters such as cooking time and volume increase were determined according to Demir et al. (2010). In order to determine the cooking time of the Erişte samples, 7-8 minutes after the sample was started to be cooked in the beaker, a piece with a collet holder was taken and squeezed between glass plates. This process was continued for one minute until the light colored uncooked part in the center of the oppressed glass between the glass plates was no longer visible and the time elapsed from the beginning until that moment

was determined. Volume increase of samples was determined by the volume difference of water overflow when the cooked and dry Erişte sample was put into the water full graduated cylinders. Erişte samples (10 g) were cooked in 100 ml boiling water for 5 min and drained for 5 min. The drained Erişte were weighed to determine the cooking weight gain, which was expressed as a percentage of the initial dry matter. The cooked Erişte was collected and dried in an air oven at 115 °C. The remaining solids were weighed to determine the cooking loss which was expressed as a percentage of the initial dry matter (Li et al., 2018).

Microbiological Analysis

Total bacteria, yeast and mold, Coliforms and *Staphylococcus* spp. analyses were made for determining microbial contamination levels of Erişte samples. Petri dishes were incubated for the mesophilic aerobic bacteria count at 30°C for 48 hours by using Plate Count Agar (PCA, Merck), for yeast and mold count at 30°C for 72 hours by using Potato Dextrose Agar (PDA, Merck), for coliform bacteria at 37°C for 24 hours by using Fluoracult Violet Red Bile Agar (FVRB, Merck) and for *Staphylococcus* spp. at 37°C for 24 hr by using Baird Parker Agar (BPA, Merck). The number of bacteria in the sample was designated as CFU/g by determining the number of colonies developing after incubation (FDA Manuel, 1995). Mueller Hinton Broth (Merck) broth was used for the activation of *B. clausii* cultures. Petri dishes were incubated at 37ºC for 24 h and counting results were obtained after spreading and cultivation in MYP Agar (Merck) and Mueller Hinton Agar (Merck) media (Urdaci et al., 2004).

Sensory Analysis

For sensory analysis, 100 g Erişte samples were simmered at 95°C in 500 ml water for 20 min. and drained for 20 s to remove excess water. The evaluation of the opinions of 8 panelists who are working at Food Engineering Department of Giresun University, Turkey was reported for sensory properties. They were asked to score the resulting cooked Erişte based on color, taste, chewiness, cooking properties and odor criterion using a 5-point scale.

Statistical analysis

The obtained data from all the analysis were exposed to the analysis of variance in the study by using IBM SPSS Statistic 19.0 software (IBM Corporation, Somers, NY, USA). Duncan's multiple range tests at ($p \leq 0.05$) level was used to compare between means (Demir et. al., 2010).

Results and Discussion

Chemical Properties of Erişte

The chemical properties of the Erişte samples produced are shown in Table 1. As shown in Table 1, when the humidity values of control-plain Erişte (A) and control group vegetables (C) Erişte were compared, it was observed that there was a decrease in moisture content of vegetable-flavored group. It was deduced that this decrease in moisture content was related to the increase in the amount of dry matter due to the addition of vegetable powders. When the moisture values of probiotic plain (B) and probiotic vegetable (D) Erişte samples were examined, it was observed that the vegetable Erişte samples showed a better drying rate compared to the plain groups. This was thought to be due to both the addition of probiotic and the addition of vegetable powders. When the moisture content levels of both groups (plain and with vegetable powders adjuvant) were compared, the addition of vegetable powders reduced the moisture values. Moisture content is an important criterion in the accesses and informs about whether the drying process is complete or not. At the same time, the moisture content in the final product is a very important parameter in terms of product safety and commercial value. The moisture content of all Erişte samples was determined below the 13% (Rajam et al., 2015). Similar results were obtained with moisture levels reported by Rajam et al. (2015). From these data, it can be said that the drying was done well, the formulation was in conformity with the standards. Rajam et al. (2015) reported that the moisture content of Erişte samples was found to be between 4.34-6.67%, similar to our study results.

Table 1. Chemical properties of Erişte (noodle) samples

Chemical analyzes	Groups*			
	A	B	C	D
Moisture (%)	10.17 ±0.47^{d}	9.16 ±0.48^{c}	7.49 ±0.17^{a}	8.57 ±0.21^{b}
Water activity	0.54 ±0.02^{b}	0.51 ±0.00^{b}	0.46 ±0.00^{a}	0.54 ±0.02^{b}
Acidity (%)	0.17 ±0.04^{b}	0.12 ±0.01^{a}	0.19 ±0.00^{b}	0.31 ±0.00^{c}
Protein (%)	10.56 ±1.08^{a}	10.74 ±0.54^{a}	12.15 ±0.72^{b}	12.23 ±1.12^{b}
Ash (%)	1.09 ±0.05^{a}	1.00 ±0.09^{a}	2.13 ±0.07^{b}	2.12 ±0.09^{b}

*A: control-plain Erişte; B: probiotic-plain Erişte ; C: control-vegetables Erişte; D: probiotic-vegetable Erişte; a-d: Means in the same row with different letters differ significantly at *$P \leq 0.05$.

It was found that water activity values were related to the moisture content when the water activity values given in Table 1 of the Erişte samples were examined. As expected, the lowest water activity value in the control-vegetable Erişte samples was determined with the lowest value of moisture (7.79 %). Also, it was found that the control-plain group Erişte samples with the highest moisture content at 10.17 % have been the highest water activity value. When the acidity values of the samples shown in Table 1 were examined, it is seen that acidity values have increased in the Erişte samples enriched with the addition of vegetable powder. It was thought that this situation is related to the presence of high organic acids in vegetable powders. The data obtained from all Erişte samples indicates that the acidity values are above the 0.05 %. It was thought that this may be caused by lactic acid bacteria contaminated from the environment in raw milk used in production. Baiano et al. (2008), in a similar study, was determined that the acidity values of the samples were between 0.054 and 0.076 % in the Traditional Italian pasta. It was determined that the highest protein content was obtained in probiotic vegetable Erişte samples when protein ratios (%) of the produced Erişte samples were evaluated. Hu et al. (2007) similar to the values obtained in our study, reported that the protein ratio of Erişte samples ranged from 10.70 to 1.63 %. According to the Filip and Vidrih (2015), the protein content of plain and enriched pasta should be at least 12% and protein values obtained in our study were suitable for the notification. As shown in Table 1, the ash values of the Erişte samples (%) were found range from 1.00-2.13 %. The increase in the ash values of the samples with the addition of vegetable powder to Erişte showed parallelism. It is more suitable to use flour obtained from wheat, which has 1.4% or less ash content in Erişte production. In general, for Erişte production, flours with ash less than 0.5% are required. However, this ratio should be 0.4% or less for better quality Erişte (Hou and Kruk, 1998). Izydorczyk et al. (2005) stated that the ash values of the Erişte samples were found to be 0.44-1.06 % similar to our study.

Color Parameters of Erişte

The color values (*L*, a** and *b**) of the Erişte samples were shown in Table 2. Erişte color is significantly affected by protein and ash content of flour with the addition of eggs (Pomeranz, 1998). In terms of Erişte quality and consumer preferences, the bright yellow appearance of Erişte is an important parameter (Ozyurt et al., 2015). When the color values of the produced Erişte samples were examined, it was determined that the addition of vegetable powder caused significant changes in color values ($p \leq 0.05$).

The highest *L** values were found with the probiotic-plain samples and control-plain samples. The lowest *L** values (higher brownness) were found with the control-vegetables Erişte and probiotic-vegetable Erişte. It can be explained by the higher content of ash and dietary fibre. The highest *a** values were determined to control-vegetable Erişte, and the lowest *a** value was found with the control-plain Erişte samples. The highest *b** values were observed in samples B. Despite the high value of the *b** parameter is desirable for pasta color scoring. Ugarčić-Hardi et al. (2007), in a similar study, reported that *L** values were as 63.8-78.9; *a** values were as -3.4 - 1.5; *b** values were as 12.9-27.0 and *ΔE** values were as 2.3-15.2 for fresh Erişte. In the same study, *L** values were as 66.8-76.6; a values were as (-2.4)-1.7; *b** values were as 12.7-26.4 and *ΔE** values were as 3.8-9.8 for dried Erişte. Demir et al. (2010), in another similar study, determined that *L** values were as 81.078-85.547; *a** values were as 0.373 – 0.381; *a** values were as 12.907-16.254 for some raw materials used in Erişte samples.

Table 2. Color values of Erişte (noodle) samples

Color values	Groups*			
	A	B	C	D
L*	62.22 ±2.19^{b}	72.52 ±2.17^{bc}	43.09 ±0.99^{a}	48.28 ±2.79^{a}
a*	6.36 ±0.36^{a}	7.14 ±0.50^{b}	11.57 ±0.16^{d}	9.98 ±0.29^{c}
b*	34.65 ±1.76^{bc}	38.79 ±1.91^{c}	31.59 ±1.29^{b}	22.94 ±1.99^{a}

*A: control-plain Erişte; B: probiotic-plain Erişte; C: control-vegetables Erişte; D: probiotic-vegetable Erişte; a-d: Means in the same row with different letters differ significantly at *$P \leq 0.05$.

Cooking Characteristics

The results of cooking analyzes of the Erişte samples were shown in Table 3. The cooking quality of Erişte is one of the important criteria for the evaluation of Erişte quality. The average cooking time of Erişte samples was found between 7 and 9 min. The shortest cooking time was determined as 7 min in the control group vegetable Erişte. Optimal cooking time is mainly based on water transfer and starch gelatinization. The weak protein network facilitates the penetration of water into starch (Sozer and Kaya, 2008). The volume increase was found to be between 201.67 and 208.67 % on average. A good quality pasta should draw at least 2 times its weight and swell 3-4 times its volume (Pınarlı et al., 2004). All Erişte samples analyzed were found to be good quality in terms of volume increase. Similarly, Demir et al. (2010) reported that volume increase values were found to be between 201.79 and 211.87 %. Weight increase values of Erişte samples were determined between 193.66-202.33%. As known, the higher the amount of protein, the lower the water absorption value. This is because of the strong protein network is the prevention of water diffusion of starch granules. The high protein content is not effective on the quality of baking of Erişte alone. Protein quality, as well as protein amount, is very important (Shewry, 2007). Similarly, Li et al. (2018) was stated that weight increase values of Erişte samples were determined between 159 – 203 %.

Microbial Properties of Erişte

Microbiological analysis (total mesophilic aerobic bacteria-TMAB, total coliform, and yeast-mold and *S. aureus*) results of Erişte samples were shown in Figure 2. As shown in Figure 2, there was a higher number of TMAB numbers of probiotic groups than in control groups. This result is thought to be caused by the *B. clausii* strains used for the production of probiotic Erişte. Also, when compared to the plain groups and the vegetable powder-added Erişte samples in terms of TMAB count, it was determined that vegetable powder supplemented groups contained a higher number of TMAB. This situation is considered to be a result of the addition of vegetable powder because of lactic acid bacteria. The maximum acceptable limit is (6.0 CFU/g) for the TMAB in the fresh noodles (Ghaffar et al., 2009). It may be considered as a reference point between spoiled and unspoiled noodles.

Table 3. Cooking properties of Erişte (noodle) samples

Cooking properties	Groups*			
	A	B	C	D
Cooking time (min)	9.00 ±0.00^{b}	9.00 ±0.00^{b}	7.00 ±0.00^{a}	8.00 ±0.00^{ab}
Volume increase (%)	203.00 ±2.64^{ab}	208.67 ±7.09^{b}	201.67 ±2.68^{a}	202.00 ±2.64^{a}
Weight increase (%)	198.66 ±2.51^{ab}	193.66 ±4.04^{a}	202.33 ±7.50^{bc}	200.66 ±3.78^{b}
Cooking loss (%)	7.86 ±0.50^{a}	8.05 ±0.51^{ab}	8.74 ±1.93^{b}	8.77 ±0.22^{b}

*A: control-plain Erişte; B: probiotic-plain Erişte; C: control-vegetables Erişte; D: probiotic-vegetable Erişte ; a-c: Means in the same row with different letters differ significantly at *$P \leq 0.05$.

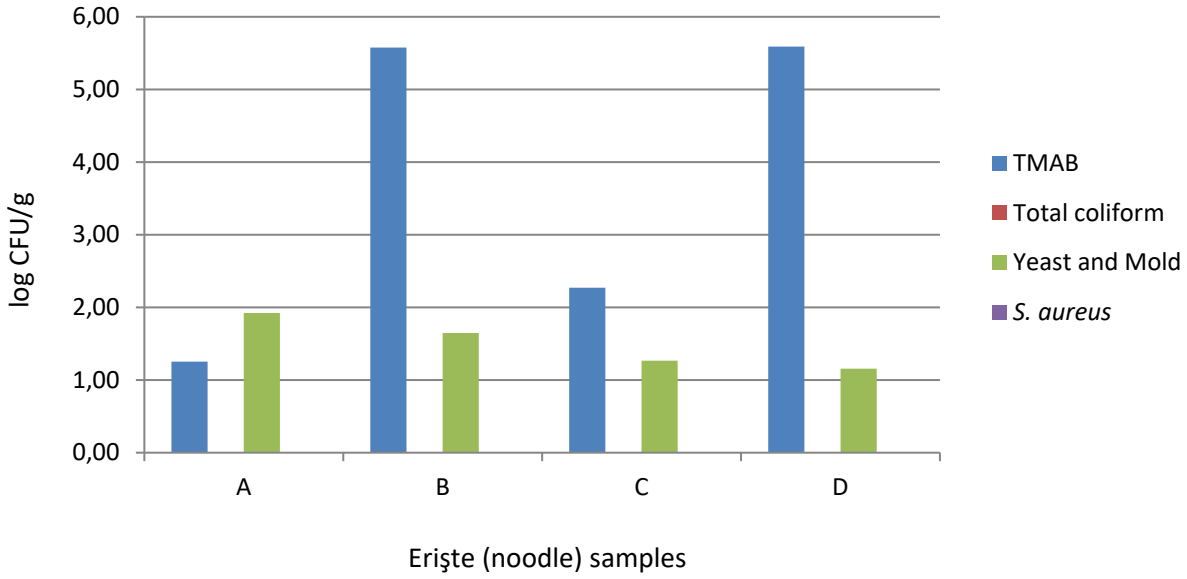

Figure 2. Microbial properties of Erişte (noodle) samples

It was determined that none of the samples were found to contain *Staphylococcus aureus* and coliform group microorganisms in our study. It may be due to the lower water activity and hygienic handling and packaging of products. When the samples of prepared Erişte were evaluated in terms of the number of yeast-mold, no significant differences were determined between the samples. It is thought that the drying process applied to the Erişte after shaping the dough reduces the moisture content in the dough and the decrease in the yeast and mold content of the Erişte as a result of the low water activity values. Yeast and mould count was much lower than the acceptable limit for fresh pasta, i.e. 4 log CFU/g (WHO, 2000).

In order to improve the functional properties of Erişte samples, the addition of *B. clausii* has been investigated and it has been determined that *B. clausii* maintains the presence of probiotic properties in all process stages. The number of *B. clausii* of probiotic Erişte samples was shown in Figure 3. As shown in Figure 3, it was determined that the number of *B. clausii* was 9.99-11.06 log CFU/g in Erişte dough on average; between the values of 9.35 – 10.11 log CFU/g after shaping the Erişte; between 5.97 and 6.95 log CFU/g values after drying (50 °C) the Erişte samples; after cooking (at 90 °C during 10 min) the Erişte samples, it was determined that they ranged between 5.02 - 5.10 log CFU/g values. These values were sufficient to maintain the resistance of probiotic microorganisms in the gastrointestinal (GIS) system.

Sensory Properties of Erişte

The color, taste, chewiness, cooking properties and odor sensory properties of the produced Erişte samples were evaluated by an expert jury of 8 people using a scale of 1-5 points and the consumer favorability was determined. The results obtained as a result of sensory analysis were shown in Figure 4. Among the samples, sample B had the highest total sensory score (4.28), followed by samples A (4.20) and D (3.73) while samples C (3.61) had the lowest score. Samples B had a very pleasant flavor. Samples C had significantly lower values or all sensory parameters than the other samples. As a result, the probiotic Erişte sample groups (plain and vegetable supplement) were more favorable than others.

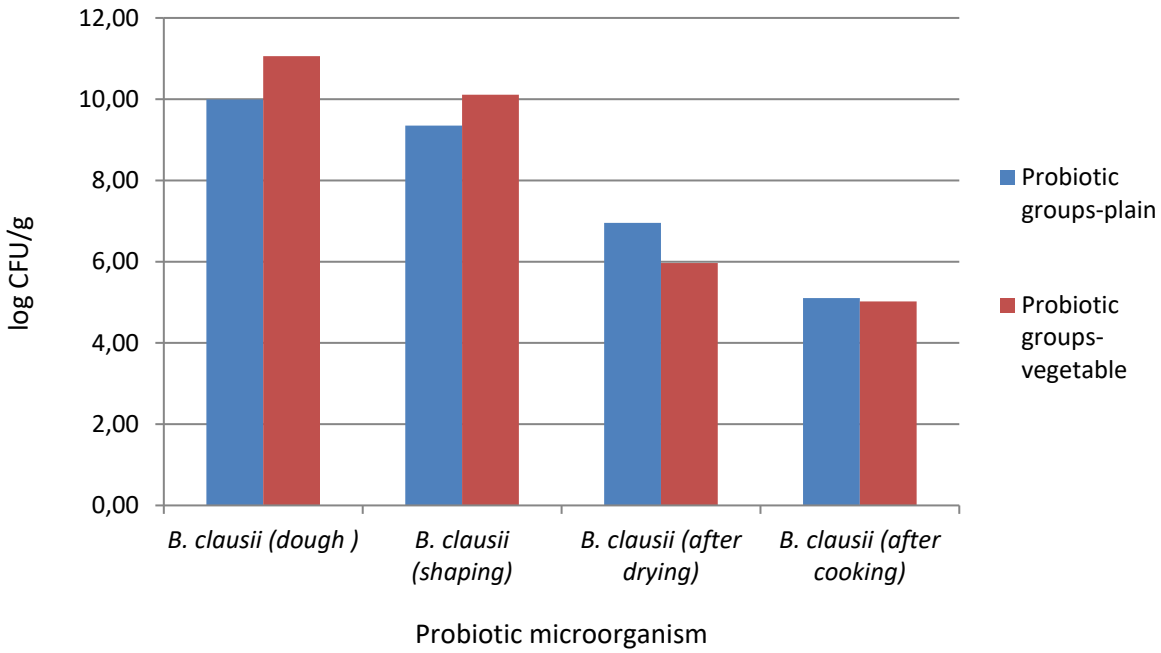

Figure 3. The number of *B. clausii* of probiotic Erişte (noodle) samples

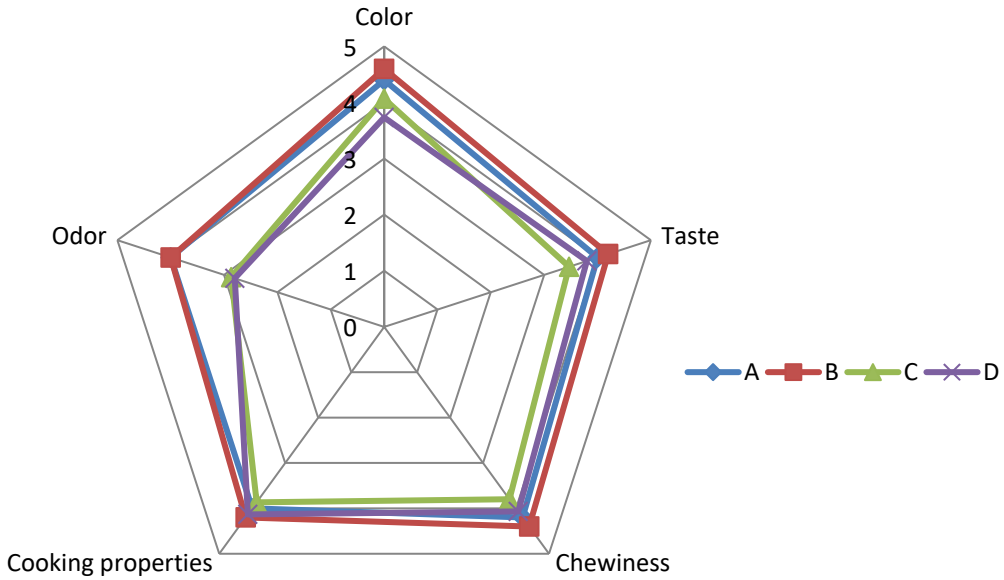

Figure 4. Sensory properties of Erişte (noodle) samples

Conclusion

In this study, it is aimed to increase the production potential of probiotic Erişte, which are beneficial to the consumer health by maintaining or improving the intestinal microbial balance of individuals and developing a functional new product. For this purpose, the production of Erişte with the addition of *B. clausii* strains and enriched with vegetable aromas was carried out. As a result, it has been determined that the enriched Erişte products are in the nature of a probiotic and functional new product in accordance with the purpose of the study. It was found that the Erişte were chemical, physical and microbiologically acceptable. In terms of sensory properties, it was established that plain-probiotic Erişte were generally more favorable than plain-control group and vegetable products. The obtained results will guide future studies and contribute to the literature for about functional product development.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: All authors declare that this study does not include any experiments with human or animal subjects.

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Isolation and identification of *Staphylococcus aureus* obtained from cheese samples

Elif Bozcal

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Istanbul University, Faculty of Science, Department of Biology, Basic and Industrial Microbiology Section, 34134, Istanbul, Turkey

ORCID IDs of the authors:
E.B. 0000-0003-2836-778X

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Correspondence: Elif BOZCAL
E-mail: elif.bozcal@istanbul.edu.tr

ABSTRACT

Milk and dairy products including cheese are one of the most significant food commodities in terms of the food industry. However, a contaminated food product could conduce a variety of food borne bacterial infections. Although *Staphylococcus aureus* is known as normal flora members of the humans, it`s often isolated from the community and hospital-acquired infections. Therefore, investigation of *Staphylococcus aureus* from cheese samples was aimed in this study. A total of nineteen (n=19) white cheese was collected from various outdoor markets in Istanbul. All cheese samples were evaluated quantitatively. Phenotypic identification tests including Gram staining, oxidase, catalase, mannitol, and DNase were performed. The presumptive *Staphylococcus aureus* colonies (n=47) were analyzed by the 16S rRNA PCR and sequencing. And the sequences were deposited into the National Center for Biotechnology Information. According to the nucleotide BLAST analysis, a total of 47 *Staphylococaceae and Enterococcaceae* members including *Staphylococcus aureus* (n=3), *Staphylococcus carnosus* (n=1), *Macrococcus caseolyticus* (n=1), *Enterococcus faecalis* (n=25), *Enterococcus faecium* (n=12*), Enterococcus durans* (n=4), *and Enterococcus gallinarum* (n=1) were identified. Regarding methicillin susceptibility testing, two of out of three *Staphylococcus aureus* were detected as methicillin-resistant.

Keywords: *Staphylococcus aureus*, 16S rRNA, cheese, PCR

Introduction

The white cheese is the most consumed cheese type in Turkey and the cheese consumption per capita was determined as 8.7 kg/person in 2017 and 9.2 kg in 2020 (Temelli et al.,2006; Ataseven, Z, 2017; www.statista.com) Cheese is such a nourishing food that could provide an environment to the bacteria for growing and multiplication including *Salmonella*, *Escherichia*, and *Staphylococcus* because of the contamination. From the production of cheese to the point of sale, an inadequate sanitization procedure of equipment and utensils lead to contamination of the cheese products and this affects not only food quality but also public health (Donnely, 1990; Aguilar et al., 2016).

Staphylococcus aureus (*S. aureus*) is known as normal flora member of the human skin, however, some strains of the *S. aureus* is the main reason of the infections and intoxications in terms of consumption of the contaminated milk, dairy products and other foods (Kadiroglu et al., 2014; Bingöl and Toğay, 2017). Staphylococcal food intoxication is a gastrointestinal disease that occurs due to the toxin produced *S. aureus*. When food or ingredients is contaminated by the enterotoxigenic strain of *Staphylococcus* spp., Staphylococcal food poisoning could be induced on the occasion of *Staphylococci* growth and enterotoxin production (Hennekinne et al., 2012; https://www.ndhealth.gov/Disease). Moreover, pathogenic strains of *S. aureus* could cause skin lesions, septicaemia, and meningitis in humans and it`s responsible for bovine mastitis in animals (Younis et al., 2003; Baran et al., 2017). The transmission of *S. aureus* to dairy products such as milk and cheese could occur via mastitis, mammary glands or animal, skin (Saka and Gulel, 2018). There may be a risk of contamination from personnel and equipment during the production of dairy products. In other words, transmission can be occurred also by animal to animal during milking as well as by the food-handlers, human to food contamination route (Kümmel et al 2016; Monte et al., 2018). Methicillin-resistant *S. aureus* (MRSA) is one of the most significant bacteria in terms of human global health due to the responsible for both community and hospital-acquired infections (Harrison et al., 2014). Moreover, livestock-associated MRSA (LA-MRSA) infections originated from livestock such as pigs, goats, and dairy cattle could transmit to the humans who is working in farms and abattoirs where raw meat processed. LA-MRSA could be occurred by handling contaminated meats. Therefore, LA-MRSA could be also the reason for human infections (Cuny et al., 2015).

Although, the isolation of the MRSA from animal and food origin were investigated frequently, the adverse effect of MRSA in dairy products illness is relatively low (Herrera et al., 2016). Hence, identification of *S. aureus* in cheese samples is important for both the food industry and public health. In this study, it was aimed to identify *S. aureus* in white cheese samples sold in outdoor markets in Istanbul.

Materials and Methods

Sample Collection and Bacteriological Analysis

A total of nineteen (n=19) white cheese was collected from outdoor markets in Istanbul in April 2018 and September 2019. The color and pH value of each cheese samples were recorded (Creamy and white, pH:6.8-7.5). The cheese samples were analyzed quantitatively by homogenizing 25 g cheese and 225 ml peptone water (Peptone:10 g/L, NaCI:5.0 g/L pH: 7.2±0.2) within 24-hour. The 10-fold serial dilutions were spread on Baird-Parker Agar Medium supplemented with Egg Yolk Enrichment (Becton Dickinson). Typical colonies (dark gray to black colonies with clear zones) were selected and counted for further identification analysis followed by the 24-h for 37 ºC incubation. Phenotypic identification tests including Gram staining, oxidase testing of cytochorome oxidase with indicator (tetramethyl-p-phenylenediamine) conversion to the indophenols catalase (A slide drop with 3% H_2O_2 onto the presumptive *S.aureus* isolates on microscope slides), mannitol fermentation (mannitol-fermentation as a carbohydrate source in the presence of phenol red as a pH indicator to detect mannitol-fermenting Staphylococci), and DNase (DNA hydrolysis test composed of growing microorganism in the DNAse test agar medium that produces Deoxyribonuclease when the DNA is broken down resulting with clear zone and green color fades) were performed. The presumptive (typical colonies) *S. aureus* colonies (n=47) were taken into consideration for further identification analysis.

Genomic DNA Isolation and 16S rRNA Sequencing

The genomic DNA isolation of the presumed *S. aureus* colonies (n=47) was performed by using GeneAll® (South Korea) genomic DNA isolation kit according to the manufacturer`s instructions. Isolated genomic DNA samples were stored at –20 ºC until PCR analysis. The 16S rRNA PCR analysis was performed according to the Frank et al. (2008). The 16S rRNA gene were amplified in a 50 µl reaction volume including 1xPCR buffer (Maximo, GeneON), 0.2 mM of each dNTPs, 2.5 mM $MgCl_2$, and 0.5 µM of each primer (16S rRNA:27F-AGAGTTTGATCCTGGCTCAG and 1492R-GGTTACCTTGTTACGACTT) (Suardana, 2014). The PCR reaction was performed as following conditions: 2 min initial denaturation at 95 °C, 25 cycles of denaturation 1 min at 95 °C, annealing at 55°C for 1 min, and extension at 72°C for 2 min and 10 min final extension at 72°C. The obtained PCR

amplicons (~1465 bp) were evaluated by agarose gel electrophoresis (1.0 %) and screened by a transilluminator implemented in WiseDoc Gel Doc System. The purification of 16S rRNA gene amplicons was performed by BMLabosis (Ankara, Turkey) using the ExoSap-IT (Affymetrix) kit. Later on, samples were sent to Macrogen (Amsterdam, The Netherlands) for the unidirectional sequencing via ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA), and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The obtained reads were aligned and trimmed using the SILVA (Quast et al., 2013). All 16S rRNA gene sequences (n=47) were deposited into the NCBI GenBank followed by the nucleotide BLAST analysis (NCBI Accession No: MK791580-MK79194 and MN629248-MN629279) (Table 1).

Methicillin susceptibility testing

In order to detect MRSA identified by 16S rRNA sequencing, the agar screening method was performed according to the Brown et al.,2008. Briefly, the density of the *S. aureus* isolates was arranged to the 0.5 McFarland standard. After that, a spot inoculation (10 µl) of *S. aureus* into the Mueller Hinton Agar medium (HiMedia) including 4% NaCI (Conda) and 6 mg/L methicillin (Sigma) was performed. Plates were incubated at 37°C for 24-hour. The growth of any single colonies on methicillin plate is evaluated as resistant (Brown and Yates,1986; Brown et al., 2008).

Results and Discussion

The preparation and consumption of the cheese products with unhygienic conditions could lead to the proliferation of the *S. aureus* in cheese and it can be posing a high risk for public health. Detection, enumeration, and identification of the *S. aureus* especially coagulase positive and methicillin - resistant strains are significant. While coagulase-positive *S. aureus* strains can produce an enterotoxin, coagulase-negative isolates could able to produce enterotoxin (Nunes et al., 2015; Yildirim et al., 2019). Therefore, coagulase - negative *S. aureus* strains should be taken into consideration. In Turkey, there have been several studies that indicate the prevalence and presence of *S. aureus* strains in various cheese samples. The detection percentage were ranging from 20.2% to 92% (Yücel and Anıl, 20011; Gökmen et al., 2013; Bingöl and Toğay, 2017). The high percentage of the detection could indicate health risk in the cheese samples which has been consumed widely in Turkey. In our study, out of 19 white cheese samples, three (n=3) (15%) *S. aureus* were identified and two of them were reported as methicillin-resistant (Table 1). Similarly, the detection percentage of MRSA is not high in Turkey. For example, Saka and Gulel (2018) reported MRSA was 9 %. In another study, the detection percentage was 1.70 %, even though MRSA was investigated from 175 milk and dairy products (Ektik et al., 2017). Nevertheless, these data could show that a serious health problem.

All cheese samples were evaluated quantitatively in this study. The enumeration results were $1.6x10^4$ CFU/g (CE_1), $9.77x10^1$ CFU/g (CE_2), $3.1x10^3$ CFU/g (CE_3), $1.51x10^6$ CFU/g (CE_4), $6.35x10^7$ CFU/g (CE_5), $2.53x10^7$ CFU/g (CE_6), $1.63x10^5$ CFU/g (CE_7), $6.78x10^4$ CFU/g (CE_8), $8.05x10^5$ CFU/g (CE_9), $1.68x10^3$ CFU/g (CE_10), $1.27x10^3$ CFU/g (CE_11), $3.40x10^4$ CFU/g (CE_12), $2.51x10^7$ CFU/g (CE_13), $1.40x10^7$ CFU/g (CE_14), $2.34x10^7$ CFU/g (CE_15), $2.18x0^7$ CFU/g (CE_16), $1.67x0^6$ CFU/g (CE_17), $1.70x0^6$ CFU/g (CE_18), $1.30x10^5$ CFU/g (CE_20). The microbiological criteria in terms of the presence of coagulase-positive *Staphylococcus* species in cheese products established by the Food and Drug Administration (FDA) is 10^2-10^3 CFU/g was acceptable (https://www.fda.gov/media/74723/download). At the same time, Turkish Food Codex Microbiological Criteria takes into consideration the same reliability limits (10^2-10^3 CFU/g) in cheese products (Turkish Official Journal, 2011) However, the presence of *Staphylococcus* species more than 10^4 CFU/gr in cheese product considered to be risky according to the compliance Policy Guide of FDA (Kadiroğlu et al., 2014; https://www.fda.gov/media/74723/download). In this study, 15 out of the 19 cheese samples included more than 10^4 CFU/g presumed *Staphylococcus* species could be considered as hazardous for public health. The number of *Staphylococcus* (CFU) or concentration of enterotoxin can be shown a determining factor of risk situation. In other words, the enterotoxigenic strains of *Staphylococcus* is necessary to grow before the toxin production at detectable levels. Thereby, to cause an infection, a high dose of *Staphylococcus* is required (Food Safety Authority of Ireland 2011; Pollitt et al., 2018).

Table 1. Phenotypic characteristics and 16S rRNA genotypic identification of *S. aureus, S. carnosus, E. faecalis,* and M. *caseolyticus, E. faecium, E.durans,* and *E. gallinarum* isolates obtained from cheese samples

No	ID	16S rRNA	GenBank_ Accession No	Gram_Reaction morphology	O	C	M	D	Methicillin (R/S)
CE_2	CE_2	*Staphylococcus carnosus* CE_2	MK791585	(+)-coccus	(-)	(+)	(+)	(+)	
CE_3	CE_3_2	*Enterococcus faecalis* CE_3_2	MK791587	(+)-coccus	(-)	(+)	(+)	(-)	
CE_1	CE_1_1	*Enterococcus faecalis* CE_1_1	MK791580	(+)-coccus	(-)	(+)	(+)	(+)	
CE_1	CE_1_2	*Enterococcus faecalis* CE_1_2	MK791581	(+)-coccus	(-)	(-)	(+)	(+)	
CE_1	CE_1_3	*Enterococcus faecalis* CE_1_3	MK791582	(+)-coccus	(-)	(+/-)	(+)	(-)	
CE_1	CE_1_4	*Enterococcus faecalis* CE_1_4	MK791583	(+)-coccus	(-)	(+/-)	(+)	(-)	
CE_1	CE_1_5	*Enterococcus faecalis* CE_1_5	MK791584	(+)-coccus	(-)	(+/-)	(+)	(-)	
CE_4	CE_4_2	*Enterococcus faecalis* CE_4_2	MK791592	(+)-coccus	(-)	(+)	(+)	(+)	
CE_4	CE_4_3	*Enterococcus faecalis* CE_4_3	MK791593	(+)-coccus	(-)	(+/-)	(+)	(-)	
CE_4	CE_4_4	*Enterococcus faecalis* CE_4_4	MK791594	(+)-coccus	(-)	(+/-)	(+)	(+)	
CE_3	CE_3_3	*Enterococcus faecalis* CE_3_3	MK791588	(+)-coccus	(-)	(+)	(+)	(+)	
CE_3	CE_3_4	*Staphylococcus aureus* CE_3_4	MK791589	(+)-coccus	(-)	(+)	(-)	(-)	S
CE_3	CE_3_5	*Enterococcus faecalis* CE_3_5	MK791590	(+)-coccus	(-)	(+)	(+)	(+)	
CE_4	CE_4_1	*Enterococcus faecalis* CE_4_1	MK791591	(+)-coccus	(-)	(+)	(+)	(-)	
CE_3	CE_3_1	*Macrococcus caseolyticus* CE_3_1	MK791586	(+)-coccus	(-)	(+)	(-)	(-)	
CE_5	CE_5_1	*Enterococcus faecium* CE_5_1	MN629248	(+)-coccus	(-)	(-)	(-)	(-)	
CE_5	CE_5_3	*Enterococcus durans* CE_5_3	MN629249	(+)-coccus	(-)	(-)	(-)	(-)	
CE_5	CE_5_4	*Enterococcus faecium* CE_5_4	MN629250	(+)-coccus	(+)	(-)	(-)	(-)	
CE_6	CE_6_1	*Enterococcus faecium* CE_6_1	MN629251	(+)-coccus	(-)	(-)	(-)	(-)	
CE_6	CE_6_2	*Enterococcus faecium* CE_6_2	MN629252	(+)-coccus	(-)	(-)	(+)	(-)	
CE_6	CE_6_3	*Enterococcus faecium* CE_6_3	MN629253	(+)-coccus	(+)	(-)	(+)	(-)	
CE_7	CE_7_2	*Enterococcus faecium* CE_7_2	MN629254	(+)-coccus	(-)	(-)	(-)	(-)	
CE_8	CE_8_1	*Enterococcus faecium* CE_8_1	MN629255	(+)-coccus	(+)	(-)	(-)	(-)	

CE_8	CE_8_2	*Enterococcus durans* CE_8_2	MN629256	(+)-coccus	(-)	(-)	(-)	(-)	
CE_8	CE_8_3	*Enterococcus faecium* CE_8_3	MN629257	(+)-coccus	(-)	(-)	(-)	(-)	
CE_9	CE_9_2	*Enterococcus faecium* CE_9_2	MN629258	(+)-coccus	(+)	(-)	(-)	(-)	
CE_10	CE_10_1	*Enterococcus faecium* CE_10_1	MN629259	(+)-coccus	(-)	(-)	(-)	(-)	
CE_10	CE_10_2	*Enterococcus faecium* CE_10_2	MN629260	(+)-coccus	(-)	(-)	(-)	(+)	
CE_11	CE_11_1	*Enterococcus faecium* CE_11_1	MN629261	(+)-coccus	(+)	(-)	(-)	(-)	
CE_11	CE_11_2	*Enterococcus durans* CE_11_2	MN629262	(+)-coccus	(-)	(-)	(-)	(-)	
CE_11	CE_11_3	*Enterococcus durans* CE_11_3	MN629263	(+)-coccus	(+)	(-)	(-)	(-)	
CE_12	CE_12_2	*Enterococcus faecalis* CE_12_2	MN629264	(+)-coccus	(-)	(-)	(+)	(-)	
CE_12	CE_12_3	*Enterococcus faecalis* CE_12_3	MN629265	(+)-coccus	(-)	(-)	(+)	(-)	
CE_12	CE_12_4	*Staphylococcus aureus* CE_12_4	MN629266	(+)-coccus	(-)	(+)	(+)	(+)	R
CE_13	CE_13_1	*Staphylococcus aureus* CE_13_1	MN629267	(+)-coccus	(-)	(+)	(+)	(+)	R
CE_14	CE_14_2	*Enterococcus faecalis* CE_14_2	MN629268	(+)-coccus	(-)	(-)	(+)	(+)	
CE_14	CE_14_3	*Enterococcus faecalis* CE_14_3	MN629269	(+)-coccus	(+)	(-)	(+)	(+)	
CE_15	CE_15_1	*Enterococcus faecalis* CE_15_1	MN629270	(+)-coccus	(+)	(-)	(+)	(+)	
CE_15	CE_15_3	*Enterococcus faecalis* CE_15_3	MN629271	(+)-coccus	(-)	(-)	(+)	(+)	
CE_16	CE_16_2	*Enterococcus faecalis* CE_16_2	MN629272	(+)-coccus	(-)	(-)	(+)	(+)	
CE_17	CE_17_1	*Enterococcus gallinarum* CE_17_1	MN629273	(+)-coccus	(-)	(-)	(+)	(+)	
CE_18	CE_18_1	*Enterococcus faecalis* CE_18_1	MN629274	(+)-coccus	(-)	(-)	(+)	(+)	
CE_18	CE_18_2	*Enterococcus faecalis* CE_18_2	MN629275	(+)-coccus	(+)	(-)	(+)	(+)	
CE_18	CE_18_3	*Enterococcus faecalis* CE_18_3	MN629276	(+)-coccus	(+)	(-)	(+)	(+)	
CE_20	CE_20_1	*Enterococcus faecalis* CE_20_1	MN629277	(+)-coccus	(-)	(-)	(+)	(-)	
CE_20	CE_20_3	*Enterococcus faecalis* CE_20_3	MN629278	(+)-coccus	(-)	(-)	(+)	(-)	
CE_20	CE_20_4	*Enterococcus faecalis* CE_20_4	MN629279	(+)-coccus	(-)	(-)	(+)	(-)	

O:Oxidase, **C**:Catalase, **M**:Mannitol fermentation, **D**:DNAse, **Methicillin:** Methicillin Susceptibility, **S: S**usceptible, **R**: Resistant **(-):** Negative reaction, **(+)** : Positive reaction, **(+/-)** : Late positive

Presumptive *S. aureus* isolates (isolate IDs: CE_12_4 and CE_13_1) were compatible with the phenotypic identification tests including oxidase, catalase, mannitol fermentation, and Dnase. However, presumptive *S. aureus* isolate (ID: CE_3_4) was mannitol fermentation and DNAse tests were negative (Table 1). Although phenotypic tests for the isolate CE_3_4 were not coherent, some of strains of the *S. aureus* could show a negative reaction for the DNase and mannitol fermentation tests (Kateete et al., 2010). According to the 16S rRNA identification results, presumptive isolates (IDs: CE_13_1, CE_12_4 and CE_3_4) were identified as *S. aureus.* In accordance with phenotypic identification tests for the isolates including CE_2, CE_1_1, CE_4_2, CE_4_4, CE_3_3, and CE_3_5 were considered as *S. aureus.* However, the 16S rRNA identification test showed that these isolates were identified as CE_2 (*S. carnosus*), CE_1_1 (*E. faecalis)*, CE_4_2 (*E. faecalis)*, CE_4_4 (*E. faecalis*, CE_3_3 (*E. faecalis)*, and CE_3_5 (*E. faecalis).* Therefore, our results showed that some of the phenotypic identification tests did not correspond to the genotypic identification test. Considering the phenotypic results in Table 1, it was seen that only 47 of the phenotypic test results did not indicate *S. aureus*. On occasion, phenotypic tests can be variable under some conditions. For instance, *E. faecalis* is catalase- positive under the acquisition of heme however, *E. faecalis* strains are catalase negative (Frankkenberg et al., 2002). The 16S rRNA analysis showed that the other *Staphylococaceae* members including *Staphylococcus carnosus* (n=1), and *Macrococcus caseolyticus* (n=1) were reported in this study. Moreover, *Enterococcus faecalis* (n=25) *Enterococcus faecium (n=12), Enterococcus durans (n=4), and Enterococcus gallinarum (n=1)* belonging to the *Enterococcaceae* family was reported in this study (Table 1). Although *E. gallinarum* was reported from clinical samples in Turkey (Özseven et al., 2011), *E. gallinarum* can be isolated during cheese making and ripening procedure. In Italy*, E. gallinarum* was reported a low abundance in artisanal Italian goat's cheese during ripening procedure (Suzzi et al., 2011).

S. carnosus is generally isolated from meat products or fish and it`s known as meat starter culture (Bückle et al., 2017). Similarly, in Turkey, *S. carnosus* was reported from Turkish fermented sausage (Nazli, 1998). Another study that was carried on in France, *S. carnosus* was detected only in dry sausage samples (Coton et al., 2010). The detection of *S. carnosus* in our study could show the contamination of cheese samples. *M. caseolyticus* was also identified in various dairy and meat food sources related to flavor development (Mazhar et al., 2018). Besides, *M. caseolyticus* can be isolated from bovine milk, chicken, and humans. In Switzerland, *M. caseolyticus* was isolated from bovine mastitis milk (Schvendener et al., 2017). However, to best our knowledge*, M. caseolyticus* has not been detected from white cheese samples in Turkey before. As distinct from *S. carnosus* and *M. caseolyticus, E. faecalis* is known as a flora member of the gastrointestinal tract in humans and animals (Abdeen et al., 2016). However, the presence of *E. faecalis* in food sources such as cheese could show fecal contamination and/or inadequate hygienic measures in cheese samples. Moreover, the transmission of *E. faecalis* to the human by consumption of dairy products could cause various infections (Anderson et al., 2016). Similarly, various antibiotic - resistant *Enterococci* such as *E. faecium* has been reported from nosocomial-acquired patients (Sanders et al., 2010). Along with the harmful effects of *Enterococci,* these species are also known to have probiotic potential. Because *Enterococci* has a tolerance to the salts and acids thereby, *Enterococci* could adapt to various foods and could involve the fermentation process of cheese. (Hanchi et al., 2018). And another striking feature of *Enterococci* including *E. faecalis*, *E. faecium*, and *E. durans* has lipolytic activity and production of aromatic compounds (Amaral et al., 2016). In Turkey, *E. faecium* has been used for cheese production as a starter culture. And they were concluded that *E. faecium* FAIR-E 198 could be used as a starter culture (Göncüoglu et al., 2009).

Conclusion

In conclusion, *S. aureus, E. faecalis, E. faecium, E. durans, E. gallinarum*, *S. carnosus*, and *M. caseolyticus* were identified by phenotypic and genotypic identification methodologies. Phenotypic identification tests results should be validated by genotypic identification tests. The detection of MRSA in our study could show the significance of the methicillin resistance in cheese samples for public health. To prevent the transmission of *S. aureus* to cheese products, hygiene and sanitation precautions should be taken during production and sales of the cheese. Also, critical control points should be determined. According to our data, the presence of *S. aureus* and *Enterococci* in cheese products could give an opinion about transmission strategies of these bacteria needed to be studied.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Determination of colistin resistance in *Escherichia coli* isolates from foods in Turkey, 2011-2015

Mustafa Güzel[1], M. Dilek Avşaroğlu[2], Yeşim Soyer[3]

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[1]Hitit University, Faculty of Engineering, Department of Food Engineering, Çorum, Turkey

[2] Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kırşehir, Turkey

[3]Middle East Technical University, Faculty of Engineering, Department of Food Engineering, Ankara, Turkey

ORCID IDs of the authors:

M.G. 0000-0002-4750-7488

M.D.A. 0000-0002-3106-9209

Y.S. 0000-0001-9687-9715

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Correspondence: Yeşim SOYER

E-mail: ysoyer@metu.edu.tr

ABSTRACT

Antimicrobial resistance of pathogenic microorganisms is an emerging public health concern. Intensive use of antibiotics in food animals might increase antimicrobial resistance in foodborne pathogens. Colistin is a last resort antibiotic for treatment of multidrug resistant (MDR) Gram negative pathogens. The recent antimicrobial resistance studies revealed a mobile antimicrobial resistance gene (*mcr*) that provides resistance to colistin. Furthermore, the gene has been found in different genera. Therefore, the aim of this study was to determine colistin resistance of *Escherichia coli* isolates (N=48) isolated in between 2011 and 2015 from food samples in Turkey. In addition, 5 *mcr* genes and their variants were screened by performing PCR on resistant isolates. 4 *E. coli* isolates were found resistant to colistin above the epidemiological cut-off value (Minimum inhibitory concentration (MIC) > 2mg/L). None of the resistant isolates had the *mcr* genes. Further studies with human and food isolates should be conducted to figure out which gene or genes are responsible for colistin resistance.

Keywords: Antimicrobial resistance, Colistin, *Escherichia coli*

Introduction

Antimicrobial resistance is one of the main public health concerns. Ineffective antibiotics might mean longer treatment, even death in some cases such as cancer treatment, organ transplant, surgeries, and dialysis (CDC, 2013). In the United States (US), more than two million of drug resistant case occurred, results in 23000 deaths, annually (CDC, 2013). It was estimated that the economic cost of drug resistant infections was around 35 billion dollars (CDC, 2013). One of the main reasons of antimicrobial resistance is to overuse of antibiotics in farm animals.

Colistin is a last resort antibiotic due to its nephrotoxic effect for humans. It is mainly used against MDR Gram negative infections (Ordooei et al., 2015). A pathogen is considered as MDR when it is resistant to 3 or more antibiotics (Tang et al., 2017). Colistin is also used in veterinary medicine to treat animal digestive disorders. Before the study of Liu et al. (2016), it was believed that colistin resistance occurred through chromosomal mutations, and thus, was only transferred vertically. However, Liu et al. (2016) discovered a transferrable, plasmid mediated gene (*mcr-1*) that causes colistin resistance in *E. coli*. The gene was dated back to mid 2000's and most likely spread from farm animals in China (Wang et al., 2018a).

After the identification of the gene, a number of reports showed that *mcr-1* has already spread globally, from South America (Arcilla et al., 2016; Fernandes et al., 2016), North America (McGann et al., 2016; Mulvey et al., 2016), Africa (Olaitan., 2016), to Japan (Suzuki et al., 2016) and South Asia (Tse and Yuen, 2016). Plasmid mediated colistin resistance has been found in almost all of the European countries (Campos et al., 2016; Doumith et al., 2016; Falgenhauer et al., 2016; Malhotra-Kumar et al., 2016; Prim et al., 2016; Zurfuh et al., 2016).

Although *mcr-1* gene has dominantly been found in *E. coli* (Arcilla et al., 2016), the gene was also identified in different species such as; *Enterobacter aerogenes* (Zeng et al., 2016), *Enterobacter cloacae* (Zeng et al., 2016), *Citrobacter freundii* (Li et al., 2017), *Citrobacter braakii* (Sennati et al., 2017), *Klebsiella pneumonia* (Liu et al., 2016), and *Salmonella enterica* (Webb et al., 2016). In addition, resistant isolates found in poultry, cattle (Hu et al., 2016), fowl (Yang et al., 2018), turkey, and pigs (Perrin-Guyomard et al., 2016) as well as environmental samples (Zurfuh et al., 2016). What is more, *mcr-1* was found in a number of different plasmid replicon types, which further contributes the dissemination of resistance (Wang et al., 2018b; Ye et al., 2016).

Recently, several variants of *mcr-1*, which were also provide resistance to colistin, were discovered. One of the genes, *mcr-2*, was found in Belgium (Xavier et al., 2016). The gene was on a 35k base plasmid, and was more prevalent than *mcr-1* in colistin resistant bacteria. It was also reported that the plasmid harboring *mcr-2* has a higher conjugation rate than original *mcr-1* harboring plasmid (Xavier et al., 2016). Yin et al. (2017) reported the discovery of *mcr-3*, another plasmid encoded colistin resistance variant of *mcr-1*. After the first report, *mcr-3* gene was found in *Salmonella* isolates from human patients in Denmark (Litrup et al., 2017; Roer et al., 2017), and *E. coli* isolates from cattle in Spain (Hernandez et al., 2017). In both studies, there were isolates carried both *mcr-1* and *mcr-3* gene. Soon after the discovery of *mcr-3*, Carattoli et al. (2017) were discovered the existence of *mcr-4* in *Salmonella* and *E. coli* isolates. The gene was found in pathogens that were isolated in Italy, in Spain, and in Belgium in a time frame between 2013 to 2016 (Carattoli et al., 2017). Borowiak et al. (2017) defined a transposon associated resistance gene, *mcr-5*, in *Salmonella* Paratyphi B. Yang et al. (2016) identified *mcr-7* in a IncI2 type plasmid in a *K. pneumoniae* isolate, in China. Wang et al. (2018) discovered mcr-8 in NDM producing *K. pneumoniae*. Lastly, *mcr-9* was identified in a *Salmonella* Typhimurium, in the US (Carroll et al., 2019). To date, more than 40 *mcr* genes and variants has been described. Additionally, co-occurrence of different *mcr* genes has been reported in several studies (Garcia et al., 2018; Hernandez et al., 2017; Yang et al., 2016).

Studies showed the animal to human transmission of *mcr-1* (Hasman et al., 2016; Liu et al., 2016; Ye et al., 2016). Considering the rapid dissemination of the gene and the importance of colistin in medicine, public health implications are severe. Therefore, the purpose of this study was to determine the colistin resistance, and to investigate the existence of *mcr-1* to *mcr-5* genes and their variants by screening the *E. coli* strains previously isolated from food samples collected in Van and Ankara, Turkey.

Materials and Methods

In this study, 48 *E. coli* strains that were isolated in between 2011-2015 from foods in previous studies, were screened (Table 1). Detailed description of Van samples was given by Kyere et al. (2015).

Isolation and Conformation of E. coli

A total of 28 samples were isolated in Van, 2011, and the rest were isolated in Ankara (n:20), 2015. *E. coli* isolates had been held in -80˚C prior the experiments, and was a part of METU Food Safety Lab collection. In both experiments, subsampling and isolation were performed following the *E. coli* isolation method of the Food and Drug Administration (FDA) (Feng et al., 2011).

Table 1. Van *E. coli* isolates, their sources, isolation locations, and phenotypic resistances

METU IDs	Specific food source	City Collected	Antibiotics resistant to
MET-K1-001	Raw milk	Van	NR
MET-K1-002	Herby Cheese	Van	NR
MET-K1-003	Raw milk	Van	Gentamycin
MET-K1-004*	Raw milk	Van	NR
MET-K1-005	Raw milk	Van	NR
MET-K1-006	Raw patty meat	Van	NR
MET-K1-007	Chicken wings	Van	AMP, FOX, NA
MET-K1-008	Salted cheese	Van	NR
MET-K1-009	Chicken drumstick	Van	AMP, AMC, SF, SXT, TE, CN, S
MET-K1-010	Chicken drumstick	Van	AMP, AMC, SF, SXT, TE, CN, S
MET-K1-011	Chicken drumstick	Van	AMP, SF, SXT, NA, TE, CN, S
MET-K1-012	Turkey wings	Van	AMP, TE
MET-K1-013	Chicken drumstick	Van	NR
MET-K1-014*	Chicken drumstick	Van	AMC, SF, SXT, NA, TE, S, K
MET-K1-015*	Chicken drumstick	Van	AMP, AMC, SF, SXT, NA, TE, S
MET-K1-016	Chicken drumstick	Van	AMP, AMC, SF, SXT, NA, TE, S
MET-K1-017	Chicken drumstick	Van	AMP, AMC, SF, SXT, NA, TE
MET-K1-018	Chicken drumstick	Van	AMP, SF, SXT, NA, TE, S, K, C
MET-K1-019	Chicken drumstick	Van	AMP, KF, SF, SXT, NA, TE, CN, C
MET-K1-020	Chicken drumstick	Van	NR
MET-K1-021	Chicken drumstick	Van	AMP, SF, SXT, TE, S
MET-K1-022	Chicken drumstick	Van	AMP, KF, SF, SXT, NA, TE, S, K, C
MET-K1-023	Raw milk	Van	NR
MET-K1-024*	Herby cheese	Van	AMP, AMC, SF, NA, TE, S, C
MET-K1-025	Chicken drumstick	Van	AMP
MET-K1-026	Herby cheese	Van	NR
MET-K1-027	Raw milk	Van	NR
MET-K1-028	Chicken drumstick	Van	AMP, SF, S, C

* Strains that exhibited colistin resistance.

AMP: Ampicillin, AMC: Amoxycillin/Clavulanic Acid, SF: Sulfafurazole, SXT: Sulphamethoxazole/Trimethoprim, TE: Tetracycline, S: Streptomycin, K: Kanamycin, C: Chloramphenicol Cro: Ceftriaxone, Eft: Ceftiofur, , Imp: Imipenem, Ak: Amikacin, Cn: Gentamicin, , Cip: Ciprofloxacin, N: Nalidixic acid, Fox: Cefoxitin, Kf: Cephalothin, Etp: Ertapenem, NR: Not resistant

Briefly, food samples were collected from the markets in city center of Van and Ankara, for *E. coli* isolation (Table 1). The samples then were transferred to the laboratory at the Food Engineering Department of Yuzuncu Yil University in Van and Food Engineering Department of METU. 25 g of each food sample was weighted aseptically, and was transferred to 225 mL of buffered peptone water for enrichment of *E. coli*, followed by homogenization using a stomacher. Homogenates were incubated at 36˚C for 18 h for cell enrichment, then 20 µL of each homogenate was sub-cultured on Endo Agar. After 18-24 h of incubation at 36˚C in an incubator, suspected colonies were isolated for further confirmation. In case of Van samples, suspected *E. coli* isolates were stored at −20˚C in brain heart infusion (BHI) broth with 15% (v/v) glycerol prior to transport to the Food Engineering Department at Middle East Technical University (METU), Ankara, Turkey.

Determination of Antimicrobial Resistance

The existence of *mcr-1* to *mcr-5* in the isolates were carried out in two steps. Initially, phenotypic colistin resistance of *E. coli* isolates was determined by MIC. Then borderline susceptible and resistance isolates was screened by PCR for the

existence of *mcr-1, mcr-2, mcr-3, mcr-4, mcr-5* genes (Rebelo et al., 2018).

Phenotypic Characterization of Antimicrobial Resistance

MIC testing was conducted with broth dilution method based on CLSI standards and EUCAST criteria (CLSI, 2014; EUCAST, 2016). For this, series of concentrations of colistin were prepared from commercial lyophilized colistin sulphate (Sigma-Aldrich), and added to the cation adjusted Muller Hilton Broth (CAMHB) in the test tubes, respectively. To standardize the inoculum density, 0.5 McFarland standard was used as turbidity standard. For every test, negative control (without antibiotic) was prepared.

PCR Screening for mcr Genes

After the MIC testing, isolates with 2 mg/L or higher colistin resistance was checked for *mcr-1* to *mcr-5* genes. PCR analysis was done described by Rebelo et al. (2018) by using the primers *mcr-1*-F (5′-GGCACCAGTATTGGCCTGCT-3′), *mcr-1*-R (5′- CATATGCGCCACAATGTGTTG -3′), *mcr-2-F* (CAAGTGTGTTGGTCGCAGTT), *mcr-2-R* (TCTAGCCCGACAAGCATACC), mcr-3-F (AAATAAAAATTGTTCCGCTTATG), *mcr-3-R* (AATGGAGATCCCCGTTTTT), mcr-4-F (TCACTTTCATCACTGCGTTG), *mcr-4-R* (TTGGTCCATGACTACCAATG) *mcr-5-F* (ATGCGGTTGTCTGCATTTATC), *mcr-5-R* (TCATTGTGGTTGTCCTTTTCTG). *E. coli* NTCC 13846 strain was used as positive control for *mcr-1* gene.

Conditions of PCR was as follows; 94 °C for 15 minutes for initial denaturation, 94 °C for 30 seconds, 58 °C for 90 seconds, 72 °C for 60 seconds for 25 cycle, and a final elongation at 72 °C for 10 minutes. Samples were taken from Eppendorf tubes, placed into gel, and ran in electrophoresis for 45 minutes. The gel was placed into staining-bath contains Ethidium Bromide solution for 30 minutes, rinsed in water, and visualized in BioRad Molecular Imager Gel-Dox XR.

Results and Discussion

In total, 48 *E. coli* strains isolated from various sources in two different cities were tested. 20 samples were isolated from poultry in Ankara, 2015. Rest of the samples were isolated from various sources (17 poultry, 6 raw milk, 4 cheese, and 1 meat) in Van, 2011 (Table 1) (Kyere et al., 2015). 11 samples were susceptible to antibiotics. Two samples were resistant to one antibiotic while the rest of the samples showed multi-resistance of two or more antibiotics. Furthermore, three of the samples were resistant to eight antibiotics. All resistant isolates were collected from food samples and were non-pathogenic. Van isolates were discussed in detail by Kyere et al. (2015). Unlike Van isolates, only three of the Ankara isolates were susceptible to antibiotics, phenotypically (Table 2). The rest were resistant to two or more antibiotics. Furthermore, 10 of the isolates were resistant to five or more antibiotics. Antibiotic resistance of Ankara isolates further characterized with PCR screening of antibiotic resistance genes. Majority of the isolates carried more than one antibiotic resistance gene (Table 3). Nine of the Ankara isolates carried Extended Spectrum β-Lactamase (bla_{TEM1}) gene. Although three isolates showed phenotypic resistance, resistance genes could not been determined.

MIC results revealed that 4 isolates were resistant to colistin (MIC ≥ 2mg/L) (Table 4). 3 of these isolates were resistant to at least two more antibiotics. All of the colistin resistant samples were isolated in Van, 2011. Among them two of the samples were isolated from dairy product (one cheese and one raw milk), the other two were isolated from poultry products. However, PCR screening of isolates for *mcr* genes were negative. The results implied that resistant isolates had a different resistance mechanism (e.g. vertical gene transfer) than plasmid mediated resistance. Another possible explanation might be the existence of other *mcr* genes. In this study, we screened samples only for *mcr-1* to *mcr-5*. However, there are 4 more *mcr* genes *mcr-6*, *mcr-7*, *mcr-8*, *mcr-9*, and their variants confer colistin resistance. Previous studies showed that currently ratio of plasmid mediated colistin resistance, due to *mcr-1*, is relatively low. For example, El Garch et al. (2017), reported that only 45 of 292 colistin resistant isolates had the *mcr-1* gene. Negative results might be associated with this low ratio.

Although *mcr* genes and their variants has already spread to the ecosystem, prevalence of *mcr-1* in food samples relatively low compared to wastewater and animal feces. Chen et al. (2017) reported the prevalence of *mcr-1* gene in colistin resistant bacteria from food samples as 36%, while the prevalence of *mcr-1* was 51% and 71% in animal feces and water, respectively. This difference might be due to the variety of gene acquisition mechanisms. For example, while plasmids that carry *mcr-1* in *Salmonella* food isolates were conjugative, plasmids in *Salmonella* animal isolates were not. It was suggested that *mcr-1* acquisition in food *Salmonella* isolates probably due to a cross species conjugation from *E. coli*, instead of *Salmonella* animal samples (Cui et al., 2017).

Table 2. Ankara *E. coli* isolates, their sources, isolation locations, and phenotypic resistances

METU IDs	Specific food source	City Collected	Antibiotics resistant to
MET A1-001	Chicken Breast	Ankara	CRO, EFT, AMP, AMC, FOX, KF
MET A1-002	Chicken Thigh	Ankara	AMP, AMC, FOX, KF
MET A1-003	Chicken Wing	Ankara	SF, SXT, C, CN, K, S, CIP, N, AMP, T
MET A1-004	Chicken Thigh	Ankara	CIP, N
MET A1-005	Chicken Thigh	Ankara	SF, SXT CN, K, CIP, N, AMP, T
MET A1-007	Chicken Wing	Ankara	S, AMP, KF
MET A1-008	Chicken Breast	Ankara	SF, SXT, C, K, S, CIP, N, AMP, T, KF
MET A1-009	Chicken Rib	Ankara	KF
MET A1-010	Chicken Wing	Ankara	SF, SXT, C, CN, S, CIP, N, AMP, T
MET A1-011	Chicken Wing	Ankara	SF, S, CIP, N, T
MET A1-012	Chicken Wing	Ankara	SF, K, CIP, N, T
MET A1-014	Chicken Wing	Ankara	NR
MET A1-015	Chicken Thigh	Ankara	SF, SXT, C, S, CIP, N, AMP, T
MET A1-016	Chicken Wing	Ankara	SF, SXT, C, CN, S, CIP, N, AMP, T, KF
MET A1-017	Chicken Rib	Ankara	NR
MET A1-018	Chicken Thigh	Ankara	NR
MET A1-019	Chicken Wing	Ankara	SF, SXT, C, K, S, CIP, N, AMP. T, KF
MET A1-020	Chicken Thigh	Ankara	SF, T
MET A1-021	Chicken Thigh	Ankara	CIP, N, ETP

AMP: Ampicillin, AMC: Amoxycillin/Clavulanic Acid, SF: Sulfafurazole, SXT: Sulphamethoxazole/Trimethoprim, TE: Tetracycline, S: Streptomycin, K: Kanamycin, C: Chloramphenicol Cro: Ceftriaxone, Eft: Ceftiofur, Imp: Imipenem, Ak: Amikacin, Cn: Gentamicin, , Cip: Ciprofloxacin, N: Nalidixic acid, Fox: Cefoxitin, Kf: Cephalothin, Etp: Ertapenem, NR: Not resistant

Table 3. Specific source and resistance profiles of *E. coli*, isolated in Ankara, 2015

Isolate ID	Specific Source	Phenotypic Resistance	Resistance genes
MET A1-001	Chicken Breast	CroEftAmpAmcFoxKf	*bla*$_{CMY-2}$
MET A1-002	Chicken Thigh	AmpAmcFoxKf	ND
MET A1-003	Chicken Wing	SfSxtCCnKSCipNAmpT	*bla*$_{TEM-1}$, *flo, aadA1, aadA2, aphA1-Iab, dhfrI, tetA, sul1*
MET A1-004	Chicken Thigh	CipN	ND
MET A1-005	Chicken Thigh	SfSxtCnKCipNAmpT	*bla*$_{TEM-1}$, *aadA1, aadA2, aphA1-Iab, tetA*
MET A1-007	Chicken Wing	SAmpKf	*bla*$_{TEM-1}$, *aadA1*
MET A1-008	Chicken Breast	SfSxtCKSCipNAmpTKf	*bla*$_{TEM-1}$, *cat1, aphA1-Iab*
MET A1-009	Chicken Rib	Kf	ND
MET A1-010	Chicken Wing	SfSxtCCnSCipNAmpT	*bla*$_{TEM-1}$, *flo, aadA1, dhfrI, tetA, sul1*
MET A1-011	Chicken Wing	SfSCipNT	*tetA*
MET A1-012	Chicken Wing	SfKCipNT	*aadA1, aadA2, tetA*
MET A1-014	Chicken Wing	Susceptible	*aadA2*
MET A1-015	Chicken Thigh	SfSxtCSCipNAmpT	*bla*$_{TEM-1}$, *flo, aadA1, aphA1-Iab, tetA, aadA2, sul1*
MET A1-016	Chicken Wing	SfSxtCCnSCipNAmpTKf	*flo, aadA1, aphA1-Iab, dhfrI, tetA, sul1*
MET A1-017	Chicken Rib	Susceptible	*bla*$_{TEM-1}$
MET A1-018	Chicken Thigh	Susceptible	*bla*$_{TEM-1}$, *aphA1-lab*
MET A1-019	Chicken Wing	SfSxtCKSCipNAmpTKf	*bla*$_{CMY-2}$, *cat1*
MET A1-020	Chicken Thigh	SfT	*tetA*
MET A1-021	Chicken Thigh	CipNEtp	*bla*$_{TEM-1}$, *aadA2*

Cro: Ceftriaxone, Eft: Ceftiofur, Sf: Sulfafurazole, Sxt: Sulphamethaxazole/trimethoprim, C: Chloramphenicol, Imp: Imipenem, Ak: Amikacin, Cn: Gentamicin, K: Kanamycin, S: Streptomycin, Cip: Ciprofloxacin, N: Nalidixic acid, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, T: Tetracycline, Fox: Cefoxitin, Kf: Cephalothin, Etp: Ertapenem, ND: Not determined

Table 4. Colistin resistant *E. coli* isolates, their sources, other resistances, and MIC values

Isolate ID	Specific Source	Antibiotics resistant to	MIC
MET-K1-004	Raw milk	Susceptible	≥2mg/L
MET-K1-014	Chicken drumstick	AMC, SF, SXT, NA, TE, S, and K.	≥2mg/L
MET-K1-015	Chicken drumstick	AMP, AMC, SF, SXT, NA, TE, and S.	≥2mg/L
MET-K1-024	Herby cheese	AMP, AMC, SF, NA, TE, S, and C.	≥2mg/L

AMP: Ampicillin, AMC: Amoxycillin/Clavulanic Acid, SF: Sulfafurazole, SXT: Sulphamethoxazole/Trimethoprim, TE: Tetracycline, S: Streptomycin, NA: Nalidixic Acid, K: Kanamycin, C: Chloramphenicol

Nearly all plasmid mediated colistin studies showed that resistant isolates had multidrug resistance (MDR) including Extended Spectrum β-Lactamase (*bla*$_{ESBL}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, *bla*$_{TEM1}$) (Li et al., 2016; Zeng et al., 2016; Zurfuh et al., 2016), Carbapanemases (Sun et al., 2016), and New Delhi Metallo β-Lactamase (*bla*$_{NDM-5}$, *bla*$_{NDM-9}$) (Borowiak et al., 2017; Yao et al., 2016). While our multidrug resistant isolates did not have *mcr* genes, the reports highlight the possible significant challenge in treatment of MDR infections.

Conclusion

MDR pathogens can be associated with increased treatment durations to life threatening conditions. There are currently a few antibiotics deployed against MDR infections. Colistin is a last line of defense against MDR Gram negative pathogens. Plasmid mediated colistin resistance disseminated rapidly between the several sources, bacterial species and continents. The absence of colistin treatment might have severe consequences. Previous studies showed the importance of farm animals and foods as gene transfer media. As a result, studies should focus on reducing the unnecessary antibiotic usage, and alternative treatments, especially in veterinary medicine.

Foodborne pathogen infections are still a serious threat for human health. In this regard, antibiotic resistance of foodborne pathogens is a critical concern. Colistin has been used for treatment of farm animals across all European countries. Considering the fact that plasmid mediated colistin resistance has spread globally, our results do not show or imply that *mcr* genes was not present in Van and Ankara, Turkey. Further studies with more isolates should be conducted. More sources such as hospitals, wastewater, and poultry farms should be monitored.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Tulumba tatlısı yapımında çeşitli kavurga unların kullanımı ve bazı karakteristik özelliklerin belirlenmesi

İlyas Çelik, Gözde Tümer

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Pamukkale Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Denizli, Türkiye

ORCID IDs of the authors:
İ.Ç. 0000-0002-8434-8797
G.T. 0000-0002-7899-6237

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Correspondence: İlyas ÇELİK
E-mail: ilyasc@pau.edu.tr

ÖZ

Bu çalışmada, kavurga buğday (KB), kavurga arpa (KA), %50 kavurga arpa+ %50 kavurga buğday (KAB) unları, standart una %5, %10 ve %20 oranlarında ikame edilerek tulumba tatlıları üretilmiştir. Tüm tulumba tatlılarında bazı fiziksel, kimyasal ve duyusal özellikler belirlenmiştir. Fiziksel özelliklerde; tatlılarda en düşük şurup çekme miktarı KAB ve %20 ikame oranında, en yüksek spesifik hacim KA ikameli, en düşük boy/en oranı KB ikameli tatlıda belirlenmiştir (p<0.05). Kimyasal özelliklerde; en düşük nem miktarı kontrol örneğinde görülürken, artan ikame oranlarıyla birlikte yağ ve protein içerikleri kontrole göre sırasıyla %15.39, %8.64 azalmıştır (p<0.05). Mineral madde bakımından magnezyum içeriği %20 ikamede, sodyum içeriği %5 ikamede en yüksek değerler vermiştir (p<0.05). Duyusal değerlendirmede en düşük puanlama %20 ikame oranlı tatlılarında elde edilmiştir. Tulumba tatlılarda artan ürün çeşitliliği, elde edilen sonuçlar dikkate alındığında % 10'a kadar kavurga unları tulumba tatlısı yapımında kullanılabilinir.

Anahtar Kelimeler: Kavurga unu, Tulumba tatlısı, Mineral madde, Kalori

ABSTRACT

Use of various roast (kavurga) flours in production of tulumba dessert and some characteristics of desserts

In this study, wheat flour was substituted with either wheat roast (kavurga) (KB), barley kavurga (KA) or an equal mixture of barley and wheat kavurga (KAB) flours by 5, 10 and 20% in the production of tulumba desserts. Some physical, chemical and sensory properties of tulumba desserts were determined. In terms of the physical properties, the lowest syrup uptake was determined in the desserts with KAB at a substitution rate of 20%. The highest specific volume was found in the desserts with KA while the lowest length/width ratio was in the KB substituted desserts (p<0.05). Among chemical properties, the lowest moisture content was obtained in control desserts containing no kavurga flour, and increasing substitution rate reduced the fat and protein contents of tulumba desserts by 15.39% and 8.64%, respectively (p<0.05). In addition, calorie values of desserts with kavurga flours decreased as substitution rate increased and the lowest was at the substitution rate of 20% (p<0.05). The magnesium content of tulumba desserts was the highest at a 20% substitution rate while the highest sodium content was at a 5% substitution rate (p <0.05). In sensory evaluation, the lowest scoring was obtained in desserts with a 20% substitution rate. Results of this study showed an increased product variety in tulumba desserts and indicated that up to 10% of kavurga flour can be used in the production of tulumba desserts.

Keywords: Kavurga flour, Tulumba dessert, Mineral subtance, Calorie

Giriş

Kavurga; Türk kültürüne ait, tahıldan yapılan geleneksel ve yöresel bir kuruyemiş çeşididir. Unutulmaya yüz tutmuş Türk kuruyemişlerinden olan kavurga, genellikle evlerde yıkanmış tahılların yanmaz bir tava ya da sac üzerinde kavrulması ile yapılmaktadır. Özellikle tok tutması, hazmının kolay olması, protein yönünden zengin oluşu ve vücudu soğuktan koruması gibi özellikleriyle daha çok kışın sert ve uzun geçtiği doğu ve iç Anadolu gibi karasal bölgelerde bilinmektedir. Ayrıca kavrulmadan gelen lezzetiyle birlikte kilo problemi olanlar için de yüksek lif içeriğinden dolayı ideal bir besindir (Karaoğlu ve Kotancilar, 2005; Sevimli ve Sönmezdağ, 2017).

Kavurma işlemi, üründe renk özelliklerini geliştirme, raf ömrünü uzatma, lezzetini arttırma gibi olumlu etkiler sağlarken, tahıl ve bakliyatların besleyici olmayan özelliklerinin azaltılmasına da yardımcı olmaktadır. Bir diğer olumlu özelliği ise, işlem sırasında oluşan nişastanın jelatinizasyonu ve protein denatürasyonu ile ürünün sindirilebilirliğinin artmasıdır (Sharma ve ark., 2011).

Türk mutfak kültüründe tatlılar Osmanlı'dan günümüze her zaman önemli bir yere sahip olmuştur. Hamur tatlıları, mayalı (lokma) ya da mayasız hamurların kızartılması (tulumba) veya fırınlanması (baklava, revani) sonrasında genellikle şerbetlendirilmesi ile tüketilmektedir (Çağlar ve Özaltın, 2013; Ertaş ve Karadağ, 2013)

Tulumba tatlısı, hamur işi tatlılar arasında ticari olarak sürümü yüksek, üretim maliyeti düşük ve halkımız tarafından sevilerek tüketilen geleneksel tatlı çeşitlerimizden biridir. Üretiminde; pişirilerek hazırlanmış hamura kademeli yumurta homojen bir şekilde yedirilerek yapılmakta ve elde edilen hamur tulumba kalıplarından belirli boyutlarda soğuk yağ içerisine bırakılmaktadır. Kızartılan tulumbalar da soğuk şerbette beklettikten sonra süzülmekte ve servis edilmektedir. Gelenekselin dışında eklenen katkı maddeleri ile farklı formülasyonların oluşturulması sağlanarak tulumba tatlısının tat ve görünüşünde birçok değişiklik elde edilebilmektedir (Doğan ve Yurt, 2002; Özen, 2006; Özen ve ark., 2009).

Tulumba tatlısı, içerdiği yüksek şeker ve yağ içeriği düşünüldüğünde yüksek kalorili tatlılardan biridir. Şeker içeriği fazla olan besinlerin tüketimi, diyetle alınan enerji miktarını arttırmakta ve yetersiz fiziksel aktivitenin de eklenmesiyle alınan fazla kalori, kilo artışına neden olmaktadır. Bu durumun obezite, kalp- damar hastalıkları, diyabet, hipertansiyon ve kanser gibi hastalıklar için önemli bir risk faktörü olduğu bilinmektedir (Akyol ve ark., 2008; Anon, 2020).

Bu çalışma ile arpa, buğday ve her ikisini karışımından elde edilen kavurga unların tulumba tatlı üretiminde kullanılması, ürün çeşitliliğin arttırılması, bazı fiziksel ve kimyasal, kalori değerlerindeki değişim ve duyusal özelliklerin belirlenmesi, en uygun uygulama çeşidi ve ikame oranın belirlenmesi amaçlanmıştır.

Materyal ve Metot

Materyal

Çalışmada tulumba tatlısı üretimi için; baklavalık un (protein; %11.24, kül; %0,50, yağ; %1.32, yaş öz; %35.25, Zeleny sediment; 30.25 mL), içilebilir nitelikte su, ayçiçeği yağı, tuz, sitrik asit, katı yağ ve yumurta; şerbet yapımı için; içilebilir nitelikte su, sitrik asit ve şeker; ikame olarak kullanılan kavurga unu üretimi için ise yerel pazardan temin edilen arpa ve buğday kullanılmıştır.

Metot

Denemenin kuruluşu

Çalışma, tulumba üretiminde kullanılan standart baklavalık una; kavurga buğday (KB), kavurga arpa (KA) ve %50 KA + %50 KB (KAB) unlarının her birinden %5, %10 ve %20 oranlarında ikame edilerek sonuçların kontrol örneğiyle karşılaştırılması şeklinde oluşturulmuştur. Deneme 2 tekerrürlü olarak yürütülmüştür.

Kavurga unu üretimi

Arpa ve buğdaylar görünür yabancı maddelerden temizlendikten sonra, ön denemelerle belirlenen buğday için 120-140°C sıcaklıkta 7 dakika, arpa için ise daha yüksek sıcaklıkta 130-145°C sıcaklıkta 8 dakika olacak şekilde pilot tipi kahve kavurma makinesinde (Has Garanti, İzmir) kavurma işlemi gerçekleşmiştir. Kavrulan arpa ve buğdaylar laboratuar tipi değirmende 30 saniye yavaş, 1,5 dakika hızlı devirde öğütme işlemine tabi tutulmuş, 500 µm tel elekten geçirilerek üretimde kullanılmak üzere cam kavanozda buzdolabı şartlarında saklanmıştır.

Tulumba tatlısı üretimi

Tulumba tatlılarının üretimi için, kontrol örneği ve kavurga unu ikameli örneklerde kullanılan formülasyon 100 g buğday unu esas alınarak oluşturulmuştur. Kullanılan kontrol örneği formülasyonuna göre 100 g buğday unu, 64.8 g yumurta, 2.8 g katı yağ, 1.08 g sitrik asit, 2.1 g tuz ve 170 mL su kullanılmıştır. Kavurga unu ikameli örneklerde ise; aynı formülasyonda kullanılan standart una % 5, % 10 ve % 20 oranlarında KB, KA ve KAB ikameleri yapılmıştır.

Şerbetin hazırlanması; 500 mL suya 1000 g şeker ilavesi yapılarak kaynamaya bırakılmış, kaynayan suya 0.8 g sitrik asit ilavesi yapılarak 4 dakika daha kısık ateşte bekletilip, oda sıcaklığında soğutulmuştur.

Üretimde öncelikle; tuz, sitrik asit, katı yağ suya ilavesi sonra kaynatılmış, kaynamış suya un ilavesi yapılarak karıştırılmak

suretiyle 7 dakika pişirme işlemi ile katı lapa haline getirilmiştir. Sıcaklığı 45-50°C dolaylarına düşen hamura homojen bir şekilde teker teker yumurta ilavesi yapılmış ve tahta kaşıkla yumurtanın yedirilmesi sağlanmıştır. İstenen yarı akıcı yapı oluşması sonrası içi yağlanan tulumba kalıbına doldurularak soğuk yağın içerisine şekilli olarak 3.0 - 3.5 cm uzunluğunda parçalar halinde bırakılmıştır (Özen ve ark., 2009). İlk 10 dakika sonrasında yağ sıcaklığı 130°C sıcaklığına ulaşmış ve işlem sonrasında sıcaklık 180°C dolaylarında olmuştur. Kızartma işlemi toplam 20 dakika sürmüştür. Kızartılmış tulumba tatlandırılması için sıcakken direkt soğuk şerbetin içerisinde bekletilerek şerbetlendirilmiştir.

Fiziksel analizler

Yağ çekme yüzdesinin belirlenmesi için, kızartılmadan önce hamur kütlesi ve kızartılmadan sonra tatlıların kütlesi (g); şurup çekme yüzdesinin belirlenmesinde, kızartmadan sonra tatlıların kütlesi ve şuruplandıktan sonra tatlıların kütlesi (g) dikkate alınmıştır. Kızartılan ürünler oda sıcaklığına geldikten sonra kitle (g) ölçümleri yapılmış ve kolza tohumu ile yer değiştirme esasına dayanarak hacim (mL) değerleri belirlenmiştir. Bulunan değerlerin oranlanmasıyla spesifik hacim (mL/g) değerleri elde edilmiştir. Tatlıların çap ile boy/en oranının hesaplanmasında dijital mikrometre kullanılmıştır (Elgün ve ark., 2012).

Kimyasal analizler

Üretilen tulumbalarda; nem, 550 ^{0}C'de yakma ile %kül miktarı, soxhalet ekstraksiyon metodu ile %yağ miktarı (Elgün ve ark., 2012), protein miktarı Kjeldahl metodu kullanılarak ACCC 46-12 (1995)'e bağlı olarak belirlenmiştir. Örneklerin hepsinde azot çeviri faktörü 6.25 olarak alınmıştır. (Elgün ve ark., 2012).

Elde edilen verilerden karbonhidrat değerleri hesaplanmış ve tatlıların enerji değerleri, Enerji (kkal/100 g) = 4 (% karbonhidrat + % protein) + 9 (% yağ) formülüne göre hesaplanmıştır (Karaağaoğlu ve ark., 2008).

Mineral madde analizinde analiz için 0.5g tartılan numunelere 10 mL HNO3 eklenerek mikrodalga cihazında yaş yakma işlemi gerçekleştirilmiştir. Sonrasında örnekler 50 mL'lik balon jojeye alınarak mavi bant filtre cihazından süzülmüş ve geri kalan hacim saf su ile tamamlanmıştır. Elde edilen süzüntüler ICP-OES cihazına verilerek P (µg/g), Mg (µg/g), Ca (µg/g), Na (µg/g), K (µg/g), Zn (µg/g) ve Fe (µg/g) mineraller belirlenmiştir (Kaçar ve İnal, 2008).

Duyusal analiz

Tatlılar, renk, koku, gözenek yapısı, tekstür, çiğnenebilirlik, lezzet ve genel beğeni özellikleri açısından Gıda Mühendisliği Bölümü öğrencileri ve öğretim elemanları arasından eğitilmemiş panelistlerce 1 (Aşırı kötü) – 7 (Mükemmel) kutucuklarından oluşan hedonik skala kullanılarak değerlendirilmiştir (Onoğur-Altuğ ve Elmacı, 2011).

İstatistiksel analizler

Elde edilen bulgular IBM SPSS Statistics 22 programı ile analiz edilmiştir. Sonuçlar arasındaki önemlilik farklılık LSD testi ile ortaya konulmuştur (Arbuckle, 2014).

Bulgular ve Tartışma

Tulumba tatlılarında belirlenen yağ ve şurup çekme yüzdeleri, spesifik hacim ve boy/en değerleri Tablo 1'de gösterilmiştir. Kavurga unu çeşidi ve ikame oranı tulumba tatlısında yağ çekme yüzdesini istatistiksel açıdan önemli derecede etkilemezken artan ikame oranlarıyla birlikte bir miktar artış görülmüştür. En yüksek şurup çekme yüzdesi KB ikameli tatlılarda gözlenirken, üç kavurga unu çeşidinde de artan ikame oranı şurup çekme yüzdesini düşürmüştür. Bu sonuç tüketici açıdan daha az şeker tüketimi anlamına gelmekte ve alınan günlük kalori değerinde de düşüşe neden olacaktır. Yüzde 20 ikame oranı en düşük değer elde edilmiş ve kontrole göre aralarındaki fark istatistiksel açıdan önemli bulunmuştur (p<0.05). İkame oranı spesifik hacimde istatistiksel olarak önemli bir fark oluşturmamış, kullanılan kavurga unu çeşidi bakımından KA tulumba tatlılarının spesifik hacimleri diğer katkılara göre istatistiksel olarak önemli ölçüde daha yüksek bulunmuştur (p<0.05). En düşük boy/en oranı ise KB ikameli örneklerde belirlenmiştir (p<0.05).

Doğan ve Yurt (2002) yaptıkları çalışmada, tulumba tatlısının üretiminde % 4.5 civarında kullanılan yağsız soya unu kullanımının tulumba tatlısının hacmini % 3' e kadar arttırdığını belirtmişlerdir. Ayrıca gluten ilavesinin de hacim artışına neden olduğunu ve bu durumun undaki protein miktarının su kaldırma kapasitesini arttırmasıyla ilgili olduğunu da vurgulamışlardır.

Tulumba tatlılarında belirlenen yüzdece nem, protein, yağ ve kül miktarları Tablo 2' de gösterilmiştir. Kullanılan kavurga unu çeşidi tatlıların nem miktarlarında istatistiksel olarak önemli bir fark oluşturmamış, artan ikame oranıyla nem miktarlarında artış görülmüş ve % 10 ile % 20 ikamelerde kontrole göre nem değerleri belirgin derecede yüksek bulunmuştur (p<0.05). Tatlıların protein miktarlarında kullanılan kavurga unu çeşidi önemli bir fark oluşturmazken, artan ikame oranıyla birlikte protein miktarlarında düşüş belirlenmiştir. En düşük protein miktarı % 20 ikamede görülürken kontrolle

aralarındaki fark önemlidir (p<0.05). Artan ikame oranıyla birlikte tulumba tatlılarının yağ miktarlarında düşüş gözlenmiş, en yüksek yağ içeriği kontrol örneğinde belirlenirken diğer ikame oranlarıyla arasındaki fark istatistiksel olarak önemli bulunmuştur (p<0.05). Tatlıların kül içeriklerinde, kullanılan kavurga unu çeşidi ve ikame oranı istatistiksel olarak önemli bir fark oluşturmamıştır. Tatlıların kalori değerleri hesaplandığında en yüksek değer kontrol tulumba elde edilirken, artan ikameyle birlikte kalori değerlerinde düşüş görülmüştür. En düşük değer belirgin şekilde %20 ikameli tulumbada elde edilmiştir (p<0.05).

Üretilen tulumba tatlılarında belirlenen mineral madde analiz sonuçları Tablo 3'de verilmiştir. Kullanılan kavurga unu çeşidi tatlıların fosfor (P), magnezyum (Mg), kalsiyum (Ca), sodyum (Na), potasyum (K), çinko (Zn) ve demir (Fe) içeriklerinde istatistiksel olarak önemli bir fark oluşturmamıştır (p<0.05). En yüksek P, Mg, K, Zn ve F değerleri KB ikameli örneklerde görülmüş, Na içeriği ise en yüksek KA ikameli örneklerde belirlenmiştir. Artan ikame oranı yalnızca Mg ve Na içeriklerindeki artış istatistiksel olarak önemli derecede olmuştur. Mg değeri kontrole göre % 20 ikamede en yüksek bulunmuştur. Mg elementi, kasların güçlenmesi, protein sentezi ve enzim sistemi aktivitesinde, hücrelerin büyümesinde ve yenilenmesinde önemli rol oynamaktadır (Elin,1988). Na değeri ise en yüksek % 5 ikamede görülürken en düşük kontrol örneğinde belirlenmiştir (p<0.05).

Tablo 1. Farklı kavurga unu çeşidi ve ikame oranı kullanılarak hazırlanan tulumba tatlılarında fiziksel analiz sonuçları*

Table 1. Physical analysis results of tulumba desserts with different types of kavurga flour and substitution rate

KavurgaUnu Çeşidi	Yağ Çekme (%)	Şurup Çekme (%)	Spesifik Hacim (mL/g)	Boy/En
KB	71.83 ±5.05^{a}	121.76 ±17.1^{5a}	2.28 ±0.28^{b}	1.70 ±0.11^{b}
KA	70.40 ±3.18^{a}	111.65 ±10.78^{ab}	2.72 ±0.29^{a}	1.90 ±0.11^{a}
KAB	72.85 ±8.16^{a}	98.24 ±14.90^{b}	2.27 ±0.1^{7b}	1.86 ±0.13^{a}
İkame Oranı (%)				
0	69.94 ±6.75^{a}	121.42 ±9.00^{a}	2.35 ±0.17^{a}	1.76 ±0.17^{a}
5	71.32 ±4.06^{a}	116.52 ±22.85^{ab}	2.45 ±0.18^{a}	1.82 ±0.20^{a}
10	70.37 ±4.35^{a}	104.95 ±10.67^{ab}	2.38 ±0.51^{a}	1.83 ±0.08^{a}
20	75.15 ±6.8^{3a}	99.30 ±15.48^{b}	2.50 ±0.39^{a}	1.87 ±0.10^{a}

*Aynı sütunda farklı harfle işaretlenmiş değerler birbirinden farklıdır (p <0.05).
KB: Kavurga buğday unu**, KA:** Kavurga arpa unu, **KAB:** %50KA+%50KB unu

Tablo 2. Farklı kavurga unu çeşidi ve ikame oranı kullanılarak hazırlanan tulumba tatlılarında kimyasal ve kalori analiz sonuçları*

Table 2. Chemical analysis and ,calori results of tulumba desserts with different types of kavurga flour and substitution rate

Kavurga Unu Çeşidi	Nem (%)	Protein (%)	Yağ (%)	Kül (%)	Kalori (kcal)
KB	15.14 ±3.17^{a}	9.90 ±0.88^{a}	33.64 ±3.75^{a}	1.353 ±0.074^{a}	502.36 ±29.46^{a}
KA	14.09 ±2.51^{a}	9.97 ±0.54^{a}	34.87 ±3.40^{a}	1.423 ±0.073^{a}	512.30 ±24.05^{a}
KAB	16.36 ±1.32^{a}	9.48 ±0.37^{a}	34.71 ±3.64^{a}	1.335 ±0.053^{a}	502.77 ±22.15^{a}
İkame Oranı (%)					
0	13.07 ±2.21^{b}	10.19 ±0.46^{a}	38.52 ±0.98^{a}	1.389 ±0.065^{a}	534.76 ±10.83^{a}
5	14.85 ±2.64^{ab}	10.00 ±0.78^{ab}	33.76 ±3.02^{b}	1.398 ±0.067^{a}	494.93 ±20.57^{b}
10	16.39 ±2.03^{a}	9.60 ±0.59^{ab}	32.77 ±2.94^{b}	1.361 ±0.051^{a}	492.85 ±21.40^{b}
20	16.48 ±2.06^{a}	9.31 ±0.40^{b}	32.59 ±3.02^{b}	1.333 ±0.108^{a}	491.70 ±22.15^{b}

*Aynı sütunda farklı harfle işaretlenmiş değerler birbirinden farklıdır (p <0.05).
KB: Kavurga buğday unu, **KA:** Kavurga arpa unu, **KAB:** %50KA+%50KB unu

Tulumba tatlılarında yapılan duyusal değerlendirme sonuçları Tablo 4' de verilmiştir. Tatlılarda yapılan renk, koku, gözenek, kırılganlık, çiğnenebilirlik, lezzet ve genel beğeni değerlendirmesinde artan ikame oranı genel olarak puanlamayı düşürmüş, % 20 ikame en düşük puanları alırken, kontrol örneği ile aralarındaki fark istatistiksel olarak önemli bulunmuştur (p<0.05). Kullanılan kavurga unu çeşidi bakımından koku, kırılganlık, çiğnenebilirlik ve lezzet değerlendirmesinde en yüksek puanlamayı KAB tulumba alırken, genel beğeni açısından KA tulumba en yüksek puanlamayı almıştır (p<0.05). En düşük puanlamalar ise koku, kırılganlık, çiğnenebilirlik, lezzet ve genel beğeni bakımından KB ikameli örnekler, diğer tulumbalarla aralarındaki fark istatistiksel açıdan önemli bulunmuştur (p<0.05).

Tablo 3. Farklı kavurga unu çeşidi ve ikame oranı kullanılarak hazırlanan tulumba tatlılarında mineral madde analiz sonuçları.

Table3. Mineral subtance analysis results of tulumba desserts with different types of kavurga flour and substitution rate

Kavurga Unu Çeşidi	P (µg/g)	Mg (µg/g)	Ca (µg/g)	Na (µg/g)	K (µg/g)	Zn (µg/g)	Fe (µg/g)
KB	1365.82 ±18^{a}	581.03 ±38.53^{a}	766.91 ±74^{a}	3989.94 ±1318^{a}	2138.25 ±369^{a}	21.92 ±4.49^{a}	16.65 ±4.60^{a}
KA	1293.16 ±194^{a}	570.42 ±41.18^{a}	766.25 ±81^{a}	5001.11 ±2647^{a}	2045.39 ±336^{a}	19.22 ±3.03^{a}	15.08 ±2.65^{a}
KAB	1286.10 ±171^{a}	569.12 ±34.63^{a}	756.63 ±74^{a}	3945.22 ±1349^{a}	2076.24 ±335^{a}	19.33 ±2.02^{a}	14.86 ±3.07^{a}
İkame Oranı (%)							
0	1256.20 ±260^{a}	556.24 ±41.7^{1b}	792.53 ±122^{a}	3091.02 ±2401.51^{b}	1972.14 ±573^{a}	18.19 ±0.36^{a}	16.45 ±4.21^{a}
5	1328.49 ±129^{a}	558.48 ±20.54^{ab}	767.75 ±38^{a}	5664.51 ±2399.92^{a}	2093.95 ±150^{a}	22.20 ±3.29^{a}	15.28 ±1.75^{a}
10	1317.25 ±201^{a}	573.07 ±37.84^{ab}	746.54 ±68^{a}	4209.72 ±581.34^{ab}	2115.32 ±304^{a}	19.77 ±3.96^{a}	14.85 ±3.97^{a}
20	1358.17 ±116^{a}	606.30 ±27.49^{a}	746.24 ±41^{a}	4283.10 ±279.99^{ab}	2165.09 ±215^{a}	20.47 ±4.22^{a}	15.55 ±4.19^{a}

*Aynı sütunda farklı harfle işaretlenmiş değerler birbirinden farklıdır (p <0.05).
KB: Kavurga buğday unu, **KA:** Kavurga arpa unu, **KAB:** %50KA+%50KB unu

Tablo 4. Farklı kavurga unu çeşidi ve ikame oranı kullanılarak hazırlanan tulumba tatlılarında yapılan duyusal analiz sonuçları*

Table 4. Sensory analysis results of tulumba desserts with different types of kavurga flour and substitution rate

Kavurga unu çeşidi	Renk (1-7 P)	Koku (1-7 P)	Gözenek (1-7 P)	Kırılganlık (1-7 P)	Çiğnenebilirlik (1-7 P)	Lezzet (1-7 P)	Genel Beğeni (1-7 P)
KB	4.30 ±0.58^{a}	4.39 ±0.29^{b}	4.46 ±0.53^{a}	4.44 ±0.35^{b}	4.50 ±0.38^{c}	4.46 ±0.32^{b}	4.48 ±0.35^{b}
KA	4.48 ±0.76^{a}	4.46 ±0.28^{b}	4.48 ±0.63^{a}	4.60 ±0.41^{ab}	4.81 ±0.39^{ab}	4.88 ±0.47^{a}	4.84 ±0.50^{a}
KAB	4.43 ±0.63^{a}	4.76 ±0.27^{a}	4.73 ±0.47^{a}	4.79 ±0.34^{a}	4.86 ±0.40^{a}	4.89 ±0.41^{a}	4.70 ±0.44^{ab}
İkame Oranı (%)							
0	4.90 ±0.33^{a}	4.75 ±0.21^{a}	5.05 ±0.14^{a}	4.80 ±0.40^{a}	4.83 ±0.48^{a}	4.98 ±0.31^{ab}	4.90 ±0.25^{a}
5	4.87 ±0.35^{a}	4.63 ±0.23^{a}	4.83 ±0.19^{a}	4.87 ±0.27^{a}	5.02 ±0.28^{a}	5.07 ±0.29^{a}	4.98 ±0.34^{a}
10	4.28 ±0.33^{b}	4.50 ±0.36^{ab}	4.42 ±0.41^{b}	4.58 ±0.21^{a}	4.67 ±0.27^{ab}	4.70 ±0.25^{b}	4.68 ±0.15^{a}
20	3.55 ±0.24^{c}	4.27 ±0.27^{b}	3.92 ±0.45^{c}	4.18 ±0.23^{b}	4.38 ±0.35^{b}	4.22 ±0.33^{c}	4.12 ±0.40^{b}

*Aynı sütunda farklı harfle işaretlenmiş değerler birbirinden farklıdır (p <0.05).
KB: Kavurga buğday unu, **KA:** Kavurga arpa unu, **KAB:** %50KA+%50KB unu

Sonuç

Kavurga, ülkemizde daha çok Nevşehir, Aksaray, Malatya, Çankırı, Emirdağ, Sivas, Yozgat, Kayseri, Erzurum, Kars gibi Anadolu'da kışın uzun ve sert geçtiği bölgelere özgü bir yemiş türüdür. Tok tutması, proteince zengin olması ve kavurmadan gelen lezzetiyle alternatif bir çerez gıdadır.

Kavurga çeşit unları kullanılarak tulumba tatlısına yeni özellikler kazandırılması, fiziksel, kimyasal ve duyusal özellikleri ortaya konulan bu çalışmayla, tulumba tatlısına eklenen kavurga unuyla birlikte tatlıların (artan katkı oranına paralel olarak) standart tulumba tatlısından daha az şerbet çektiği ve yağ içerdiği belirlenmiştir. Aynı zamanda artan kavurga unu kullanım oranıyla tulumba tatlılarının kalori değerleri de%8,05 azalma göstermiştir. Yine artan ikame oranıyla tatlıların magnezyum içeriği kontrol örneğine göre %8,99 artmış, özellikle %20 ikamede bu artış belirgin olarak görülmüştür. Kavurga unlu tulumba tatlılarının, duyusal değerlendirilmede düşük puanlandırılmasının nedeninin tulumba tatlısında alışık olunmayan duyusal özelliklerden kaynaklandığı düşünülmektedir. Sonuç olarak, kavurga unlu tulumba tatlısı da dahil, birçok unlu mamül ürünlerin hazırlanmasında formülasyona dahil edilip kullanılabileceği düşünülmektedir.

Etik Standart ile Uyumluluk

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Investigation of the physicochemical, nutritional properties and antioxidant activities of commercial and traditional pomegranate molasses samples

Sema Özmert Ergin

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Mehmet Akif Ersoy University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Burdur, Turkey

ORCID IDs of the authors:
S.Ö.E. 0000-0001-7742-8185

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Correspondence: Sema ÖZMERT ERGİN
E-mail: sozmert@mehmetakif.edu.tr

ABSTRACT

Pomegranate molasse is a pomegranate product which has become widespread in recent years and can be added to some foods with its unique taste and aroma. In this study, three commercial sold in the market and three pomegranate molasses made by traditional method taken from Burdur (Turkey) public market were investigated. The pH, water soluble dry matter results of the samples were found to comply with the standards. Viscosity values range from 191.35 to 13000 mPa. According to the color analysis, it was detected that the color of the traditional pomegranate molasses was darker than the commercial ones. The phenolic content of samples varies between 10.40-931.56 mg GAE/L. The highest quantity of antioxidant substance was found in the commercial C2 sample. Total aerobic mesophilic bacteria count was carried out to evaluate the hygienic quality of pomegranate molasses samples. It was seen that the most bacteria number was at C1 commercial pomegranate molasse. It was concluded that it would be beneficial to elaborate pomegranate molasse standards and in traditional production, more healthy and reliable products can be obtained with the informations.

Keywords: Antioxidant activity, Hygienic quality, Nutrition, Pomegranate molasse

Introduction

Pomegranate is a fruit of the *Punicaceae* family, *Punica granatum* Linnaeus species, suitable for growing in temperate climates. Pomegranate, an important commercial product in Asia, North Africa, Mediterranean and Middle East countries, is mostly grown in the Mediterranean Region in Turkey (Sarkosh et al., 2006). Therapeutic effects of pomegranate fruit have been known for centuries. Anthocyanins, flavonoids, hydrolyzable tannins, organic acids, vitamins, minerals in pomegranate provide its antioxidant and antimicrobial effects (Vardin and Fenercioğlu, 2003; Lansky and Newman, 2007). Pomegranate fruit has preventive effect on diabetes, cancer and cardiovascular diseases. In addition, it is useful in the treatment of diseases such as ulcer, diarrhea, dysentery, hemorrhoids and food poisoning (Viuda-Martos et al., 2010; Prashanth et al., 2001). The pomegranate products made from pomegranate (pomegranate juice, pomegranate juice concentrate, pomegranate vinegar, pomegranate wine, pomegranate molasse) are also beneficial for human health.

Pomegranate molasse is a pomegranate product used in salads, as sweetener and flavoring sauce in some meals (raw meatballs, kısır, dolma and etc.) (Maskan, 2009). In parallel to widespread pomegranate production in Turkey, pomegranate molasse is among the products that have increased production and consumption in recent years. According to the Turkish Standarts (Anonymous, 2001) pomegranate molasse is defined as 'it is a sour food product that produced by pressing pomegranate fruit, clarifying pomegranate juice and evaporating open or under vacuum technique and used to flavoring some foods'.

Pomegranate molasse can be obtained commercially or traditionally. The traditional production stages of pomegranate molasse are; washing pomegranate, shredding, pressing, boiling pomegranate juice, cooling, filtering and bottling (Karabıyıklı and Kışla, 2012). No sugar or additive is added in the production by traditional method. In commercial production, there are pasteurization of pomegranate juice, enzyme addition, clarification, filtration, evaporation stages. In these stages, glucose/fructose syrup, citric acid, antioxidant agents, colorant and preservatives can be added to the product (Karaca, 2011; Kışla and Karabıyıklı, 2013). Pomegranate molasse is a high nutritional value product and has a strong antioxidant effect. In this regard, it has the function of preventing diabetes, cancer and cardiovascular diseases (Incedayı et al., 2010; El-Darra et al., 2017). In addition, pomegranate molasse is a product that rich in terms of phenolic compounds and minerals as potassium, magnesium, calcium, zinc (Fadavi et al., 2005).

In this study, some physicochemical properties such as pH, dry matter content, viscosity, color and phenolic content, antioxidant capacity and hygienic quality of the pomegranate molasses samples produced by commercial and traditional methods were investigated.

Materials and Methods

Supply of Pomegranate Molasses Samples

In this study, a total of six pomegranate molasses samples were used for analysis, three of which were produced by commercial method and three of which were produced by traditional method. The commercial pomegranate molasses samples (C1, C2, C3) were purchased from a local supermarket in Burdur (Turkey). And traditional pomegranate molasses samples (T1, T2, T3) were purchased from different producers in the public market in Burdur. T1 is produced in Kahramanmaraş, T2 in Antalya and T3 in Burdur. All of the samples used in the study were produced in 2017. The samples were stored at 4 ^{0}C during the analysis, commercial ones at glass bottles, and traditional ones at plastic bottles.

Physicochemical Analysis

The pH values of the pomegranate molasses samples were determined by the digital pH meter (Mettler Toledo S20K-Kit). The dry matter quantities of the samples were determined according to TS 4890 refractometer method (Anonymous, 1986). The Brookfield RVDV-11 + PX was used to determine the viscosity values and the results were given in milipascal. L* brightness, a* redness and b* yellowness values of commercial and traditional pomegranate molasses samples were determined with color measurement device (Konika Minolta Chroma Meter Cr-400/410). The L* value is between 0-100 (black-white) and refers to brightness. The positive a* value represents red color, the positive b* value is yellow color; negative a* represents green color and negative b* is blue color (Legua et al., 2016).

Determination of Total Phenolic Content

The total phenolic contents of pomegranate molasses samples were detected by Folin-Ciocalteau method suggested by Singleton et al. (1965). Firstly, extracts were prepared for phenolic content analysis. For this, 2 grams of the samples were taken and 10 mL of 96% ethanol was added on it. It was mixed with the homogenizer for two minutes and kept in a water bath at 45 ^{0}C overnight. After this time, it was centrifuged at 4000 rpm for 5 minutes. Then dried at 45 ^{0}C in the evaporator. The prepared extracts were dissolved in 1 mL of methanol and used in the analysis. 40 µL of extract was taken into the test tube containing 2.4 mL of distilled water. 200 µl of Folin-Ciocalteau reagent and 600 µL sodium carbonate

were added on it. To this, 760 µL distile water is added and vortexed. After standing at room temperature for 2 hours, the absorbance was read at 765 nm spectrophotometer. The same processes were applied to the gallic acid solutions prepared in different concentrations for the calibration curve. The absorbance of the extract solution was read from the drawn gallic acid calibration curve and the total phenolic content was calculated as the gallic acid equivalent (mg GAE/L).

Determination of Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity method was used to determine the antioxidant activity of the samples. Accordingly, 2 grams of the sample were homogenized with 10 ml of 96% ethanol. 1 mL of this mixture was taken and 1 mL of DPPH solution was added on it. After waiting for 30 minutes in a dark environment, the absorbance was measured at 517 nm (Sahu et al., 2013).

Determination of Total Aerobic Mesophilic Bacteria

Total aerobic mesophilic bacteria count was performed as the hygienic quality parameter in the pomegranate molasses samples. For this, rapid microbiological analyzer was used (Biomerux, Tempo). The results were given as cfu/mL (colony forming unit) (Anonymous, 2009).

Statistical Analysis

All analyzes except for color measurement and total aerobic mesophilic bacteria were performed in three replicates. The results were analyzed with SPSS 16.0 statistical program and shown as mean±standard deviation. Spearman correlation test was used between the phenolic content and antioxidant content of samples.

Results and Discussion

Physicochemical Analysis

In order to determine the physicochemical characterization of pomegranate molasses samples; pH values, dry matter quantities, viscosity values and color measurement values were investigated. The results of the physicochemical analysis of pomegranate molasses samples are shown in Table 1.

As the seen in the table, the pH values of pomegranate molasses samples have changed between 1.79 and 2.77. Incedayı et al., (2010) reported that the pH values of seven different commercial pomegranate molasses samples were between 0.87 and 1.98. And in the study of Kaya and Sozer (2005), this value determined as 2.05. The pH value may vary depending on the type of pomegranate fruit, sugar content, amount of organic acid, region where it grows. In addition, different applications in the production of pomegranate molasse effect the pH value, hence the sourness-sweetness status (Melgarejo and Artes, 2000; Tehranifar et al., 2000).

According to Turkish Standart of pomegranate molasses, it is stated that the dry matter quantity of pomegranate molasse as at least 68% (Anonymous, 2001) (Table 2). All of the samples used in the study comply with the standard. The sample with the lowest quantity of dry matter is C3 (69.90%) and the highest is C1 (86.23%). Yılmaz et al. (2007) reported that the moisture content of commercial pomegranate molasses samples was 24.4% on average in their study. Therefore, the dry matter quantities of these samples is around 75.6%.

The resistance of liquid foods against fluidity is viscosity. Foods with high viscosity value are more dense and flow rates are slow. The viscosity values of the pomegranate molasses samples in the study vary between 191.35 and 13000 mPa. The sample with the highest viscosity is C1 (13000 mPa) and the lowest one is C3 (191.35 mPa). These results are similar to the results of dry matter quantity. Among the samples, only C1 contains fructose syrup. It is thought that high viscosity and dry matter content of C1 can be caused by fructose syrup. The average viscosity values are calculated as 5344.11±6761 in commercial pomegranate molasses samples, 2593.48±1071 in traditional pomegranate molasses samples. Incedayi et al. (2010) found the viscosity values of pomegranate molasses samples are between 200-1800 mPa. In a study using commercial pomegranate molasses, it was reported that the viscosity values were between 176-2900 mPa (Akpınar Bayızıt et al., 2016). In natural pomegranate molasses, low dense and high dense consistency are not desired. Low dence molasse can not provide the desired aroma, also nutritious value is low. And high dense pomegranate molasse can not show homogenous distribution when used as sauce. The results of the study confirm that viscosity is an important parameter in the production of pomegranate molasse. It is expected that the appropriate viscosity values will be included in the pomegranate molasse standards with studies to do about it.

Table 1. Physicochemical analysis results of pomegranate molasses samples

Sample	pH	Dry matter quantity (%)	Viscosity (mPa)	Color values		
				L*	a*	b*
C1	1.91 ±0.00	86.23 ±0.15	13000.00 ±875.00	22.09	0.14	0.37
C2	1.79 ±0.00	82.70 ±0.20	2841.66 ±14.43	21.17	1.19	0.42
C3	2.77 ±0.01	69.90 ±0.26	191.35 ±1.06	20.85	0.34	0.36
T1	2.13 ±0.00	84.13 ±0.20	1683.33 ±7.21	20.71	0.19	0.40
T2	1.89 ±0.00	83.30 ±0.20	3773.80 ±20.61	20.67	0.24	0.19
T3	1.90 ±0.01	81.30 ±0.36	2323.33 ±55.48	19.73	0.16	0.41

Data are reported as mean values±SD of three measurements

Table 2. General composition of the pomegranate molasses (Anonymous, 2001)

Components	**Amount**
Water soluble dry matter, %, minimum	68.0
Titration acidity (as citric acid), %, minimum	7.5
pH	3.0
HMF, mg/kg, maximum	50
Saccharose, preservative agent, coloring agent	Not allowed

The results of color analysis of commercial and traditional pomegranate molasses samples are shown in Table 1. L* refers to brightness (lightness-darkness), a* redness and b* yellowness. The L* brightness values of the samples are in the range of 19.73-22.09. Relation to the L* value, ranging from 0-100, values close to 0 indicates darkness and values close to 100 indicates lightness. According to this, the darkest sample is T3 and the lightest sample is C1. Orak (2009) found the L* values of the pomegranate juice and pomegranate molasse obtained from this as 10.24, 4.92 respectively. It is stated in the study that the color is darkened as a result of heat treatment. In addition, anthocyanins which give red color to the fruit are decomposed as a result of the heat treatment and a darker color emerges with non-enzymatic browning reactions (Cemeroglu and Artık, 1990). a* shows low values with decreasing in red color. In our study, the highest a* value is seen in C2 (1.19). In a study L*, a* and b* values of pomegranate molasses samples were found as 1.88, 0.57 and -0.31 respectively. It is reported that these values represent darkness, light red and blue color (Yılmaz et al., 2007). Maskan (2006) stated that all color parameters (L*,a*,b*) have decreased over time as a result of the heat treatment of pomegranate juice. In the study of Orak (2009), a* value decreased from 12.33 to 1.82 and the value of b* decreased from 2.38 to 1.60 in the process of product pomegranate molasse from juice. L*, a*, b* values of grape molasses were measured as 18.87, 4.99, -1.61 (Ertaş and Coklar, 2008). According to these results, it can be said that grape molasses are darker than the pomegranate molasses, the red color is more and the blue color is more dominant.

Total Phenolic Content

The phenolic substances are compounds play an important role in human health and found naturally in fruits and vegetables (especially in red color) (Tzulker et al., 2007). Phenolic contents of pomegranate juice include phenolic acids, hydrolysable tannins and anthocyanins. Gallic acid, elagic acid, chlorogenic acid, coumaric acid, α-punicalagin, β-punicalagin, delfinidine-3,5-diglycoside, cyanidine-3,5-diglycoside, pelargonidine-3,5-diglycoside, cyanidin-3-glycoside, delfinidin-3-glycoside, pelargonidin-3-glycoside are some of them (Poyrazoglu et al., 2002; Alighourchi et al., 2008). Table 3 shows the total phenolic content of commercial and traditional pomegranate molasses samples and the corresponding calibration curve in Figure 1. Accordingly, the sample with the highest phenolic content is C3 (931.56 mg GAE/L). C3 is in the second row with regard to antioxidant substance. Incedayı et al (2010) reported that the total amount of polyphenol in commercial pomegranate molasses samples ranged between 551.61 and 9695.17 mg GAE/kg. It is expected that the content of phenolic substance will be increased in pomegranate molasse which is a concentrate product. In a study, the total phenolic content of pomegranate juice and pomegranate molasse obtained from this were compared. Total phenolic content of pomegranate molasse was found to be three times higher than the pomegranate juice (Orak, 2009). In our study, the average phenolic content of commercial pomegranate molasses samples was found to be higher (490.22 ±278) than the traditional pomegranate molasses samples (24.11 ±4.06).

Figure 1. Total phenolic content analysis calibration curve

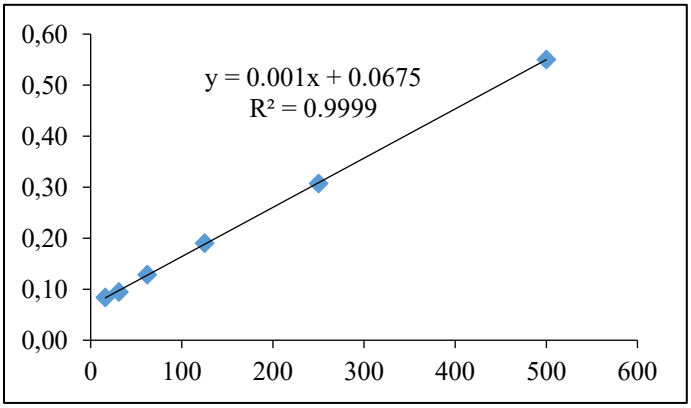

Table 3. Total phenolic content of pomagranate molasses samples

Sample	Total phenolic content (mg GAE/L)
C1	528.71 ±21.87
C2	10.40 ±9.45
C3	931.56 ±14.43
T1	28.63 ±8.89
T2	22.98 ±11.78
T3	20.74 ±26.23

Data are reported as mean values±SD of three measurements

Total Antioxidant Content

It is also known that foods with high phenolic content are high in antioxidant activity. The consumption of foods with antioxidant content is important in protecting the cells by preventing oxidation reactions in the body (Karadeniz et al., 2005). Pomegranate fruit is one of the foods with high antioxidant effect. In the study, the antioxidant content of pomegranate molasses are between 0.68-735.28 µmol TE/g and C2>C3>T1>T3>T2>C1. The average quantity of antioxidant substances in commercial pomegranate molasses samples is 356.40±367 and 1.64±1.60 in traditional pomegranate molasses samples (Table 4). The calibration curve for the analysis of antioxidant capacity is shown in Figure 2. In order to reduce costs in the production of pomegranate molasse, there may be adulterations. Fruit juice, colorant, thickener, antioxidant, glucose-fructose syrup can be added (El Darra et al., 2017). This makes it difficult to compare the antioxidant activity of commercial and traditional pomegranate molasses samples. In addition, the fact that they are not indicated on the label can pose a risk to consumers (Boggia et al., 2013). In a study in which antioxidant capacities of commercial pomegranate molasses were measured by DPPH method, the lowest value was 140.22; the highest value is 471.85 µmol TE/g (Akpınar Bayızıt et al., 2016). According to this, the antioxidant capacity of C1 sample in our study is very low. In a study using the same method, the quantity of antioxidant substance in pomegranate juice ranged between 8.98-15.47 µmol TE/g (Kaur et al., 2014). Oztan (2006) was found the antioxidant capacities of freshly squeezed pomegranate juice, commercial pomegranate juice and commercial pomegranate molasse samples as 52.12, 46.24, 54.8 µmol TE/g respectively.

Table 4. Total antioxidant content of pomagranate molasses samples

Sample	Total antioxidant content (µmolTE/g)
C1	0.68 ±0.14
C2	735.28 ±32.8
C3	333.24 ±29.58
T1	3.49 ±0.55
T2	0.70 ±0.07
T3	0.73 ±0.08

Data are reported as mean values±SD of three measurements

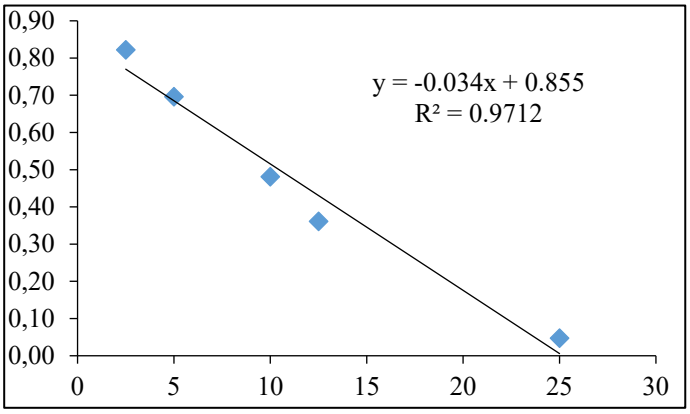

Figure 2. Total antioxidant content analysis calibration curve

Correlation Between Phenolic and Antioxidant Contents

Spearman test was used to determine whether there is a correlation between the phenolic and antioxidant contents of the samples. It is expected that the sample with high phenolic content to have high antioxidant activity. According to Table 5, there was no significant correlation between phenolic content and antioxidant activity ($p > 0.05$). This may be due to the different phenolic content of different pomegranate species. In addition, various additives used in pomegranate molasses especially commercial ones, may also affect the results.

Total Aerobic Mesophilic Bacteria Count

Figure 3 shows the total aerobic mesophilic bacteria count (TAMB) found in pomegranate molasses samples. The total aerobic mesophilic bacteria count was determined by TEMPO TVC rapid test method. The total number of live bacteria ensures the hygienic evaluation of the product. In general, foods with a total aerobic mesophilic bacteria count is at 10^6-10^8/g are risky in terms of consumption and their hygienic qualities are low (Anonymous, 2005). Looking at the table, it is observed that all of the pomegranate molasse samples are below these values. The sample with the highest number of aerobic mesophilic bacteria is commercial C1 sample ($4.9x10^4$ cfu/mL). It has been reported that pomegranate molasses have antimicrobial properties and are mostly due to phenolic compounds, especially hydrolyzable tannins (Gullon et al., 2016). In a study in which pomegranate molasse was used as a sauce, pomegranate molasses samples showed antimicrobial effect on foods and prevented microbial growth in foods. In this study, it is also stated that the antimicrobial effect of the pomegranate molasses samples produced by the traditional method is higher than the samples produced by commercial method (Karabıyıklı and Kışla, 2012). In a study, washing water with pomegranate molasse was used for washing some vegetables and its antimicrobial effect was investigated. As a result of this study, it was found that the levels of *Listeria monocytogenes* decreased 1.96-2.97 log cfu/g in the vegetables (Kang and Song, 2017). In another study, the traditional pomegranate molasse applied to parsley leaves and ready-to-eat salads. It was reported that the pomegranate molasse had a significant antibacterial effect against *Salmonella typhimurium* and enhanced the microbial safety of these foods (Faour-Klingbeil and Todd, 2018).

Conclusions

Pomegranate molasse is a pomegranate product with high nutritional value known for its flavor-enhancing effect when used in foods. The production technique of pomegranate molasse affects its nutritional value, hygienic quality, sensory properties and taste. In this study, pomegranate molasses samples produced by commercial and traditional methods were investigated. It was observed that all samples comply with the standards in terms of pH and dry matter quantity. The commercial C1 sample with the highest viscosity was determined to contain fructose syrup. The adulterations that can be done in production can interfere to a healthy comparison and evaluation. Similarly, adulterations such as colorant, thickener and antioxidant addition may also change the results. In the study, the sample with the highest phenolic content is C3 and the highest antioxidant content is C2. The hygienic quality of pomegranate molasses samples used in the study is high. Although it is thought that the hygienic quality of the samples produced by traditional method will be low, the total number of aerobic mesophilic bacteria was found to be highest in commercial C1 sample. Pomegranate molasse which has positive effects on human health should take place in diet more. In terms of commercial production, pomegranate molasse standards should be expanded, more detailed and adulterations should be prevented. It will be useful to include the parameters of viscosity and hygienic quality in pomegranate molasse standards. On the other hand, traditional production of pomegranate molasses should be supported. Producers should be informed about the hygienic conditions during production stages.

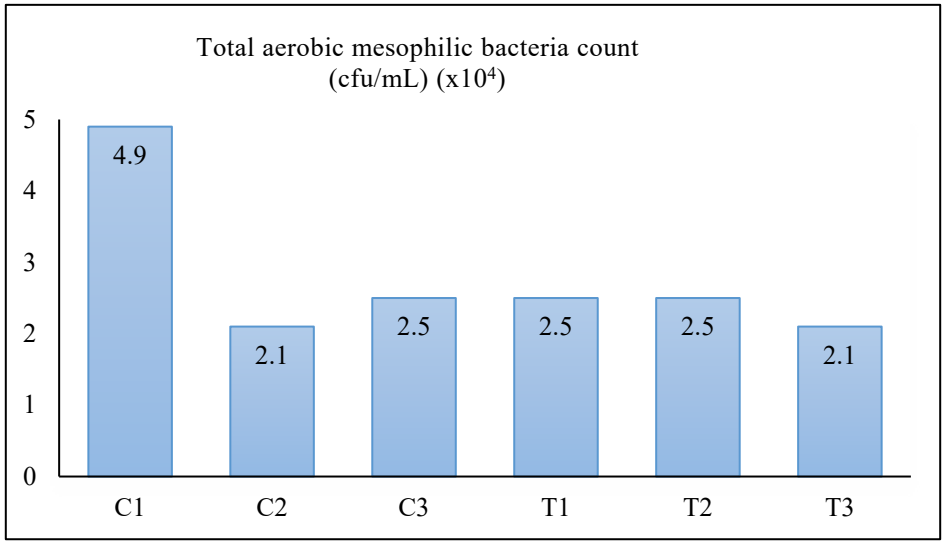

Figure 3. TAMB count of pomegranate molasses samples

Table 5. The correlation values between total phenolic and antioxidant contents of molasses

			Phenolic content	Antioxidant content
Spearman's rho	Phenolic content	Correlation Coefficient	1.000	-0.257
		Sig. (2-tailed)	.	0.623
		N	6	6
	Antioxidant content	Correlation Coefficient	-0.257	1.000
		Sig. (2-tailed)	0.623	.
		N	6	6

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Gıda bilimlerinde Excel kullanımı 1: Doğrusal regresyon

Cansu Leylak[1], Merve Yurdakul[2], Sencer Buzrul[1]

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[1] Konya Gıda ve Tarım Üniversitesi, Gıda Mühendisliği Bölümü, Melikşah Mah. Beyşehir Cad. No:9, 42080 Meram, Konya, Türkiye

[2] Konya Gıda ve Tarım Üniversitesi, Biyomühendislik Bölümü, Melikşah Mah. Beyşehir Cad. No: 9, 42080 Meram, Konya, Türkiye

ORCID IDs of the authors:
C.L. 0000-0003-2393-0545
M.Y. 0000-0002-5597-4692
S.B. 0000-0003-2272-3827

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Correspondence: Sencer BUZRUL
E-mail: sencer.buzrul@gidatarim.edu.tr

ÖZ

Excel genellikle kullandığımız bilgisayarlarda Microsoft® Office'in bir parçası olarak yüklü olarak gelmekte ve deneysel verilerle uğraşan hemen hemen herkes Excel'in basit de olsa kullanımına aşina olmaktadır. Gıda bilimlerinde de deneysel verileri işlemek, dönüştürmek, grafik haline getirmek ya da herhangi bir modelle tanımlamak için Excel'i kullanmak çok yaygındır. Doğrusal regresyon sadece düz bir çizgiyi veriye uydurmak için kullanılır gibi yanlış bir kanı vardır. Ancak, ikinci dereceden bir polinom da ya da bir eğri de doğrusal regresyon kullanılarak veriye uydurulabilir: eğer deneysel verileri tanımlamak için kullanılan model parametresine/parametrelerine göre doğrusalsa bu modelin parametresi/parametreleri doğrusal regresyon kullanılarak bulunabilir. Excel'deki veri çözümleme aracının içerisinde yer alan regresyon uygulaması parametrelerine göre doğrusal modeller için kullanılabilir. Bu çalışmanın amacı doğrusal regresyon kullanılarak parametrelerinin elde edilebileceği modellerin deneysel verilere Excel kullanılarak nasıl uygulanacağını örnekler üzerinde göstermektir. İlk örnekte Excel'in içinde yer alan doğrusal model kullanılarak mikrobiyolojik veriler üzerinde veri çözümleme aracının uygulaması ve sonuçların yorumlanması gösterilmiştir. İkinci örnekte gaz kromatografi verisini tanımlamak için Excel'in içinde yer almayan ancak kullanıcı tarafından denklemi bilinen bir modelin, üçüncü örnekte ise mikrobiyal büyüme hızını pH'a göre tanımlamak için kullanıcının kendi yarattığı bir modelin uygulamaları gösterilmiştir. Bu çalışmanın gıda mühendisliği ve gıda bilimleri alanında çalışanlar için önemli katkıları olacağı değerlendirilmektedir.

Anahtar Kelimeler: Matematik modeller, Veri Çözümleme, Excel, Doğrusal Regresyon

ABSTRACT

Use of Excel in food science 1: Linear regression

Excel is usually included in the computer package as a part of Microsoft® Office. Almost everyone who deals with the experimental data is more or less familiar with the use of Excel. In food science, it is very common to use Excel to process, transform, sketch or define experimental data with any model. There is a wrong opinion as linear regression can only be used to fit a linear line to the data. However, a second order polynomial or a curved data could also be modelled by using the linear regression: if the model which is used to define the experimental data is linear according to its parameter(s), the parameter(s) of this model can be obtained by using linear regression. Regression application in data analysis tool in Excel could be used for linear models according to its parameters. The aim of this study was to show the application of models to the experimental data by using Excel with examples, where model parameters can be obtained by using linear regression. In the first example, using the linear model in Excel, the application of the data analysis tool on the microbiological data and the interpretation of the results were shown. In the second example, the application of a model that is not included in Excel but its equation is known by the user was shown to define the gas chromatography data. In the third example, the application of a model created by the user to define the microbial growth rate according to pH was shown. It is considered that this study would have important contributions for those working in the field of food engineering and food science.

Keywords: Mathematical models, Data Analysis, Excel, Linear Regression

Giriş

Fen bilimleri ve mühendislik alanlarında, özellikle biyoloji, kimya, biyoteknoloji ve gıda bilimlerinde, deneysel verilerin uygun bir matematik modelle tanımlanması yaygın bir uygulamadır. Bir veya birden çok matematik modelin veriye uydurulması için farklı bilgisayar programları mevcut olup, bu programların kullanımı belli bir deneyim ve uzmanlık gerektirmektedir. Dahası bu programlar (örneğin SigmaPlot ve Microcal Origin gibi programlar) çoğunlukla ücretlidir. Öte yandan kullandığımız bilgisayar ister masaüstü ister dizüstü olsun genel olarak Excel bunların içinde, Microsoft® Office'in bir parçası olarak, yüklü olarak gelmekte ve deneysel verilerle uğraşan hemen hemen herkes Excel'in basitte olsa kullanımına aşina olmaktadır.

Excel hem doğrusal hem de doğrusal olmayan modeller için kullanılabilir. Ancak doğrusal modeller için "Veri Çözümleme" aracının doğrusal olmayan modeller için "Çözücü" aracının kullanılması gerekmektedir. Bu araçların kullanımı her ne kadar çok karmaşık olmasa da belli protokollerin yerine getirilmesini gerektirmektedir. Bu çalışmanın amacı doğrusal modellerin diğer bir deyişle doğrusal regresyon kullanılarak model parametrelerinin elde edilebileceği modellerin deneysel verilere Excel kullanılarak nasıl uygulanacağını göstermektir. Ayrıca model özetinin nasıl yorumlanması gerektiği, modelin veriye uygun olup olmadığının değerlendirilmesi ve grafiksel gösterimi de ele alınacaktır.

Modellerde Doğrusallık

Modeller karmaşık deney verilerinin basit matematiksel ifadelerle tanımlanmasını sağlarlar ve çok farklı şekillerde karşımıza çıkabilmektedirler (logaritmik, üstel veya türevsel denklem). Bunların tamamına kısaca matematik modeller denmektedir. Örneğin $y = f(x)$ denklemini düşünürsek burada x bağımsız değişken olup, araştırıcı tarafından kontrol edilebilir, y bağımlı değişkendir çünkü x'e bağlıdır ve araştırıcı tarafından ölçülür, f ise fonksiyon olup, veriyi tanımlamak için bir veya birden fazla parametreye sahip olabilir (Brown, 2001). Çok basit bir denklem olan $y = ax + b$ denkleminde a ve b model parametreleri, x ve y de sırasıyla bağımsız ve bağımlı değişkenlerdir. Peki, bu modelin doğrusallığı hakkında ne diyebiliriz? Hemen herkes bu modelin doğrusal olduğunu söyleyecektir. Gerçekten de bu model hem bağımsız değişkenine yani x'e göre hem de parametrelerine (a ve b) göre doğrusaldır.

Öte yandan $y = Ax^2 + Bx + C$ denklemine bakarsak parametrelerine göre bu modelin de doğrusal olduğunu görürüz. Ancak, x'e göre bu model doğrusal değildir. Regresyonda amaç model parametrelerini elde etmek olduğundan ve yukarıda örnek olarak verdiğimiz her iki modelde parametrelerine göre doğrusal olduğundan her iki modele de doğrusal regresyon uygulanabilir. Bir modelin parametrelerine göre doğrusal olup olmadığını anlamanın en kolay yolu modelin ilgili parametreye göre kısmi türevini almaktır (van Boekel ve Zwietering, 2007). Eğer model parametresi kısmi türevde yer almıyorsa model o parametreye göre doğrusaldır.

Örneğin $y = Ax^2 + Bx + C$ modelinin A parametresine göre kısmi türevi $\frac{\partial y}{\partial A} = x^2$, B parametresine göre kısmi türevi $\frac{\partial y}{\partial B} = x$, C parametresine göre kısmi türevi ise $\frac{\partial y}{\partial C} = 1$'dir. Görüldüğü gibi her üç parametrede kısmi türevlerinde yer almadığından model parametrelerine göre doğrusaldır ve veriyi bu modelle tanımlamak için doğrusal regresyon kullanılabilir. Genellikle doğrusal regresyon sadece düz bir çizgiyi veriye uydurmak için kullanılır gibi yanlış bir kanı vardır. Oysa görüldüğü gibi ikinci dereceden bir polinom da ya da bir eğri de doğrusal regresyon kullanılarak veriye uydurulabilir.

Excel Kullanarak Deneysel Verilere Doğrusal Regresyon Analizi Yapmak

Bu bölümde basitten karmaşığa üç farklı örnek üzerinde Excel'de "Veri Çözümleme" aracını kullanarak doğrusal regresyon ile deneysel verileri tanımlamayı göstereceğiz. Ancak, veri çözümleme aracı Excel'de yüklü değilse (Microsoft Office Standard 2016 Excel'de "Veri" sekmesine tıkladığınızda sağ üste veri çözümleme görünmüyorsa), sırasıyla Dosya > Seçenekler > Eklentiler > Excel Eklentileri (Git) sekmelerinden sonra çıkan ekrandan "Çözümleme Araç Takımı" ve "Çözümleme Araç Takımı - VBA" işaretleyerek yüklenmelidir.

Listeria monocytogenes'in İnaktivasyonunun Doğrusal Modelle Tanımlanması

İlk örneğimizde sıvı besiyerinde ısıyla inaktive edilen ve hastalık yapıcı bir bakteri olan *L. monocytogenes*'i ele alacağız. *L. monocytogenes* pastörizasyon sıcaklıklarına (71.7°C'de 15 saniye veya 62.8°C'de 30 dakika) karşı çok hassas olduğundan (Ray, 2014) bu bakterinin daha düşük bir sıcaklıktaki (58°C'de) inaktivasyonu örnek olarak kullanılmıştır. Şekil 1'de *L. monocytogenes*'in mL'de ölçülen canlı hücre sayısı ($\log_{10}$ KOB/mL, KOB: koloni oluşturan birim) zamana bağlı olarak gösterilmektedir. Örneğimizdeki bağımsız değişken zaman (dakika) iken bağımlı değişken canlı hücre sayısıdır ($\log_{10}$KOB/mL).

Zaman ve zamana karşı ölçülen canlı hücre sayısı x ve y olacak şekilde bir Excel sayfasında tanımlanır (Şekil 1). Daha sonrasında verilerin regresyon uygunluğunu saptamak amacıyla dağılım grafiği çizilir. Doğrusal model kullanarak bu veriyi tanımlamak mümkündür:

$$\log_{10} N(t) = \log_{10} N_0 - k \cdot t \quad (1)$$

Burada $\log_{10}N(t)$ bakterinin t zamandaki sayısı, $\log_{10}N_0$ bakterinin başlangıçtaki sayısı ($t = 0$), k ise inaktivasyon hızıdır (zaman^{-1}). Yani doğrusal modelin iki parametresi $\log_{10}N_0$ ve k'dır.

Tablo 1. Örnek 3'te yer alan modellerin uyumlarının karşılaştırılması

Table 1. Comparison of goodness-of-fit of the models in Example 3

Model	Ayarlı R^2	Standart hata
2'nci dereceden polinom	0.9031	0.0430
3'üncü dereceden polinom	0.9411	0.0335
Alternatif model	0.9399	0.0338

Grafik üzerindeki verilere sağ tıklanarak "Eğilim çizgisi ekle"den Excel'in içindeki "Doğrusal model" seçilir ve en altta yer alan "Grafik üzerinde Denklemi görüntüle" ile "Grafik üzerinde R-kare değerini görüntüle" kısımları işaretlenir. Grafik üzerinde model uyumu (kesikli mavi çizgiler), model denklemi ($y = -0.9794x + 10.287$) ve R-kare değeri ya da belirleme katsayısı ($R^2 = 0.9932$) görünmektedir (Şekil 2). Bu durumda, Denklem 1 ile grafik üzerinde görünen denklemi eşleştirirsek *L. monocytogenes*'in başlangıçtaki ($t = 0$) sayısını $\log_{10}N_0 = 10{,}287$ $\log_{10}$KOB/mL ve inaktivasyon hızını $k = 0.9794$ dakika^{-1} olarak bulabiliriz.

Burada birçok Excel kullanıcısı model parametrelerini ($\log_{10}N_0$ ve k) ve R^2 değerini elde etmekle istediğimiz sonuca ulaştığımızı düşünecektir. Ancak, mevcut durum istediğimiz sonucu elde edebilmekten çok uzaktadır. Birincisi model parametreleri belirsizlikleri (standart hata veya güven aralığı) ile elde edilmemiştir ve bu halleriyle hiçbir anlam taşımamaktadırlar (van Boekel, 1996; 2008). İkincisi her ne kadar birçok bilimsel çalışmada hala model uyumunu belirlemede kullanılsa da R^2 değeri model uyumunu anlamak için yeterli değildir (Ratkowsky, 2004). Daha doğrusu R^2 değerinin 1'e yakın olması ($R^2 > 0.90$) model uyumunun iyi olduğunu göstermez (Montgomery ve Runger, 2011) ancak R^2 değeri düşükse ($R^2 < 0.70$) model uyumuyla ilgili bir sorun olduğu aşikardır (Granato ve ark., 2014). Herhangi bir model uyumu için yüksek R^2 değeri elde edilmesi durumunda o modelin uyumunu değerlendirmek için başka uyum ölçütlerini de gözden geçirmek en doğru yoldur (Granato ve ark., 2014).

İşte tam da bu noktada "Veri Çözümleme" aracını kullanmak istediğimiz sonuçlara ulaşmayı mümkün kılacaktır. Bunun için Excel'de veri çözümleme aracı açılır ve çıkan pencereden regresyon seçilir (Şekil 3a). Regresyon altında Y giriş aralığı olarak *Listeria monocytogenes'in* canlı hücre sayısı (Excel hücreleri B2:B13) ve X giriş aralığı süre (Excel hücreleri A2:BA13) olarak seçilir (Şekil 3b). Veriler uygun şekilde girildikten sonra başka hiçbir değişiklik yapmadan Tamam'a basılır ve yeni bir Excel çalışma sayfasında Şekil 4'te görünen sonuçlar elde edilir. Görüldüğü gibi uygulanan doğrusal regresyon sonucu birçok bilgi ekranda belirmiştir.

Sonuçları değerlendirmeye "Özet Çıkışı"nın en altındaki tablodan başlarsak, grafik gösteriminde elde edilen parametrelerin bu sefer standart hataları ($\log_{10}N_0 = 10.29 \pm 0.08$ ve $k = 0.98 \pm 0.026$) ve % 95 güven aralıkları ($\log_{10}N_0 = 10.29 \pm 0{,}19$ ve $k = 0.98 \pm 0.06$) ile birlikte bulunduğu görülebilir. Parametre belirsizliklerini elde etmek özellikle gıda bilimlerinde önemlidir (Dolan ve Mishra, 2013) ve % 95 güven aralığı parametre değerinin % 95 ihtimalle olacağı aralığı belirtmektedir. Sonuçlarda görülen *p*-değerinin küçük olması elde edilen parametrelerin istatiksel olarak anlamlı olduğunu göstermektedir. Genel olarak $p > 0.05$ ise parametreler istatiksel olarak anlamsızdır ve verilerimizi tanımlamak için anlamsız parametrelere sahip modeli kullanmak geçerli bir uygulama değildir. Örneğimizde $\log_{10}N_0$ parametresinin *p*-değeri 3×10^{-17}, k parametresinin *p*-değeri ise 3.7×10^{-12}'dir. Her iki parametrenin de *p*-değeri < 0.05 olduğundan her iki parametrede anlamlıdır. Parametrelerin standart hata ve güven aralığı da *p*-değeri ile orantılıdır. Diğer bir deyişle *p*-değeri ne kadar küçükse standart hata/güven aralığı o kadar küçüktür. Aynı tabloda yer alan *t*-değeri ise parametre değerinin standart hataya bölümüdür ve sayısal olarak büyük olması parametrenin anlamlı olduğunu gösterir.

Özet çıkışının ortasında ANOVA tablosu yer almaktadır (Şekil 4). Burada *df* (degrees of freedom) serbestlik derecesi demektir. Toplam 12 verimiz olduğundan (12 zamanda elde edilen 12 canlı hücre sayısı) toplamın serbestlik derecesi 12–1 = 11'dir. Veri sayısı özet çıkışının ilk tablosunun hemen altında "Gözlem" olarak da gösterilmektedir (Şekil 4). Regresyonda veya modelde iki parametremiz ($\log_{10}N_0$ ve k) olduğundan regresyonun serbestlik derecesi 2–1 = 1'dir. Farkın serbestlik derecesi ise bu ikisinin farkıdır yani 11 – 1 = 10'dur. Farkın serbestlik derecesini veri sayısını (12) modelin parametre sayısından (2) çıkartarak da hesaplamak mümkündür (12–2 = 10). Tabloda görülen *SS* (Sum of squares) karelerin toplamı anlamını taşımaktadır. Örneğimiz için toplamın *SS*'si aşağıda verilmiştir:

$$SS_{Toplam} = \sum_{n=1}^{12} \left(y_{veri} - y_{veri,ortalama}\right)^2 \quad (2)$$

Burada her bir y verisini y verilerinin ortalamasından ($y_{veri,ortalama} = 7{,}59$) çıkartarak karelerini alıp bütün bunları toplarsak $SS_{Toplam} = 34{,}53$ olarak hesaplanabilir. Regresyonun (modelin) SS'si aşağıdaki gibi hesaplanabilir:

$$SS_{Model} = \sum_{n=1}^{12}(y_{model} - y_{veri,ortalama})^2 \quad (3)$$

Model denklemi ve model parametreleri kullanılarak her bir zaman için y_{model} hesaplanırsa buradan SS_{model} = 34.29 olarak bulunabilir. SS_{Toplam} veri noktalarının ortalama değerden sapmasını ölçerken SS_{Model} ise modelden elde edilen veri noktalarının ortalama değerden sapmasını ölçer. Farkın SS'si $SS_{Toplam} - SS_{Model}$ = 0.24 olarak elde edilir. Öte yandan farkın *SS*'sini aşağıdaki gibi hesaplamak ta mümkündür:

$$SS_{Fark} = \sum_{n=1}^{12}(y_{veri} - y_{model})^2 \quad (4)$$

Regresyonda amaç farkların karelerinin (SS_{Fark}) toplamının en aza indirilmesidir. Dolayısıyla SS_{Fark}'ın mümkün olan en düşük değerde olması istenir. Denklem 2, 3 ve 4 arasındaki bağıntıyı bulmak çok da zor değildir:

$$SS_{Toplam} = SS_{Model} + SS_{Fark} \quad (5)$$

Tabloda görülen *MS* (Mean square) ortalama kare değeri anlamına gelir *SS* değerinin serbestlik derecesine bölümü ile elde edilir yani:

$$MS_{Model} = \frac{SS_{Model}}{df_{Model}} = \frac{34,29}{1} = 34,29 \quad (6)$$

$$MS_{Fark} = \frac{SS_{Fark}}{df_{Fark}} = \frac{0,24}{10} = 0,024 \quad (7)$$

F-değeri bu ikisinin oranıdır: MS_{Model}/MS_{Fark} = 34.29/0.024 = 1451.27. Eğer *F*-değeri örneğimizdeki gibi yüksek bir sayı ise bağımsız değişkenin (*x* ya da örneğimizde zaman) bağımlı değişkeni (*y* ya da örneğimizde canlı hücre sayısı) tanımlamak için etkisi olduğu söylenebilir. Öte yandan "anlamlılık *F*-değeri" ne kadar düşük ise (örneğimizde bu değer 3.7×10^{-12}'dir) bağımsız değişkenin bağımlı değişkeni tanımlamak için kullanılabileceği anlamı çıkar.

Özet çıkışının en üstündeki tabloda modelin uyum iyiliğini gösteren ölçümler görülmektedir. Grafik gösterimi sadece R^2 değerini verirken burada R^2'ye ilaveten ayarlı R^2 (adjusted R^2) ve modelin standart hatasını da görmekteyiz. R^2 değeri bir altta yer alan ANOVA tablosundaki değerler kullanılarak hesaplanabilir:

$$R^2 = 1 - \frac{SS_{Fark}}{SS_{Toplam}} = 1 - \frac{0,24}{34,53} = 0,9932 \quad (8)$$

Ayarlı R^2 ise aşağıdaki denklemden hesaplanabilir ve 0.9925 olarak bulunabilir.

$$R^2_{ayarlı} = 1 - (1 - R^2) \cdot \frac{df_{Toplam}}{df_{Fark}} \quad (9)$$

Ayarlı R^2 sadece iki durumda R^2'ye eşit olabilir: (i) modelin tek bir parametresi varsa yani $df_{Toplam} = df_{Fark}$ ise; (ii) model mükemmel uyumlu ise yani R^2 = 1 ise. Onun dışında ayarlı R^2 her zaman R^2'den düşük çıkacaktır. Bazı kaynaklarda R^2 yerine ayarlı R^2 değerinin kullanılmasının daha uygun olduğu belirtilmekle birlikte (Davey ve Amos, 2002) model uyumunun en uygun göstergesi modelin standart hata değeridir (Ratkowsky, 2004). Standart hata (SH) aşağıdaki gibi hesaplanır:

$$SH = \sqrt{MS_{Fark}} = \sqrt{0,024} = 0,1537 \quad (10)$$

Standart hataya ortalama karesel hataların karekökü de (RMSE: root mean square error) denir. R^2 model tarafından açıklanan bağımlı değişken varyansının yüzdesini temsil ederken standart hata veri noktalarının regresyon (model) çizgisinden düştüğü ortalama mesafeyi gösteren mutlak bir ölçüdür. Diğer deyişle, daha küçük standart hata değerleri daha iyidir çünkü gözlemlerin yerleştirilen hatta (model çizgisine) daha yakın olduğunu gösterir. Standart hatanın birimi *y*'nin birimi ile aynıdır. Örneğimizde modelin 0.1537 $\log_{10}$KOB/mL'lik bir standart hatası vardır ve mikrobiyolojide 0.5 $\log_{10}$KOB/mL'den az hatalar makul olarak değerlendirildiğinden (Jarvis, 1989; Mossel ve ark., 1995) modelimiz bu veriyi tanımlamak için son derece uygundur.

Kısaca belirtmek gerekirse Excel'de veri çözümleme aracını kullanmayı ve elde edilen sonuçları (özet çıkışını) yorumlamayı bilirsek model parametrelerinin istatistiksel olarak anlamlı olup olmadığını ve modelin veriyi tanımlamak için yeterli olup olmadığını anlamak çok kolaylaşır. Dahası veri çözümleme bizi birçok farklı hesaptan kurtararak (*SS* hesaplamaları, R^2, ayarlı R^2 ve standart hata değerleri) zamandan tasarruf sağlamaktadır.

Gaz Kromatografi Verisinin Van Deemter Modeli İle Tanımlanması

İkinci örneğimizde Şekil 5'te görünen verilerin [bağımsız değişken akış hızı (mL/dakika) iken bağımlı değişken tabaka yüksekliğidir (mm)] van Deemter modeli ile tanımlamasını ele alacağız. Akış hızına karşı tabaka yüksekliği *x* ve *y* olacak şekilde yeni bir Excel sayfasında tanımlanan verilerin dağılım grafiği çizilir (Şekil 6). İlk örneğimizde Excel'in içindeki bir denklemi (doğrusal model) kullanma imkanı varken bu örneğimizde bunu yapmak mümkün değildir çünkü Excel'in içerisindeki hiçbir model bu veriyi tanımlayamamaktadır.

Kullanacağımız van Deemter modeli aşağıdaki şekildedir:

$$y = Ax + B/x + C \quad (11)$$

Burada *y* tabaka yüksekliği (mm), *x* akış hızı (mL/dakika), *A*, *B* ve *C* ise model parametreleridir. Her şeyden önce model parametrelerine göre doğrusaldır ve bu nedenle bu parametreler doğrusal regresyonla elde edilebilir. Bu da Excel'de veri çözümleme aracını kullanmak anlamına gelmektedir. Denklem 11'de hem *x* hem de 1/*x* yer aldığından hemen *x* sütununun yanına yeni bir sütun eklenerek 1/*x* değerleri Excel'de hesaplatılır (Şekil 7). Veri çözümlemeden regresyona girildikten sonra Y çıkış aralığı için C2:C14 seçilir. Denklemimizde iki *x* değeri yer aldığından (*x* ve 1/*x*) X çıkış aralığı için bunların her ikisi de seçilir (A2:A14 + B2:B14) ve özet çıkışı elde edilir (Şekil 8). Özet çıkışından model parametrelerinin anlamlı ve modelin verilerle uyumlu olduğu (standart hata = 0,11) anlaşılmaktadır. Ancak, grafik üzerinde görsel olarak da bu uyumluluğu göstermek uygun olacaktır. Bunun için D kolonuna model yazılır ve her bir akış hızı için plaka yüksekliği Excel'e hesaplatılır. Daha sonra grafik üzerindeki verilere sağ tıklayıp "Veri Seç"ten "Ekle"ye gelinir. Çıkan pencereye seri adı olarak "model" yazılır. Seri X değeri olarak *x* seçilir (A2:A14), seri Y değeri olarak ise hesaplatılan model verileri (D2:D14) girilir. Böylece model verileri (turuncu olarak görülen daireler) deney verileri (daha önce grafik üzerinde gördüğümüz mavi daireler) ile birlikte gözlemlenebilir, ancak bu göz yanılmasına yol açacağından model verilerini görsellik olarak daha uygun hale getirmek yerinde olacaktır. Bunun için model verilerine (turuncu daireler) sağ tıklayıp "Seri Grafik Türünü Değiştir"e gelip açılan pencereden model için dağılım yerine "Düz Çizgilerle Dağılım" seçilir. Böylece deney verileri ve bu verileri tanımlamak için kullanılan modelin uyumu aynı grafik üzerinde görülebilir (Şekil 9).

Mikrobiyal Büyüme Hızının pH'a Göre Tanımlanması

Son örneğimizde Şekil 10'da görünen bir bakterinin pH'ya (*x*) karşılık büyüme hızını (*y*) gösteren veriler vardır. Diğer iki örneğimizde olduğu gibi grafik çizerek başlamak en doğru yaklaşım olacaktır. Verilere dikkatlice bakınca Excel'in içinde yer alan 2'nci ve 3'üncü dereceden polinom modellerle verinin tanımlanabileceği anlaşılmaktadır (Şekil 11). Ancak, her iki model de veriyi tanımlama açısından sorunludur. Şöyle ki, 2'nci dereceden polinom modelde büyüme hızı (μ) 6<pH<7 aralığında (ki bu aralıkta herhangi bir deneysel veri bulunmamaktadır) önce artıp sonra azalmaktadır. Büyüme hızı yaklaşık olarak pH = 6.5'te de maksimuma ulaşmaktadır. Diğer yandan aynı aralıkta 3'üncü dereceden polinom modelde büyüme hızı hafifçe azalıp sonra artmaktadır ve yine yaklaşık olarak pH=6.5'te bu aralığın en düşük büyüme hızı gözlemlenmektedir (Şekil 11). Veriye baktığımızda genel olarak pH artıkça büyüme hızının da arttığı görülmektedir dahası mikrobiyoloji bilgimiz genel olarak bakteriler için pH=7'de asidik pH'lara göre büyüme hızının daha fazla olması gerektiğini söylemektedir. Bu durumda optimum pH'yı 7 olarak kabul edip yeni bir değişken olarak $pH' = (7 - pH)^2$ tanımlanırsa ve bu değişken baz alınarak parabolik regresyon yapılırsa [$\mu = a(pH')^2 + b$] daha önce uygulanan iki polinom modele alternatif olabilir. Burada hem 2'nci hem de 3'üncü dereceden polinom modeller parametrelerine göre doğrusaldır. Dahası alternatif olarak yazmış olduğumuz model de parametrelerine göre doğrusal olduğundan Excel'de veri çözümleme aracını kullanmak mümkündür. Burada dikkat edilecek husus 2'nci dereceden polinom model için x^2'yi Excel'de hesaplatmak ve veri çözümleme-regresyon'da X giriş aralığına hem *x*'li hem de x^2'li sütunları girmek; 3'üncü dereceden polinom modelde için ise x^2 ve x^3'ü Excel'de hesaplatarak X giriş aralığına *x*'li, x^2'li ve x^3'lü sütunları girmektir. Şekil 12'de her üç modelin veriye uyumlulukları gösterilmektedir. Tablo 1'de de özet çıkışlarından elde edilen ayarlı R^2 ve standart hatalar verilmiştir. İkinci dereceden polinom model en kötü uyumu göstermektedir. Öte yandan, her ne kadar 3'üncü dereceden polinom model en iyi uyumu vermiş gibi görünse de (en yüksek ayarlı R^2 ve en düşük standart hata değerine sahiptir) alternatif model mikrobiyolojik olarak daha gerçekçi sonuç vermektedir. Bunun nedeni yukarıda da değindiğimiz gibi bakterilerin genellikle pH=7 değerinde büyüme hızlarının maksimum olmasıdır. Alternatif model bize bunu sağlamaktadır (Şekil 12). Oysa 2'nci polinom modelde büyüme hızı yaklaşık 6.5 pH değerinde maksimumken, 3'üncü dereceden polinom modelde büyüme hızı pH=6.5'de azalmakta sonra tekrar artmaktadır. Burada "istatistiksel olarak daha iyi sonuç veren ancak daha fazla parametre içeren bir model mi (4 parametreye sahip 3'üncü dereceden polinom model) yoksa istatistiksel olarak biraz daha kötü ancak daha az parametreye sahip aynı zamanda mikrobiyolojik olarak gerçekçi bir model mi (2 parametreye sahip alternatif model)" sorusunun cevabını da vermiş bulunuyoruz. Alternatif model ile 3'üncü dereceden polinom model arasındaki uyumun farkının az olması (Tablo 1) ve alternatif modelin daha az parametreyle gerçekçi sonuçlar elde etmesi bu modeli tercih sebebi haline getirmektedir.

Sonuç

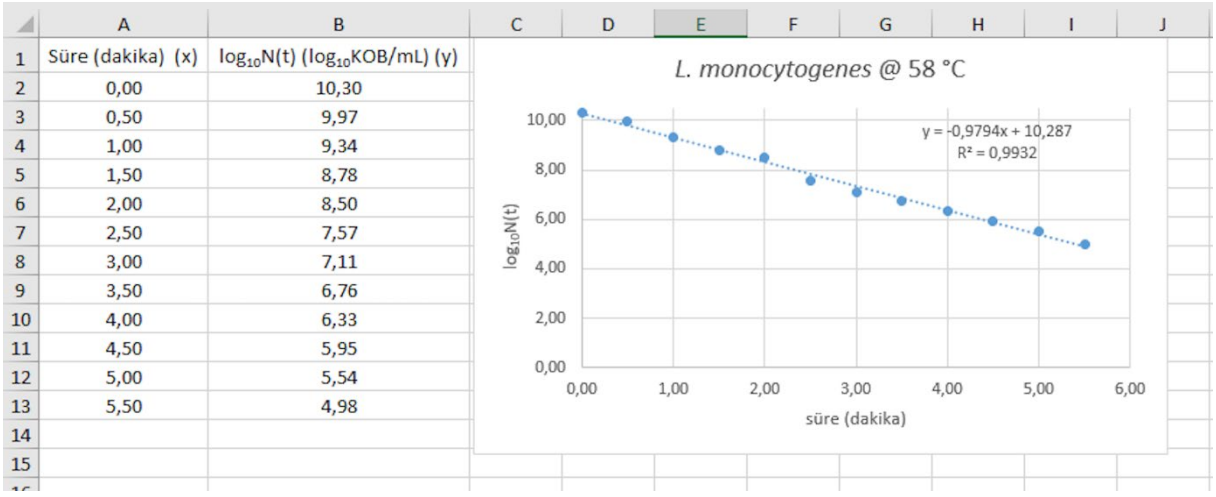
Eğer deneysel verileri tanımlamak için kullanılan herhangi bir model parametresine/ parametrelerine göre doğrusalsa bu modelin parametresi/parametreleri doğrusal regresyon kullanılarak bulunabilir. Excel'de yer alan veri çözümleme uygulaması parametrelerine göre doğrusal modeller için kullanılabilecek güçlü bir doğrusal regresyon aracıdır. Bu çalışmada basitten karmaşığa üç örnekle Excel'in doğrusal regresyonda nasıl kullanılacağı açıklanmaya çalışılmıştır. İlk örnekte hem

verilerin hem de model parametrelerin doğrusal olduğu ve Excel'in içinde yer alan bir modelin kullanılarak mikrobiyolojik veriler üzerinde veri çözümleme aracının uygulaması ve sonuçların yorumlanması gösterilmiştir. İkinci örnekte gaz kromatografi verisini tanımlamak için Excel'in içinde yer almayan ancak bilinen bir modelin, üçüncü örnekte ise mikrobiyal büyüme hızını pH'a göre tanımlamak için kullanıcının kendi yarattığı bir modelin uygulamaları gösterilmiştir. Bu çalışmanın gıda mühendisliği ve gıda bilimleri alanında çalışanlar için önemli katkıları olacağı değerlendirilmektedir.

	A	B
1	Süre (dakika) (x)	$\log_{10}$N(t) ($\log_{10}$KOB/mL) (y)
2	0,00	10,30
3	0,50	9,97
4	1,00	9,34
5	1,50	8,78
6	2,00	8,50
7	2,50	7,57
8	3,00	7,11
9	3,50	6,76
10	4,00	6,33
11	4,50	5,95
12	5,00	5,54
13	5,50	4,98
14		
15		
16		

Şekil 1. Sıvı besiyerindeki *Listeria monocytogenes*'in 58 °C'deki inaktivasyon verilerinin Excel'e girilmiş hali. Orijinal veriler Hassani ve ark. (2005)'dan alınmıştır.

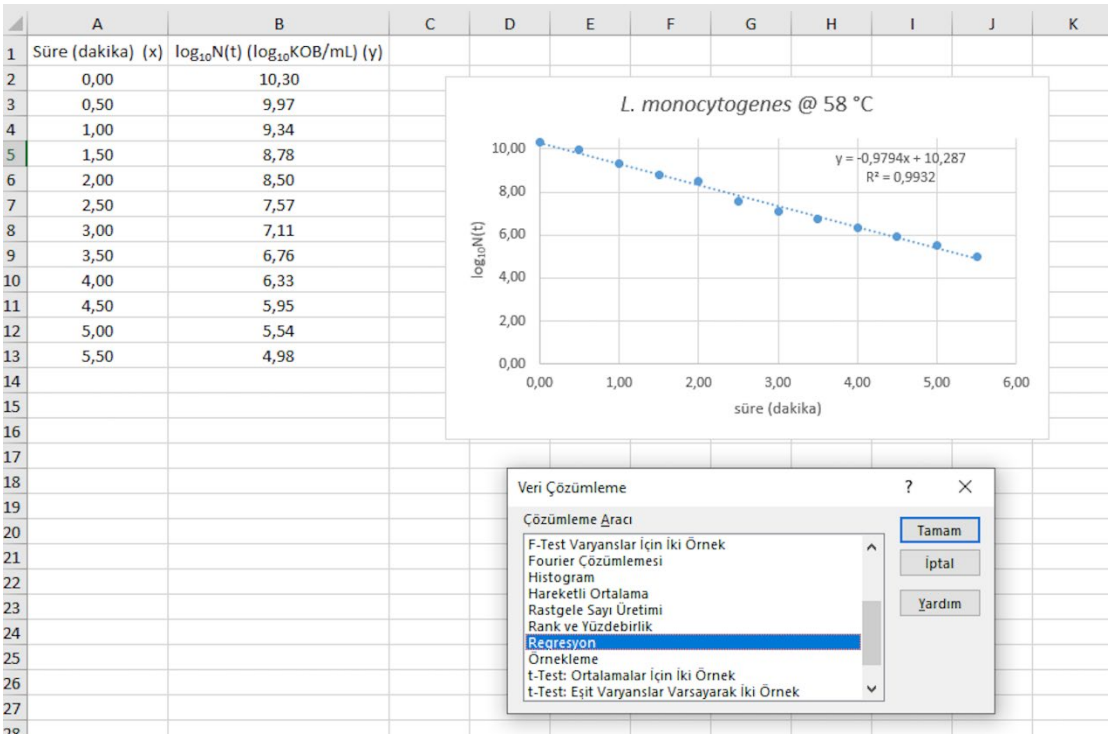
Figure 1. Inactivation data of *Listeria monocytogenes* in broth at 58 °C inserted in Excel. Original data were from Hassani et al. (2005).



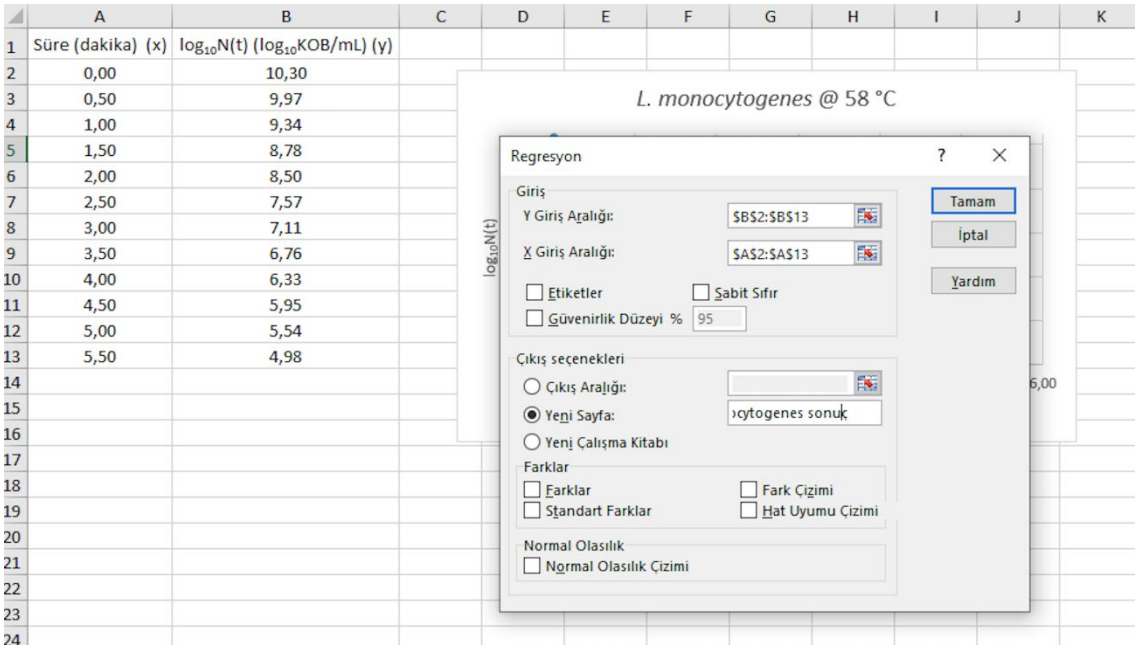
Şekil 2. Şekil 1'de gösterilen verinin Excel'de grafikleştirilmiş hali ve Excel'in içindeki doğrusal modelin ($y = ax + b$) bu veriye uygulanışı.

Figure 2. Sketching the graph of the data in Excel given in Figure 1 and application of the linear model ($y = ax + b$) in Excel to the data.

(a)



(b)



Şekil 3. Excel'de Şekil 1'de gösterilen veriye "Veri Çözümleme" aracını kullanarak doğrusal regresyon uygulanması.

Figure 3. Application of linear regression to the data given in Figure 1 by using "Data Analysis" tool in Excel.

	A	B	C	D	E	F	G	H	I
1	ÖZET ÇIKIŞI								
2									
3	*Regresyon İstatistikleri*								
4	Çoklu R	0,996572442							
5	R Kare	0,993156631							
6	Ayarlı R Kare	0,992472295							
7	Standart Hata	0,153713209							
8	Gözlem	12							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Anlamlılık F*			
12	Regresyon	1	34,29021416	34,29021416	1451,26867	3,70418E-12			
13	Fark	10	0,236277506	0,023627751					
14	Toplam	11	34,52649167						
15									
16		*Katsayılar*	*Standart Hata*	*t Stat*	*P-değeri*	*Düşük %95*	*Yüksek %95*	*Düşük 95,0%*	*Yüksek 95,0%*
17	Kesişim	10,2874359	0,083469499	123,2478456	3,03387E-17	10,10145426	10,47341753	10,10145426	10,47341753
18	X Değişkeni 1	-0,979370629	0,025708289	-38,09552034	3,70418E-12	-1,036652266	-0,922088993	-1,036652266	-0,922088993
19									
20									
21									

Şekil 4. Şekil 1'de gösterilen veriye uygulanan doğrusal regresyonun Excel'deki "Özet Çıkışı".

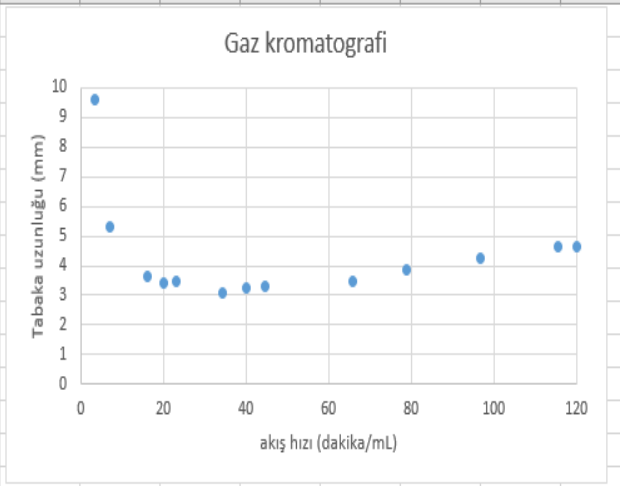
Figure 4. "Summary Output" of the application of linear regression to the data given in Figure 1 in Excel.

	A	B
1	Akış hızı (mL/dakika) (x)	Tabaka uzunluğu (mm) (y)
2	3,4	9,59
3	7,1	5,29
4	16,1	3,63
5	20	3,42
6	23,1	3,46
7	34,4	3,06
8	40	3,25
9	44,7	3,31
10	65,9	3,5
11	78,9	3,86
12	96,8	4,24
13	115,4	4,62
14	120	4,67
15		
16		

Şekil 5. Gaz kromatografi verileri. Orijinal veriler Moody (1982)'den alınmıştır.

Figure 5. Gas chromatography data. Original data were from Moody (1982).

	A	B
1	Akış hızı (mL/dakika) (x)	Tabaka uzunluğu (mm) (y)
2	3,4	9,59
3	7,1	5,29
4	16,1	3,63
5	20	3,42
6	23,1	3,46
7	34,4	3,06
8	40	3,25
9	44,7	3,31
10	65,9	3,5
11	78,9	3,86
12	96,8	4,24
13	115,4	4,62
14	120	4,67
15		
16		



Şekil 6. Şekil 5'te gösterilen verinin Excel'de grafikleştirilmiş hali.

Figure 6. Sketching the graph of the data in Excel given in Figure 5.

	A	B	C
1	Akış hızı (mL/dakika) (x)	(1/x)	Tabaka uzunluğu (mm) (y)
2	3,40	0,29	9,59
3	7,10	0,14	5,29
4	16,10	0,06	3,63
5	20,00	0,05	3,42
6	23,10	0,04	3,46
7	34,40	0,03	3,06
8	40,00	0,03	3,25
9	44,70	0,02	3,31
10	65,90	0,02	3,50
11	78,90	0,01	3,86
12	96,80	0,01	4,24
13	115,40	0,01	4,62
14	120,00	0,01	4,67
15			
16			

Şekil 7. Şekil 5'te gösterilen *x* verisinin Excel'de 1/*x*'e dönüştürülmüş hali.

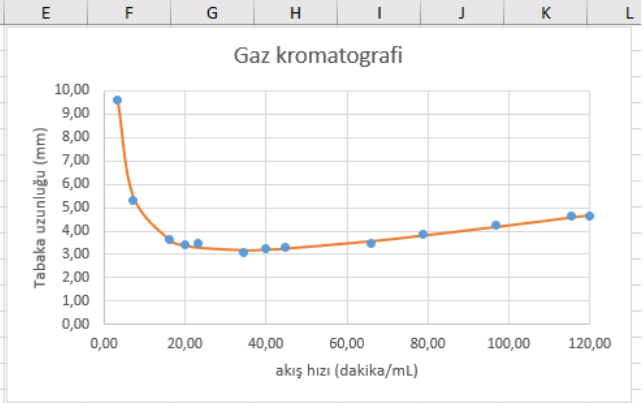
Figure 7. Transformation of the *x* data given in Figure 5 to 1/*x* in Excel.

	A	B	C	D	E	F	G	H	I
1	ÖZET ÇIKIŞI								
2									
3	*Regresyon İstatistikleri*								
4	Çoklu R	0,998405078							
5	R Kare	0,9968127							
6	Ayarlı R Kare	0,99617524							
7	Standart Hata	0,106506046							
8	Gözlem	13							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Anlamlılık F*			
12	Regresyon	2	35,47636462	17,73818231	1563,725771	3,28939E-13			
13	Fark	10	0,113435378	0,011343538					
14	Toplam	12	35,5898						
15									
16		*Katsayılar*	*Standart Hata*	*t Stat*	*P-değeri*	*Düşük %95*	*Yüksek %95*	*Düşük 95,0%*	*Yüksek 95,0%*
17	Kesişim	1,568074804	0,075571995	20,74941642	1,49738E-09	1,399689904	1,736459703	1,399689904	1,736459703
18	X Değişkeni 1	0,02435858	0,000966832	25,19423027	2,22317E-10	0,022204345	0,026512816	0,022204345	0,026512816
19	X Değişkeni 2	26,72786047	0,487411668	54,8363164	9,85846E-14	25,64183959	27,81388134	25,64183959	27,81388134
20									
21									
22									

Şekil 8. Şekil 7'de gösterilen veriye uygulanan doğrusal regresyonun [van Deemter modeli ($y = Ax + B/x + C$)] Excel'deki "Özet Çıkışı".

Figure 8. "Summary Output" of the application of linear regression [van Deemter model ($y = Ax + B/x + C$)] to the data given in Figure 7 in Excel.

	A	B	C	D
1	Akış hızı (mL/dakika) (x)	(1/x)	Tabaka uzunluğu (mm) (y)	ymodel = Ax+B/x+C
2	3,40	0,29	9,59	9,51
3	7,10	0,14	5,29	5,51
4	16,10	0,06	3,63	3,62
5	20,00	0,05	3,42	3,39
6	23,10	0,04	3,46	3,28
7	34,40	0,03	3,06	3,17
8	40,00	0,03	3,25	3,20
9	44,70	0,02	3,31	3,24
10	65,90	0,02	3,50	3,56
11	78,90	0,01	3,86	3,80
12	96,80	0,01	4,24	4,17
13	115,40	0,01	4,62	4,57
14	120,00	0,01	4,67	4,67
15				
16				
17				A = 0,024
18				B = 26,73
19				C = 1,57
20				



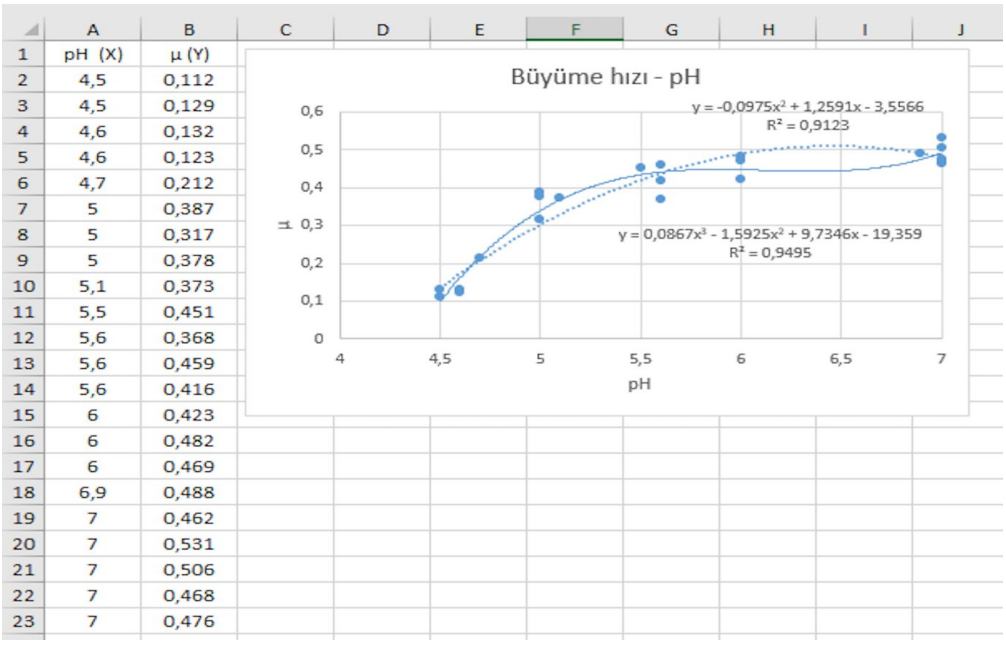
Şekil 9. Şekil 7'de gösterilen veriye uygulanan van Deemter modelinin ($y = Ax + B/x + C$) uyumunun Excel'de grafik üzerinde gösterilmesi. Mavi daireler deney verilerini turuncu çizgi ise model uyumunu belirtmektedir.

Figure 9. Showing the application of the van Deemter model ($y = Ax + B/x + C$) on the graph to the data given in Figure 7 in Excel. Blue circles represent experimental data orange line represents model fit.

	A	B
1	pH (X)	μ (Y)
2	4,5	0,112
3	4,5	0,129
4	4,6	0,132
5	4,6	0,123
6	4,7	0,212
7	5	0,387
8	5	0,317
9	5	0,378
10	5,1	0,373
11	5,5	0,451
12	5,6	0,368
13	5,6	0,459
14	5,6	0,416
15	6	0,423
16	6	0,482
17	6	0,469
18	6,9	0,488
19	7	0,462
20	7	0,531
21	7	0,506
22	7	0,468
23	7	0,476
24		

Şekil 10. Bir bakterinin pH'ya karşılık büyüme hızı (μ) verileri. Orijinal veriler Baranyi ve Roberts (1995)'dan alınmıştır.

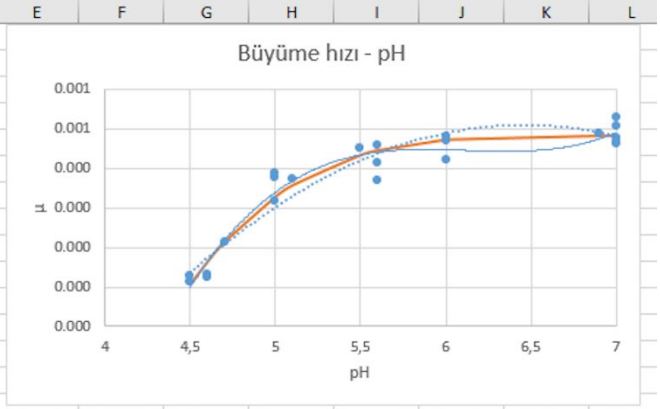
Figure 10. pH versus growth rate (μ) data of a bacterium. Original data were from Baranyi and Roberts (1995).



Şekil 11. Şekil 10'da gösterilen verinin Excel'de grafikleştirilmiş hali. Mavi daireler deney verilerini, noktalı mavi çizgi veriye uygulanan 2'nci derece polinom modeli ($y = ax^2 + bx + c$), düz mavi çizgi ise uygulanan 3'nci derece polinom modeli ($y = ax^3 + bx^2 + cx + d$) belirtmektedir.

Figure 11. Sketching the graph of the data in Excel given in Figure 10. Blue circles represent experimental data, dotted blue lines represent 2nd order polynomial model ($y = ax^2 + bx + c$) and solid blue lines represent 3rd order polynomial model ($y = ax^3 + bx^2 + cx + d$).

	A	B	C	D
1	pH	[(7-pH)^2]^2 (x)	μ (y)	ymodel = ax+b
2	4,5	39,0625	0,112	0,107
3	4,5	39,0625	0,129	0,107
4	4,6	33,1776	0,132	0,163
5	4,6	33,1776	0,123	0,163
6	4,7	27,9841	0,212	0,213
7	5	16	0,387	0,328
8	5	16	0,317	0,328
9	5	16	0,378	0,328
10	5,1	13,0321	0,373	0,356
11	5,5	5,0625	0,451	0,433
12	5,6	3,8416	0,368	0,444
13	5,6	3,8416	0,459	0,444
14	5,6	3,8416	0,416	0,444
15	6	1	0,423	0,471
16	6	1	0,482	0,471
17	6	1	0,469	0,471
18	6,9	1E-04	0,488	0,481
19	7	0	0,462	0,481
20	7	0	0,531	0,481
21	7	0	0,506	0,481
22	7	0	0,468	0,481
23	7	0	0,476	0,481
24				



Şekil 12. Şekil 10'da gösterilen veriler için Excel'de alternatif modelin yazılması ve uyumu. Mavi daireler deney verilerini, noktalı mavi çizgi veriye uygulanan 2'nci derece polinom modeli ($y = ax^2 + bx + c$), düz mavi çizgi uygulanan 3'nci derece polinom modeli ($y = ax^3 + bx^2 + cx + d$), turuncu kalın çizgi ise alternatif modeli belirtmektedir.

Figure 12. Formulating the alternative model for the data given in Figure 10 in Excel. Blue circles represent experimental data, dotted blue lines represent 2nd order polynomial model ($y = ax^2 + bx + c$), solid blue lines represent 3rd order polynomial model ($y = ax^3 + bx^2 + cx + d$) and thick orange lines represent the alternative model.

Etik Standart ile Uyumluluk

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Gıda bilimlerinde Excel kullanımı 2: Doğrusal olmayan regresyon

Merve Yurdakul[1], Cansu Leylak[2], Sencer Buzrul[2]

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[1] Konya Gıda ve Tarım Üniversitesi, Biyomühendislik Bölümü, Melikşah Mah. Beyşehir Cad. No:9, 42080 Meram, Konya, Türkiye iye

[2] Konya Gıda ve Tarım Üniversitesi, Gıda Mühendisliği Bölümü, Melikşah Mah. Beyşehir Cad. No:9, 42080 Meram, Konya, Türk

ORCID IDs of the authors:
M.Y. 0000-0002-5597-4692
C.L. 0000-0003-2393-0545
S.B. 0000-0003-2272-3827

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Correspondence: Sencer BUZRUL
E-mail: sencer.buzrul@gidatarim.edu.tr

ÖZ

Gıda bilimlerinde deneysel verilerin matematiksel modellerle tanımlanabilmesi için doğrusal olmayan regresyona sıklıkla ihtiyaç duyulmaktadır. Excel'in içerisinde yazılımda yüklü olan doğrusal olmayan birçok model olmasına karşın Excel bunları doğrusal hale dönüştürmekte ve verilere doğrusal olmayan regresyon yerine doğrusal regresyon uygulamaktadır. Oysa Excel'de yer alan "Çözücü" aracı kullanılarak doğrusal olmayan regresyon uygulamak mümkündür. Bu çalışmanın amacı Excel'deki Çözücü aracını kullanarak deneysel verilere doğrusal olmayan regresyonun nasıl uygulanacağını örnekler üzerinden göstermektir. Bu amaç doğrultusunda sırasıyla iki, üç ve dört parametreli doğrusal olmayan modeller, üç farklı veri setine Çözücü kullanılarak uygulanmıştır. İlk örnekte yeşil zeytinde bulunan violaksantin pigmentinin zamana bağlı olarak değişimi üstel model kullanılarak tanımlanmış, ikinci örnekte ise *Escherichia coli* bakterisinin sıcaklıkla inaktivasyonu üç parametreli doğrusal olmayan bir modelle açıklanmaya çalışılmıştır. Son örneğimizde *Listeria monocytogenes* bakterisinin büyümesi yine doğrusal olmayan bir modelle tanımlanırken model uyumunu gösteren değerler de model parametreleriyle birlikte hesaplanmıştır. Çözücü aracının tek olumsuz yanı parametre değerlerini standart hata veya güven aralıklarıyla birlikte hesaplayamamasıdır. Onun dışında doğrusal olmayan regresyon yapmak için kullanılan ücretli yazılımlardan herhangi bir farkı yoktur. Bu çalışmanın gıda mühendisliği veya gıda bilimi alanında çalışan ve bilgisayarlarında Excel yüklü olan ancak diğer ücretli yazılımlara sahip olmayan birçok araştırmacıya faydalı olacağı değerlendirilmektedir.

Anahtar Kelimeler: Doğrusal olmayan modeller, Excel, Regresyon, Çözücü

ABSTRACT

Use of Excel in food science 2: Non-linear regression

Nonlinear regression is often required in order to define experimental data with mathematical models in food science. Although there are many non-linear models in Excel by default, Excel linearizes them and applies linear regression to the data instead of nonlinear regression. However, it is possible to apply non-linear regression by using the "Solver" tool in Excel. The objective of this study was to show how to apply non-linear regression to experimental data by using the Solver tool in Excel. For this purpose, non-linear models having two, three and four parameters, were applied to three different data sets using Solver, respectively. In the first example, the change of the violaxanthin pigment in green olives with respect to time was described using the exponential model, and in the second example, the heat inactivation of *Escherichia coli* was tried to be explained with a three-parameter non-linear model. In our last example, the growth of *Listeria monocytogenes* was again described by a non-linear model, while the goodness-of-fit indices were calculated together with the model parameters. The only disadvantage of the Solver tool was that it cannot calculate parameter values along with the standard errors or confidence intervals. Apart from that, there was no difference between shareware used for non-linear regression. It is considered that this study would be beneficial for many researchers having Excel installed in their computers but without other sharewares and working in the field of food engineering or food science.

Keywords: Non-linear models, Excel, Regression, Solver

Giriş

Excel her ne kadar finans alanında çalışanlar için hesap çizelgesi programı olsa da fen bilimleri ve mühendislik alanlarında da sıklıkla kullanılmakta ve kullanıcılar için büyük kolaylıklar sağlamaktadır. Model parametrelerinin doğrusal olup olmamasına bağlı olarak uygun bilgisayar programları kullanılarak doğrusal regresyon veya doğrusal olmayan regresyon uygulanır. Örneğin $y = a \cdot e^x$ modeli [y bağımlı değişken, x bağımsız değişken, a model parametresi, e ise Euler sayısıdır (2.7182818)] parametresine göre doğrusaldır çünkü $\frac{\partial y}{\partial a} = e^x$'dir. Yani a parametresi kısmi türevin içinde yoktur. Bu nedenle $y = a \cdot e^x$ modeli için doğrusal regresyon kullanılarak a parametresi bulunur. Öte yandan, $y = e^{ax}$ modeli doğrusal değildir çünkü $\frac{\partial y}{\partial a} = x \cdot e^{ax}$'dir. Kısmi türevin içinde a parametresi olduğundan model a parametresine göre doğrusal değildir ve bu model için doğrusal olmayan regresyon uygulanmalıdır.

Doğrusal (parametresine göre doğrusal) modeller için Excel'de yer alan "Veri Çözümleme" aracının içerisindeki regresyon uygulaması kullanılarak kolayca doğrusal regresyon yapılabilir. Doğrusal regresyon konusunda yeterli bilgisi olmayanlar daha önceki makalemiz Leylak ve ark. (2020)'dan faydalanabilirler. Söz konusu makalede Excel'de doğrusal regresyon örnekler üzerinde açıklanmıştır. Peki, doğrusal olmayan modeller için Excel kullanmak mümkün müdür? Excel'de x-y şeklindeki veriler grafik haline getirilip bu verilere farklı doğrusal veya doğrusal olmayan modeller kullanılarak eğilim çizgisi eklenebilir. Excel'in içinde fabrika ayarı olarak bazı doğrusal olmayan modeller yer almaktadır. Ancak, Excel bu modelleri doğrusallaştırmakta başka bir deyişle doğrusal hale getirmekte ve parametre değerlerini doğrusal hale getirilmiş modellere doğrusal regresyon uygulayarak hesaplamaktadır.

Burada bir örnek vermek okuyucuyu aydınlatmak açısından yerinde olacaktır. Yeşil zeytinde bulunan violaksantin maddesinin zamana bağlı olarak değişimini ele alalım. Şekil 1'de gösterilen veriyi tanımlamak için uygun model üstel model olabilir. Excel'in içinde bu model $y = a \cdot e^{bx}$ şeklinde yer almaktadır ve bu model a parametresine göre doğrusal, b parametresine göre ise doğrusal değildir. Diğer bir deyişle a'ya göre kısmi türev alındığında a parametresi türev için yer almamakta, b'ye göre kısmi türev alındığında ise b parametresi kısmi türev içinde yer almaktadır. Dolayısıyla bu veriyi bu modelle tanımlayabilmek için doğrusal olmayan regresyon kullanılmalıdır. Excel'de söz konusu veriyi grafik haline getirip eğilim çizgisi ekleden üstel model seçilirse Şekil 2'de gösterilen model uyumu ve sonuçlar elde edilmektedir. Bu sonuçlara göre a = 1.875, b = – 0.067 ve R^2 = 0.9407'dir. Modelin doğal logaritmasını ($\log_e$ ya da ln) alırsak $\ln y = \ln a + bx$ elde ederiz yani modeli doğrusal hale getirmiş oluruz. Bu modeli şu şekilde yazabiliriz $y' = a' + bx$ ve bu haliyle model parametrelerine (a' ve b) göre doğrusaldır. Yani doğrusal regresyon kullanılabilir. Excel'de ln$y$'ye (y') karşılık x grafiğini çizip eğilim çizgisi ekleden doğrusal model eklenirse Şekil 3'teki model uyumu ve sonuçlar elde edilir: b = –0.067, $\ln a$ = 0.6286 → $a = e^{0.6286}$ = 1.875 ve R^2 =0.9407'dir. Görüldüğü gibi her iki durumda da aynı sonuçlar elde edilmiştir: Yani Excel doğrusal olmayan bir modeli doğrusal hale getirip parametre değerlerini bu modele göre hesaplamaktadır. Öte yandan, aynı veriyi aynı modelle SigmaPlot (Versiyon 12.0) programı ile doğrusal olmayan regresyon uygulayıp tanımlarsak a =1.975, b= –0.072 ve R^2 = 0.9660 elde ederiz. Şekil 4'te verinin SigmaPlot'ta doğrusal ve doğrusal olmayan regresyon uygulanarak tanımlanması gösterilmektedir. Görüldüğü gibi her ne kadar sonuçlar yakın olsa da doğrusal olmayan regresyon daha yüksek R^2 değerine yani daha iyi model uyumuna sahiptir. SigmaPlot, R^2 değerinin dışında model uyumu anlamak için başka ölçütleri de hesaplamaktadır: Bütün bunlar da göstermektedir ki, örneğimiz için doğrusal olmayan regresyon sonuçları doğrusal regresyon sonuçlarına göre daha iyi sonuç vermektedir (gösterilmeyen sonuçlar). Dahası, verileri doğrusal hale getirmek çok tekrarlı deneylerdeki (buradaki deney iki tekrarlı yapılmıştır.) ~~hata~~ tekrarlar arasındaki fark yapısını değiştirmektedir; verileri doğrusal hale getirmek düşük x değerlerinde tekrarlar arasındaki farkları küçültürken x büyüdükçe tekrarlar arasındaki farklar da büyümektedir (Şekil 2 ve Şekil 3). Bu nedenle doğrusal olmayan modelleri doğrusal hale getirip parametre değerlerini elde etmek tavsiye edilen bir uygulama değildir (Motulsky ve Ransnas, 1987).

Tam burada "Doğrusal regresyon ile doğrusal olmayan regresyon arasındaki fark nedir?" sorusunu sormak uygun olacaktır. Doğrusal regresyonda analitik çözüm varken doğrusal olmayan regresyonda bu mümkün değildir. Dahası, doğrusal olmayan regresyonda parametrelerin başlangıç değerlerini kullanıcının girmesi beklenir. Kullanılan program girilen bu değerlerden başlayarak belli sayıda iterasyon yaparak parametre değerlerini bulmaya çalışır (Kemmer ve Keller, 2010).

Excel'de "Çözücü" Aracı Kullanılarak Deneysel Verilere Doğrusal Olmayan Regresyon Uygulamak

Bu bölümde üç farklı örnek üzerinde Excel'de doğrusal olmayan regresyon ile deneysel verileri tanımlamayı göstereceğiz. Ancak, çözücü aracı Excel'de yüklü değilse (Microsoft Office Standard 2016 Excel'de "Veri" sekmesine tıkladığınızda sağ üste çözücü görünmüyorsa), sırasıyla Dosya > Seçenekler > Eklentiler > Excel Eklentileri (Git) sekmelerinden sonra çıkan ekrandan "Çözücü" işaretlenerek yüklenmelidir.

Yeşil Zeytinde Bulunan Violaksantin Pigmentinin Zamana Bağlı Olarak Değişiminin Tanımlanması

İlk örneğimizde Şekil 1'de verileri gösterilen, yeşil zeytinde bulunan violaksantin maddesinin zamana bağlı olarak değişimini ele alalım. Bu verilerin üstel model ($y = a \cdot e^{bx}$) ile tanımlanabileceğini biliyoruz. Dahası Excel'in içinde bulunan bu modeli kullanırsak Excel'in doğrusal olmayan regresyon yerine doğrusal regresyonla parametreleri (*a* ve *b*) hesaplayacağını ve bunun yetersiz çıkarımlara neden olacağını da biliyoruz. Bu nedenle burada Excel'de çözücü aracını kullanarak model parametrelerini hesaplatmayı göstereceğiz.

İlk aşamada dağılım grafiği çizilir. F sütununa (F1 hücresi) parametre, G sütununa (G1 hücresi) ise parametre değerleri yazılır. Parametreler F2 ve F3 hücrelerine a ve b olacak şekilde sırayla girilir. Daha sonra G2 hücresine tıklanıp 'Formüller' sekmesinden "Ad tanımla" seçeneği seçilerek açılan pencerede Tamam'a tıklanır. Excel otomatik olarak G2 hücresini "a" olarak tanımlar. Aynı işlemler G3 hücresi için tekrarlanır ve b parametresi de tanımlanır (Şekil 5).

Parametreler tanımlandıktan sonra C sütununa model yazılır. Türkçe Excel'de *e* sayısı "ÜS" olarak ifade edildiğinden $y = a \cdot e^{bx}$ modeli Excel'de ymodel = a*ÜS(-b*A2) şeklinde yazılıp bütün süreler (A2:A16) için ymodel hesaplatılır (Şekil 6). Parametrelere ad tanımlamamızın nedeni modeli yazarken parametrelerin modelde hücre (G2 ve G3) olarak değil a ve b olarak görünmesidir. Bu aşamada parametrelerin başlangıç değerlerini girmek gerekecektir. Bu, Çözücü'nün iterasyona nereden başlaması gerektiğini söylemekle aynı anlama gelmektedir. Parametre değerlerini gerçek değerlere ne kadar yakın yazarsak Çözücü daha az iterasyonla yani daha hızlı bir şekilde sonuca ulaşacaktır. Öte yandan, uygun parametre değerleri girilmezse Çözücü herhangi bir sonuca ulaşamayabilir. Örneğimizde *a* parametresinin değerinin 2'ye yakın olacağını tahmin etmek zor değildir çünkü $x = 0$ iken $y = a$'dır. Ancak *b* parametresinin tahmini o kadar da kolay değildir. Örneğimizde her iki parametre değeri de 1 olacak şekilde yazılarak bu değerler için ymodel'e karşılık süre serisi aynı grafik üzerinde gösterilmiştir (Şekil 7).

Regresyonda amaç hataların karesinin toplamının minimize edilmesi olduğundan, C sütununa hataların karesi yani $(y\text{-ymodel})^2$ eşitliği girilir ve hesaplatılır. Hesaplanan değerler toplanarak D18 hücresindeki değer elde edilir (Şekil 7). Sonrasında "Veri" sekmesinden Çözücü'ye tıklanır ve açılan ekranda "Hedef ayarla" kısmına hataların karesi (D18 hücresi) seçilir. Amacımız D18 hücresinin minimize edilmesi olduğundan "Hedef" "En Küçük" tıklanır. Bu amaca ulaşmak için yani hataların karesinin toplamını minimize etmek için a ve b parametrelerinin değiştirilmesi gerekmektedir. Bu nedenle "Değişken Hücreleri Değiştirerek" kısmına parametreler (G2:G3) seçilir ve "Çöz"e tıklanır. Çözücü en küçük toplam $(y\text{-ymodel})^2$ değerini bulmak için iterasyon yapar ve en uygun *a* ve *b* parametrelerinin değerlerini belirler (Şekil 8). Görüldüğü gibi Excel Çözücü aracı SigmaPlot'la aynı sonucu elde etmiştir ($a = 1.975$, $b = -0.072$). Bu noktada çözücünün olumsuz bir yanından bahsetmek gerekirse parametre değerleri bu yöntemde standart hataları veya güven aralıklarıyla birlikte hesaplanamamaktadır. Parametre değerlerinin belirsizliğini elde etmek önemlidir (Dolan ve Mishra, 2013) ve bunu Excel kullanarak yapmak da mümkündür (Lambert ve ark., 2012). Ancak bu çalışmada amacımız Excel'de doğrusal olmayan regresyonu göstermek olduğundan başka bir yöntemle parametrelerin standart hatalarını hesaplamak irdelenmemiştir.

Escherichia coli Bakterisinin Isıyla Inaktivasyonunun Tanımlanması

İkinci örneğimizde *E. coli* bakterisinin 56,6 °C'de inaktivasyonunu inceleyeceğiz. Şekil 9'da gösterilen veriler için uygun model aşağıda gösterilmiştir:

$$\log_{10} N(t) = \log_{10} N_0 - \log_{10}\{1 + e^{[k\cdot(t-S)]}\} \quad (1)$$

Burada $\log_{10}N(t)$ bakterinin *t* zamandaki sayısı, $\log_{10}N_0$ bakterinin başlangıçtaki sayısı ($t = 0$), *k* inaktivasyon hızı (zaman^{-1}), *S* ise inaktivasyonun gözlemlenmeye başladığı zamandır. Yani modelin üç parametresi $\log_{10}N_0$, *k* ve S'dir. İlk örneğimizde anlatıldığı üzere A ve B sütunundaki verilerle dağılım grafiği çizilir ve parametreler tanımlanır. C sütununa model yazılır: ymodel = logN0-LOG10((1+ÜS((k*(A2-S))))) (Şekil 9).

Mevcut durumda parametre değerlerine yine 1 yazılabilir. D sütununda ise karelerin hatası hesaplatılır ve yazılan model süreye karşılık aynı grafik üzerinde gösterilir (Şekil 10). Çözücü'den parametre değerleri değiştirilerek (G2:G4) D17 hücresi (hataların karesinin toplamı) minimize edilir ve parametre değerleri elde edilir (Şekil 11). Çözücü burada logN0, k ve S parametrelerine farklı değerler atayıp, iterasyon yaparak toplam $(y\text{-ymodel})^2$ değerini en küçük olarak elde edecek şekilde bu parametrelerin değerlerini belirlemektedir. Minimize edilen toplam $(y\text{-ymodel})^2$ değeri için logN0 = 9.47, k = 0.71 ve S = 8.61 olarak elde edilmiştir. Şekil 11'de görüldüğü gibi $(y\text{-ymodel})^2$ toplamı minimize edilerek oluşturulan model ve deney verileri oldukça uyumludur.

Listeria monocytogenes'in *Büyümesinin Tanımlanması*

Son örneğimizde *L. monocytogenes*'in 30 °C'de %9 tuz içeren sıvı besiyerinde büyümesini gösteren verileri tanımlayacağız. Şekil 12'de gösterilen verileri tanımlamak için uygun bir model olan Gompertz denklemini kullanacağız:

$$\log_{10} N(t) = \log_{10} N_0 + (\log_{10} N_{max} - \log_{10} N_0) \cdot e^{-e^{\left\{\frac{\mu \cdot e}{[\log_{10} N_{max} - \log_{10} N_0]} \cdot (\lambda - t) + 1\right\}}} \quad (2)$$

Burada $\log_{10}N(t)$ bakterinin t zamandaki sayısı, $\log_{10}N_0$ bakterinin başlangıçtaki sayısı ($t = 0$), $\log_{10}N_{max}$ bakterinin ulaşabileceği azami sayı ($t \to \infty$), μ büyüme hızı (logKOB/mL/zaman), λ ise büyümenin gözlemlenmeye başladığı zamandır. Modelin parametreleri $\log_{10}N_0$, $\log_{10}N_{max}$, μ ve λ'dır.

Diğer iki örneğimizde olduğu gibi grafik çizilerek başlamak en doğru yaklaşımdır. Bu örnekte diğerlerine ek olarak model uyumunu gösteren R^2, ayarlı R^2 ve RMSE değerlerinin Excel kullanılarak hesaplanması da açıklanacaktır. Bu değerler hakkında daha detaylı bilgi için okuyucuyu yine bir önceki makalemize yönlendirmekte fayda görüyoruz (Leylak ve ark., 2020).

Öncelikle parametreler (logN00, logNmax, μ, λ), parametrelerin hemen altına sırasıyla yort (y değerlerinin ortalaması), df (serbestlik derecesi), R^2 (R^2), ayarlıR^2 (ayarlı R^2) ve RMSE yazılır. Bu yazılanların hepsi "Formüller > Ad tanımla" kullanılarak tanımlanır ve C sütunu içerisine model oluşturulur:

ymodel=logN00+(logNmax-logN00)*ÜS(-ÜS(μ*ÜS(1)/(log Nmax-logN00)*(λ-A2)+1)) Modelin parametre sayısı diğer iki örneğimize göre fazla olduğundan başlangıç parametre değerlerini belirlemek burada daha önemlidir. Kullanıcının bütün parametre değerlerine 1 girmesi durumunda ymodel tanımsız olacağından bu sefer bütün parametrelere 1 girilmemiştir. Modelde yer alan logN00 başlangıçtaki, logNmax ise azami bakteri sayısını göstermektedir. Verimize ve grafiğimize (Şekil 12) göre başlangıç miktarı 3.9 $\log_{10}$, azami miktar ise 8.9 $\log_{10}$'dur. Dolayısıyla, logN00 ve logNmax değerlerine yakın değerler örneğin 3 ve 8 yazabiliriz. Diğer iki parametreden büyüme hızını (μ) veriye veya grafiğe bakarak anlamaya çalışmak çok kolay değildir ve bu durumda daha önceki örneklerde yaptığımız gibi μ değerine 1 yazılabilir. Öte yandan, büyümenin gözlemlendiği (λ) zaman grafikten (Şekil 12) az çok anlaşılabilir durumdadır ve bu nedenle bu değere de 10 yazılabilir. Sonuç olarak parametre değerlerine veriye ve grafiğe bakarak sırasıyla 3, 8, 1 ve 10 girilebilir (Şekil 13).

Model denklemi oluşturulduktan sonra yort=ortalama (B2:B:34) Excel'e hesaplatılarak 6,14 olarak bulunur. D sütununda (y-ymodel)^2 değerleri ve E sütununa ise (y-yort)^2 değerleri hesaplatılır. Serbestlik derecesi (df) veri sayısı – modeldeki parametre sayısı olduğundan df=BAĞ_DEĞ_SAY(A2:A34)-BAĞ_DEĞ_SAY(I2:I5) formülü ile hesaplanır ve 29 (=33-4) olarak bulunur. R^2 değeri R^2 =1-TOPLA((B2:B34-C2:C34)^2)/TOPLA ((B2:B34-yort)^2)) formülünden hesaplatılır ancak bu formül dizi formülü olduğundan (her bir işlemin sırayla yapılması gerektiğinden) R^2 formülü seçilir ve "Ctrl+Shift+Enter" yapılarak {} içerisine alınır. Ayarlı R^2 değeri ayarlıR^2 =1-((1-R^2)*(BAĞ_DEĞ_SAY(B2:B34)-1)/df) formülünden hesaplanır. RMSE değeri ise =(KAREKÖK(TOPLA ((B2:B34-C2:C34)^2)/df)) formülü kullanılarak hesaplatılır ancak aynı R^2 değerinde olduğu gibi yine formül seçilir ve "Ctrl+Shift+Enter" yapılarak {} içerisine alınır.

Çözücü' ye gelinerek "Hedef ayarla" kısmına R^2 (I8 hücresi); ayarlıR^2 (I9 hücresi) veyahut RMSE (I10 hücresi) seçilebilir. Burada dikkat edilmesi gereken husus şudur: Uyumlu bir model için R^2 ve ayarlı R^2 değerlerinin yüksek olması istendiğinden I8 hücresi veya I9 hücresi seçildiğinde "Hedef" "En Büyük" tıklanır. Yani amacımız R^2 ve ayarlı R^2 değerlerinin maksimize edilmesidir. Öte yandan, "Hedef ayarla" kısmına RMSE (I10 Hücresi) seçilmesi durumunda RMSE değerinin uyumlu bir model için küçük olması istendiğinden "Hedef" "En Küçük" tıklanır. Bu durumda amacımız RMSE değerinin minimize edilmesidir. Hangi yöntem (maksimizasyon veya minimizasyon) seçilirse seçilsin aynı sonucu elde etmek mümkündür: Parametre değerleri logN00 = 3.95, logNmax = 8.85, μ = 0.1714, λ = 19.82 olarak bulunmuştur. Model uyumunu gösteren R^2 = 0.9984, ayarlıR^2 = 0.9981 ve RMSE = 0.0908 olarak bulunmuştur (Şekil 14). Bu değerlerden anlaşıldığı gibi model veriye iyi bir uyum sağlamaktadır ve bu grafik üzerinde de görülebilmektedir.

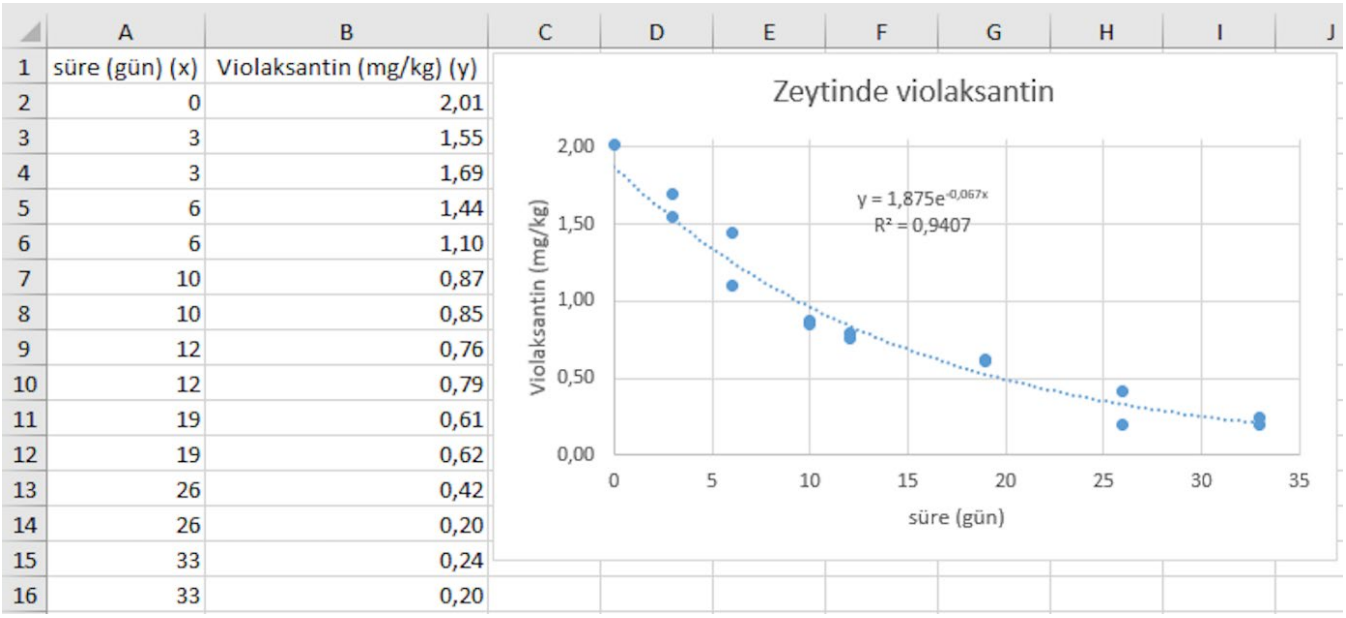
Sonuç

Eğer deneysel verileri tanımlamak için kullanılan herhangi bir model parametresine/ parametrelerine göre doğrusal değilse bu modelin parametre değerini/değerlerini elde etmek için doğrusal olmayan regresyon kullanılması gerekmektedir. Excel'de yer alan Çözücü aracını kullanarak doğrusal olmayan regresyon uygulamak mümkündür ve bu çalışmada üç farklı örnek üzerinden Excel'de doğrusal olmayan regresyonun nasıl kullanılacağı açıklanmaya çalışılmıştır. Excel'deki Çözücü aracı parametre değerlerinin standart hataları hariç ücretli yazılımlarla elde edilen sonuçların aynısını bulmaktadır.

	A	B
1	süre (gün) (x)	Violaksantin (mg/kg) (y)
2	0	2,01
3	3	1,55
4	3	1,69
5	6	1,44
6	6	1,10
7	10	0,87
8	10	0,85
9	12	0,76
10	12	0,79
11	19	0,61
12	19	0,62
13	26	0,42
14	26	0,20
15	33	0,24
16	33	0,20
17		

Şekil 1. Yeşil zeytinde bulunan violaksantin pigmentinin zamana bağlı değişim verileri. Orijinal veriler Mínguez-Mosquera ve Gandul-Rojas (1994)'dan alınmıştır.

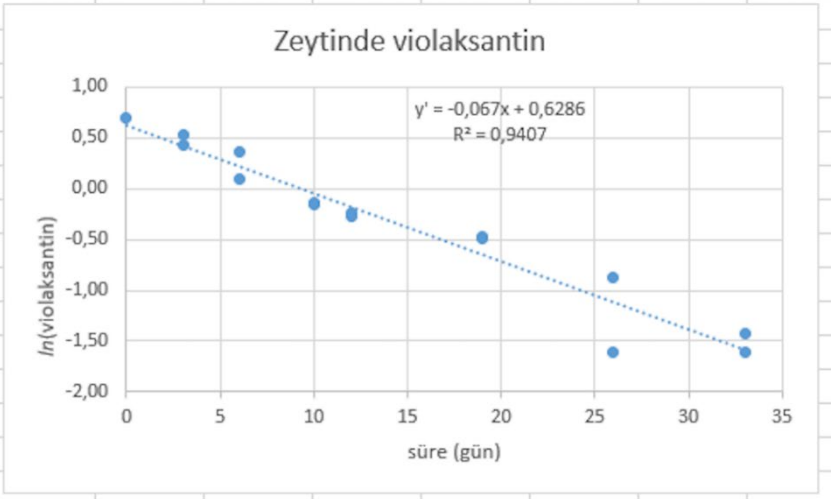
Figure 1. Change of violaxanthin pigment in green olives with respect to time. Original data are from Mínguez-Mosquera and Gandul-Rojas (1994).



Şekil 2. Şekil 1'de gösterilen verilere Excel'in içindeki üstel modelin ($y=a\cdot e^{bx}$) uygulanışı.

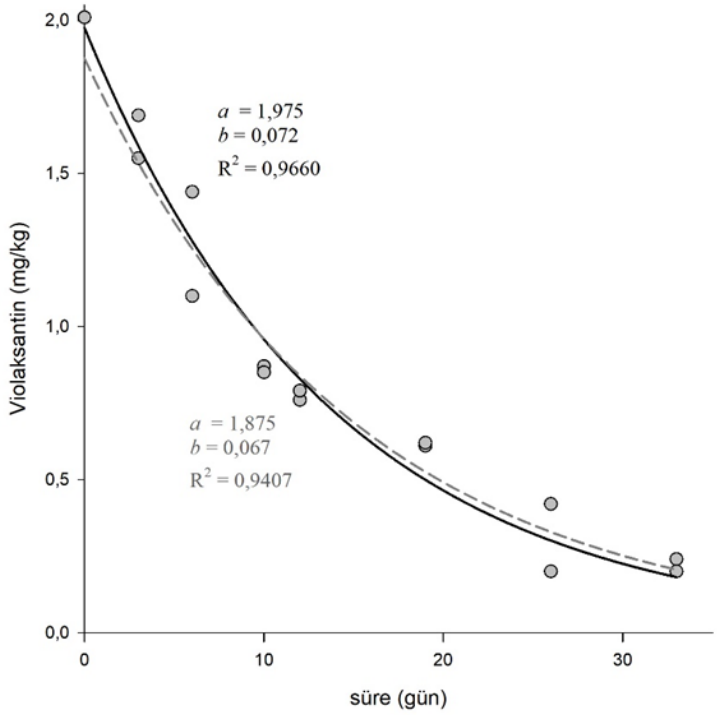
Figure 2. Application of exponential model ($y=a\cdot e^{bx}$) to the data given in Fig. 1 in Excel.

	A	B
1	süre (gün) (x)	lny (y')
2	0	0,70
3	3	0,44
4	3	0,52
5	6	0,36
6	6	0,10
7	10	-0,14
8	10	-0,16
9	12	-0,27
10	12	-0,24
11	19	-0,49
12	19	-0,48
13	26	-0,87
14	26	-1,61
15	33	-1,43
16	33	-1,61



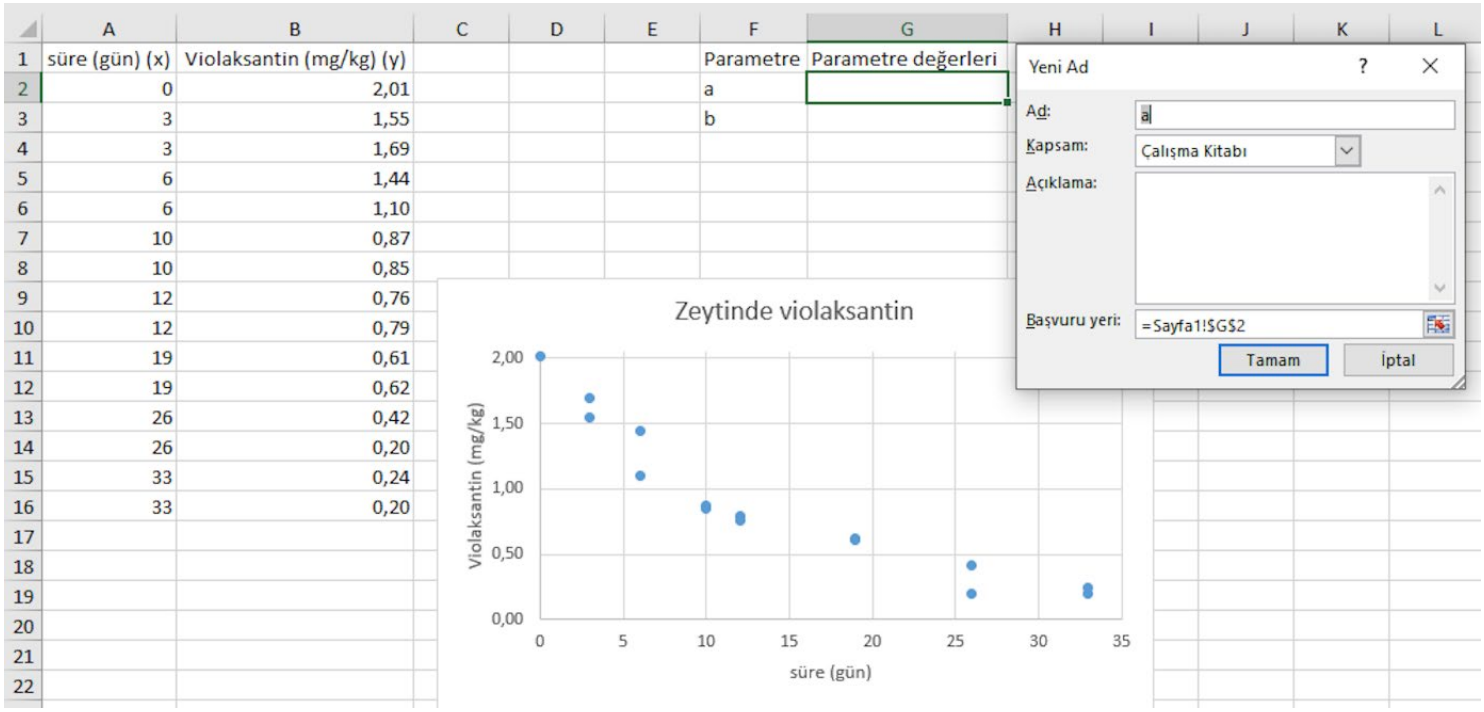
Şekil 3. Şekil 1'de gösterilen verilerin doğrusallaştırılması ve Excel'in içindeki doğrusal modelin ($y'=a'+bx$) bu doğrusal hale getirilmiş verilere uygulanışı.

Figure 3. Linearization of data given in Fig. 1 and application of the linear model ($y'=a'+bx$) to the linearized data given in Excel.



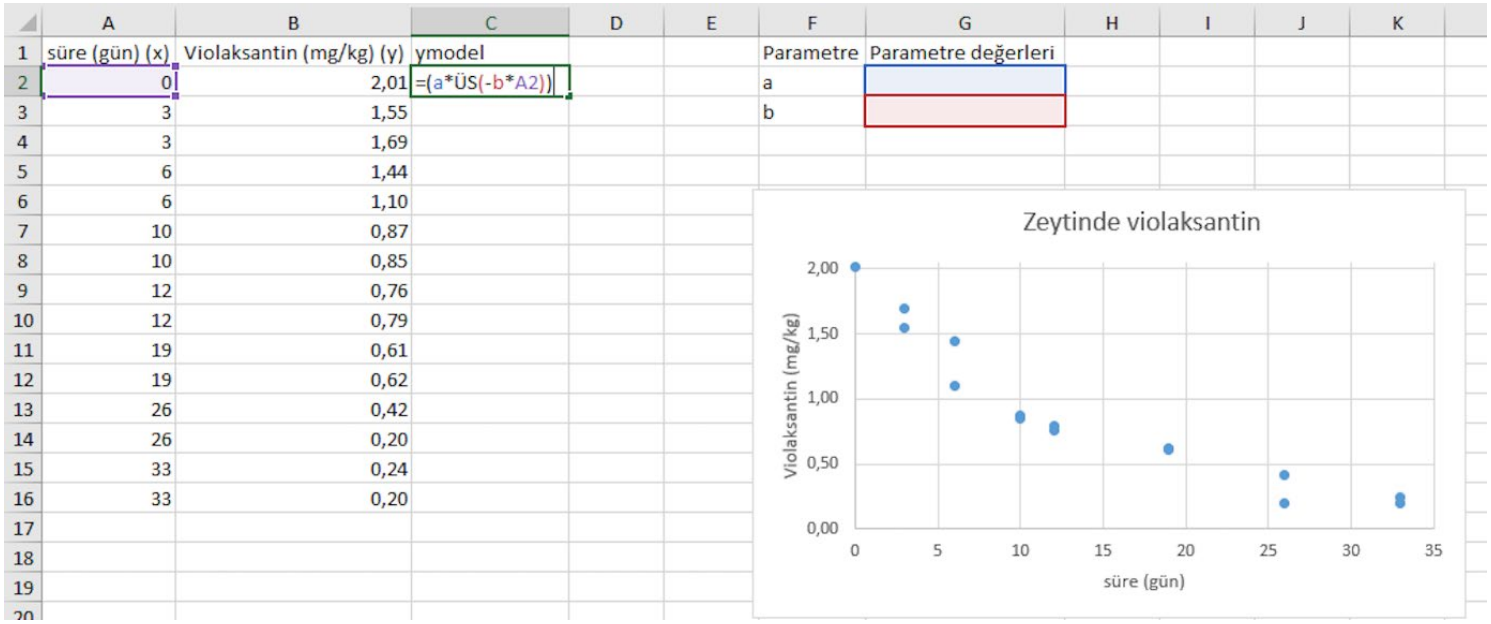
Şekil 4. Şekil 1'de gösterilen verilere SigmaPlot'ta üstel modelin ($y=a\cdot e^{bx}$) uygulanışı. Gri kesikli çizgi doğrusal regresyon uyumunu, siyah düz çizgi ise doğrusal olmayan regresyon uyumunu göstermektedir.

Figure 4. Application of exponential model ($y=a\cdot e^{bx}$) to the data given in Fig. 1 in SigmaPlot. Gray dashed line indicates the fit of linear regression, black solid line indicates the fit of non-linear regression.



Şekil 5. Excel'de Şekil 1'de gösterilen veriler için üstel model ($y=a\cdot e^{bx}$) parametre adlarının tanımlanması.

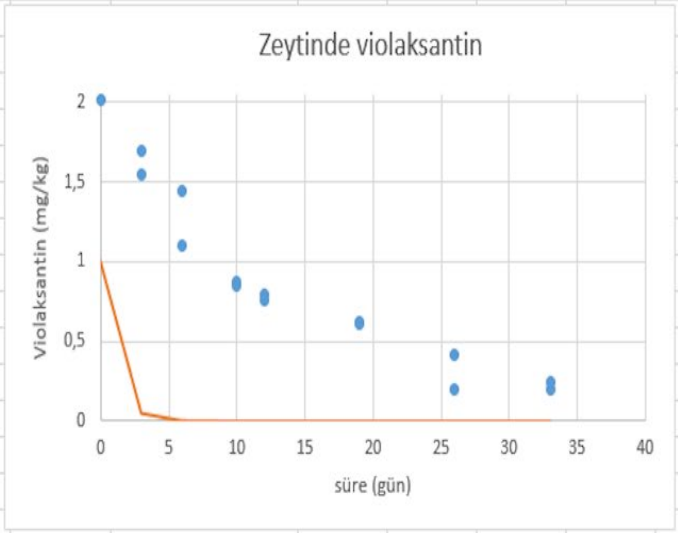
Figure 5. Defining the names of the parameters of exponential model ($y=a\cdot e^{bx}$) given in Figure 1 in Excel.



Şekil 6. Şekil 5'te tanımlanan parametrelerin kullanılarak üstel model ($y=a\cdot e^{bx}$) denkleminin Excel'de oluşturulması.

Figure 6. Generating the equation of exponential model ($y=a\cdot e^{bx}$) by using defined parameters in Fig. 5 in Excel.

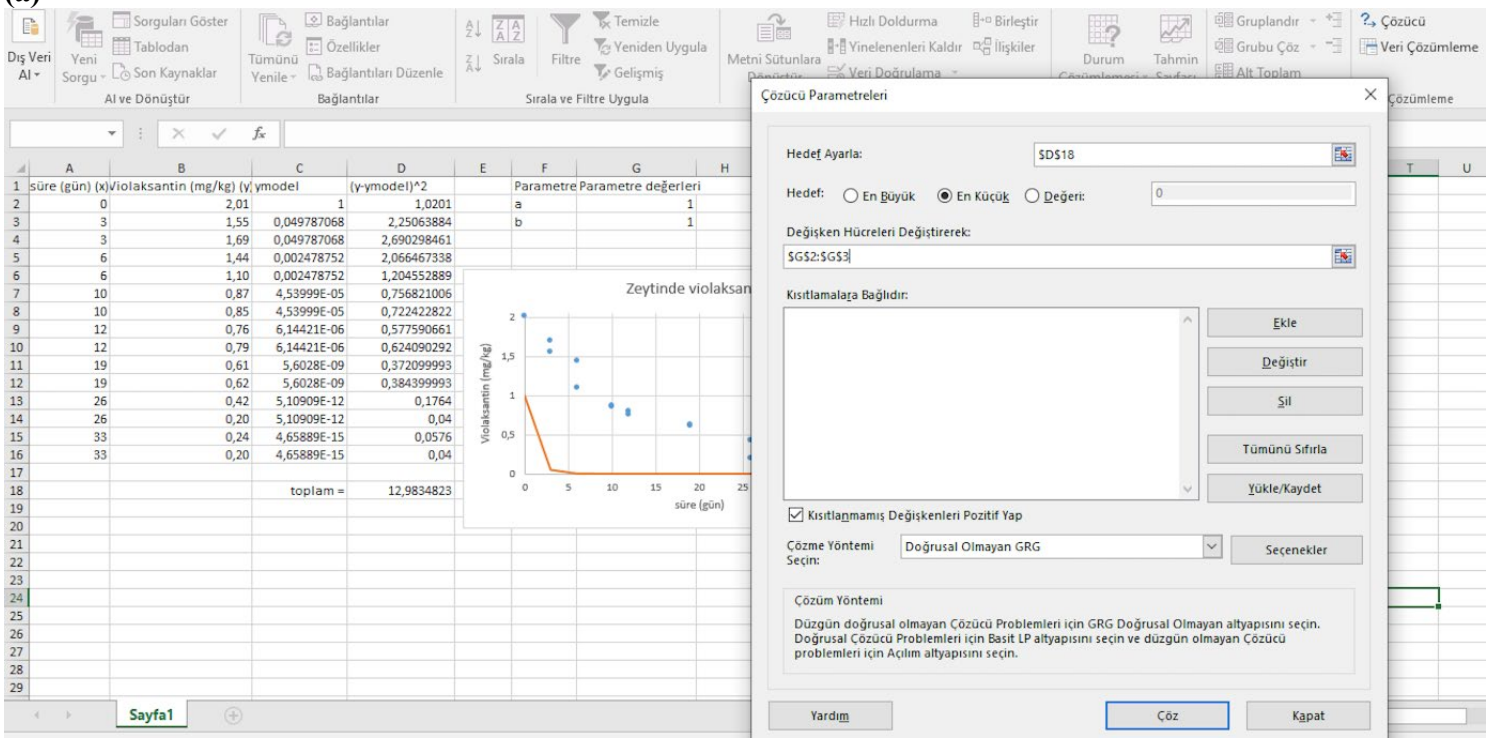
	A	B	C	D	E	F	G	H	I	J	K
1	süre (gün) (x)	Violaksantin (mg/kg) (y)	ymodel	(y-ymodel)^2		Parametre	Parametre değerleri				
2	0	2,01	1	1,0201		a	1				
3	3	1,55	0,049787068	2,25063884		b	1				
4	3	1,69	0,049787068	2,690298461							
5	6	1,44	0,002478752	2,066467338							
6	6	1,10	0,002478752	1,204552889							
7	10	0,87	4,53999E-05	0,756821006							
8	10	0,85	4,53999E-05	0,722422822							
9	12	0,76	6,14421E-06	0,577590661							
10	12	0,79	6,14421E-06	0,624090292							
11	19	0,61	5,6028E-09	0,372099993							
12	19	0,62	5,6028E-09	0,384399993							
13	26	0,42	5,10909E-12	0,1764							
14	26	0,20	5,10909E-12	0,04							
15	33	0,24	4,65889E-15	0,0576							
16	33	0,20	4,65889E-15	0,04							
17											
18			toplam =	12,9834823							
19											
20											
21											



Şekil 7. Hataların karesi denkleminin [(y-ymodel)^2] oluşturulması ve parametreler için başlangıç değerlerinin girilmesi.

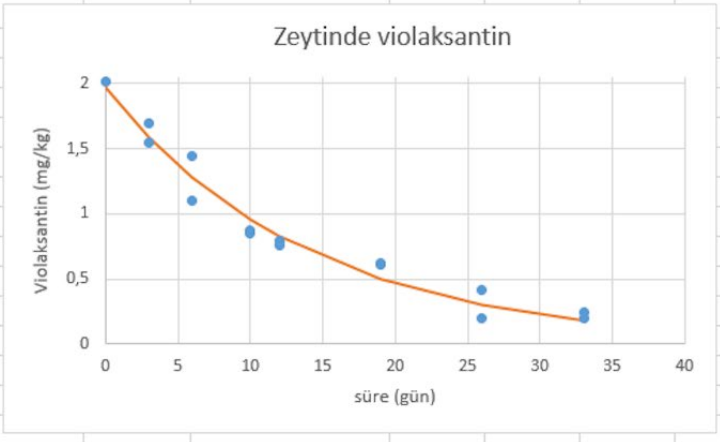
Figure 7. Generating the residual square equation [(y-ymodel)^2] and entering initial values for the parameters.

(a)



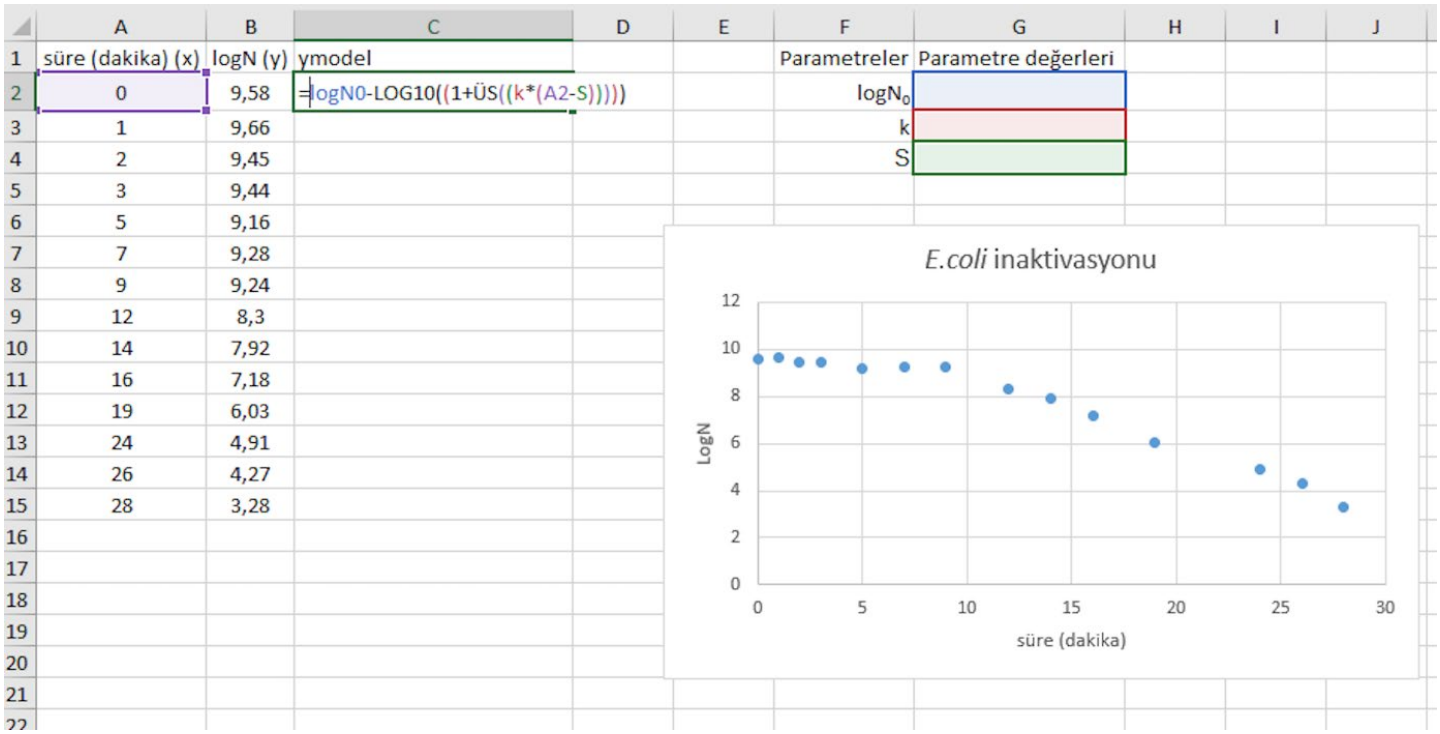
(b)

	A	B	C	D	E	F	G
1	süre (gün) (x)	Violaksantin (mg/kg) (y)	ymodel	(y-ymodel)^2		Parametre	Parametre değerleri
2	0	2,01	1,975026797	0,001223125		a	1,975026797
3	3	1,55	1,589607534	0,001568757		b	0,072364938
4	3	1,69	1,589607534	0,010078647			
5	6	1,44	1,279401431	0,0257919			
6	6	1,10	1,279401431	0,032184874			
7	10	0,87	0,957846813	0,007717063			
8	10	0,85	0,957846813	0,011630935			
9	12	0,76	0,82878269	0,004731058			
10	12	0,79	0,82878269	0,001504097			
11	19	0,61	0,499398023	0,012232797			
12	19	0,62	0,499398023	0,014544837			
13	26	0,42	0,300921325	0,014179731			
14	26	0,20	0,300921325	0,010185114			
15	33	0,24	0,181325595	0,003442686			
16	33	0,20	0,181325595	0,000348733			
17							
18			toplam =	0,151364354			
19							
20							



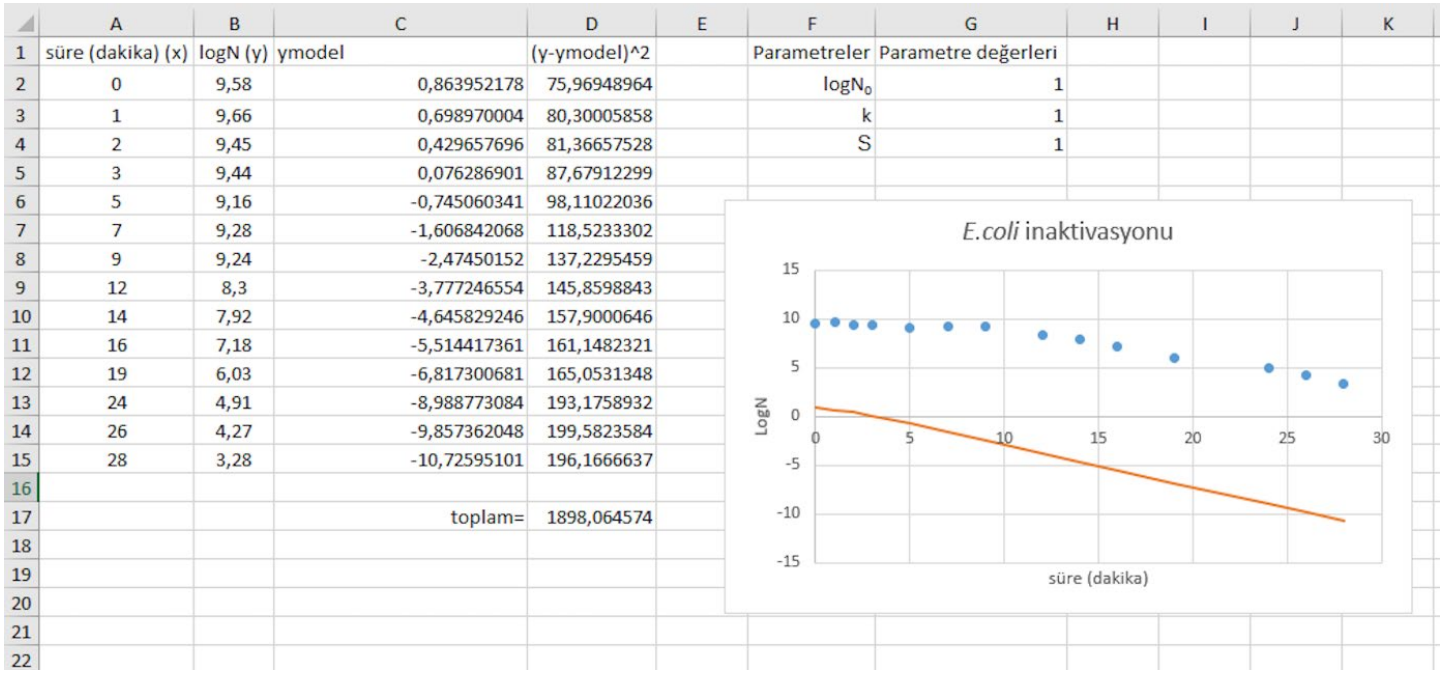
Şekil 8. Excel' deki Çözücü aracı kullanılarak hataların karesi toplamının minimize edilmesi (a), Çözücü'nün bulduğu parametre değerleri ve model uyumu (b).

Figure 8. Minimizing the sum of residual squares by using the Solver tool in Excel (a), Parameter values estimated by the Solver and model fit (b).



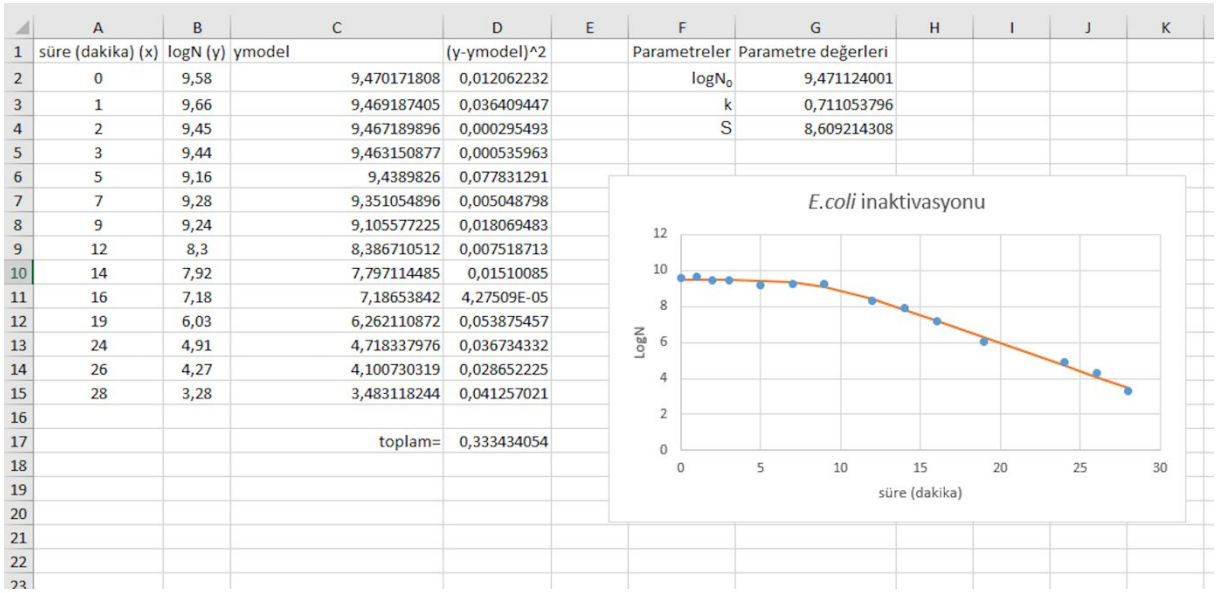
Şekil 9. Sıvı besiyerindeki *E. coli*'nin 56.6 °C'de inaktivasyon verileri ve tanımlanan parametreler kullanılarak model denkleminin Excel'de oluşturulması. Orijinal veriler Valdramidis ve ark. (2005)'dan alınmıştır.

Figure 9. Inactivation data of *E. coli* in broth at 56.6 °C and creating the model equation by using the defined parameters in Excel. Original data are from Valdramidis et al. (2005).



Şekil 10. Hataların karesi denkleminin [(y-ymodel)^2] oluşturulması ve parametreler için başlangıç değerlerinin girilmesi.

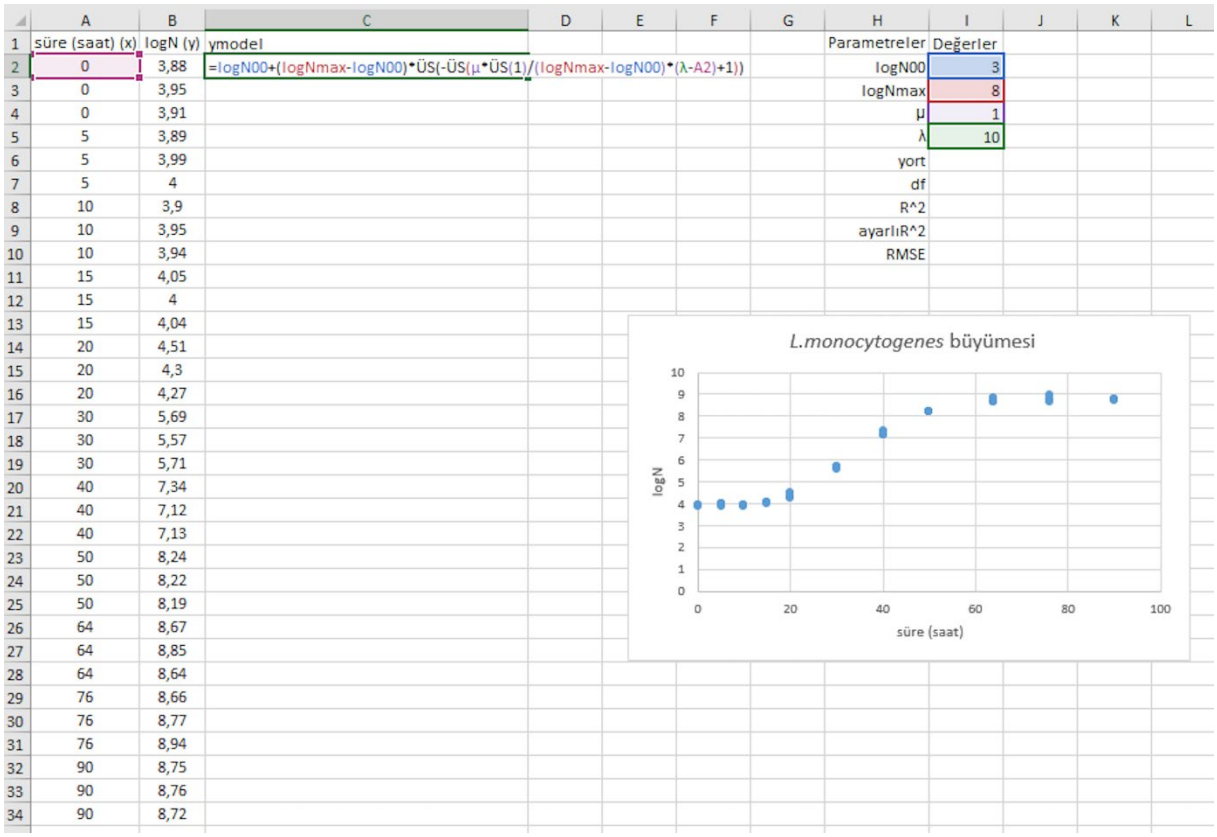
Figure 10. Generating the residual square equation [(y-ymodel)^2] and entering initial values for the parameters.



	A	B	C	D	E	F	G	H	I	J	K
1	süre (dakika) (x)	logN (y)	ymodel	(y-ymodel)^2		Parametreler	Parametre değerleri				
2	0	9,58	9,470171808	0,012062232		$logN_0$	9,471124001				
3	1	9,66	9,469187405	0,036409447		k	0,711053796				
4	2	9,45	9,467189896	0,000295493		S	8,609214308				
5	3	9,44	9,463150877	0,000535963							
6	5	9,16	9,4389826	0,077831291							
7	7	9,28	9,351054896	0,005048798							
8	9	9,24	9,105577225	0,018069483							
9	12	8,3	8,386710512	0,007518713							
10	14	7,92	7,797114485	0,01510085							
11	16	7,18	7,18653842	4,27509E-05							
12	19	6,03	6,262110872	0,053875457							
13	24	4,91	4,718337976	0,036734332							
14	26	4,27	4,100730319	0,028652225							
15	28	3,28	3,483118244	0,041257021							
16											
17			toplam=	0,333434054							
18											
19											
20											
21											
22											
23											

Şekil 11. Çözücü'nün bulduğu parametre değerleri ve model uyumu.

Figure 11. Parameter values estimated by Solver and model fit.

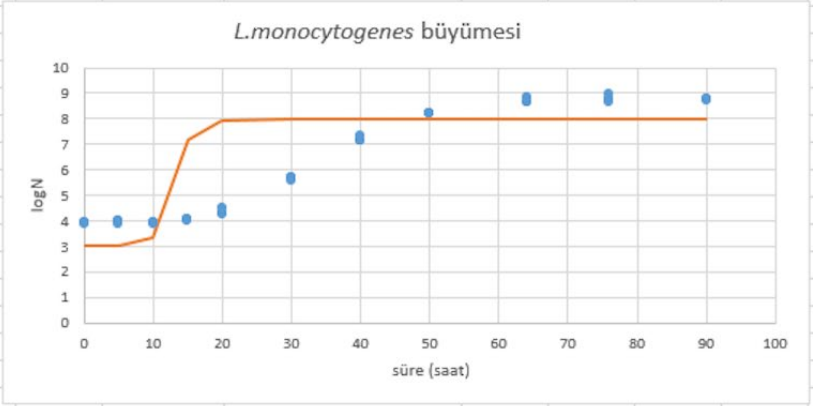


	A	B	C	D	E	F	G	H	I	J	K	L
1	süre (saat) (x)	logN (y)	ymodel					Parametreler	Değerler			
2	0	3,88	=logN00+(logNmax-logN00)*ÜS(-ÜS(μ*ÜS(1)/(logNmax-logN00)*(λ-A2)+1))					logN00	3			
3	0	3,95						logNmax	8			
4	0	3,91						μ	1			
5	5	3,89						λ	10			
6	5	3,99						yort				
7	5	4						df				
8	10	3,9						R^2				
9	10	3,95						ayarlıR^2				
10	10	3,94						RMSE				
11	15	4,05										
12	15	4										
13	15	4,04										
14	20	4,51										
15	20	4,3										
16	20	4,27										
17	30	5,69										
18	30	5,57										
19	30	5,71										
20	40	7,34										
21	40	7,12										
22	40	7,13										
23	50	8,24										
24	50	8,22										
25	50	8,19										
26	64	8,67										
27	64	8,85										
28	64	8,64										
29	76	8,66										
30	76	8,77										
31	76	8,94										
32	90	8,75										
33	90	8,76										
34	90	8,72										

Şekil 12. *L. monocytogenes*'in 30°C'de % 9 tuz içeren sıvı besiyerinde büyüme verileri ve tanımlanan parametreler kullanılarak model denkleminin Excel'de oluşturulması. Orijinal veriler Lambert ve ark. (2012)'dan alınmıştır.

Figure 12. Growth data of *L. monocytogenes* at 30°C in broth containing 9 % salt and creating the model equation by using the defined parameters in Excel. Original data are from Lambert et al. (2012).

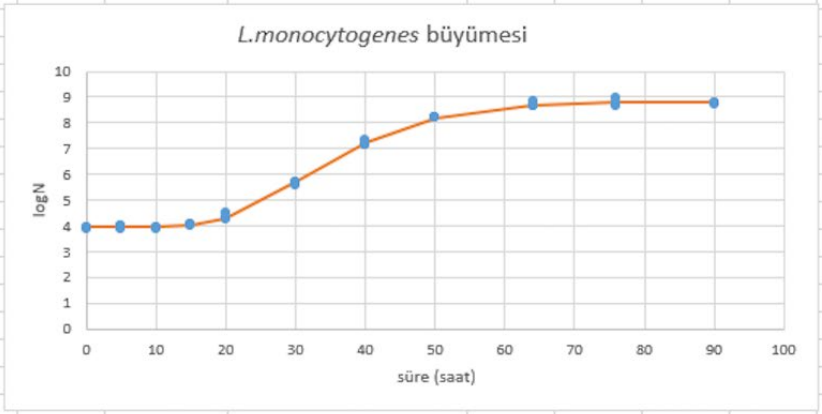
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	süre (saat) (x)	logN (y)	ymodel	(y-ymodel)^2	(y-yort)^2			Parametreler	Değerler				
2	0	3,88	3	0,7744	5,098017			logN00	3				
3	0	3,95	3	0,9025	4,786814			logNmax	8				
4	0	3,91	3	0,8281	4,963444			μ	1				
5	5	3,89	3	0,7921	5,052959			λ	10				
6	5	3,99	3	0,9801	4,613383			yort	6,137878788				
7	5	4	3	1	4,570526			df	29				
8	10	3,9	3,329940179	0,324968199	5,008101			R^2	0,322405995				
9	10	3,95	3,329940179	0,384474181	4,786814			ayarlıR^2	0,228944753				
10	10	3,94	3,329940179	0,372172985	4,830671			RMSE	1,841651243				
11	15	4,05	7,178965942	9,790427868	4,359238								
12	15	4	7,178965942	10,10582446	4,570526								
13	15	4,04	7,178965942	9,853107187	4,401095								
14	20	4,51	7,941166166	11,77290126	2,649989								
15	20	4,3	7,941166166	13,25809105	3,377798								
16	20	4,27	7,941166166	13,47746102	3,488971								
17	30	5,69	7,9997423	5,334909493	0,200595								
18	30	5,57	7,9997423	5,903647645	0,322486								
19	30	5,71	7,9997423	5,242919801	0,18308								
20	40	7,34	7,999998878	0,435598519	1,445095								
21	40	7,12	7,999998878	0,774398025	0,964562								
22	40	7,13	7,999998878	0,756898047	0,984304								
23	50	8,24	7,999999995	0,057600002	4,418914								
24	50	8,22	7,999999995	0,048400002	4,335229								
25	50	8,19	7,999999995	0,036100002	4,211201								
26	64	8,67	8	0,4489	6,411638								
27	64	8,85	8	0,7225	7,355601								
28	64	8,64	8	0,4096	6,260611								
29	76	8,66	8	0,4356	6,361095								
30	76	8,77	8	0,5929	6,928062								
31	76	8,94	8	0,8836	7,851883								
32	90	8,75	8	0,5625	6,823177								
33	90	8,76	8	0,5776	6,87552								
34	90	8,72	8	0,5184	6,66735								
35													



Şekil 13. Hataların karesi denklemlerinin [(y-ymodel)^2 ve (y-yort)^2] oluşturulması ve girilen parametre başlangıç değerleri için hesaplanan model uyumu göstergeleri (R^2, ayarlı R^2 ve RMSE değerleri) ve model uyumu.

Figure 13. Generating the residual square equations [(y-ymodel)^2 and (y-yort)^2] and calculated goodness-of-fit indices and model fit for the entered initial values of the parameters.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	süre (saat) (x)	logN (y)	ymodel	(y-ymodel)^2	(y-yort)^2			Parametreler	Değerler				
2	0	3,88	3,950546738	0,004976842	5,098017			logN00	3,950546656				
3	0	3,95	3,950546738	2,98922E-07	4,786814			logNmax	8,849351703				
4	0	3,91	3,950546738	0,001644038	4,963444			μ	0,171391773				
5	5	3,89	3,95061838	0,003674588	5,052959			λ	19,82387617				
6	5	3,99	3,95061838	0,001550912	4,613383			yort	6,137878788				
7	5	4	3,95061838	0,002438544	4,570526			df	29				
8	10	3,9	3,955390382	0,003068094	5,008101			R^2	0,998353949				
9	10	3,95	3,955390382	2,90562E-05	4,786814			ayarlıR^2	0,998126908				
10	10	3,94	3,955390382	0,000236864	4,830671			RMSE	0,090770391				
11	15	4,05	4,016973474	0,001090751	4,359238								
12	15	4	4,016973474	0,000288099	4,570526								
13	15	4,04	4,016973474	0,000530221	4,401095								
14	20	4,51	4,288739585	0,048956171	2,649989								
15	20	4,3	4,288739585	0,000126797	3,377798								
16	20	4,27	4,288739585	0,000351172	3,488971								
17	30	5,69	5,69466069	2,1722E-05	0,200595								
18	30	5,57	5,69466069	0,015540288	0,322486								
19	30	5,71	5,69466069	0,000235294	0,18308								
20	40	7,34	7,237620796	0,010481502	1,445095								
21	40	7,12	7,237620796	0,013834652	0,964562								
22	40	7,13	7,237620796	0,011582236	0,984304								
23	50	8,24	8,149532586	0,008184353	4,418914								
24	50	8,22	8,149532586	0,004965656	4,335229								
25	50	8,19	8,149532586	0,001637612	4,211201								
26	64	8,67	8,653926447	0,000258359	6,411638								
27	64	8,85	8,653926447	0,038444838	7,355601								
28	64	8,64	8,653926447	0,000193946	6,260611								
29	76	8,66	8,786061352	0,015891465	6,361095								
30	76	8,77	8,786061352	0,000257967	6,928062								
31	76	8,94	8,786061352	0,023697107	7,851883								
32	90	8,75	8,832556911	0,006815644	6,823177								
33	90	8,76	8,832556911	0,005264505	6,87552								
34	90	8,72	8,832556911	0,012669058	6,66735								



Şekil 14. Çözücü'nün bulduğu parametre değerleri, model uyumu göstergeleri (R^2, ayarlı R^2 ve RMSE değerleri) ve model uyumu.

Figure 14. Parameter values estimated by Solver, goodness-of-fit indices (R^2, adjusted R^2 and RMSE values) and model fit.

Not

Bu çalışmada masaüstü bilgisayara yüklü Windows 10'un altında yer alan Excel 2016 kullanılmıştır ve burada gösterilen örnekler talep edilmesi halinde e-posta ile gönderilebilir.

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Kaynaklar

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Units should be prepared in accordance with the International System of Units (SI).

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Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

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All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before

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References

Reference System is APA 6th Edition

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The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Note: All second and third lines in the APA Bibliography should be indented.

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Table 2.

Material Type	Reference List/Bibliography
A book in print	**Baxter, C. (1997).** *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	**Haybron, D.M. (2008).** Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
An eBook	**Millbower, L. (2003).** *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015).
An article in a print journal	**Carter, S., Dunbar-Odom, D. (2009).** The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
Preview article in a journal with DOI	**Gaudio, J.L., Snowdon, C.T. (2008).** Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
Websites - professional or personal sites	***The World Famous Hot Dog Site.* (1999, July 7).** Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015).
Websites - online government publications	**U.S. Department of Justice. (2006, September 10).** Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.2015).
Photograph (from book, magazine or webpage)	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015).
Artwork - from library database	**Clark, L. (c.a. 1960's).** *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor.
Artwork - from website	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015).