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Original Article/Özgün Araştırma

Modelling of the growth of *Listeria monocytogenes* in cooked ground beef stored under different storage conditions for 21 days

Farklı depolama koşullarında 21 gün depolanan pişmiş sığır kıymasında *Listeria monocytogenes* gelişiminin modellenmesi

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Abstract

Objective: The aim of this study was to evaluate the growth behavior of *L. monocytogenes* in cooked ground beef stored under aerobic and vacuum packaging conditions for 21 days and to compare the performance of different mathematical models in describing its growth kinetics under these storage conditions.

Materials and methods: Cooked ground beef samples were inoculated with *L. monocytogenes* and stored at 4 °C under aerobic and vacuum packaging conditions for 21 days. Microbial counts were monitored during storage and the obtained data were fitted to Henderson, Baranyi, Gompertz, Logistic, Richards, and Stannard models.

Results and discussion: *L. monocytogenes* growth was observed under both packaging conditions, although the growth curves differed between aerobic and vacuum storage. Among the tested models, the Richards model provided the best fit under both conditions, achieving R^2 values of 0.9984 (aerobic) and 0.9942 (vacuum), followed by the Logistic model with R^2 values of 0.9970 and 0.9922, respectively. The Gompertz model also showed acceptable performance under aerobic storage (R^2=0.9727), whereas the Baranyi and Stannard models did not adequately describe the growth behavior of *L. monocytogenes*.

Conclusion: Packaging conditions significantly affected the growth dynamics of *L. monocytogenes* in cooked ground beef. Richards and Logistic models were found to be more suitable for predicting *L. monocytogenes* growth under the evaluated storage conditions.

Keywords: meat, *L. monocytogenes*, predictive modeling

Öz

Amaç: Bu çalışmanın amacı, aerobik ve vakumlu paketleme koşullarında 21 gün boyunca depolanan pişmiş kıymada *L. monocytogenes*'in gelişim davranışını değerlendirmek ve farklı matematiksel modellerin bu gelişim kinetiğini açıklamadaki performansını karşılaştırmaktır.

Materyal ve metot: Pişmiş kıyma örnekleri *L. monocytogenes* ile inoküle edilmiş ve 4 °C'de aerobik ve vakumlu paketleme koşullarında 21 gün boyunca depolanmıştır. Depolama süresince mikrobiyal sayımlar yapılmış ve elde edilen veriler Henderson, Baranyi, Gompertz, Lojistik, Richards ve Stannard modellerine uygulanmıştır.

Bulgular ve tartışma: *L. monocytogenes* gelişimi her iki paketleme koşulunda da gözlenmiştir, ancak gelişim eğrileri aerobik ve vakumlu depolama arasında farklılık göstermiştir. Test edilen modeller arasında Richards modeli her iki koşulda da en yüksek uyumu sağlamış; aerobik depolamada R^2=0.9984, vakumlu depolamada ise R^2=0.9942 değerine ulaşmıştır. Lojistik model sırasıyla R^2=0.9970 ve R^2=0.9922 değerleriyle ikinci sırada yer almıştır. Gompertz modeli özellikle aerobik depolamada kabul edilebilir sonuçlar verirken (R^2=0.9727), Baranyi ve Stannard modelleri *L. monocytogenes* gelişimini yeterli düzeyde açıklayamamıştır.

Sonuç: Paketleme koşulları, pişmiş kıymada *L. monocytogenes'*in gelişim dinamikleri üzerinde önemli etkiye sahiptir. Richards ve Lojistik modellerinin, değerlendirilen depolama koşullarında *L. monocytogenes* gelişimini tahmin etmek için daha uygun modeller olduğu sonucuna varılmıştır.

Anahtar kelimeler: et, *L. monocytogenes*, tahminsel modelleme

1. Introduction

Meat products are an important source of human nutrition and meet nutritional needs with proteins, vitamins, and essential fatty acids (Domínguez et al., 2019). Animal-derived meat contains essential amino acids, vitamins, and minerals and provides a suitable environment for the development of microorganisms (İşeri and Erol, 2009). High water activity, rich nutrient content, and various pH levels make meat a suitable substrate for microbial growth (Toldrá and Barat, 2017). Minced meat is sensitive to microbial contamination and spoilage. During the processing of fresh meat, the release of intracellular water, which forms a suitable medium for microorganisms, into the external environment can lead to widespread microbial problems in minced meat products (Kalaba et al., 2021). Despite its high nutritional value, ground meat is subject to criticism owing to commercial fraud as well as pathogenic microorganisms (Doroudian et al., 2024). Factors that cause contamination and cross-contamination of raw meat after slaughter can increase health risks in meat products (Yang et al., 2024).

Listeria monocytogenes is a globally important foodborne pathogen that causes epidemics and sporadic listeriosis (Yang et al., 2024). *L. monocytogenes* poses a great risk both in nature and in foodstuffs because it can grow under both anaerobic and aerobic conditions and in a wide range of temperatures (- 1.5 °C to 45 °C), pH (4.0-9.6) and water activity (≥0.92) (Zhang et al., 2021). Pregnant women, newborns, the elderly, and individuals with weakened immune systems are at high risk for infection resulting in meningitis, septicaemia, miscarriage, and febrile enteritis (Cavalcanti et al., 2022). Many studies have shown that ready-to-eat meat and poultry products are sources of *L. monocytogenes*. For this reason, the listeriosis outbreak poses a threat to public health and the economy (Zhang et al., 2021).

Beyond the use of traditional preservation methods and additives, investigating the effects of different preservation methods on the growth of *L. monocytogenes* may offer an alternative approach to meet the demand for healthy meat products. Therefore, it is important to evaluate how packaging, cooling, and freezing methods affect the growth of *L. monocytogenes* in meat and meat products (Zhou et al., 2010; EFSA, 2011).

In recent years, concerns regarding food quality and safety have increased. Foods can easily be contaminated with microorganisms that cause infection, intoxication, and spoilage. Owing to the expansion of the food industry, managing risks and protecting consumer health is becoming increasingly difficult (Njage et al., 2017). Therefore, it is important to understand microbial behavioural parameters (Van Impe et al., 2005) to understand microbial growth dynamics and the responses of microorganisms to certain environmental conditions (Dorota et al., 2014; Ross and McMeekin, 2003).

Growth, inactivation parameters, and toxin production can be predicted based on predictive models (Stavropoulou and Bezirtoglou, 2019; Schlundt et al., 2020). It is thought that the answers provided by the models can support food businesses and regulatory bodies in data-based decision making. Predictive microbiology aims to mathematically represent the reality of a microbiological process and measure the effects of environmental factors (Valdramidis, 2016).

Although predictive microbiology has been widely applied to raw meat and fish products, data on cooked ground beef remain limited. Cooked products are of particular concern because thermal processing eliminates competing microflora, creating a selective environment that may favour post-process contaminants such as *L. monocytogenes*. Furthermore, the combined influence of aerobic and vacuum packaging on *L. monocytogenes* growth kinetics in this matrix has not been systematically compared using a broad panel of primary models. In this study, six primary growth models (Henderson, Baranyi, Gompertz, Logistic, Richards and Stannard) were selected to provide a comprehensive comparative evaluation, as these models span a range of mathematical complexities and have been widely cited in predictive microbiology literature, allowing identification of the most suitable descriptor for the specific growth curves observed in cooked ground beef. This research therefore aimed to evaluate the effects of aerobic and vacuum packaging on the behavior of *L. monocytogenes* inoculated in cooked ground beef through predictive microbiology modeling, providing data that may support risk assessment and shelf-life management in the meat industry.

2. Materials and methods

2.1. Material

In this study, 24-hour post-mortem beef (*Longissimus thoracis* et *lumborum*) obtained from a local market in Burdur, with fat and connective tissues removed, was used. After passing through a 2 mm grinder plate, the meat was brought to the laboratory under cold chain and aseptic conditions and frozen. To prevent differences that might arise from beef, the meat used in this study was obtained from the same batch. The *L. monocytogenes* strain ATCC 19115 used in this study was obtained from the Department of Food Processing, Food Agriculture and Livestock Vocational School, Burdur Mehmet Akif Ersoy University.

2.2. Preparation of meat model

Ground beef was thawed at 4 °C for 12 hours. Following the addition of 10% pure water and 2% NaCl, the thawed beef was portioned equally among the experimental groups. After incorporating these ingredients, the samples were placed in plastic centrifuge tubes with screw caps. Each experimental group consisted of 50 g of ground beef packed into each tube without leaving any air gaps. An additional tube was included to monitor the core temperature. Once the tubes were placed in a water bath at 60°C, the temperature was increased to 85 °C. The core temperature of the experimental groups was monitored with a thermocouple, and cooking was stopped when it reached 74 °C. After removing the cooking liquid, the cooked samples were stored at 4 °C for 21 days. (Soyuçok et al., 2024).

2.3. Contamination of cooked ground samples with *L. monocytogenes*

Tubes containing *L. monocytogenes* grown at 35 °C for 18 h were centrifuged at 5,000 rpm for 5 min. After the medium residues were removed, the remaining pellets were washed 3 times with the same volume of 0.9% physiological saline. At the end of the washing process, the remaining pellet was dissolved in an equal volume (10 ml) of 0.1% peptone water. After the watery part released by the samples cooled to room temperature at the end of the cooking process was aseptically removed, the sample was placed back into the tube with the help of sterile forceps in a sterile cabinet, and 1 ml of the bacterial suspension was added. The meat sample was thoroughly coated in the tube containing the contamination liquid and its surface was thoroughly contaminated. The samples were contaminated for approximately 30 s, and a contamination level of approximately 4.5 log CFU/g was targeted. The prepared samples were stored in sterile tubes for aerobic storage conditions. Other samples were placed in food-contact safe 120 µm thick PA/PE vacuum bags (165 × 200 mm) and vacuumed for 9–11 seconds using a vacuum packing device (Packtech, Pt-vkm-ctr, China) with a vacuum force of 0.80 bar. The bags were then hermetically sealed with a 3 mm sealing strip. The process was carried out using a device with a vacuum pump capacity of 5 L/min. The vacuum-packaged samples were stored under appropriate conditions until the time of analysis. Cooked samples were stored at 4°C for 21 days after packaging (Soyuçok et al., 2024).

2.4. Determination of *L. monocytogenes* counts

A 25 g meat sample was weighed, and 225 ml of peptone water was added. Serial dilutions were prepared using this 1/10 dilution and for *L. monocytogenes* counting, serial dilutions were inoculated onto PALCAM Listeria-Selective agar (Merck, Germany) medium using the surface spreading method. Petri dishes were incubated at 35 °C for 24 hours and typical (olive green-grey colored, black centered, black zoned) colonies were counted (Hitchins et al., 2016). After the vacuum packaging was contaminated, the tubes containing the ground beef were vacuumed and stored.

2.5. Kinetic modelling of *L. monocytogenes* growth in cooked ground beef

The experimental data derived from the microbiological analysis were interpreted using various models frequently employed in the literature to describe microbial growth curves (Table 1). The parameters of these models were determined through a nonlinear regression procedure using the Curve Fitting Toolbox of MATLAB software package (MATLAB R2016a 64-bit, MathWorks Inc. USA). The coefficient of determination (R^2), adjusted R^2 (adj-R^2), sum of squares error (SSE), and root mean square error (RMSE) were used to evaluate the goodness of fit of the selected mathematical models to the experimental data. A model is considered more suitable if it exhibits higher values of R^2 and adj-R^2, along with lower values of the SSE and RMSE.

Table 1. Microbial growth models.

Model name	Model equation	Reference
Stannard	a*(1+exp(-(l+k*x)/p))^(-p)	Zwietering et al., 1990
Richards	a*((1+v*exp(k*(t-x)))^(-1/v))	Annadurai et al., 2000
Henderson	a*exp(-k*x)	McMinn et al, 2005
Baranyi	No+u*log(1+((exp((Nmax-Nno)/u))-1)/(exp(u*x)))	Baranyi and Roberts, 1994
Gompertz	Nmax*exp(-(exp(-(a*(x-t)))))	Zwietering et al., 1990
Logistic	K/(1+((K-No)/No)*exp(-r*x))	Wachenheim et al., (2003)

3. Findings and Discussion

Before inoculation, all ground beef samples were screened for the presence of *L. monocytogenes*, and no naturally occurring contamination was detected. The growth of *L. monocytogenes* was evaluated using six different mathematical models, and the suitability of these models was analysed using statistical metrics, such as root mean square error (RMSE), total square error (SSE), and coefficient of determination (R^2). In this study, these metrics were examined separately under vacuum and aerobic conditions, and the obtained results were compared (Table 2). The model with the lowest RMSE value under vacuum conditions was determined using the Richards model, and the value of this model was calculated as 0.1338. This was followed by the Logistic model with 0.1345, Gompertz model with 0.1409, and Henderson model with 0.2927. Under aerobic conditions, the lowest RMSE value was again observed to belong to the Richards model with 0.0964, whereas the value of the Logistic model was calculated as 0.1162, and the Gompertz and Henderson models were calculated as 0.2481. In terms of the total squared error (SSE) values, the Logistic model showed the best performance under vacuum conditions, with 0.0723. The Gompertz model came second with a value of 0.0794, while the Henderson model had higher error rates (0.4283) and the Richards model (0.4537). Under aerobic conditions, the lowest SSE value was determined to belong to the Richards model with 0.0279, while the SSE value of the Logistic model was 0.054, and that of the Gompertz and Henderson models was 0.3078. Coefficient of determination (R^2) analysis was used to evaluate the level of fit of the models to the data. Under aerobic conditions, the Richards model achieved the highest R^2 value of 0.9984, followed by the Logistic model (0.997), Henderson model (0.9827), and Gompertz model (0.9727). Under vacuum conditions, the Richards model again had the highest R^2 value of 0.9942, followed by the Logistic model with 0.9922, Gompertz model with 0.9914, and Henderson model with 0.9536. The graphs presented in Figure 1 and Figure 2 illustrate the progression of *L. monocytogenes* over time. When this graph is examined, it is seen that *L. monocytogenes* behaviour does not fit the Stannard and Baranyi models, whereas it is found to be more suitable for the Richards and Logistic models.

Table 2. Error metrics of the models.

	Henderson	Baranyi	Gompertz	Logistic	Richards	Stannard
Aerobic storage						
R^2	0.9827	0.2767	0.9727	0.997	0.9984	0.4419
Adj-R^2	0.9792	-0.4465	0.9792	0.9954	0.9969	-0.1163
SSE	0.3078	12.8703	0.3078	0.054	0.0279	9.9318
RMSE	0.2481	2.0713	0.2481	0.1162	0.0964	1.9195
Vacuum storage						
R^2	0.9536	0.3618	0.9914	0.9922	0,9942	0.2945
Adj-R^2	0.9443	-0.2764	0.9871	0.9882	0.9884	-0.411
SSE	0.4283	5.8906	0.0794	0.0723	0.4537	6.5118
RMSE	0.2927	1.4013	0.1409	0.1345	0.1338	1.4733

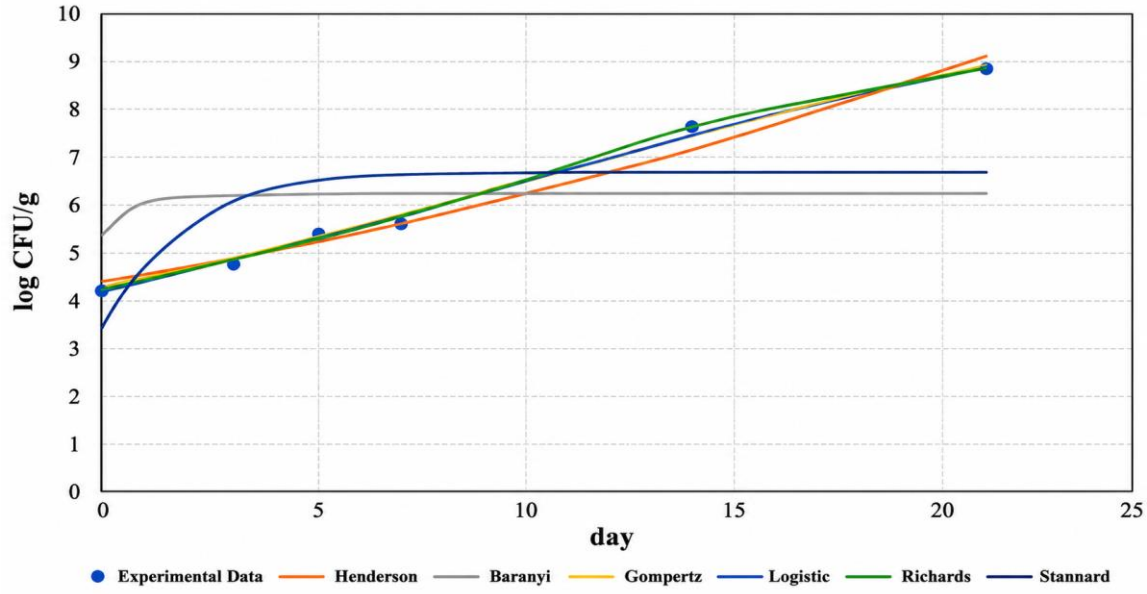

Figure 1. *L. monocytogenes* growth in ground beef during aerobic storage

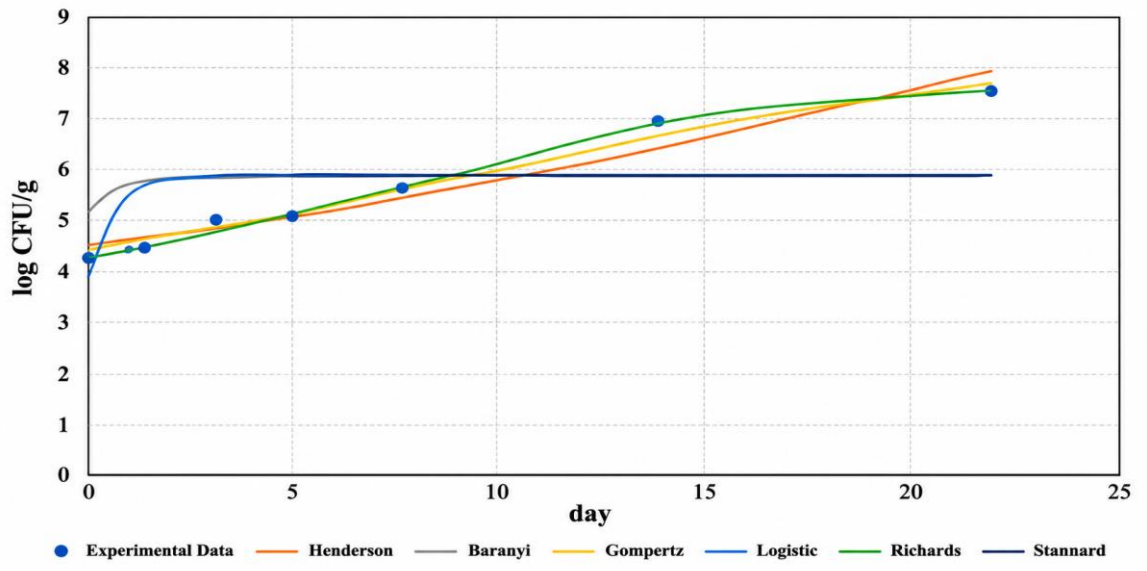

Figure 2. *L. monocytogenes* growth in ground beef during vacuum storage

When the time-growth behaviour modelling of *L. monocytogenes* development was examined, it was found that it did not fit the Stannard and Baranyi models in both aerobic and vacuum packaging systems, whereas it was found to be more suitable for the Richards and Logistic models. Ding et al. (2011) examined the growth data of *S. aureus* in Kimbab using modified Gompertz model and logistic models and found that R^2 values varied between 0.9740 and 0.9975. In the study, the R^2 value of the Gompertz model of *L. monocytogenes* growth was found to be 0.9727 in aeorbic storage, while it was found to be 0.9914 in vacuum storage. In the logistic model, the R^2 value was found to be 0.997 in aeorbic storage, while it was found to be 0.9922 in vacuum storage. When the results were compared, similar data were obtained for the modelling systems.

In a different study conducted by Öztürk et al. (2020) investigated the microbiological and chemical effects of artichoke leaf extract on sardine meatballs stored at +4°C were investigated. The R^2 values of the parameters measured using a

nonlinear regression model on the microbiological and chemical effects of the artichoke leaf extract on fish meatballs varied between 0.845 and 0.958, and the model compatibility was high. In this study, it was determined that the R^2 value varied between 0.927 and 0.998 in aerobic packaging in the Henderson, Gompertz, Logistic and Richards models, and the R^2 value varied between 0.953 and 0.994 in vacuum packaging. The data presented and obtained were similar.

In a study modelling the development of *Pichia anomala* in olive fermentation, researchers used 4 models, namely modified Gompertz, modified logistic, modified Richards-Stannard, and Baranyi-Roberts models. Although the development of *P. anomala* was well-fitted to all models, it was stated that the most suitable models were modified Gompertz and Richards-Stannard models (Arroyo et al., 2005). In this study, the Henderson, Baranyi, Gompertz, Logistic, Richards, and Stannard models were tested, and the most suitable modelling system was found to be Richards and Logistic. Although the Gompertz system was compatible with the aerobic packaging system, it was not compatible with vacuum packaging. When the results were compared, it was thought that the different systems showing compatibility might be due to the different growth curves of each bacterium.

Ratkowsky and Baranyi models were used to evaluate the growth of *L. monocytogenes* in fishery products stored under vacuum and aerobic conditions. The researchers stated that the developed models provided reliable estimates of *L. monocytogenes* in fishery products (Bolívar et al., 2018). In this study, we determined that *L. monocytogenes* did not comply with the Baranyi system in development modelling. When the two studies were compared, it was thought that this was because the same bacteria may show different growth curves in different environments.

In their study of the effects of pH and potassium sorbate on *S. aureus* growth, Giannuzzi et al. (1999) determined R^2values between 0.837 and 0.939 using the Gompertz model. In this study, the R^2 values were determined to be 0.9727 and 0.9914 in the Gompertz model in aerobic and vacuum packaging, respectively. The Gompertz model yielded compatible results in both the studies.

Menezes et al. (2018) found the average R^2 value for sliced salami to be 0.979 using the Baranyi and Roberts model, while the R^2 value of salami samples containing 0.4% thyme essential oil was determined to be 0.941. In the same study, the researchers found the average R^2 value of control salami samples to be 0.975 and the R^2 value of salami samples containing 0.4% thyme essential oil to be 0.950 using modified Gompertz modelling. In the study conducted, while the R^2 values in the Baranyi model were found to be between 0.2767 and 0.3618, no agreement was achieved, whereas the modelling system was found to be suitable with R^2 values of 0.9727 and 0.9914 in the Gompertz model. This shows that each modelling system may lead to differences in the shape of the growth curve or in the quantitative evaluation of its contribution.

4. Conclusions

L. monocytogenes was able to grow in cooked ground beef under both aerobic and vacuum packaging conditions during the 21 day storage period. However, the extent of growth and the shape of the growth curves varied according to the packaging system. Among the evaluated mathematical models, the Richards and Logistic models provided the best fit to the experimental data under both aerobic and vacuum conditions. Although the Gompertz model showed acceptable performance, particularly under aerobic storage, its fit was lower under vacuum packaging. In contrast, the Baranyi and Stannard models did not adequately describe the growth behavior of *L. monocytogenes* in either packaging system. These findings indicate that packaging conditions significantly influence the growth dynamics of *L. monocytogenes* in cooked ground beef and that Richards and Logistic models may be more suitable tools for predicting its behavior under the evaluated storage conditions.

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5. Kaynaklar

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