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Prevalence of *Listeria monocytogenes* in ready-to-eat foods, and growth boundary modeling of the selected strains in broth as a function of temperature, salt and nisin

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ABSTRACT

The objectives of this study were to determine the prevalence of *Listeria monocytogenes* in ready-to-eat (RTE) foods, and the growth boundaries of two strains of *L. monocytogenes* isolated from RTE foods and one ATCC7644 strain under different temperature (4, 20, and 37°C), salt (0, 1, 2, 3, and 4% w/v), and nisin (0, 50, 100, 200, and 400 µg/mL) levels with different inoculum sizes (3, 4, 5, and 6 $\log_{10}$ CFU/mL). One hundred thirty-three retail RTE foods were examined and a total of 39 isolates were identified. The positive *Listeria* spp. and *L. monocytogenes* samples were 29.3% and 12.8%, respectively. Dairy and fruit-based RTE foods were *L. monocytogenes* negative whereas seafood-based (26.7%), meat-based (19.4%), poultry-based (16%), and vegetable-based (10%) RTE foods contained *L. monocytogenes*. It was shown that nisin was more effective than NaCl to avoid growth. Moreover, protective effect of NaCl on nisin activity was observed. As inoculum size increased probability of growth also increased. Suppressing the growth by using temperature, NaCl and nisin was more difficult in RTE-isolates strains (strains 120 and 137) than that of ATCC7644 strain. A mathematical model based on logistic regression could be used to determine growth limits of *L. monocytogenes* strains with a concordance of > 91%.

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Introduction

Listeria monocytogenes is a Gram positive, facultative anaerobe, psychrotrophic, motile at least at 30°C, rod-shaped (0.5 µm wide and 1–2 µm long), halotolerant, non-encapsulated, non-spore-forming foodborne pathogen responsible for human listeriosis.[1–4] Listeriosis is a serious illness that occurs after the consumption of contaminated foods with *L. monocytogenes*, mainly in pregnant women, neonates, immunocompromised individuals, the elderly, and humans with underlying illness such as kidney disease, cancer, diabetes, and HIV AIDS patients.[5,6] Listeriosis can be classified into two different forms: i) severe invasive listeriosis and ii) noninvasive febrile gastroenteritis. Invasive listeriosis is commonly seen in immunocompromised individuals. More severe symptoms like sepsis, meningitis, endocarditis, encephalitis, meningoencephalitis, septicemia, and brain infection can occur in this type of listeriosis. Invasive listeriosis is responsible for 90% of hospitalization and about 20–30% case fatality rate .[7]

L. monocytogenes may persist for long periods in food processing areas, and different environments such as soil, water, silage, sewage, and the intestinal tract of many mammals and humans.[1] Additionally, different food types such as vegetables, raw milk, and dairy products especially soft

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cheeses made from raw milk, poultry, raw meat, and processed meat products, raw fish, seafood products, ready-to-eat (RTE) foods and juices can be contaminated with *L. monocytogenes* .[8] In recent times, a large number of *L. monocytogenes* outbreaks have been linked to RTE foods since RTE foods may be a suitable substrate for the pathogen to grow. Moreover, *L. monocytogenes* can grow at refrigeration temperatures, low pH, and high salinity until consumption.[9] In general, RTE foods may not be subjected to further cooking or processing to destroy *L. monocytogenes* before consumption.[10] The regulatory agencies have identified that only three conditions have been described where RTE foods do not favor the growth of *L. monocytogenes*. These are i) pH≤ 4.4, ii) a_w≤ 0.92, and iii) pH≤ 5.0 and a_w≤ 0.94.[11] Although there are several legislations in many countries to limit the prevalence of *L. monocytogenes* in RTE foods, there is no common consensus on the acceptable levels of *L. monocytogenes* in foods.[12,13] While EU regulation prohibits *L. monocytogenes* in 25 grams RTE foods for infants and RTE foods for special medical purposes, they follow a policy that states the number of *L. monocytogenes* should be less than 100 CFU/g of food placed on the market during its shelf-life.[14] The U.S. Food and Drug Administration (FDA) does not allow the presence of *L. monocytogenes* in 25 g of RTE foods.[13] Similarly, Turkey enforces no detectable level of viable *L. monocytogenes* cells in 25 g of RTE foods.[15]

Nisin is the most common bacteriocin used in processed foods such as meat products, dairy products, and vegetables.[16] Effective use of nisin against bacteria depends on different factors especially if it is used in combination with other hurdles such as pH, a_w, and preservatives.[17] Salt (NaCl), on the other hand, is used not only to control the bacterial growth but also to improve the flavor, especially in processed meat products.[18] Growth boundary determination of foodborne pathogens based on probabilistic modeling offers detailed information on the growth limits under various conditions such as temperature, pH, a_w, etc.[19] Although many modeling studies on *L. monocytogenes* exist, new ones are required to better understand under which conditions *L. monocytogenes* could potentially be able to grow. Therefore, the objectives of this study were to determine (i) the prevalence of *L. monocytogenes* in RTE foods, (ii) growth boundaries of selected strains under different temperature, salt, and nisin levels with different inoculum sizes.

Materials and methods

Sample collection

In this study, a total of 133 retail RTE foods were randomly purchased from various supermarkets, patisseries, restaurants, open bazaars, and street hawkers in Ankara, Turkey between November 2020 and September 2021. RTE foods were classified into six categories: 20 were dairy-based, 31 meat-based, 25 poultry-based, 30 vegetable-based, 12 fruit-based and 15 fish-based. All samples except for vegetable- and fruit-based ones had undergone heat treatment. Each RTE food samples was stored at 4°C during the sale. RTE foods sold by the street hawkers and restaurants were unpackaged, while samples collected from other places were packaged and nonfrozen. All RTE foods were checked for expiry dates and all samples were placed in a cold box at 4°C and processed immediately on the sampling day.

Isolation and biochemical identification of Listeria monocytogenes

Detection of the *L. monocytogenes* was carried out in accordance with the International Organization for Standardization standard protocol[20] with two-stage enrichment. *L. monocytogenes* ATCC7644, *L. innocua* ATCC12612, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC6538 and *Rhodococcus equi* ATCC6939 were used as references strains. Briefly, 25 g of each food sample were added to 225 mL ½ Fraser broth (Merck) and homogenized in a stomacher (Seward 400, USA) for 3 min. After incubation at 30 ± 1°C for 24 ± 2 h, 0.1 mL of the primary enrichment culture was transferred into 10 mL full Fraser broth and incubated at 37°C for 48 ± 2 h. To isolate *Listeria* spp.,

a loopful each of the ½ and full Fraser broth were streaked on both ALOA agar (Merck) and PALCAM agar (Merck), and the plates were incubated at 37°C for 24–48 h. Then, five suspected colonies each from ALOA and PALCAM agar plates were picked and subjected to morphological and biochemical tests including by Gram staining, catalase reactions, oxidase tests, carbohydrate utilization, CAMP tests, and motility at 20–25°C. Bacterial isolates were stored in Brain Heart Infusion (BHI) broth (Merck) with 30% sterile glycerol (Merck) at – 80°C.

Genomic DNA extraction and PCR identification of Listeria monocytogenes

Following the biochemical tests, *L. monocytogenes* isolates were confirmed by polymerase-chain reaction (PCR) analysis. Bacterial cultures were grown in Tryptic Soy Broth supplemented with 0.6% of yeast extract (TSB-YE) (Merck) for both the 16S rRNA gene and listeriolysin (*hlyA*) gene determination. Genomic DNA extraction and PCR amplifications were used as described by Sanlibaba et al.[3] The primer pairs including forward U1 (5′–CAGCMGCCGCGGTAATWC–3′) and reverse LI1 (5′–CTCCATAAAGGTGACCCT–3′) were used to amplify a 938 bp region in the 16S rRNA gene of the *Listeri*a genus.[21] Additionally, the primer pairs 5′–CATTAGTGGAAAGATGGAATG–3′ (forward) and 5′–GTATCCTCCAGAGTGATCGA–3′ (reverse) were used to amplify a 730 bp region of the *hly*A gene of *L. monocytogenes* strains.[22] *Escherichia coli* ATCC25922 and *L. monocytogenes* ATCC7644 were used as negative and positive controls, respectively. Amplification conditions were slightly modified and were as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min, then final extension step at 72°C for 5 min, as described previously.[22] Amplified DNA was separated using 1% agarose gel electrophoresis (w/v) in 1X TBE buffer stained with ethidium bromide solution (2.5 µL/100 mL), and then, the gel was visualized under a UV transilluminator (SYNGENE, Biosystems UK). A 1000 bp DNA ladder (Thermo Scientific) was used as a reference marker.

Preparation of nisin solution and determination of minimum inhibitory concentration

Nisin stock solutions were prepared with Nisaplin (2.5% nisin A, Product Number: N5764, Sigma) by adding 0.020 g or 0.200 g to 10 mL of 0.02 N HCl. These solutions were kept at 4°C for a maximum of 24 h.[23] Minimum inhibitory concentration (MIC) assay was used to determine the antimicrobial activity of nisin against *L. monocytogenes* strains. MIC assays were carried out in 96 well microtiter plates in triplicate as described by Lynch et al. .[24]

Experimental design for determination of growth rate of L. monocytogenes

To determine the growth rates of 4 strains of *L. monocytogenes* isolated from RTE meals and an ATCC7644 strain, a microplate reader (BioTek Elisa Reader, Biotek Inc., USA) with 96 wells were used. The optical density (OD) was measured at 600 nm and growth rates were determined by using the formula below[25]:

$$t_{OD} = \lambda + \frac{2.303 \cdot \left[\log_{10}N_{OD} - \log_{10}N_0\right]}{\mu} \quad (1)$$

where t_{OD} is the time (h) when OD value is equal to 0.15, that is, time to detection (TTD), λ is the lag time (h), $\log_{10}N_{OD}$ is the number of *L. monocytogenes* ($\log_{10}$CFU/mL) at OD = 0.15, $\log_{10}N_0$ is the initial number of *L. monocytogenes* ($\log_{10}$CFU/mL) and µ is the specific growth rate (h^{-1}). To verify the growth rate obtained from Eq.(1), colony counts were also performed and three growth rate models were fitted to the data – see below. For the microbiological plate counts, at appropriate time intervals 1 mL of sample was added to 9 mL of sterile peptone water (0.5%) and mixed for about 15 s. Serial 10-

fold dilutions were prepared in peptone water and 0.1 mL of aliquots from each dilution were spread plated in duplicate. All the experiments were repeated twice with duplicate measurements (n = 4).

ÖK-BUZ GRoFiT, an Excel freeware file in which Baranyi,[26] modified Gompertz,[27] and three phase linear model[28] is inserted, was downloaded from https://mmf.gidatarim.edu.tr/en/ok-buz-grofit and used for modeling the microbial count.[29] There are two versions of this tool. Second version of the tool is available both in Turkish (https://mmf.gidatarim.edu.tr/ok-buz-grofit) and in English. The Baranyi model in ÖK-BUZ GRoFiT is a 4-parameter alternative to the one in DMFit which has six parameters. Nevertheless, almost identical fits for these Excel tools could be obtained with the growth data having lag, exponential, and stationary phases. Two strains with the highest growth rates and the ATCC7644 strain were used for growth/no growth studies.

Experimental design for determination of growth boundaries of L. monocytogenes

A full factorial design was used to test the growth ability of *L. monocytogenes* in TSB with different combinations of temperature (4, 20, and 37°C), sodium chloride (0, 1, 2, 3, and 4% *w/v*), nisin (0, 50, 100, 200, and 400 µg/mL), and initial concentration (3, 4, 5, and 6 $\log_{10}$CFU/mL). Three replications were done for each combination which resulted in 900 data points (3 temperature levels × 5 salt levels × 5 nisin levels × 4 initial concentration levels × 3 replications). Selected temperature levels represented the refrigeration, ambient, and abuse (optimum growth) temperatures. Salt and nisin concentrations were the ones that are generally used or allowed levels (except 400 µg/mL for nisin) in foods. It should be noted that the inoculum sizes of 5 and 6 $\log_{10}$CFU/mL were higher than the expected contamination of *L. monocytogenes* in foods and higher growth probability with these inoculum sizes should be expected.[30] Inoculum sizes of 3 and 4 $\log_{10}$CFU/mL were also used to determine growth boundaries under more realistic conditions.

Preparation of broth and growth evaluation

Uninoculated samples were served as negative controls and broths (TSB) with no salt or nisin inoculated with 10^3, 10^4, 10^5, and 10^6 CFU/mL bacteria were used as the positive control. Microbial growth was monitored weekly by measuring OD at 600 nm up to 8 weeks. Same microplate reader with 96 wells were used for growth evaluation. At higher initial inocula (10^5 and 10^6 CFU/mL), 0.1 increase in the OD values were considered as growth.[31] Growth was also verified by plate counts for some wells. At lower inocula (10^3 and 10^4 CFU/mL), growth was monitored both by checking the OD values and by plate counts.

Model development

Two models were proposed:

$$\text{logit}(p) = a_0 + a_1 \cdot T + a_2 \cdot NaCl + a_3 \cdot nisin + a_4 \cdot IC \tag{2}$$

or

$$\begin{aligned}\text{logit}(p) = {} & b_0 + b_1 \cdot T + b_2 \cdot NaCl + b_3 \cdot nisin + b_4 \cdot IC + b_5 \cdot T \cdot NaCl + b_6 \cdot T \cdot nisin + b_7 \cdot T \cdot IC \\ & + b_8 \cdot NaCl \cdot nisin + b_9 \cdot NaCl \cdot IC + b_{10} \cdot nisin \cdot IC\end{aligned} \tag{3}$$

where, p is the probability of growth in the range of 0–1 (growth = 1, no growth = 0), $\text{logit}(p) = \ln[p/(1-p)]$ a_0–a_4, b_0–b_{10} are the coefficients of the model to be estimated, T is the temperature (°C), $NaCl$ is the salt concentration (% *w/v*), $nisin$ is the nisin concentration (µg/mL), and IC is the initial concentration ($\log_{10}$CFU/mL) of *L. monocytogenes*.

SPSS (Version 22, Chicago, IL, USA) was used for logistic regression and goodness-of-fit of the models were compared by using (i) $-2 \cdot \ln L$ with L the likelihood in its optimum, (ii) Hosmer–Lemeshow (H-L) statistic (iii) maximum rescaled R^2 statistic and (iv) percent concordant.[32] The fitted growth/no growth boundaries for p= .1, 0.5, and 0.9 were calculated by using Microsoft® Excel.

The first model was the simplest possible model with the main effects (temperature, salt, nisin, and initial concentration). The second one was more complex one with bilateral interaction terms. If the interaction terms were insignificant (P > .05), they were removed from the model and regression was repeated without those terms, that is, remaining coefficients in the model were all significant (P ≤ .05).

Results

Prevalence of Listeria monocytogenes *in RTE food samples*

The identification of the 39 isolates was made according to the 16S rRNA sequence results (data not shown). The positive *Listeria* spp. and *L. monocytogenes* samples were 39/133 and 17/133, respectively. All *L. monocytogenes* strains showed a positive result for the presence of the virulence-associated *hly*A gene. Results of the prevalence of *Listeria* spp. and *L. monocytogenes* in the positive samples are shown in Table 1. In particular, *L. monocytogenes* isolated from RTE foods was highlighted in 4/15 seafood-based, 6/31 meat-based, 4/25 poultry-based, 3/30 vegetable-based. However, dairy and fruit-based RTE foods were *L. monocytogenes* negative.

MIC results

Seventeen *L. monocytogenes* strains isolated in this study were tested for susceptibility to nisin using MIC assays. Nisin can inhibit *L. monocytogenes*; MIC ranging from 128 to 4096 μg/mL were obtained. Four *L. monocytogenes* strains were selected among the strains with the highest, middle and lowest MIC values (Table 2). Among these selected strains, 120 and 151 was isolated from meat-based RTE foods, 137 from vegetable-based RTE foods, and also 241 from seafood-based RTE foods. While *L. monocytogenes* 120 was the most resistant strain to nisin (4096 μg/mL), strains 137 and 241 exhibited the lowest MIC values of 512 μg/mL. Strain 151 also displayed low susceptibility to nisin (2048 μg/mL). However, *L. monocytogenes* ATCC7644 was sensitive to nisin with an observed MIC value of 128 μg/mL.

Growth rate determination

Value of N_{OD} was determined by enumerating the number of *L. monocytogenes* in each well when the OD_{600} value was about 0.15. Although N_{OD} values changed with respect to strains, they were between 8.0 and 8.5 $\log_{10}$. Figure 1 shows the fit of Eq.(1) that is, how the growth rates (μ) were calculated for three strains (120, 137, and ATCC7644) and Table 3 lists the growth rates of each strain. The highest and lowest growth rates were obtained as 2.21 and 2.04 h^{-1} for strains 120 and 151, respectively.

Table 1. Presence of *Listeria* spp. and *L. monocytogenes* in RTE food products.

		Listeria spp.		*L. monocytogenes*	
RTE Food Groups	No	No	%	No	%
Dairy-based	20	4	20	—	—
Meat-based	31	8	25.8	6	19.4
Poultry-based	25	10	40	4	16
Vegetable-based	30	11	36.7	3	10
Fruit-based	12	—	—	—	—
Seafood-based	15	6	40	4	26.7
Total	**133**	**39**	**29.3**	**17**	**12.8**

Table 2. Minimum inhibitory concentration (MIC) values of nisin against *L. monocytogenes* strains*.

Bacterial Code	MIC (µg/mL) Values
77	512
100	512
104	1024
107	2048
114	512
115	512
116	1024
120	**4096**
122	2048
137	**512**
150	512
151	**2048**
152	2048
197	2048
217	1024
219	512
241	**512**
ATCC7644	128

* For each strain, identical MICs values were obtained in three independent determinations.

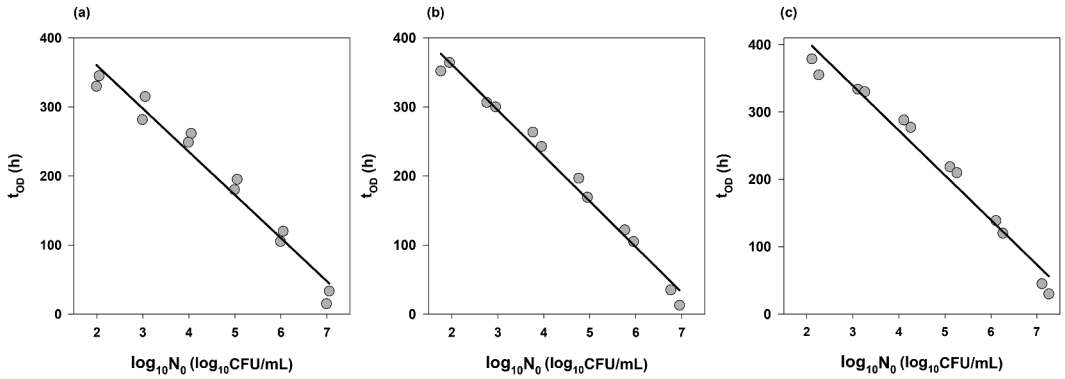


Figure 1. Time-to-turbidity as a function of logarithm of the inoculum size (gray circles) and the fit of Eq.(1) (black lines) to determine the growth rates of *L. monocytogenes* strains 120 (a), 137 (b) and ATCC7644 (c) in TSB.

Table 3. Growth rates of *L. monocytogenes* strains in TSB calculated by using Eq.(1) from OD measurements.

Strain	Growth rate (h^{-1}) ± SE	R^2_{adj}
120	2.21 ± 0.13	0.9614
137	2.09 ± 0.09	0.9849
151	2.04 ± 0.04	0.9899
241	2.07 ± 0.11	0.9708
ATCC7644	2.08 ± 0.11	0.9685

Plate count method was also applied for three strains (120, 137, and ATCC7644) to compare the growth rates of time-to-detection (TTD) data with colony counts. The fit of three models (modified Gompertz, Baranyi, and three-phase linear) used to describe the growth data of *L. monocytogenes* 137 is shown in Figure 2. All models produced reasonable fits with R^2_{adj}>0.97 for the other strains and closest growth rates to TTD experiments were the ones obtained from the fit of three-phase linear

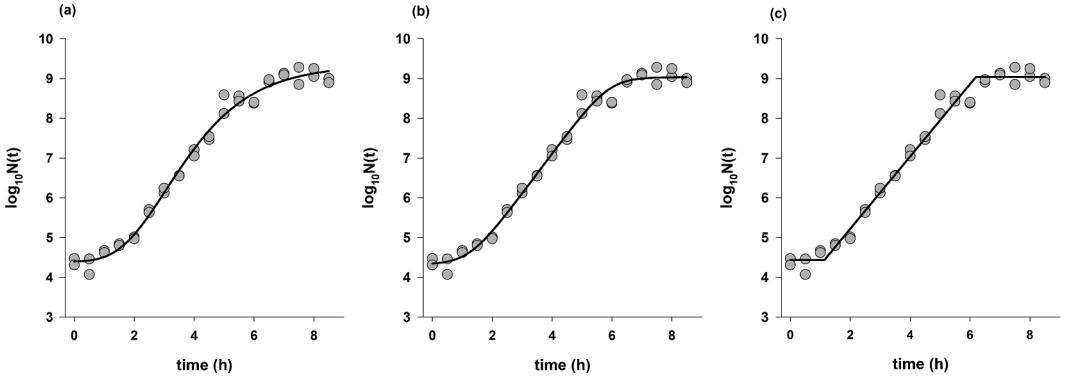


Figure 2. Plate count or growth data (gray circles) of *L. monocytogenes* 137 in TSB and the fit (black lines) of modified Gompertz equation (a), Baranyi model (b) and three phase linear model (c).

Table 4. Growth rates of *L. monocytogenes* strains in TSB calculated by using three phase linear model from colony count data.

Strain	Growth rate (h^{-1}) ± SE	R^2_{adj}
120	2.10 ± 0.11	0.9739
137	2.25 ± 0.09	0.9868
ATCC7644	2.04 ± 0.10	0.9754

Table 5. Growth rates of *L. monocytogenes* strains in TSB calculated by using Baranyi and Gompertz models from colony count data.

	Baranyi		Gompertz	
Strain	Growth rate (h^{-1}) ± SE	R^2_{adj}	Growth rate (h^{-1}) ± SE	R^2_{adj}
120	2.36 ± 0.16	0.9744	2.89 ± 0.21	0.9811
137	2.32 ± 0.09	0.9903	2.66 ± 0.13	0.9896
ATCC 7644	2.30 ± 0.15	0.9765	2.76 ± 0.19	0.9822

model (Table 4). According to the model fit, highest growth rate was 2.25 h^{-1} for the strain 137, followed by 2.10 h^{-1} for the strain 120 and 2.04 h^{-1} for the ATCC7644 strain. The growth rates obtained for the Baranyi and Gompertz models were given in Table 5. The information obtained from MIC study combined with the growth rate study, and the strains with higher MIC and growth rates (120, 137, and ATCC7644) were further used for growth/no growth study.

Effects of temperature, salt, nisin, and initial inoculum on the growth of L. monocytogenes

Growth responses of three strains with the inoculum levels of 10^3 and 10^6 CFU/mL are shown in Fig.3 and 4, respectively. In general, same response (growth or no growth) was observed for all three replicates; however, there were some exceptions. For example, at 4°C, without nisin and salt two out of three responses for the strain 120 were 1 (growth) and the one was 0 (no growth) meaning that growth probability was 67% (Figure 3b). For the strain 137, one out of three responses was 1 (growth) and two were 0 (no growth) indicating the probability of growth was 33% at 4°C without nisin and with 3% salt (Figure 3d). Differences between the replicates could also be seen in Figures 4b, 4d and 4f.

It was observed that the temperature had a significant impact on the growth of *L. monocytogenes*: at 37°C, the use of high salt and nisin concentration was not enough to avoid the growth even at the lowest inoculum (10^3 CFU/mL) – results not shown. On the other hand, high nisin concentration (≥200 µg/mL) was very effective at 20°C in the absence or presence of salt when the initial inoculum

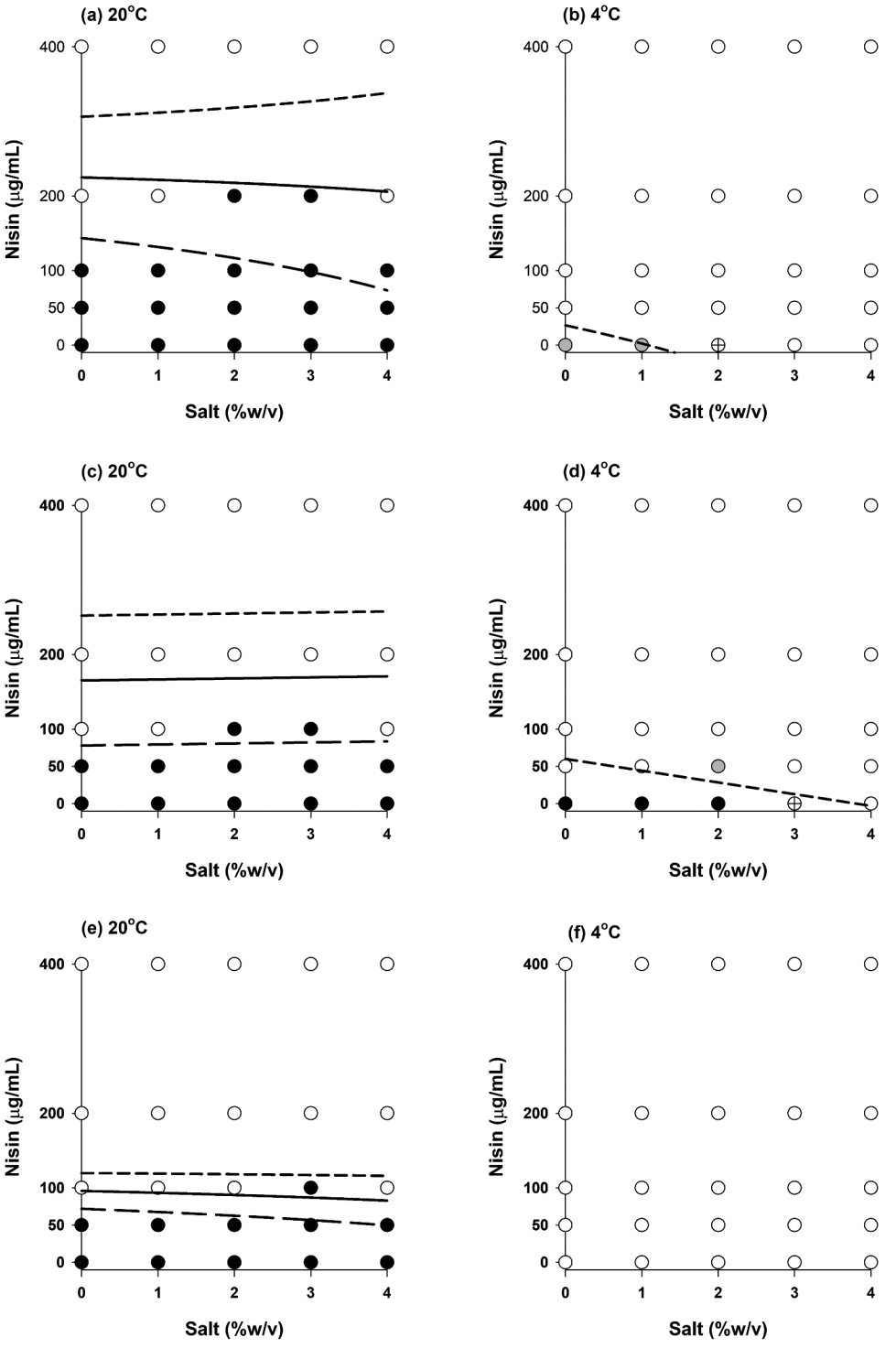


Figure 3. Growth/no growth interfaces of *L. monocytogenes* (10^3 CFU/mL) strains (a and b: strain 120, c and d: strain 137, e and f: ATCC7644 strain) in TSB after 8 weeks. White circles: no growth in all three replications, black circles: growth in all three replications, gray circles: two growth-one no growth observations and crossed circle: one growth-two no growth observations. Short dashed line, solid line and long dashed line represent the growth probabilities of 0.1, 0.5 and 0.9, respectively according to .Eq.(3) with the coefficient values given in Table 7

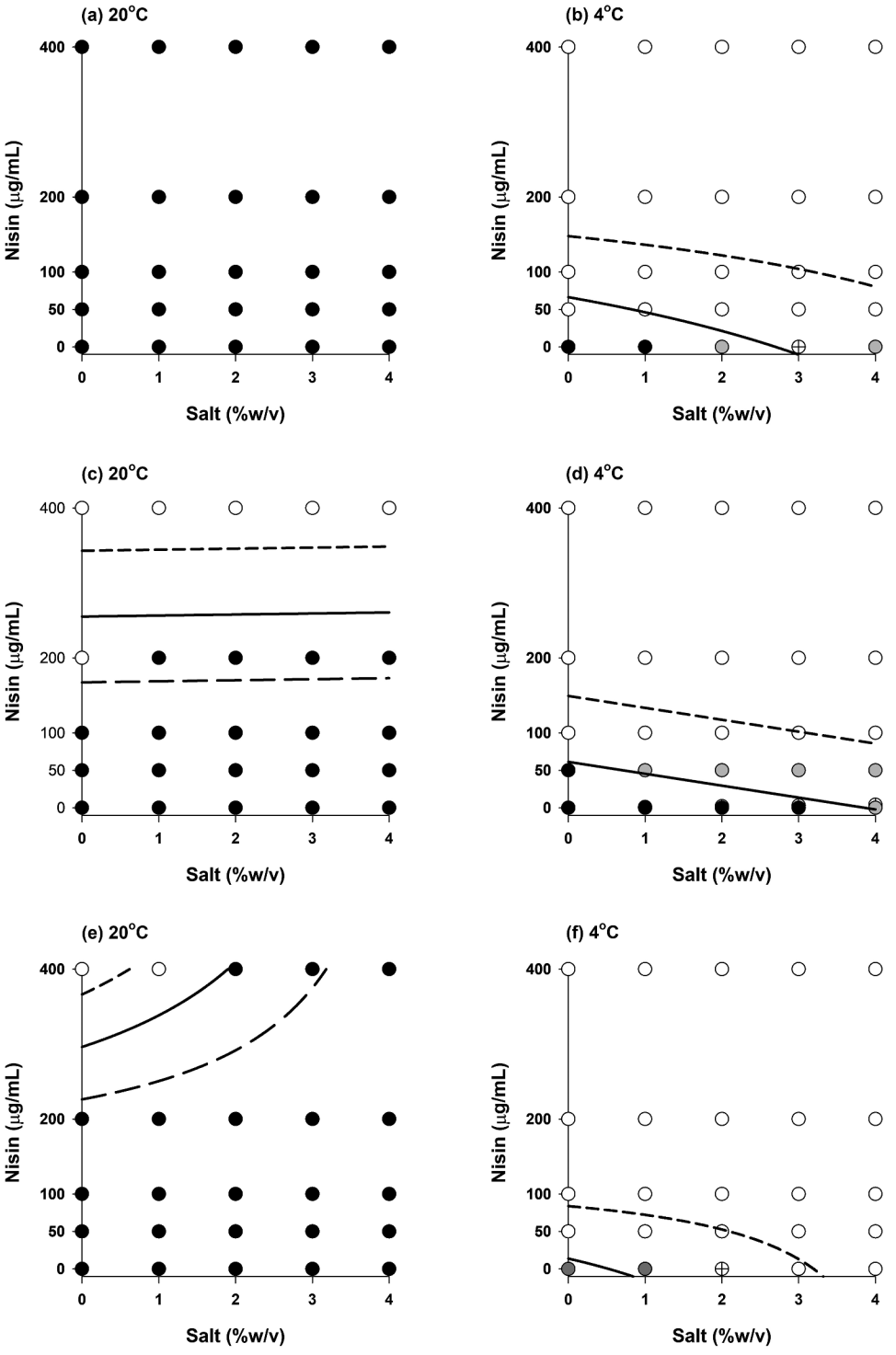


Figure 4. Growth/no growth interfaces of *Listeria monocytogenes* (10^6 CFU/mL) strains (a and b: strain 120, c and d: strain 137, e and f: ATCC7644 strain) in TSB after 8 weeks. White circles: no growth in all three replications, black circles: growth in all three replications, gray circles: two growth-one no growth observations and crossed circle: one growth-two no growth observations. Short dashed line, solid line and long dashed line represent the growth probabilities of 0.1, 0.5 and 0.9, respectively according to .Eq.(3) with the coefficient values given in Table 7

Table 6. Estimated coefficients of the simple model [Eq.(2)].

Strain	Coefficient	Estimate	SE	P value
120	a_0	−7.020	1.011	<0.001HT
	a_1	0.459	0.0399	<0.001HT
	a_2	−0.296	0.119	0.013
	a_3	−0.0214	0.00218	<0.001HT
	a_4	1.044	0.180	<0.001HT
137	a_0	−4.753	0.704	<0.001HT
	a_1	0.349	0.0294	<0.001HT
	a_2	−0.0812	0.0952	0.349
	a_3	−0.0248	0.00249	<0.001HT
	a_4	0.712	0.131	<0.001HT
ATCC7644	a_0	−17.377	2.188	<0.001HT
	a_1	0.619	0.0647	<0.001HT
	a_2	0.0729	0.135	0.590
	a_3	−0.0256	0.00306	<0.001HT
	a_4	2.279	0.334	<0.001HT

was less than 10^6 CFU/mL for all strains in general. Refrigeration temperature (4°C) changed the results drastically: even at the lowest nisin (50 µg/mL) concentration, growth was not observed for the strains 120 and 137 (Figure 3b and Figure 3d) whereas no growth was observed for the ATCC7644 strain without nisin and salt during storage of 8 weeks (Figure 3f).

Growth boundary determination

Models proposed [Eq.(2) and (3)] were applied to the data, and coefficient estimates, their standard errors and P values for Eq. (2) and (3) are all given in Table 6 and Table 7, respectively. The simplest model with only the main effects [Eq.(2)] had an insignificant coefficient (P > .05) of *NaCl* terms [a_2 in Eq.(2)] for the 137 and ATCC7644 strains (Table 6). On the other hand, for the complex model [Eq.(3)] all main effects retained in the equation when the insignificant coefficients were removed from the model. All goodness-of-fit indices were better for the second model (Table 8 and Table 9) and therefore, Eq.(3) was further used to plot the predicted growth/no growth boundaries of *L. monocytogenes*. Boundaries for likely to grow probability (p= .9), equal probability of growth and no growth (p= .5), and unlikely to grow probability (p= .1) are also displayed in Figures 3 and 4.

Discussion

In this study, the prevalence of *L. monocytogenes* in RTE foods was as 12.8%. Previous studies reported that the percentage of positive samples of *L. monocytogenes* in RTE foods was 36.73% in Nigeria[33] and 33.3% in Italy.[34] On the other hand, our results showed higher prevalence of *L. monocytogenes* strains in RTE foods compared to some other studies conducted in different regions, such as 2.6% in Estonia,[35] 6.87% in China,[36] 1.7% in Japan,[37] 0.4% in USA,[38] 0.1% in Poland,[39] and 3.1% in Chile.[40] Previous studies in Turkey[3,41,42] documented that the prevalence of *L. monocytogenes* in RTE foods was in the range of 4.0–19.7%.

Table 7. Estimated coefficients of the complex model [Eq.(3)].

Strain	Coefficient	Estimate	SE	P value
120	b_0	−6.640	1.027	<0.001HT
	b_1	0.472	0.041	<0.001HT
	b_2	−0.664	0.179	<0.001HT
	b_3	−0.027	0.003	<0.001HT
	b_4	1.091	0.186	<0.001HT
	b_5	—	—	—
	b_6	—	—	—
	b_7	—	—	—
	b_8	0.0026	0.0009	0.004
	b_9	—	—	—
	b_{10}	—	—	—
137	b_0	−4.118	0.726	<0.001HT
	b_1	0.303	0.032	<0.001HT
	b_2	−0.505	0.162	0.002
	b_3	−0.025	0.003	<0.001HT
	b_4	0.740	0.134	<0.001HT
	b_5	0.027	0.008	0.001
	b_6	—	—	—
	b_7	—	—	—
	b_8	—	—	—
	b_9	—	—	—
	b_{10}	—	—	—
ATCC7644	b_0	−49.60	8.398	<0.001HT
	b_1	2.894	0.514	<0.001HT
	b_2	−0.824	0.296	0.005
	b_3	−0.152	0.028	<0.001HT
	b_4	7.970	1.412	<0.001HT
	b_5	—	—	—
	b_6	—	—	—
	b_7	−0.390	0.083	<0.001HT
	b_8	0.0064	0.0019	<0.001HT
	b_9	—	—	—
	b_{10}	0.020	0.004	<0.001HT

Table 8. Goodness-of-fit indices of the simple model [Eq.(2)].

Goodness-of-fit	120	137	ATCC7644
−2lnL	249.999	354.794	184.703
HL	13.469 (P = .097)	20.047 (P = .010)	16.744 (P = .033)
Max. rescaled R^2	0.883	0.831	0.923
Percent concordant	93.3%	91.6%	96.1%

Higher prevalence in seafood products (26.7%) than other RTE foods were observed and this result was even higher than the previous reports from Iran,[5] Greece,[43] Estonia,[44] and Nigeria.[45] The prevalence of *L. monocytogenes* RTE seafood products in Turkey was found as 12% by Sanlibaba et al.[3] The higher prevalence obtained in this study may indicate poor hygiene level by the food

Table 9. Goodness-of-fit indices of the complex model [Eq.(3)].

Goodness-of-fit	120	137	ATCC7644
−2lnL	240.896	343.180	123.178
HL	13.258 (P = .103)	12.397 (P = .134)	2.424 (P = .965)
Max. rescaled R^2	0.888	0.837	0.950
Percent concordant	93.4%	91.1%	97.6%

handlers. Incidence of *L. monocytogenes* was slightly higher in meat-based samples (19.4%) than in poultry-based samples (16%). Available data from literature indicated that the prevalence of *L. monocytogenes* was between 3.6% and 27.9%.[22,39,46] Additionally, Awaisheh[22] and Sanlibaba et al.[3] found the prevalence of *L. monocytogenes* on RTE poultry foods at the level of 15% and 10.5%, respectively. The reason for the high prevalence of *L. monocytogenes* in the RTE meat and poultry-based foods may be inadequate heat treatment to eliminate of *L. monocytogenes* or inadequate physical separation between the raw and cooked food processing areas .[39]

L. monocytogenes have been widely associated with vegetable products.[47] In this study, although *L. monocytogenes* was not detected in fruit-based RTE foods, the prevalence of this pathogen was 10% in vegetable-based RTE foods. Fruit- or vegetable-based RTE foods which may be considered as minimally or moderately processed foods, can be great health risk for consumers since they are consumed fresh without applying heat treatment.[48] The main reason of contamination of vegetable-based RTE foods by *L. monocytogenes* may be processing, packing, or poor sanitary conditions of food handlers.[8,9] It is worth noting that *L. monocytogenes* was not isolated from dairy-based RTE in this study. This pathogen can be controlled in dairy-based RTE by the appropriate implementation of good manufacturing practices and hazard analysis and critical control point systems. Many studies have published that the prevalence in dairy-based RTE foods ranged from 5.90 to 36%.[21,49,50] Moreover, consumption of soft cheeses named Queso Fresco caused *Listeria* outbreak in 2021 according to the report published by Centers for Disease Control (CDC), and 12 persons became ill, of whom 1 died.[51] Post-pasteurization contamination by *L. monocytogenes* is the main problem in dairy products .[2]

Our results revealed that the specific growth rates of *L. monocytogenes* strains isolated from RTE foods obtained by the absorbance method and the plate count method were similar (Table 3 and Table 4) which had been also observed in different studies.[52–54] Therefore, it may be possible to determine the specific growth rate by performing two instead of multiple plate count experiments with 2-fold dilution method[54] since the initial value of each strain was also determined by enumeration. Strain 120 was the most resistant one to nisin according to MIC results (Table 2), it had also the highest growth rate with respect to 2-fold dilution method (Table 3) and second highest growth rate according to colony count data (Table 4). Hence, it was included in the growth/no growth study. On the other hand, although strain 137 had middle susceptibility to nisin (Table 2) it had the second highest growth rate with respect to 2-fold dilution method (Table 3) and the highest growth rate with respect to colony count data (Table 4). Therefore, it was also included in growth boundary study together with ATCC7644 strain.

Growth response data (1: growth, 0: no growth) of three strains of *L. monocytogenes* were monitored at different temperature, salt, nisin, and inoculum concentrations for 8 weeks (56 days), and no increase in OD values of negative controls during this period was observed indicating that no contaimination was occurred during the preparation of the liquid media. On the other hand, OD values of the positive control (without salt and nisin) increased at each temperature as expected. Growth/no growth results also indicated that the nisin was more effective than NaCl to avoid growth. Moreover, protective effect of salt on nisin activity was also observed. For example, at 20°C with 2% and 3% of salt concentrations, and with 200 μg/mL of nisin growth were observed for the strain 120 but, the responses were no growth at the same temperature and nisin concentration with 0%, 1%, and 4% salt concentrations (Figure 3a). Protective effect of salt on nisin activity against *L. monocytogenes* was also observed by Boziaris et al.[55] However, contradictory results on this subject were also found in the literature. For example, it was

observed that the presence of salt (NaCl) improves the action of nisin against *Listeria*.[56,57] On the other hand, the use of 2–4% NaCl has a protective effect of nisin against *L. monocytogenes*.[58,59] Form a hurdle concept point of view, this phenomenon should be further investigated at a microscopic level.

Initial inoculum was also an important parameter. Comparison of Figures 3 and 4 revealed this fact. Probability of growth increased as the initial inoculum increased and this was in agreement with the work of Koutsoumanis and Sofos[60] who studied the combined effect of temperature, pH, water activity, and inoculum size of *L. monocytogenes*. Furthermore, suppressing the growth by using temperature, NaCl, and nisin was more difficult in RTE-isolates strains (120 and 137) than that of ATCC7644 strain (Fig. 3 and 4).

While the simple model [Eq.(2)] had only five coefficients (with an insignificant main effect), the complex model [Eq.(2)] had six coefficients for the strains 120 and 137, and 8 coefficients for ATCC7644 strain after the removal of the insignificant terms and repetition of the regression (Table 6 and Table 7). Therefore, the second model was preferable over the first one and support for this statement also comes from Table 8 and Table 9 which compared the goodness-of-fit of the models. Furthermore, HL statistics were poor for the first model with $P < .05$ for the strains 137 and ATCC7644. The model is rejected if P is below 0.05.[31] The concordance and maximum rescaled R^2 values were all >91% and >0.83, respectively, indicating good predictive power of Eq.(3). Growth/no growth boundaries were in general consistent with the experimental data (Fig. 3 and 4) and it is possible to estimate the growth probability of *Listeria* using Eq.(3) for any combination of temperature, salt, and nisin levels within the experimental range.

The strains 120 and 137 were isolated from RTE foods, however, this study was conducted in laboratory medium (broth). Use of broth can present a quick estimation of growth boundaries nevertheless, deviations from the results could be obtained in the laboratory medium compared to a real food system.[30]

Conclusion

Prevalence of *L. monocytogenes* in RTE foods sold in Turkish market was determined in this study. It was observed that some RTE foods (dairy and fruit-based) did not contain any *Listeria*. On the other hand, seafood-based, meat-based, poultry-based, and vegetable-based RTE foods had *L. monocytogenes* showing a possible cross-contamination or poor hygiene during preparation. Growth/no growth study with different levels of temperature, NaCl, nisin, and inoculum size revealed that avoiding the growth of RTE-isolated strains were more difficult than ATCC7644 strain. Furthermore, nisin was more effective than NaCl for suppressing the growth of *L. monocytogenes* and protective effect of NaCl on nisin activity was observed. This issue should be taken into consideration by the researchers and food processors before the simultaneous use of salt and nisin as chemicals in RTE foods. Probabilistic model used in this study showed high concordance and it was possible to determine the growth limits of *L. monocytogenes* accurately.

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Authors contributions

Conceptualization, S.B. and P.Ş.; methodology, E.Ş., S.B., and P.Ş.; data analyses and modeling, S.B.; writing original draft, S.B. and P.Ş.; reviewing and editing, E.Ş., S.B., and P.Ş.; supervision, P.Ş. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

Data are available upon request from the corresponding authors.

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