

Modeling the growth rate of *Listeria innocua* influenced by coriander seed essential oil and storage temperature in meat using FTIR

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Abstract

The use of natural compounds to preserve food and the application of new tools for monitoring food safety is of great interest. In this study, the growth rate, including the lag time of *Listeria innocua* in ground lamb as a function of storage temperature (4, 10, 25, and 37°C) influence of the coriander seed essential oil (CEO), as an antimicrobial agent, was modeled. Fourier-transform infrared (FTIR) spectroscopy was used to monitor chemical changes in investigated specimens that may indicate spoilage in ground lamb. Results showed the use of CEO reduced growth rate of *L. innocua*, to approximately 0.0051 (CFU/h) for a sample containing CEO and to 0.042 (CFU/h) in control samples cultivated at 4°C. Increasing the cultivation temperature from 4 to 37°C led to a 5-fold increase of the growth rate (0.042 to 0.222 in the control sample) and 28-fold growth from 0.0051 to 0.147 in a sample containing the CEO. Minimum-recorded temperatures (T_{\min}) were -0.57 and 0.60°C for the control sample and a sample containing the CEO. The comparison of FTIR spectra of samples during the shelf life indicated that the increased shelf life and bacterial growth results in decreased moisture ($1600\text{--}1650\text{ cm}^{-1}$) and protein content in the samples; while the intensity and sharpness of peaks increased in the range $1000\text{--}1100\text{ cm}^{-1}$, indicating the impact of bacterial activity on amounts of amines and amino acids. In conclusion, the CEO increased the *L. innocua* growth rate lag phase and improved the shelf life of the ground lamb. It is also recommended to use the FTIR to monitor the spoilage in meat.

Keywords: coriander essential oil; lamb meat; *Listeria innocua*; shelf life

Introduction

At present, foodborne diseases are a rising public health problem. Every year, the consumption of contaminated food with various pathogens causes many human and financial losses in countries around the world (El Sheikha, 2015). *Listeria innocua* is widely found in the environment, especially in soil and food sources. It is a Gram-positive, rod-shaped bacterium that can multiply at low and high temperatures and high salt concentrations. Fresh and frozen raw meat and meat products such as burgers

are among foods that can be contaminated by this pathogen and contribute to the transmission of bacteria to consumers (Criado *et al.*, 2019; Gómez *et al.*, 2014). Natural inhibitors such as essential oils for food preservation have now received much attention (Khezerlou *et al.*, 2019; Menezes *et al.*, 2018; Nikolić *et al.*, 2020). Essential oils are secondary products of plant metabolism, aromatic and volatile compounds isolated from various segments of plants, which have a potent antimicrobial, insecticide, and antioxidant ability that has been successful in preventing and treating bacterial infections (Azhdarzadeh *et al.*, 2018;

Foltinova *et al.*, 2019; Khezerlou *et al.*, 2019). Coriander (*Coriandrum sativum* L.) is an annual plant belonging to the Apiaceae family that probably originated from the eastern Mediterranean and spread to the world as an old spice (Laribi *et al.*, 2015). The herb and seeds of coriander include bioactive components, which are used in the pharmaceutical industries (Omidbaigi *et al.*, 2009). Coriander seed is extensively used in traditional Iranian medicine for the treatment of various disorders (Nejad Ebrahimi *et al.*, 2010) as well as in several food products like pickles, curry powder, sausages, meat, confectionery, and bakery as a condiment (Laribi *et al.*, 2015; Nejad Ebrahimi *et al.*, 2010). Šojić *et al.* (2019) evaluated the use of CEO as a natural food additive on cooked pork sausage quality. They observed that the use of CEO improved the oxidative and microbial stability of cooked pork sausages during refrigerated storage. Recently, guar gum, including extracts of some culinary spices like coriander seed was used as edible coating to prolong lemon's shelf life and showed that treated lemon had better physicochemical, biochemical, and microbiological attributes than controls (Naeem *et al.*, 2019).

To date, the use of new techniques and tools instead of traditional methods for identifying foodborne microorganisms and monitoring food safety has increased for various reasons (El Sheikha *et al.*, 2018). Predictive microbiology is applied to enhance and use mathematical models to predict the response of microorganisms to factors and measured under controlled and defined conditions (Noori *et al.*, 2014). Predictive models are significantly crucial in determining microbial behavior factors in the farm-to-consumer food chain to decide the shelf life of perishable foods. Many efforts have been made to assess risk and manage the risk of widespread diseases due to microbial pathogens (Costa *et al.*, 2019; Don *et al.*, 2018). Different levels of models are used in the predictive microbiology of foods. First-level models deal with microbial changes over time. In these models, changes in numbers of bacteria over time are calculated under specific environmental conditions, but in the second-level model, changes in initial model parameters are evaluated under the influence of ecological factors (Juneja *et al.*, 2019; Kakagianni and Koutsoumanis, 2019; Yoon *et al.*, 2019). In other words, the models describe the response of one or more parameters of the initial model such as bacterial growth lag phase time due to changes in environmental conditions such as temperature (Lianou *et al.*, 2018; Menezes *et al.*, 2018; Mishra *et al.*, 2017; Sommers *et al.*, 2018). Fourier-transform infrared (FTIR) spectroscopy is a rapid, advanced, simple, environmentally friendly, and reliable analytical technique that has recently been used in quality control and market management of various food products (Xu *et al.*, 2018; Yasmin *et al.*, 2019). Therefore, the present study aims to dynamically model the growth behavior of *L. innocua* as

a function of storage temperature in ground lamb containing CEO using FTIR to monitor chemical changes for indicating the spoilage.

Material and Methods

Bacterial strain and inoculum preparation

Listeria innocua ATCC 33090 was obtained from Mashhad University of Medical Sciences (Mashhad, Iran). It was cultured in Müller-Hinton (MH) broth (Difco, Detroit, MI, USA) in sterile falcon tubes (Becton Dickinson, Falcon™, Franklin Lakes, NJ, USA) and incubated at 37°C for 24 h, centrifuged at 9000 rpm at 7°C for 5 min. To rinse the bacterial sediment, 4 to 5 mL of physiological saline was added, vortexed, and re-centrifuged. Initial bacterial suspension of 10⁸ CFU/mL has been prepared using McFarland 0.5 Turbidity Standard, and it was diluted up to a final, desired concentration of 10⁴ CFU/g (McNamee *et al.*, 2010; Rasooli *et al.*, 2006). During the incubation period, 1 g was taken from the meat sample every 24 h and serial dilutions were made in physiological saline, inoculated on MH agar with 6.5% NaCl (Merck, Darmstadt, Germany) and incubated for the determination of the exact number of *L. innocua*.

Meat samples

Fresh ground lamb (10.48% fat, 17.26% protein, and 1.23% ash) was purchased from the local retail store and transferred to the laboratory in the shortest time following hygienic conditions in a plastic bag and rubber dry icebox. Compositional percentage of lamb meat such as fat, protein, and ash were determined according to the Official Methods of Analysis of Association of Official Analytical Chemists (AOAC, 2005). Coriander seed essential oil (CEO) was bought from Tabib Daru Co, Iran. For every 5 g of ground lamb, 0.075 g of sterile CEO (passed through a 0.22 syringe filter) and the microbial suspension was added and mixed well with the ground lamb, so the final number of bacteria was 10⁴ CFU/g. The ground lamb samples were separated and placed in sterile plastic bags and stored at 4, 10, 25, and 37°C for 8 days, and bacterial counts were monitored on days 0, 2, 4, 6, and 8 of samples. At each of the examined temperatures, a ground lamb sample without CEO that was sterilized with plasma (APLM-SP-YB-D1KW, ATV Electronic Technology Co., Suzhou, China) was used as the control sample.

FTIR

The FTIR spectrophotometer (Thermo Nicolet, Madison, WI, USA) at ambient temperature was used

for 1000–2000 cm⁻¹ with a resolution of 4 cm⁻¹ to obtain spectral information of ground lamb. For this purpose, meat samples were placed directly on the plate to completely cover the Zinc selenide crystal sensor without any air bubble remaining between the sample and the top of the crystal and spectra of control and sample containing CEO were evaluated (Akhtar *et al.*, 2018; Papadopoulou *et al.*, 2011).

Evaluation of growth parameters

Three growth curves, representing three replications at each incubation temperature (4, 10, 25, and 37°C), were obtained in the present study. Two replications of each temperature were used for the analysis of kinetic parameters, including specific growth rates (ln CFU/g h⁻¹, μ_{max}) and lag time (l, h), and the remaining replications were evaluated for the model validation (equations 1–3) (Huang, 2014). To fit data, the laboratory data on the growth of *L. innocua* were first converted as log(CFU/g). The reduced Huang equation was then utilized for the initial modeling of *L. innocua* growth in control and a sample containing 1.5% CEO. MATLAB software (The Mathworks Inc., Natick, MA, USA, version R2016a) was used to fit the data and obtain growth parameters such as maximum specific growth rate and duration of lag phase. The Suboptimal Huang Square-Root equation was used to examine the dependence of the maximum specific growth rate on temperature. Equation (4) was also used to investigate the effect of temperature on the lag time of bacterial growth (Huang, 2014; Huang *et al.*, 2012).

$$Y = Y_0 + \mu_{\max} \left\{ t + \frac{1}{4} \ln \left[\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}} \right] \right\}$$

$$Y = Y_0 + Y_{\max} - \ln \left\{ e^{Y_0} + \left[e^{Y_{\max}} - e^{Y_0} \right] e^{-\mu_{\max} B(t)} \right\} \quad \text{Equation (1)}$$

$$B(t) = t + \frac{1}{4} \ln \left(\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}} \right) \quad \text{Equation (2)}$$

$$\sqrt{\mu_{\max}} = a(T - T_{\min})^{0.75} \quad \text{Equation (3)}$$

$$\lambda = \alpha + \beta \times \ln(\mu_{\max}) \quad \text{Equation (4)}$$

Here, initial bacterial concentration = Y₀; lag time (h) = λ; specific growth rates (log CFU/g h⁻¹) = μ_{max}; maximum cell = Y_{max}; real-time number of bacterial (log(CFU/g)) = Y; regression coefficients = α, β, a.

Statistical analysis

The statistical parameters, namely the Root Mean Square Error (RMSE), the sum of squared estimate

of errors (SSE), and correlation coefficient (R²), were used to compare the efficiency of primary and secondary models and validate the model (Juneja *et al.*, 2019; Mishra *et al.*, 2017). In that *k* is the number of experimental data, *m* is the number of model parameters, *o*_{exp} are the experimental values of concentration of microbial cells, *o*_{pre} are the prediction values of concentration of microbial cells, and \bar{o} is the mean value of concentration of microbial cells.

$$R^2 = \frac{\sum (o_{\text{pre}} - \bar{o})^2}{\sum (o_{\text{exp}} - \bar{o})^2}$$

$$\text{RMSE} = \sqrt{\frac{\sum (o_{\text{exp}} - o_{\text{pre}})^2}{k - m}}$$

$$\text{SSE} = \sum (o_{\text{pre}} - o_{\text{exp}})^2$$

Results and Discussion

First, the reduced Huang model was fitted on the obtained laboratory data from the growth of *L. innocua* in raw ground lamb, and model parameters (maximum specific growth rate and duration of lag phase) were examined. It should be noted that 7logCFU/g of *L. innocua* was used as an upper limit number, that is, an indicator for the evaluation of the shelf life of investigated specimen (Sommers *et al.*, 2018). As shown in Figure 1, the lag phases shortened with increasing the incubation temperature. Figure 1 shows a concentration of 7logCFU/g of *L. innocua* in the control sample reached after 3 days of incubation at 4°C, and the same number of *L. innocua* was reached in a sample containing 1.5% CEO after 25 days of incubation at the same temperature. At 10°C, the bacterial population in a CEO sample reached 7logCFU/g after 8 days of incubation, and thus the shelf life of samples increased by 6 days compared to the control sample.

As observed in Figure 1, the antimicrobial activity of CEO greatly impacts growth parameters. According to results (Table 1), increasing the temperature from 4 to 37°C led to 5-fold increased growth rate (0.042 to 0.222 in the control sample) and 28-fold increased growth from 0.0051 to 0.147 in a sample containing CEO. Furthermore, increasing the temperature from 4 to 37°C led to a 20-fold decrease in lag time (from 18 to 0.9 h) in the control sample and a 65-fold decrease (from 90 to 1.38 h) in a sample containing seed CEO.

Table 1 presents the mean values of parameters *a* and *T*_{min} for *L. innocua* in the control sample and CEO sample. Minimum-recorded temperatures (*T*_{min}) were -0.57 and 0.60°C for the control and CEO sample,

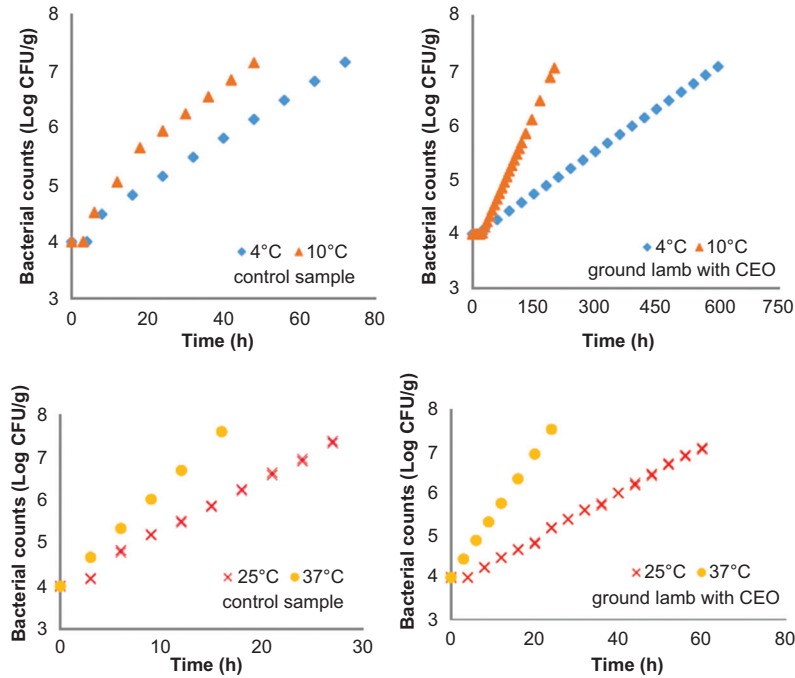


Figure 1. Effect of CEO and storage temperatures on the growth rate of *L. innocua* in ground lamb.

Table 1. Statistical parameters for *L. innocua* growth rate in ground lamb samples.

	a*	T _{min}	R ²	RMSE	SSE
Ground lamb without CEO (Control)	0.02 ± 0.01	-0.57 ± 0.10	0.97	0.21	0.0441
Ground lamb with CEO	0.023 ± 0.003	0.6 ± 0.1	0.94	0.27	0.0729

*a: regression coefficient from equation (3); T_{min}: the minimum; R²: correlation coefficient; RMSE: root mean square error; SSE: sum of squared estimate of errors; CEO: coriander essential oil.

respectively. Besides, Table 1 presents the obtained statistical parameters (RMSE, SSE, and R²) from the fitting model to the laboratory data. Statistical indices indicated high accuracy of the model in the data prediction so that R² value was 97% for the control sample, and 94% for a sample containing 1.5% CEO. The RMSE values were 0.21 for the control and 1.5% for the CEO sample, respectively.

Figure 2 shows the most important spectral peaks of FTIR for the ground lamb. Based on the obtained data, the FTIR spectrum can be divided into four categories: (1) 1600–1650 cm⁻¹, (2) 1500–1550 cm⁻¹, (3) 1200–1350 cm⁻¹, and (4) 1000–1100 cm⁻¹. Wave range of 1600–1650 cm⁻¹ was caused by hydroxyl groups (OH), moisture, and amide bond I in protein. The peaks in the wave range of 1500–1550 cm⁻¹ indicate the tensile of C–N bonds of amide groups II in the sample protein. The peak in the wave range 1220–1800 cm⁻¹ indicates the presence of a tensile C=O bond (first type amide) in the

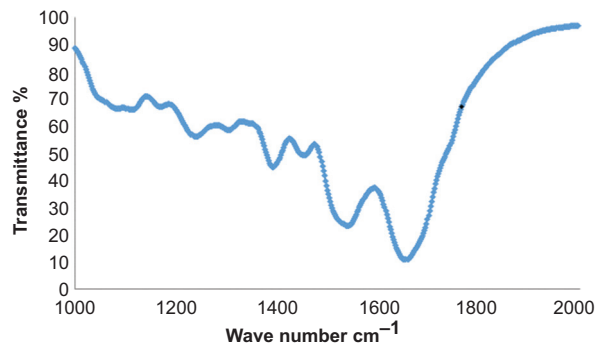


Figure 2. FTIR spectra of ground lamb.

sample. Furthermore, the peak in 1200–1350 indicates the presence of a tensile C–N bond of amine groups in the sample structure. The observed peak in 1000–1100 cm⁻¹ is related to free amino acids and amines in the sample structure (Papadopoulou *et al.*, 2011).

According to results, the use of CEO reduced the growth rate of *L. innocua*, so no bacterial growth was seen in samples containing 1.5% CEO oil during the first 90 h of incubation. However, increasing the incubation temperature increased bacterial growth rates in control samples, but CEO reduced growth rates at all incubation temperatures. Thus, the CEO increased shelf life of the ground lamb, from 3 days for the control sample to 25 days for the samples with CEO incubated at 4°C. And the shelf life was 27 h for the controls and 60 h for the CEO samples incubated at 25°C. The shelf life of samples incubated at 37°C was 16 h for the control and 24 h for CEO samples. Chouliara *et al.* (2007) investigated the impact of using orange essential oils on the shelf life of fresh chicken incubated at 4°C. The results of this study indicated that adding 1% essential oil completely prevented the bacterial growth of lactic acid bacteria for 12 days and increased the shelf life of samples up to 16 days, while the lactic acid bacteria reached a concentration of 7logCFU/g after 9 days in control samples. Mexis *et al.* (2009) examined the impact of the addition of 0.1% essential oil on tarama salad shelf life that was kept at 4°C and indicated that the concentration of lactic acid bacteria reached 6.45logCFU/g in the control sample after 24 days of storage, while it reached 5.95 in the sample containing essential oil after 40 days of storage. Sant'Ana *et al.* (2012) examined the shelf life of packed minimally processed lettuce at storage temperatures of 7 to 30°C and reported μ value between 0.02 ± 0.0001 (for 7°C) and $0.02 + 0.22$ (for 30°C) for *L. innocua*. In addition, λ value was found between $59.4 + 12.7$ (7°C) and $2.4 + 0.8$

(30°C) for *L. innocua*. Kaklan (2019) evaluated the antimicrobial effect of probiotic lactic acid bacteria against *Staphylococcus aureus* in white cheese. The result of this study showed the number of *S. aureus* has decreased by approximately 5 log units at the end of the storage time. Li *et al.* (2020) evaluated the kinetic models (Huang, Baranyi, and Reparameterized Gompertz model) to describe the growth of *Pseudomonas* spp. in Agaricus bisporus fruiting bodies. Results of this study showed the Reparameterized Gompertz model was a better fit than the Huang and Baranyi models to describe the growth of *Pseudomonas* spp., and had the lowest mean square error (MSE) and sum of squared errors (SSE) values, ranging 0.012–0.291 and 0.059–1.783, respectively.

The minimum temperature that supported the growth of *L. innocua* was about 3°C (Junttila *et al.*, 1988). The minimum temperature (T_{min}) was often below the minimum storage temperature for bacterial growth. Our study showed that T_{min} values for *L. innocua* for the control sample and CEO samples were -0.57 and 0.60 °C, respectively. Slope parameters (b) were 0.020 and 0.023 for the control sample and a sample containing CEO. The comparison of FTIR spectra of samples during the shelf life indicated that the increased shelf life and bacterial growth decreased moisture (1600–1650 cm^{-1}) and protein content in the samples, while the intensity and sharpness of peaks increased in 1000–1100 cm^{-1} , indicating the impact of bacterial activity on amounts of amines and amino acids (Figure 3). The proteolysis process due to the bacterial activity during the shelf life was the

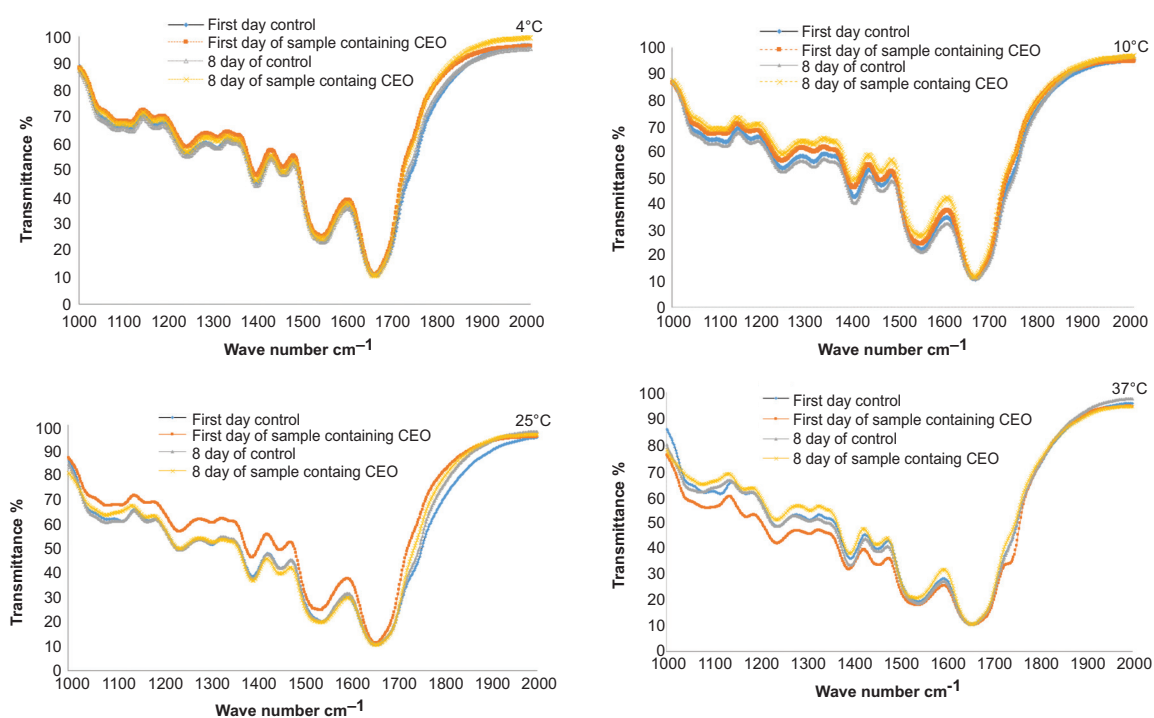


Figure 3. Effect of CEO and storage temperatures on the FTIR spectra of ground lamb.

most critical reason for changes in amounts and intensity of peaks in the FTIR spectrum (Argyri *et al.*, 2010). Furthermore, the comparison of the control sample and a sample containing the CEO indicated the lower rates of changes in intensity and sharpness of peaks in samples containing CEO at the same temperature and duration compared to the first day, indicating the inhibitory effect of CEO on the *L. innocua* activity.

Conclusions

In conclusion, the reduced Huang model can be used with high accuracy ($R^2 > 0.94$) to predict the growth rate of *L. innocua* in ground lamb. The addition of CEO in meat samples increased delayed phases of samples, so at 10°C, the bacterial population in a CEO sample reached an upper limit number 7logCFU/g after 8 days, and thus the shelf life of samples increased by 6 days compared to the control sample. While increasing the temperature from 4 to 37°C led to a 20-fold decrease in lag time (from 18 to 0.9 h) in the control sample and a 65-fold decrease (from 90 to 1.38 h) in a sample containing the CEO. Furthermore, the comparison of FTIR spectra of the control sample and a sample containing the CEO indicated the lower rates of changes in intensity and sharpness of peaks in samples containing the CEO at the same temperature and duration compared to the first day, indicating the inhibitory effect of CEO on the *L. innocua* activity. According to results, the CEO can be used as a natural antimicrobial in various meat applications in the food industry to extend the shelf life. Also, FTIR can be used to monitor chemical changes in meat, which may represent the spoilage. It is also suggested as a suitable tool to predict the shelf life of meat products at different storage temperatures.

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Conflict of interest

There is no conflict of interest.

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