Modelling of *Staphylococcus Aureus* under the Effect of *Carum Copticum* Essential Oil, pH, Temperature, and Inoculum Level

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A R T I C L E I N F O	A B S T R A C T
	Staphylococcus aureus is among the major causes of foodborne outbreaks globally. To limit its potential risks and predict its growth behaviors, it is crucial to define the growth boundaries of *Staphylococcus aureus*. So, this experiment was designed to estimate the growth behavior of *Staphylococcus aureus* in brain heart infusion (BHI) broth while affected by various concentrations of *Carum copticum* EO (0, 0.015, 0.030, 0.045%), pH (5, 6, 7), temperature (25, 35 °C), and inoculum levels (10^3, 10^5 CFU ml^{-1}). The assay was performed with 48 treatment conditions in triplicate. Visible turbidity represents growth onset was checked daily during 30 days of trial. According to the accelerated failure time (AFT) approach, a parametric survival model was chosen to predict the impact of selected variables on *Staphylococcus aureus* growth. GC-MS assay had quantified sixteen (16) compounds constituting 98.88% of pure oil. Based on our findings, the major components of essential oil were identified as thymol (57.18%), ρ-cymene (22.55%), γ-terpinene (13.07%), and trans-anethole (1.7%). The MIC value of the EO was 0.625 μl ml^{-1}. The median time to detection of bacterial growth was six days. All the predictor variables showed a significant effect on time to initiate the bacterial growth ($p < 0.05$). The ultimate model could precisely estimate the growth responses of *Staphylococcus aureus.*

Introduction

Staphylococcus aureus (*S. aureus*) is a facultative, gram-positive foodborne pathogen, which gives rise to numerous complications like gastroenteritis, cutaneous infections, furunculosis, impetigo, scalded skin syndrome, and toxic shock syndrome [1-3]. It has been distinguished as the second cause of food poisoning outbreaks worldwide and a marker of insufficient hygiene of food handlers and inadequate storage temperature [4, 5]. As long as *S. aureus* is relatively poor in competition with other microorganisms, food poisoning from raw foods is rare [6]. Staphylococcal food poisoning is caused by the ingestion of foods that contain heat-resistant staphylococcal enterotoxins A, B, C, D, E, and F (SEA-SEF). Subsequently, heat treatment of food before consumption would eliminate the bacterial cells, but not their toxins [6]. Staphylococcal intoxication is induced by ingestion of staphylococcal enterotoxins (≤ 1.0 μg), which are secreted by > 10^5 CFU g^{-1} of cell bacteria [7]. *S. aureus* has the capability of increasing and surviving in a large variety of environmental conditions for a long time because of its ability to cultivate in a broad spectrum of thermal conditions (7- 48.5 °C) and pH (4.0-10.0) [7]. Furthermore, *S. aureus* is among the antibiotic resistance bacteria, which is even served as indicator for this phenomenon [8]. So, this growing problem of the emergence of antibiotic-resistant bacteria, along with the adverse effects of many antibiotics in humans (i.e., hypersensitivity and allergic reactions) has turned this issue into a significant public health concern [9]. Therefore, surging for new treatment options such as exploring the medicinal plants for their bioactive molecules with antimicrobial properties has gained lots of interests. Plant extracts such as essential oils (EOs) can serve as safe alternatives to synthetic antibiotics [10]. The antibacterial property of EOs against various bacteria has been defined to be at concentrations between 0.2 and 10 μl ml^{-1}. But the critical point is to identify the lowest level of EO, which could prevent the growth of pathogens, as well as being organoleptically accepted when used in food [11]. Among traditional medicinal herbs, *Carum copticum*

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(syn: *Trachyspermum Ammi*), belonging to the *Apiaceae* family, is an annual herbaceous plant. It is commonly known as a flavoring agent, also known to possess anti-allergic, anti-inflammatory, antimicrobial, hypocholesteremia, and antioxidant activities [9, 12, 13]. In Iran, their fruits, commonly known as '*Zenyan*' or '*Ajwain*' have been used widely in traditional Iranian medication to cure several diseases such as gastrointestinal, inflammatory, and rheumatic complications [14]. Its EO (2.5-5%) consists of phenolic components like thymol, p-cymene, γ-terpinene, carvacrol, and β-pinene, which represent antimicrobial properties [14, 15].

Taken together, the growth of foodborne pathogens can be affected by many intrinsic and extrinsic factors. To minimize their development and limit their potential risks, it is crucial to scrutinize the environmental effects on them and realize their growth boundaries. In this regard, predictive microbiology is typically applied to develop models to predict the growth responses of particular microorganisms. The basic premise of predictive microbiology is the reproducibility of the microbial responses to environmental factors [16]. These microbial performance models were initially illustrated by [17]. After that, it gains more and more attention worldwide and has been widely studied in recent years [1, 7, 16, 18, 19]. This study was also designed to estimate the growth behavior of *S. aureus* in brain heart infusion (BHI) broth while affected by various concentrations of *C. copticum* EO, pH, temperature, and inoculum levels.

Material and Methods

Experimental Approach

To evaluate the growth responses of *S. aureus*, while affected by *C. copticum* EO, pH, temperature, and inoculum levels, the present trial was conducted in a multidimensional matrix in BHI broth (Merck). This matrix (4×3×2×2×3 equal to 144 conditions) consisted of 4 levels of the EO (0, 0.015, 0.03, and 0.045%), 3 values of pH (5, 6, and 7), 2 incubated temperature (35 and 25°C), and 2 concentrations of inoculums of the bacteria (10^3 and 10^5 CFU ml^{-1}), which were observed daily during 30 days for the possible growth.

Bacterial Strain

The bacteria which were undergone in this experiment was *S. aureus* subsp. *Aureus* ATCC 25923 (Mast International Inc-England).

Inoculum Preparation

The inoculums were ready by plating the reference bacteria on Baird Parker agar plates followed by 24 h storage at 37°C. Then, second subcultures were plated for 24 h at 37°C. After that, to acquire the optical density (i.e., absorbance) of 0.669 at 600 nm, a full loop of bacterial cells was transferred to sterile cuvettes, which had been filled with isotonic saline solution, and then placed in a spectrophotometer apparatus (Jenway 6105, Essex, England). This acquirement is equal to a cell level of 1.2×10^9 CFU ml^{-1}. Tenfold serial dilution was performed to estimate the concentration of the bacteria cells in the suspension.

Plant Material

The pure steam distillation extraction of *C. copticum* EO was acquired from the Agro-Industry corporation, Mashhad, Iran (Nader-Co®).

GC-MS Identification of EO

The EO constituents were identified by employing an Agilent Technologies HP-6890 Gas chromatography-mass spectrometry (Palo Alto, CA, USA), with a capillary column (Model HP-5MS; 30 m × 0.25 mm internal diameter, 0.25 μm membranous thickness), coupled with a mass spectrometer (Model HP 5973; Agilent Technologies, Palo Alto, CA, USA), which have an electron ionization potential (70 eV). The oven temperature was held at 50°C for about 5 min at the beginning and gradually was elevated at the rate of 3°C per min until it reached 240°C. Eventually, it was raised at the rate of 15°C per min until reached 290°C, then maintained at this degree for 3 min. The inert gas was Helium which flowed past by a speed of 0.8 ml per min. The injector mode was run in the splitless mode, so 1.0 μl of each sample was injected manually. Quantitative data were obtained by using the percentage of the area of the peaks. Retention indexes were measured for each constituent using a similar sequence of hydrocarbons (i.e., n-alkanes series) injected in the same situations as the samples. The EO ingredients were quantified by assimilating their retention indexes in proportion to the n-alkanes series (C8 to C22), with those presented in the literature [20].

In Vitro Antibacterial Assay: MIC Test

The standard tube dilution technique was applied to define the minimum inhibitory concentration of the used EO [21]. The method

was carried out by binary serial dilutions of EO in BHI broth, using 5% (v/v) dimethyl sulfoxide (DMSO, Merk, Hohenbrunn, Germany) as an emulsifying agent. To attain EO concentrations from 1 % to 0.001 %, EO was serially diluted 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.4, 0.2 and 0.1 µl 10 ml^{-1}, respectively. Subsequently, the test organism (10^6 CFU ml^{-1}) was inoculated into the test tubes with various concentrations of EO. The tubes, which included various amounts of EO but not the bacteria were assumed as the negative control tubes. Then, all tubes (both controls and tests) were stored at 37°C for 24 h. Finally, the tubes were checked daily to observe the presence or lack of turbidity. Accordingly, the tube which had the lowest amount of EO that showed no evident bacterial growth (i.e., absence of turbidity) was considered as MIC. In the current research, four distinct values of EO, which were below MIC, were chosen as analyte amounts.

Performing the Trial

At first, 3.7 g of BHI powder (Merck, Darmstadt, Germany) was diluted in 90 ml distilled water using gentle heating in a 250 ml containing flask to prepare BHI broth medium. To produce and maintaine the stability of oil emulsion in BHI broth medium throughout the study period (30 days), the revised technique elucidated by Mann C and Markham JJ (1998) [22] were applied. In this regard, an emulsifying agent named dimethyl-sulfoxide (5% v/v, from Merck, Darmstadt, Germany), along with a stabilizing agent named agar agar (0.05% w/v, from Merck, Darmstadt, Germany) was added to the broth medium of all combinations, even to combinations without EO (0.0%), to recognize any probable influence of them on the growth responses of the tested bacteria. By adding distilled water, the ultimate content of BHI broth was 100 ml. The pH values were adjusted using the normal solution of HCL as an acidifying agent and a pH meter (Jenway, Staffordshire, UK) [5-7]. Then, all flasks were sterilized at 121°C for 15 min. After getting cold, the pH degree of every tube was remeasured and readjusted by using 1 N purified sterilized NaOH (or HCl). Then, the sterile BHI broth in the flask was portioned out into 3 ml amounts in sterilized capped tubes (16 × 100 mm; Becton Dickinson, Durham, USA). After that, different amounts of filtered sterilized EO (0, 0.015, 0.030, 0.045%), were added. The tubes were infected with *S. aureus* culture (10^3

and 10^5 CFU ml^{-1}) and stored at 35 and 25°C for 30 days. Throughout this period, the tubes were verified daily for possible observable growth (turbidity). At every observation, the number of tubes exhibiting growth was recorded. A negative control (uninfected tube) was considered for each combination. All experiments were performed with three repetitions. The whole numbers of conditions were 144 (4 × 3 × 2 × 2 × 3).

Analysis of the Statistics

The time to the nearest visual turbidity (TTD) was determined as a dependent variable for further consideration in this work. Considering the combinations that did not grow during the entire period of the experiment (30 days), standard regression approaches were recognized to be incompatible. As an alternative, an event-time (survival) assay was applied, which was capable of using all the observational data regardless of whether or not growth occurred. According to the accelerated failure time (AFT) method [23], a parametric survival model was chosen to evaluate the impact of each of the predictor parameters on time to detection. The inclusive type of AFT model is as follows (equation No. 1):

$$\log(t)=(\alpha+\beta_1 x_{1i}+...+\beta_m x_{mi})+\log(\tau) \tag{1}$$

Where log (t) is stands for the Napierian logarithm of the duration of time to growth inhibition, α is an intercept term, $\beta_1 x_{1i} + ... + \beta_m x_{mi}$ is a linear regression of the m predictor variables and their constant coefficients, and $\log(\tau)$ is an error term. The AFT coefficients indicate the predicted alteration in log (t) for variations in the explanatory levels. In this survey, the Weibull, exponential, log-logistic, and log-normal distributions that could be elucidated in the AFT metric were estimated. To assess the degree of fitness of nominated distribution to the present findings, the mean square error (MSE) was calculated and matched up based on equation No. 2. The lower MSE value represents a more suitable match.

$$\text{MSE} = \frac{\sum(\text{Predicted}-\text{Observed})^2}{(n-p)} \tag{2}$$

Where n is the number of parameters to be observed, and p is the number of variables to be predicted.

To choose those predictor variables that best interpreted time to detection, a backward stepwise technique was utilized. Predictor variables that were not statistically significant were eliminated from the model one at a time, starting with the lowest significant, until the predicted regression coefficients for all remaining variables were significant at an alpha level of < 0.05.

Result
Determination of EO Constituents
The ingredients of *C. copticum* EO, along with their retention time and percentages, were summarized in Table 1. The GC-MS assay had quantified sixteen (16) compounds constituting 98.88% of pure oil. The major components were identified as thymol (57.18%), ρ-cymene (22.55%), γ-terpinene (13.07%), and trans-anethole (1.7%) (Table 1).

Table 1. Essential oil composition of *C. copticum* identified by GC-MS.

No.	Phytochemical	Percent	Retention index (RI)
1	*α-Pinene*	0.29	11.35
2	*ß-Pinene*	0.43	13.45
3	*ß-Myrcene*	0.34	14.28
4	*α-Phellandrene*	0.065	14.89
5	*α-Terpinen*	0.311	15.54
6	ρ-Cymene	22.55	16.21
7	β-Phellandrene	0.541	16.29
8	*γ-Terpinene*	13.07	17.93
9	*α-Terpinolene*	0.095	19.18
10	*α-Terpineol*	0.155	24.92
11	L-Carvone	0.908	27.97
12	*trans* -anethole	1.7	28.68
13	Thymol	57.18	29.73
14	Carvacrol	0.524	29.84
15	3-Dodecen-1-al	0.161	36.51
16	Apiol	0.566	42.73
	Total identified	98.886	

MIC Analysis
In vitro antibacterial properties of *C. copticum* EO was evaluated by standard tube dilution approach versus *S. aureus*. The antibacterial property was described as MIC value. In the present survey, the MIC value of the EO was 0.625 µl ml^{-1}. Thus, four distinct values of EO below MIC were chosen as analyte amounts.

Table 2. Accelerated failure time model of factors influencing time to detection of bacterial growth.

Variables	β (SE)	P-value	TTD ratio (95% CI)
Intercept	-0.536 (0.056)	< 0.01	
Essential oil (%):			
0	0		1
0.015	1.096 (0.052)	< 0.01	2.99 (2.70-3.31)
0.030	1.984 (0.061)	< 0.01	7.27 (6.45-8.19)
0.045	2.994 (0.071)	< 0.01	19.98 (17.38-22.97)
pH:			
7	0		1
6	0.367 (0.050)	< 0.01	1.44 (1.30-1.59)
5	0.885 (0.042)	< 0.01	2.42 (2.17-2.70)
Inoculum level (CFU/ml):			
10^5	0		1
10^3	0.305(0.048)	< 0.01	1.35 (1.24-1.47)
Temperature (℃):			
35	0		1
25	0.896 (0.044)	< 0.01	2.45 (2.24-2.67)
P	4.541 (0.327)		
1/p	0.220 (.015)		

β: Regression coefficient; SE: Standard error; CI: Confidence interval; TTD: Time to detection.

Definition of Growth/No Growth

Close to 81.25% of conditions (117 out of 144) displayed turbidity throughout the entire survey, and 18.75% of conditions (27 out of 144) did not show turbidity, so regarded as censored observations. According to the MSE value, the Weibull model represented the most suitable match to the obtained findings. The lower the value of the MSE, the better the fit. The MSE value of the Weibull model was 116.44. The MSE values were 139.98, 125.98, and 647.56 for log-normal, log-logistic, and exponential models, respectively.

Time to Detection (TTD) Assessment

The median time to detection of bacterial growth was 6 days. Kaplan-Meier survival curve for predictor variables is indicated in figures 1-4. The ultimate model displayed that all predictor variables had significant correlation ($P < 0.01$) with time to detection (Table 2).
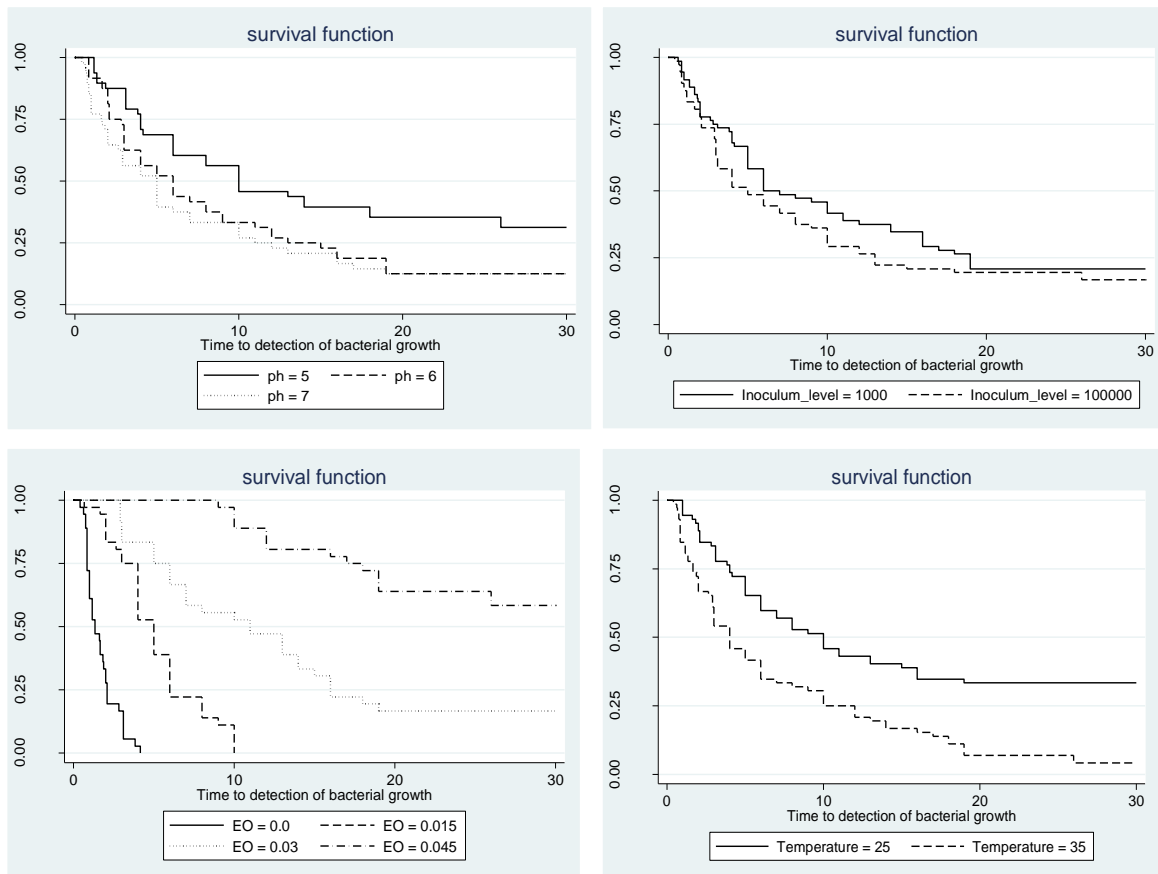

Figure 1. Kaplan-meier survival curves displaying the proportion of no growth combinations for various levels of pH, inoculum size, essential oil, and temperature.

Taken together, TTD for treatment conditions that contain 0.015%, 0.03%, and 0.045% of *C. copticum* EO was 2.99, 7.27, and 19.98 times higher than those without it, respectively. Moreover, TTD for those treatment conditions with pH values of 6 and 5 was 1.44 and 2.42 times higher than those with 7, respectively. In addition, this period for treatment conditions with an inoculum level of 10^3 was 1.35 times higher than combinations with an inoculum size of 10^5. Moreover, this period for conditions with incubator temperature of 25℃ was 2.45 times higher than combinations with incubator temperature of 35℃. The final model equation No. 3 is as below: (3)

$$TTD = \left[-\ln 0.5\right]^{0.22} \times e^{-0.54 + 0.89T25 + 0.31IL1000 + 1.09EO0.015 + 1.98EO0.03 + 2.99EO0.045 + 0.88PH5 + 0.36PH6}$$

Where "TTD" is time to detection, "ln" is the natural logarithm (logarithm to the base e), "e" is a constant coefficient roughly identical to 2.718281828, "T" is temperature," IL" is inoculum size, and" EO" is essential oil. The model well estimates the value of TTD, which described the growth behaviors of *S. aureus* as environmental conditions were modified. Figure 2 shows the relationship between the observed and predicted values by the Weibull model for time to detection (TTD) of bacterial growth in designed combinations.
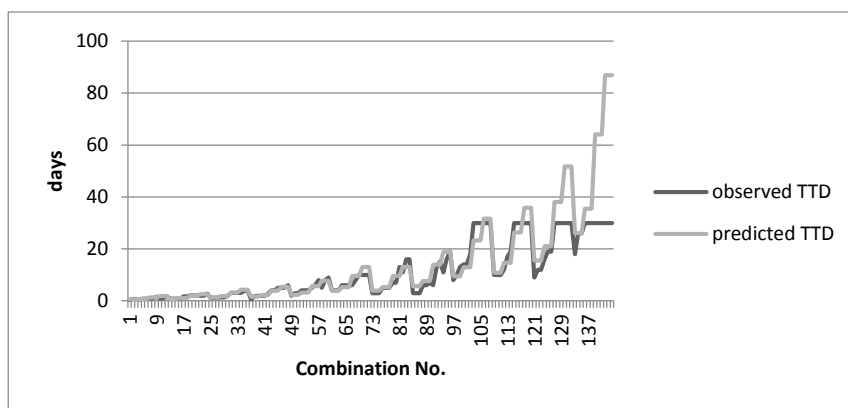

Figure 2. Observed and predicted days needed for growth onset of *S. aureus* (TTD) according to the weibull model.

Discussion

A key point for controlling *S. aureus* and other foodborne pathogens is identifying the parameters which affect their growth and manipulating those parameters to limit their potential risks [24]. With respect, multifactorial combinations have been designed to stop microbial growth, particularly *S. aureus* [6, 7, 16, 24], which was the aim of the multiple hurdle effect proposed by Leistner *et al*. [25]. After that, the concept of predictive microbiology came into existence to combine the mathematical modeling with the experimental data within a matrix of conditions that influence the growth responses of foodborne pathogens to predict the growth fate of micro-organisms [1, 6, 26, 27]. Recently, predictive modelling has been significantly expanded. However, the number of works arranged for modelling the effects of EOs, is scarce [16, 18, 26, 28]. So, the purpose of this work was to estimate the growth behaviors of *S. aureus* in BHI broth while affected by *C. copticum* EO, pH, temperature, and inoculum levels. Based on the concept of TTD in predictive microbiology, the Weibull model was nominated to estimate the growth onset of *S. aureus* in other situations. Our findings disclosed that in the ultimate model, all predicted variables had a significant correlation with TTD. Respecting the impacts of the predictor variables on *S. aureus* growth, the lower the values of temperature, pH, and inoculums, but higher levels of the EO would lead to the higher values of TTD. Similar data have been obtained by other scientists [7, 11, 16, 18]. Moreover, the evidence provided by another scientist [19] showed that elevated temperature would enhance TTD while increasing the pH and inoculum size would decrease TTD.

C. copticum EO is a potentially valuable source of phenolic compounds, which possess high levels of antimicrobial activity [18]. These phenolic components are naturally hydrophobic, hence quickly diffusing into the cell and induce destabilization and destruction of the cell wall, then lead to leakage of vital intracellular components, and eventually end it up with inactivation of enzyme mechanisms [29, 30]. There is remarkable attention to the potential application of these compounds as food additives to postpone and, or inhibit the growth of food spoilage. Among which *S. aureus* is of great significance [16]. In this research, the principal component was thymol (57.18%) and the second dominant constituent was p-cymene (22.55%). In accordance with our findings, a recent study [31] also indicated thymol (48.4%), p-cymene (21.8%) as the major components. Whereas another survey [30] reported γ-terpinene (39.8%), thymol (34.1%), and p-cymene (24.8 %) as the major compounds. Moreover, in another similar research [14], the major components were detected to be carvacrol (1%)

and p-cymene (23%). These differences may be attributed to the various geographic regions, climatic conditions, cultivation time, and genetic variations [30, 32]. The current research illustrated that the antimicrobial activities of plant EO are related to concentration, which is in good agreement with another study [31]. Well coped with our data by elevating the content of EO, the growth onset of *S. aureus*, and the number of no growth combinations raised [16]. Scientists [33] have demonstrated that the application of EOs along with other inhibitory methods like low temperature or low pH could arise synergistic effects to the current method.

Another inhibitory parameter was pH, which could significantly influence the growth onset and metabolism of microorganisms [16]. It was observed that *S. aureus* could grow at pH = 4.5, which defined the capability of *S. aureus* to cultivate at low pH [6]. Although, its growth onset at low pH can be affected by other parameters like the type of acidulants [1, 7]. It was disclosed that in acidic situations, *S. aureus* modulates its gene expression to improve defense mechanisms against acidity, which would result in growth retardation [29]. Apart from the direct impact of pH on the activity and stability of macromolecules, the hydrophobicity of EO is also higher at lower pH, which promotes their dissolution in the bilayer lipid membrane [18, 29]. Similar to our findings, the growth decline of *S. aureus* at lower pH was also demonstrated by other researchers [11, 16, 24].

Temperature is another most relevant hurdle applied to control microbial growth. *S. aureus* is a mesophilic bacterium that grows within a temperature range (7 - 48.5°C) depending on other environmental conditions, with an optimum of 30 - 37°C. Based on our findings, increasing the storage temperature positively affects the rate of proliferation. In the similar conditions, the time to detection of the bacterial growth for conditions with a storage temperature of 25 ˚C was 2.45 times higher than those of 35 ˚C. In keeping with the above observation, various researchers have shown that the growth rate of *S. aureus* at refrigeration temperatures (< 8 °C) was inhibited. And the slowest growth was noticed to be at 7°C. While, the most accelerated growth was discovered to be at 43°C [5, 7, 28]. Furthermore, it has been proven that low temperature, in combination with low pH has been more effective against *S.*

aureus [7]. To sum up, temperature mainly affects the shape of the intracellular enzymes required for metabolism hence lower temperature would lead to lower metabolic activity [1, 16].

Another significant hurdle on the growth onset of the microbial population was inoculum size, which was well documented by different scientists [11, 16]. Our findings also revealed the extension of TTD for combinations with lower inoculum levels. Moreover, it has been indicated that greater inoculum size led to growth onset at the lower pH. By elevating the inoculum size, the possibility of detecting cells in the appropriate physiological condition to initiate growth increases hence the intensity of other inhibitory parameters should be increased [11].

Conclusion
The results disclosed that the obtained TTD of bacterial growth and the value estimated by the Weibull model equation was appropriately estimated the growth onset and prevention situations of *S. aureus* as affected by EO, pH, temperature, and inoculum levels. This sort of studies can supply food manufacturers with reliable methods to predict the growth boundaries of *S. aureus*, prevent enterotoxin production along with the extension of their shelf-life, and hence contribute to the safety of relevant foods.

Acknowledgments

Conflict of Interests
The authors declare no conflict of interest.

References
1. Jamshidi A, Kazerani HR, Seifi HA, Moghaddas E. Growth limits of Staphylococcus aureus as a function of temperature, acetic acid, NaCl concentration, and inoculum level. Iranian J Vet Res 2008;9(4):353-9.
2. Jomehpour N, Eslami G, Khalili MB. The effect of Ferula assa-foetida L and Carum copticum hydroalcoholic extract on the expression levels of Staphylococcus aureus genes involved in quorum sensing. Jundishapur J microbio 2016;9(10).
3. Soltaninezhad B, Khanzadi S, Hashemi M, Azizzadeh M. Inhibition of staphylococcus aureus in hamburger using chitosan film containing the nanoemulsion of

trachyspermum ammi and bunium persicum essential oils. J Nutr Fast health 2020;8(4):231-7.

4. Cao H, Wang T, Yuan M, Yu J, Xu F. Growth and modeling of staphylococcus aureus in flour products under isothermal and nonisothermal conditions. J food Prot. 2017;80(3):523-31.

5. MedVeďoVá A, VAlík Ľ, StudenIčoVá A. The effect of temperature and water activity on the growth of Staphylococcus aureus. Czech J Food Sci. 2010;27(Special Issue 2):28-35.

6. Sutherland J, Bayliss A, Roberts T. Predictive modelling of growth of Staphylococcus aureus: the effects of temperature, pH and sodium chloride. Inter J Food Microbiol. 1994;21(3):217-36.

7. Valero A, Pérez-Rodríguez F, Carrasco E, Fuentes-Alventosa JM, García-Gimeno R, Zurera G. Modelling the growth boundaries of staphylococcus aureus: effect of temperature, pH and water activity. J Inter J Food Microbio. 2009;133(1-2):186-94.

8. Grădinaru A, Trifan A, Şpac A, Brebu M, Miron A, Aprotosoaie A. Antibacterial activity of traditional spices against lower respiratory tract pathogens: combinatorial effects of Trachyspermum ammi essential oil with conventional antibiotics. J Let Applied Microbiol. 2018;67(5):449-57.

9. Hassan W, Gul S, Rehman S, Noreen H, Shah Z, Mohammadzai I, et al. Chemical composition, essential oil characterization and antimicrobial activity of Carum copticum. J Vitam Miner 2016;5(139):2376.

10. Rabiey S, Hosseini H, Rezaei M. Use Carum copticum essential oil for controlling the Listeria monocytogenes growth in fish model system. J Brazilian J Microbio. 2014;45:89-96.

11. Shakeri G, Jamshidi A, Khanzadi S, Azizzadeh M. Modeling of Salmonella typhimurium growth under the effects of Carum copticum essential oil, temperature, pH and inoculum size. Vet Res Forum. 2017;8(1):59.

12. Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of Satureja hortensis and Trachyspermum copticum essential oil. Iranian J Microbiol. 2011;3(4):194.

13. Stanković DM. Sensitive voltammetric determination of thymol in essential oil of Carum copticum seeds using boron-doped diamond electrode. J Analyt Biochem. 2015;486:1-4.

14. Kazemi Oskuee R, Behravan J, Ramezani M. Chemical composition, antimicrobial activity and antiviral activity of essential oil of Carum copticum from Iran. J Avicenna J Phytomed. 2011;1(2):83-90.

15. Grădinaru A, Trifan A, Şpac A, Brebu M, Miron A, Aprotosoaie A. Antibacterial activity of traditional spices against lower respiratory tract pathogens: combinatorial effects of Trachyspermum ammi essential oil with conventional antibiotics. J Let Applied Microbiol. 2018;67(5):449-57.

16. Jamshidi A, Khanzadi S, Azizi M, Azizzadeh M, Hashemi M. Modeling the growth of Staphylococcus aureus as affected by black zira (Bunium persicum)

essential oil, temperature, pH and inoculum levels. Vet Res Forum. 2014;5(2):107.

17. Ratkowsky D, Ross T. Modelling the bacterial growth/no growth interface. J Let Applied Microbio. 1995;20(1):29-33.

18. Basti AA, Misaghi A, Khaschabi D, Technology. Growth response and modelling of the effects of Zataria multiflora Boiss. essential oil, pH and temperature on Salmonella typhimurium and Staphylococcus aureus. J LWT-Food Sci. 2007;40(6):973-81.

19. Zhao L, Montville T, Schaffner DW. Time-to-detection, percent-growth-positive and maximum growth rate models for Clostridium botulinum 56A at multiple temperatures. Inter J Food Microbiol. 2002;77(3):187-97.

20. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry: Allured publishing corporation Carol Stream, IL; 2007.

21. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of Syzygium jambolanum seeds. J Ethnopharmacol. 2004;91(1):105-8.

22. Mann C, Markham J. A new method for determining the minimum inhibitory concentration of essential oils. J Applied Microbiol. 1998;84(4):538-44.

23. Kleinbaum DG, Klein M. Survival analysis: Springer; 2010.

24. Giannuzzi L, Contreras E, Zaritzky N. Modeling the aerobic growth and decline of Staphylococcus aureus as affected by pH and potassium sorbate concentration. J Food Prot. 1999;62(4):356-62.

25. Leistner L. Hurdle technology applied to meat products of the shelf stable product and intermediate moisture food types. Properties of water in foods: Springer; 1985; 309-29.

26. Koutsoumanis K, Lambropoulou K, Nychas GE. A predictive model for the non-thermal inactivation of Salmonella enteritidis in a food model system supplemented with a natural antimicrobial. J Inter J Food Microbiol. 1999;49(1-2):63-74.

27. Tienungoon S, Ratkowsky D, McMeekin T, Ross T. Growth limits of Listeria monocytogenes as a function of temperature, pH, NaCl, and lactic acid. J Applied Environ Microbiol. 2000;66(11):4979-87.

28. Tassou C, Koutsoumanis K, Nychas G-J. Inhibition of Salmonella enteritidis and Staphylococcus aureus in nutrient broth by mint essential oil. J Food Res Inter. 2000;33(3-4):273-80.

29. Buldain D, Gortari Castillo L, Marchetti ML, Julca Lozano K, Bandoni A, Mestorino N. Modeling the Growth and Death of Staphylococcus aureus against Melaleuca armillaris Essential Oil at Different pH Conditions. J Antibiot. 2021;10(2):222.

30. Tadele A, Gemeda N, Lemma H, Girma B, Tesfaye B, Debella A, et al. Broad-spectrum antimicrobials from the essential oil of Trachyspermum ammi. Ethiopian J Public Health Nutr. 2020;2(2).

31. Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MR. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. J Food Chem. 2010;120(3):765-70.

32. Raja S, Ashraf M, Anjum A, Javeed A, Ijaz T, Attiq A. Antibacterial activity of essential oils extracted from medicinal plants against multi-drug resistant Staphylococcus aureus. J Ani Plant Sci. 2016;26:415-23.

33. Mohammadzadeh A. In vitro antibacterial activity of essential oil and ethanolic extract of Ajowan (Carum copticum) against some food-borne pathogens. J Global Pharma Technol. 2017;9(4):20-5.