Heat resistance of *Escherichia coli* O104:H4 in ground chicken as affected by pomegranate powder (70% ellagic acid)

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Abstract

The objective of this study was to assess the effect of commercial pomegranate on the heat resistance of *Escherichia coli* O104:H4 in ground chicken. A full 24 factorial design was used, consisting of temperature treatment with four levels (55.0, 57.5, 60.0, and 62.5°C) and pomegranate with four levels (0.0, 1.0, 2.0, and 3.0 wt/wt % containing 70% ellagic acid). The three-parameter Weibull model demonstrated that the phenolic compound causes *E. coli* O104:H4 cells to become more susceptible to heat ($p<0.01$). It was estimated that the 5.0-log reduction time would reach a minimum at a pomegranate powder concentration of 2.5%, producing a 50% decrease in lethality time, in comparison to that when no pomegranate powder is added. However, adding more than 1.0% pomegranate powder resulted only in a marginal decrease in thermal resistance. **Keywords:** Weibull, *E. coli*, minced chicken, predictive microbiology.

1. Introduction

Pagliarulo et al. (2016) reported that both pomegranate aril and peel extracts containing multiple bioactive molecules (anthocyanins, catechins, tannins, gallic, and ellagic acids) inhibited the bacterial growth of two clinical isolates of *E. coli* and *Staphylococcus aureus* that were involved in foodborne illness. Moreover, pomegranate juice and polyphenolic extracts also exhibited antiviral properties against medical and foodborne viruses (Su et al., 2011). The objective of this study was to model the effect of pomegranate powder on the thermal inactivation of *E. coli* O104:H4 in ground chicken.

2. Methodology

2.1 Inoculation of samples and experimental design

Raw ground chicken (100 g) was mixed thoroughly with pomegranate powder (0.0 to 3.0%; w/w) of 70% ellagic acid using a kitchen mixer, and then, 3 g portions were weighed into filter stomacher bags and vacuum sealed. These bags were frozen and irradiated (25 kGy; Lockheed Georgia Co., Marietta, GA, USA) to eliminate background microflora. A cocktail of three-strain mixture of *E. coli* O104:H4

(0.1 ml) was added to completely thawed ground chicken (3 g) in bags to obtain a final concentration of cells of ~9 log CFU/g. The inoculated meat was pummeled with a Mini Mix stomacher for 2 min to ensure homogeneous distribution of the organisms. A complete factorial design was employed to assess the effects of pomegranate concentration (0.0, 1.0, 2.0, 3.0%) at different heating temperatures (55.0, 57.5, 60, 62.5 °C). Combinations were repeated twice. Thermal inactivation studies were conducted in a temperature-controlled water bath. The time of heat treatments began immediately after samples were submerged in the water bath. Bags for each replicate were removed at each sampling time and instantaneously plunged in an ice-water bath. To determine the number of surviving CFU per gram, 3 ml of sterile 0.1% peptone was added to each meat sample to obtain a 1:1 (wt.vol) slurry and pummeled for 2 min with a Bag Mixer 100 MiniMix (Interscience, St. Nom, France). Decimal serial dilutions prepared in 0.1% peptone water were surface plated onto duplicate dishes containing TSA-YP (tryptic soy agar with added 0.6% yeast extract and 1% pyruvate). The plates were incubated for 48 h at 30°C before surviving *E. coli* O104:H4 cells were counted.

2.2 Predictive microbiology modelling

An omnibus mixed-effects model based on the three-parameter Weibull model was fitted to the microbial data. The log CFU concentration taken at the time *k* in the food sample *i* exposed at the environmental condition *j* (*j*=1, … 16) was then estimated as,

$$\log N_{ijk} = \log N_0 - \frac{1}{2.303}\left(\frac{t}{\chi_j}\right)^{\beta_j} + \varepsilon_{ijk}$$

$$Ln\ \chi_j = a_1 + a_2 Temp + a_3 Pmg + a_4 Temp \times Pmg + u_j$$

$$Ln\ \beta_j = b_1 + b_2 Temp + b_3 Pmg + b_4 Temp \times Pmg + v_j \qquad (1)$$

where the base 10 logarithm of the microbial concentration (CFU/g) at the time *t* (min) is represented by log *N*. Log N_0 is a a model parameter that represents the initial microbial concentration at time t=0. The scale and shape parameters of the underlying Weibull distribution are χ and β, respectively. The model assumed that χ and β could be expressed as a linear function of the environmental variables: temperature (*Temp*), pomegranate concentration (*Pmg*) and their interaction (*Temp*×*Pmg*). The random-effects terms *u* and *v* were added to the mean of the intercepts a_1 and b_1 of the polynomial expressions predicting Ln χ and Ln β, respectively. Thus, the two random effects *u*, *v* were assumed to take in random shifts subject to a given set *j* of experimental conditions. The residuals ε_{ijk} followed a normal distribution with mean zero and variance s^2.

In addition, for each of the survival curves, the lethality time needed to obtain a 5-log relative reduction ($t_{5.0}$) was estimated as,

$$t_{5.0} = \chi(5\ln 10)^{1/\beta} \qquad (2)$$

A multiple regression was conducted to find the best polynomial model that could predict the log-transformed lethality time in terms of temperature and pomegranate powder concentration.

3. Results and discussion

In the present study, *E. coli* O104:H4 was found to follow a non-log linear inactivation displaying concave curves, which suggested that sensitive members of the bacterial population perish rapidly, leaving behind more resistant microorganisms that may adapt to the combined stress of heat and pomegranate's antioxidant activity. For the Weibull's shape parameter (ln β, a parameter related to concavity), when no pomegranate was added, the higher the temperature, the lower the ln β and the greater the concavity of the survival curve (graphs not shown). At the lowest evaluated temperature of 55ºC, the ln χ (or first decimal reduction time) values neatly decreased as the pomegranate concentration increased from 0% to 3%. As a consequence, the addition of pomegranate powder to chicken also decreased the time required to reach a 5.0-log reduction. Notice in Figure 1 (left) that the main decrease in ln $t_{5.0}$ took place between 0% and 1.0% pomegranate powder. Further increments in pomegranate concentration only decrease marginally the 5.0-log lethality times (except for the difference between 2.0% and 3.0% pomegranate concentration at 62.5°C). This behavior becomes more evident in Figure 1 (right) which clearly shows that, across the studied temperatures, pomegranate powder concentrations beyond 1.0% led to similar reductions in ln $t_{5.0}$ values. In order to verify this outcome, a Tukey's comparison of means was run. The test failed to reveal any significant differences in ln $t_{5.0}$ among the treatments with 1.0, 2.0 and 3.0% pomegranate powder. Thus, the addition of 1.0% (or higher) pomegranate powder in ground chicken can attain a mean decrease of ~0.60 log (i.e., a factor of ~0.55) in the time to achieve 5.0-log reduction.

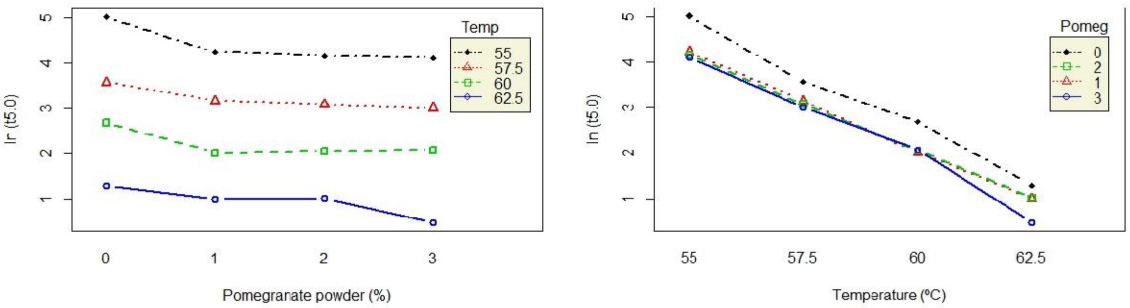

Figure 1: Effects of Temperature (ºC, left) and Pomegranate Powder Concentration (% w/w, right) on the Log-Transformed Time (min) to Achieve a 5.0 Log-Reduction in *E. coli* O104:H4 in Ground Chicken

The parameter estimates for the omnibus mixed-effect model based on the three-parameter Weibull decay function are listed in Table 1. Analysing the p-values, the mild temperatures had a stronger impact on the inactivation kinetics of *E. coli* O104:H4 than pomegranate powder concentration. The negative slopes for temperature and pomegranate as significant predictors of ln χ and ln β indicated that as temperature and pomegranate concentration increase, the counts of *E. coli* O104:H4 decrease. Nonetheless, the fact that the term temperature×pomegranate was significant for both Weibull's model parameters ln χ ($p=0.001$) and ln β ($p=0.003$; Table 1), and had in both cases positive slopes, may suggest that temperature itself has an effect on the antimicrobial properties of pomegranate. The omnibus mixed-effects model consisted of thirteen parameters – nine fixed-effect terms and four variances, and was capable of describing well all the inactivation experimental curves that arose from the combination of environmental factors.

Parameters	Mean	SE	Pr > |t|
Predictors of Ln χ			
a_1 (Intercept)	45.64	3.931	<.0001
a_2 (Temperature)	-0.773	0.067	<.0001
a_3 (Pomegranate)	-6.994	2.077	0.0009
a_4 (Temp×Pomeg)	0.118	0.035	0.0010
Predictors of Ln β			
b_1 (Intercept)	7.414	1.598	<.0001
b_2 (Temperature)	-0.127	0.027	<.0001
b_3 (Pomegranate)	-2.519	0.849	0.0033
b_4 (Temp×Pomeg)	0.044	0.014	0.0028
Log N_0	9.203	0.066	<.0001
s^2 (residuals)	0.211		

Table 1: Parameter Estimates of the Mixed-Effects Omnibus Model Predicting the Non-Loglinear Decline of *E. coli* O104:H4 in Ground Chicken as a Function of Temperature (ºC) and Pomegranate Powder Concentration (% w/w)

4. Conclusion

The positive effect of pomegranate concentration on both the shape (β) and the scale (γ) factors demonstrated that their phenolic compounds (70% ellagic acid) cause *E. coli* O104:H4 cells to become more susceptible to heat, increasing the steepness and concavity of the isothermal survival curves, so that a target inactivation level can be achieved in shorter time. It was estimated that adding 1.0% pomegranate powder to ground chicken decreases the 5.0-log reduction time by half, in comparison to when no pomegranate powder is added in the formulation. Nonetheless, adding pomegranate powder to ground chicken in concentrations higher than 1.0% (w/w) results only in a marginal decrease in thermal resistance at a constant heating temperature, as measured by the 5.0-log lethality time.

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