Heat Resistance of *Escherichia coli* O104:H4 in Ground Chicken as Affected by Pomegranate Powder (70% Ellagic Acid)

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Introduction

Pomegranate aril and peel extracts containing multiple bioactive molecules (anthocyanins, catechins, tannins, gallic, and ellagic acids) have been proven to inhibit the bacterial growth of two clinical isolates of *Escherichia coli* and *Staphylococcus aureus* that were involved in foodborne illness (Pagliarulo et al., 2016). **Thus, 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.**

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.

-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.

-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). The time of heat treatments began immediately after samples were submerged in the water bath.

-Bags were removed at each sampling time and instantaneously plunged in an ice-water bath and surviving E. coli O104:H4 cells were quantified using TSA-YP (tryptic soy agar with added 0.6% yeast extract and 1% pyruvate).

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 (**log N_{ijk}**) taken at the time **k** in the food sample **i** exposed at the environmental condition **j** (j=1, … 16) was then estimated as,

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

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

$$Ln\ \beta_j = b_1 + b_2 Temp + b_3 Pmg + b_4 Temp \times Pmg + v_j$$

Log N_0: initial microbial concentration at time t=0 (log CFU/g)

χ and **β**: Scale and shape parameters of the Weibull distribution

Temp: temperature in ºC

Pmg: pomegranate concentration (% w/w)

u and **v**: random effects added to the mean of the intercepts a_1 and b_1 of the polynomial expressions predicting **Ln χ** and **Ln β**, respectively.

e_{ijk}: residuals ~ Normal (0, s^2)

Results

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)

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		

Analysing the p-values of the model's parameter estimates (Table 1), the mild temperatures had a stronger impact on the inactivation kinetics of *E. coli* O104:H4 than the 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), and had in both cases positive slopes, may suggest that temperature itself has an effect on the antimicrobial properties of pomegranate

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. At the lowest 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 ($t_{5.0}$). Notice in Figure 1 (top) 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 behaviour becomes more evident in Figure 1 (bottom) which clearly shows that, across the studied temperatures, pomegranate powder concentrations beyond 1.0% led to similar reductions in ln $t_{5.0}$ values.

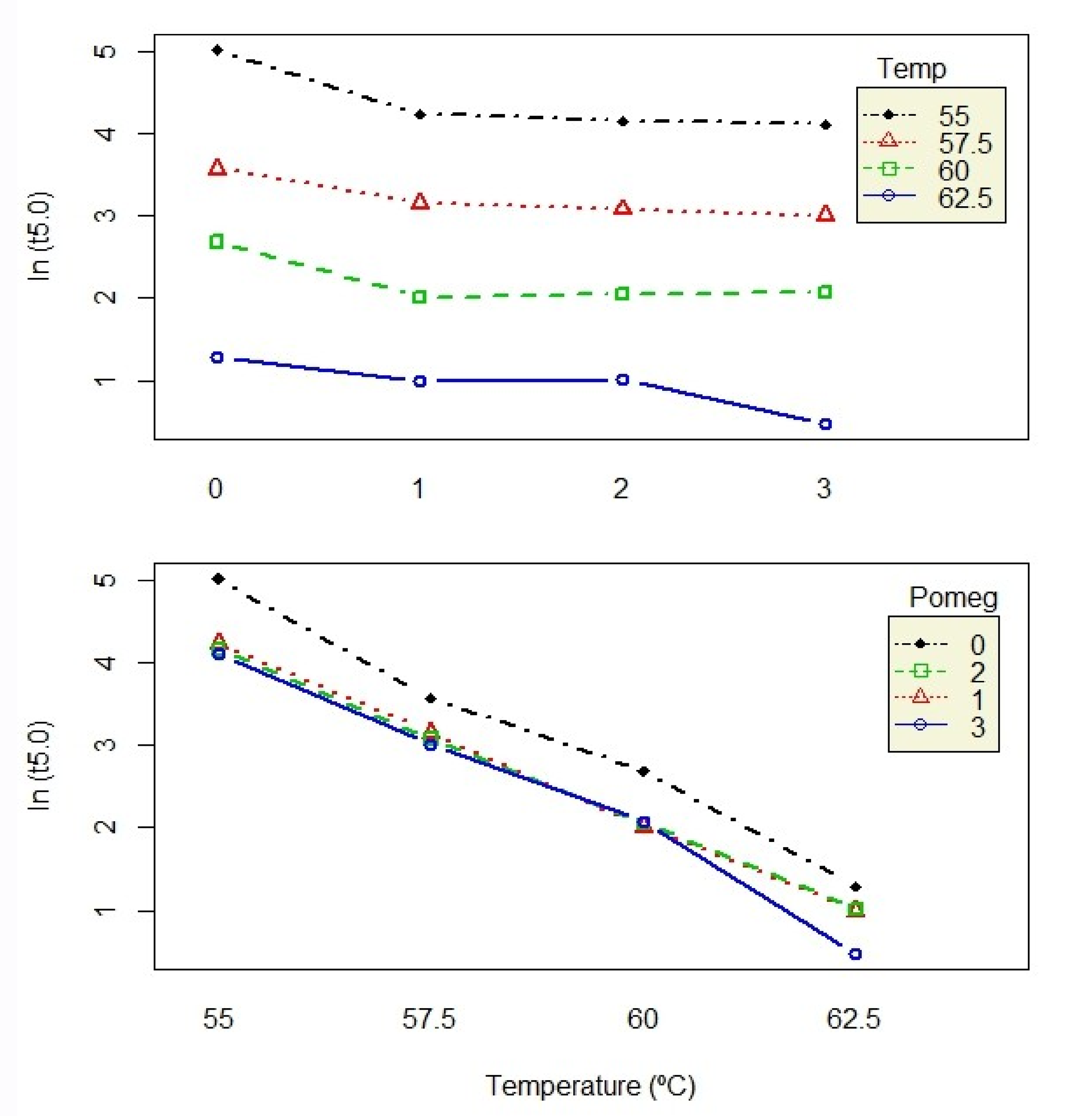

Figure 1: Effects of Temperature (ºC, top) and Pomegranate Powder Concentration (% w/w, bottom) on the Log-Transformed Time (min) to Achieve a 5.0 Log-Reduction in *E. coli* O104:H4 in Ground Chicken

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. The addition of 1.0% 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. 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.

Reference

Pagliarulo, C., De Vito, C., Picariello, G., Colicchio, R., Pastore, G., Salvatore, P., & Volpe, M.G. (2016). Inhibitory effect of pomegranate (*Punica granatum* L.) polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic *Staphylococcus aureus* and *Escherichia coli*. Food Chemistry, 190, 824-831.

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