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Research Note

Modeling the Growth of *Salmonella* in Cut Red Round Tomatoes as a Function of Temperature

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ABSTRACT

Tomato-associated *Salmonella* outbreaks have recently become a significant food safety concern. Temperature abuse of cut tomatoes may have played a role in some of these outbreaks. The purpose of this study was to develop a mathematical model to describe the growth of *Salmonella* on cut tomatoes at various temperatures. Four *Salmonella* serotypes (Typhimurium, Newport, Javiana, and Braenderup) obtained from previous tomato-linked cases of salmonellosis were used in this study. These four serotypes were cultured separately, combined into a cocktail, and inoculated onto whole red round tomatoes and allowed to dry overnight. The tomatoes were then cut into pieces and incubated at a predetermined range of temperatures (10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, and 35°C). *Salmonella* concentration was measured at specified time intervals to determine the growth curve for *Salmonella* on cut tomatoes at each temperature. The growth rates were calculated using DMFit and used to build a mathematical model to illustrate the relationship between the growth rates of *Salmonella* on tomatoes and incubation temperatures from 10 to 35°C. The resulting model compared favorably with a *Salmonella* growth model for raw poultry developed by our laboratory. The Pathogen Modeling Program underpredicted growth at low temperatures and overpredicted growth at high temperatures. ComBase predicted consistently slower growth rates than were observed in tomatoes but showed parallel increases in growth rate with increasing temperature.

Fresh vegetables and fruits are healthy, nutritious foods that are popular around the world. Concerns have arisen in recent years due to cases of salmonellosis linked to such items, including seed sprouts, cantaloupes, watermelon, and apple cider *(9)*. Between April and August of 2008, a very large multistate raw produce outbreak caused by *Salmonella* Saintpaul occurred, which was associated with several different produce items, including tomatoes, jalapeno, and serrano peppers *(6)*.

Between 1990 and 2007, at least 12 *Salmonella* outbreaks clearly linked to tomatoes have occurred in the United States *(5)*. The *Salmonella* serotypes identified in these outbreaks have included Newport, Typhimurium, Braenderup, Javiana, Anatum, and Montevideo. The varieties of tomatoes implicated in these outbreaks included red round, grape, and Roma tomatoes; most were sliced or presliced before consumption *(4)*. In some cases, contamination was traced back to the packinghouse or growing field. Sources of contamination are typically thought to include irrigation water, manure, runoff water passing through adjacent livestock farms, contaminated wash water, animal feces, or handling by workers and may occur at any point along the distribution chain *(9)*. Research has shown that *Salmonella* can enter the tomato plant from the roots or flowers *(4)* or enter the fruit itself through stem scars, small cracks in the skin, or wounds on the plant *(4)*. Asplund and Nurmi *(1)* first reported that *Salmonella* Enteritidis, *Salmonella* Infantis, and *Salmonella* Typhimurium could grow at 22 and 30°C in diced tomatoes. Shi et al. *(14)* found that the growth and persistence of *Salmonella* introduced on and into ripened tomatoes were serovar dependent, which may explain why only some salmonellae serovars are involved in tomato outbreaks. Additionally, *Salmonella* can also grow on the tomato surface, stem scars, and pulp tissues during storage time *(2, 10)*.

Tomatoes are typically cut before serving and eating. During the slicing or dicing process, bacteria on the surface of the tomato can then be transferred via the knife to the interior of the fruit, where water and available nutrients are sufficient to support bacterial growth. The 2007 U.S. Food and Drug Administration (FDA) Federal Food Code has defined cut tomatoes as a ''time/temperature control for safety'' food *(8)*, which means that tomatoes must be refrigerated once they are cut, sliced, or processed in any way *(5)*.

As cut tomatoes appear to play an important role in the *Salmonella* outbreaks, the purpose of this study was to develop a mathematical model capable of predicting the growth rate of *Salmonella* on cut tomatoes as a function of incubation temperature. Since a limited number of published reports *(2, 15)* quantify the growth of *Salmonella* on cut tomatoes at only two temperatures (22 and 30°C), data from

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those reports were not sufficient for creating a mathematical model.

MATERIALS AND METHODS

Sample preparation. Round red tomatoes were purchased from a local New Jersey supermarket, and pH values of macerated tomatoes were determined prior to every experiment. Four strains of *Salmonella* (Typhimurium, Newport, Javiana, and Braenderup) were generously provided by the Centers for Disease Control and Prevention (Atlanta, GA). These cultures were originally isolated from the fecal samples of victims of previous tomato-related outbreaks. The salmonellae were cultured at 37°C overnight in tryptic soy broth (BD Diagnostics, Sparks, MD) media *(3)*. Cultures were mixed in equal proportions to create a strain cocktail, and this mixture was diluted 100 times with 0.1% peptone water (BD Diagnostics) to reach a bacterial concentration of approximately 10^6 CFU/ml. An inoculation procedure was developed to mimic surface contamination transferred to the internal tissue upon cutting. Whole tomatoes were then dip inoculated by immersion in the diluted culture (30 s, ~25°C) and then dried overnight in a biosafety cabinet *(3, 12, 13)* to a target final inoculum level of 10^3 to 10^4 CFU per whole tomato or 10^2 CFU/g of cut tomato. A knife was flame sterilized following immersion in 95% ethanol and then used to slice whole tomatoes into pieces, which were then placed into a sterile plastic bag (sterile stomacher bag, Fisherbrand, Pittsburgh, PA). Gentle massage was applied to the outside of the bag in order to promote the spreading of *Salmonella* homogenously throughout the sample, without destroying the integrity of the fruit pieces. The bags were then transferred to a temperature-controlled water bath for incubation at the desired temperature.

Growth curves. Tomato pieces were incubated at a predetermined range of temperatures (10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, and 35°C) for a variety of time intervals (typically 5 to 12) at each temperature. Experiments were repeated two or three times to obtain suitable growth curves at each temperature. Twenty-five grams of tomato was removed and homogenized in 225 ml of 0.1% peptone water at each sampling time. The sample was then serially diluted with the same buffer and inoculated onto xylose lysine Tergitol 4 (XLT4) plates (BD Diagnostics). A time point zero was taken immediately after the tomatoes were cut and then at the appropriate time intervals during the incubation period. After 18 to 24 h of incubation, the black colonies on the XLT4 plates were counted.

Modeling. Growth curves corresponding to each temperature were constructed with the software DMFit 1.0 (Institute of Food Research, Norwich Research Park, Norwich, UK; http://www.ifr.bbsrc.ac.uk) and an Excel (Microsoft, Redmond, WA) spreadsheet add-in program, and exponential growth rates of *Salmonella* were calculated. Correlation coefficients for individual growth curves were routinely $R^2 > 0.9$. The Ratkowsky or square root model was used to describe *Salmonella* growth rate (log CFU per hour) as a function of temperature as given in the equation below:

$$\sqrt{\text{Growth Rate}} = \beta_1 T + \beta_0$$

where β_1 and β_0 are regression constants and T is the incubation temperature.

Model comparisons. Two computer programs, the Pathogen Modeling Program 7.0 (PMP) and ComBase Predictor from the ComBase Modeling toolbox, were also used to predict the growth of *Salmonella* at conditions mimicking those found in fresh cut tomatoes. The ComBase ''Salmonellae with CO_2'' model was used with CO_2 set to 0% and the physiological state parameter set for no lag time. ComBase allowed predictions at pH (4.27) and water activity (0.995) values similar to those expected for tomatoes *(15)*. PMP allowed the use of a water activity value of 0.995 but only allowed a minimum pH value of 5.6 due to a more limited range over which the PMP was developed. Predicted *Salmonella* growth rates as a function of temperature were extracted from these two models for the pH and water activity values noted above and used to construct square root models in the form shown above for purposes of comparison with our model and data. Finally, in addition to these two widely available models, a model for the growth of *Salmonella* on raw poultry developed in our laboratory *(8)* was also used for comparison.

RESULTS AND DISCUSSION

Growth curves showed starting concentrations of approximately 10^2 CFU/g of tomato, and *Salmonella* concentration increased with incubation time to a final concentration of ~10^7 to 10^8 CFU/g (data not shown). In most cases growth commenced with little or no lag time evident. The lack of a lag time was also repeatedly confirmed by the fitting results from DMFit. This finding is consistent with our observations for the growth of *Salmonella* in raw poultry *(8)* and is similar to the findings of Juneja and Marks *(11),* who reported that *Salmonella* serotypes growing in brain heart infusion have short lag times (1 to 2 h) when cultured at room temperature and above. This is in marked contrast to FDA research which documents lag times of 3 to 4 h for *Salmonella* in cut Roma tomatoes and 5 to 7 h in cut beefsteak tomatoes at 22.2°C *(15)*. It is critical to note that in the FDA experiments the tomatoes were cut and refrigerated prior to inoculation. This key difference, as well as the use of different *Salmonella* strains and tomato cultivar types, may have led to the differences observed. It should also be noted that despite differences in observed lag times, the observed growth rates were similar (see below). Finally, assuming that lag time is short or negligible is a fail-safe assumption, leading to models that are conservative, or which err on the side of safety.

The pH of red round tomatoes used in our experiments ranged from 4.0 to 4.5, with an average pH of 4.3. The two predominant acids found in fresh tomatoes are citric and malic acids. The minimum pH values at which salmonellae can initiate growth in acidified broth with citric acid or malic acid are 4.05 and 4.30, respectively *(7)*. Beuchat and Mann *(2)* recently reported that ripe tomatoes, which have a higher pH than green tomatoes, do not in fact differ in their ability to support the growth of *Salmonella*. These same investigators also observed similar growth rates in cut grape, red round, and Roma tomatoes, despite the fact that grape tomatoes have a higher pH than the other two *(2)*.

Figure 1 summarizes the results of more than 20 growth curves at 10 different temperatures. It is clear from this figure that temperature has a profound effect on the growth rate of *Salmonella* and that the relationship between the square root of the growth rate and increasing temperature is linear. The regression line shown in this figure has an R^2 value of 0.9398. The square root or Ratkowsky equation,

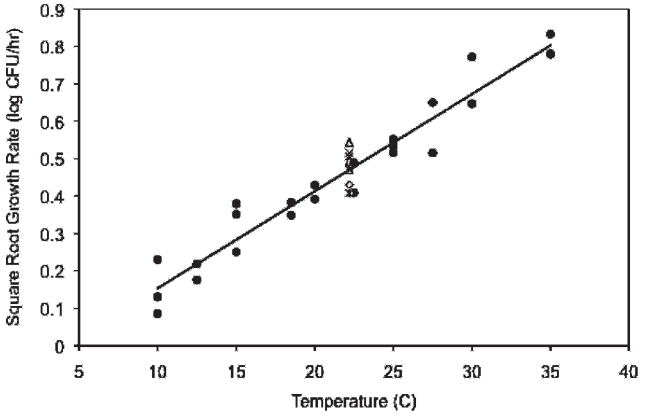

FIGURE 1. *Mathematical model for the growth of* Salmonella *on cut red round tomatoes as a function of temperature,* $\sqrt{Growth\ Rate} = 0.026T - 0.1065$, $R^2 = 0.9398$, *including the data used to create the model (●) and published growth rates of* Salmonella *on cut beefsteak tomatoes (Δ), Roma tomatoes (×), blended beef steak tomatoes (*), and Roma tomatoes (◇).*

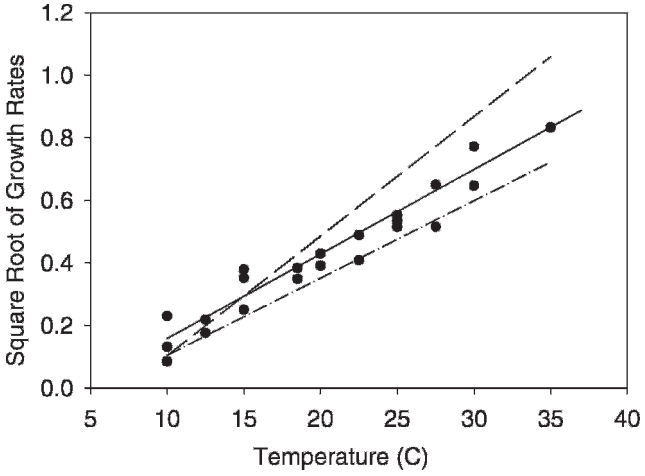

FIGURE 2. *A comparison of the regression models from ComBase (—·—·—), PMP (— —), and a predictive model for raw poultry (——) versus growth rates of* Salmonella *on cut tomatoes (●).*

including the parameters corresponding to the regression line shown in Figure 1, is

$$\sqrt{\text{Growth Rate}} = 0.026T - 0.107$$

where the parameter confidence limits are ± 0.003 on the slope and ± 0.067 on the intercept, and where growth rate has units of log CFU per hour.

Figure 1 also shows the growth rates of *Salmonella* on beef steak and Roma tomatoes, cut or blended, at 22.2°C as obtained by the FDA *(15)* for comparison. These data closely match predictions made by our model.

Figure 2 shows a comparison of the predictive models from the PMP and ComBase for *Salmonella* for pH and water activity conditions as similar to those of tomatoes as the software would allow. The predictive model derived from the PMP was

$$\sqrt{\text{Growth Rate}} = 0.038T - 0.278$$

while the model from ComBase was

$$\sqrt{\text{Growth Rate}} = 0.025T - 0.142$$

Figure 2 also shows the mathematical model for the growth of *Salmonella* on raw poultry recently published by our laboratory *(8),* where this model is

$$\sqrt{\text{Growth Rate}} = 0.027T - 0.112$$

Figure 2 shows a marked difference between the PMP-derived model and all the other models shown in this figure. The PMP-derived model has the greatest slope, with the model rising from the lowest predicted growth rate at 10°C to the highest growth rate at 35°C. This may be due to the requirement to use a pH value of 5.6 (the lowest allowed by the PMP *Salmonella* model), which is significantly higher than the pH of tomatoes (~4.2), due to differences in the strains used to create the model or differences in the statistical method used to create the model. Given the close match between the chicken-based model and the tomato data (see below), this may, however, indicate other sources for the differences in the PMP model predictions.

The model derived from ComBase and the chicken model more closely match the trend seen in the tomato data (Fig. 2). The tomato data show a generally higher growth rate at most temperatures compared to the ComBase model. This may be due to differences in strains used in each case. Since our experiments used strains obtained as human fecal isolates associated with tomato outbreaks, these strains may be more acid resistant and grow faster under the acidic conditions present in tomatoes versus *Salmonella* strains not obtained in this manner. However, the close match between the chicken-based model and the tomato data is somewhat surprising, since the pH of tomatoes is significantly less (~4.2) than that of poultry (~6). This finding suggests that the *Salmonella* strains used in these experiments are not as sensitive to the reduced pH of tomatoes as might be expected, which is also consistent with these strains being responsible for outbreaks in tomatoes.

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