MODELING THE GROWTH OF SALMONELLA SPP. ON CUT TOMATOES

By

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ABSTRACT OF THE THESIS

Modeling the growth of *Salmonella* spp. on cut tomatoes

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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 (*Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Javiana, *Salmonella* 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, 18.5, 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 10-35°C.

The resulting model compared favorably with a *Salmonella* growth model for raw poultry developed by our laboratory. The Pathogen Modeling Program under-predicted growth at low temperatures and over predicted 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.

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1. INTRODUCTION

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 (Guo, Chen, Brackett, & Beuchat, 2002). 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 (Centers for Disease Control and Prevention, 2008b).

Between 1990 and 2007, at least 12 *Salmonella* outbreaks clearly linked to tomatoes have occurred in the United States (Centers for Disease Control and Prevention, 2008a). The *Salmonella* serotypes identified in these outbreaks have included *S*. Newport, *S*. Typhimurium, *S*. Braenderup, *S*. Javiana, *S*. Anatum, and *S*. Montivideo. The varieties of tomatoes implicated in these outbreaks included red round, grape, and roma tomatoes, and most were sliced or pre-sliced before consumption (Centers for Disease Control and Prevention, 2005). 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, handling by workers, animal feces or contaminated wash water and may occur at any point along the distribution chain (Guo et al., 2002). Research has shown that *Salmonella* can enter the tomato plant from the roots or flowers (Centers for Disease Control and Prevention, 2005), or enter the fruit itself through stem scars, small cracks in the skin, or wounds on the plant itself (Centers for Disease Control and Prevention, 2005). Asplund and colleagues (Asplund & Nurmi, 1991) first reported that *Salmonella* Enteritis, *Salmonella*

Infantis and *Salmonella* Typhimurium could grow at 22°C and 30°C in diced tomatoes. Shi et al (Shi, Namvar, Kostrzynska, Hora, & Warriner, 2007) 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 involved in tomato outbreaks. Additionally, *Salmonella* can also grow on the tomato surface, stem scars and pulp tissues during storage time (Iturriaga, Tamplin, & Escartín, 2007; Beuchat, 2008).

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 FDA Federal Food Code has defined cut tomatoes as a "time/temperature control for safety" food, which means that tomatoes must be refrigerated once they are cut, sliced or processed in any way (Centers for Disease Control and Prevention, 2008a).

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 quantify the growth of *Salmonella* on cut tomatoes at only a few temperatures, data from those reports were not sufficient for creating a mathematical model.

2. LITERATURE REVIEW:

2.1 Tomato Production

Tomatoes are one of the most widely grown and consumed fresh produce items in the United States. Besides being a well-known source of lycopene, tomatoes also provide such significant essential nutrients as Vitamins A, B and C, as well as the minerals potassium, iron and calcium. Major producers of processed tomatoes in this country include California, Indiana, Michigan and Ohio. Florida and California lead the way in fresh-market production, followed by Ohio, Tennessee, Virginia and Georgia. Mexico and Canada are our largest trading partners for tomatoes, for both import and export (2006). Tomato cultivars are divided into two types, with commercial growers preferring to raise determinate cultivars which are firm and well adapted to handling, packing and long distance shipment, while indeterminate cultivars are sold in local fresh markets since they are soft for slicing (Rutledge, Wills, & Bost).

Tomato is a warm season crop which is sensitive to frost. The optimum soil temperature for seed germination is 68°F or above, while the optimum production temperature ranges from 70 to 80°F (Rutledge, Wills, & Bost). Tomato quality is reduced when temperatures fall below 68°F during the day and tomatoes are subject to chilling injuries once temperatures reach 50°F or below overnight (Rutledge, Wills, & Bost).

Marketing tomatoes to large, wholesale customers is taken into account from the beginning of production and includes the steps of planning, harvesting, packaging, transportation, distribution, warehousing and pricing (2006). Typical seeding dates are February and March, and occur in a greenhouse or hot-bed either by vacuum seeding into

growing containers or hand seeding into trays (Rutledge, Wills, & Bost). The containers are then covered with paper or glass for the tomatoes to germinate at optimal temperatures. Most tomatoes plants are transplanted from greenhouses to fields as 5- to 6-week old seedlings (2006). Plastic mulch with drip or trickle irrigation is commonly used in tomato fields to promote early germination, reduce weed pressure and to conserve moisture and fertilizer (2006). It is usually better to grow tomatoes on supports such as stakes and trellises (2006), but some fresh market tomatoes are grown as bushes without support and absorb water through furrow irrigation (Rutledge, Wills, & Bost). Soil for cultivating tomatoes should be adjusted to a pH of approximately 6.5 (Rutledge, Wills, & Bost). Weed control by way of chemicals is applied to the fields before or during planting. Sprinkler irrigation can be used to protect plants from frost while pesticides are applied through sprayers. Mature green tomatoes are harvested into polyethylene picking buckets and then dumped into plastic bulk bins. The bins are transported to packinghouses where the fruit is washed and run through a grading machine, separated according to size and color, and specimens with defects are removed (Rutledge, Wills, & Bost;2006). Some fresh market tomatoes can be harvested at the pink fruit stage and subsequently sold at retail stores as vine-ripe (Strange, Schrader, & Hartz, 2000). Tomato wash water temperature should be 10°F to prevent water and microorganism uptake (Rutledge, Wills, & Bost). Chlorine can be added to the wash water to reduce the possibility of decay during the packing and shipping processes (2006). Containers used to pack tomatoes must provide good ventilation and not be under- or over-filled, which could cause bruising and short weights (2006). Pre-cooling tomatoes to 50°F is critical to maintaining tomato quality during long distance shipping. Transporting tomatoes with

ethylene-sensitive commodities such as cucumbers, peppers or lettuce should be avoided because the ethylene released by tomatoes can reduce the quality of sensitive items (2006). Fresh-market tomatoes are sold to local grocery stores, farmers' markets, independent buyers and roadside markets at variable prices mostly affected by weather and acreage changes (Strange, Schrader, & Hartz, 2000; 2006).

Once tomatoes are contaminated with foodborne pathogens, it is difficult to eliminate these organisms. The key points of potential contamination in the supply chain include transportation and storage, repacking points, foodservice operations, retail stores and consumer handling (North American Tomato Trade Work Group, 2006). Good agricultural practices should be in place to (a) help ensure the quality of water that comes in contact with fresh produce, (b) minimize risk from manure and municipal biosolids (c) minimize animal fecal contamination, (d) insure worker health and hygiene, (e) emphasize field and harvest sanitation, (f) as well as sanitation in the packing facilities, especially as pertains to post-harvest water used during packing (North American Tomato Trade Work Group, 2006). Food safety programs for fields, greenhouses and packing facilities include field audits, packinghouse audits and accurate recordkeeping (North American Tomato Trade Work Group, 2006). The prevention of microbial contamination should be a primary concern from production to distribution at all levels.

2.2 *Salmonella* On Tomatoes

Salmonella are Gram-negative, rod-shaped bacteria recognized as one of the major food borne pathogens in the United States. The vehicles have historically been associated with *Salmonella* are poultry, meat, pork, eggs and milk (Bryan, 1981). In recent years, a number of multi-state *Salmonella* outbreaks have been linked to fruits and vegetables

such as tomatoes, seed sprouts, cantaloupes, watermelons, unpasteurized orange juice and apple cider (Iturriaga, Escartin, Beuchat, & MartinezPeniche, 2003; Golden, Rhodehamel, & Kautter, 1993).

Although tomatoes have a high water activity (0.99), their low pH (3.5~4.5) was thought by food microbiologists to constitute an unfavorable environment for *Salmonella* growth. However, the growth of *Salmonella* in tomatoes was first reported by Asplund and colleagues in 1991, who studied *S. enteritidis, S. infantis* and *S.* Typhimurium growth in tomatoes at 7, 22 and 30˚C (Asplund & Nurmi, 1991). Their results demonstrated a possible risk posed by *Salmonella* growth on cut tomatoes held at abuse temperatures. Since the first reported case in 1990, there have been at least 12 reported *Salmonella* outbreaks associated with tomatoes.

There are many published articles describing such topics as the behavior of Salmonella in and on tomatoes, the impact of different holding conditions on Salmonella survival and growth, the impact of environmental factors on Salmonella persistence and growth on tomatoes, internalization of Salmonella from the tomato surface, and inactivation of Salmonella by sanitation methods. Past tomato-linked outbreaks have demonstrated that Salmonella can grow on various kinds of tomatoes including red round, cherry, and grape. Salmonella strains involved in these outbreaks include Salmonella Montevideo, Salmonella Anatum, Salmonella Brenderup, Salmonella Javiana, Salmonella Newport, Salmonella Typhimurium and Salmonella Saintpaul (Centers for Disease Control and Prevention, 2008a). Salmonella can survive on tomato skins, stem scars and growth cracks for more than 20 hours, and it can grow rapidly on puncture wounds and tomato slices (Wei et al., 1995). Researchers have also found that survival

and growth depends on inoculum dose, inoculum site and the medium delivering the bacterium. Salmonella Montevideo can survive longer on stem scars and growth cracks than on tomato skins under the same inoculum dose of 104CFU/site (Wei et al., 1995). Salmonella serovars, irrespective of whether they're isolated from tomato-linked salmonellosis outbreaks or from other animals or clinical infections, can survive and grow on both pre- and postharvest tomatoes but the level of survival and growth are serovar dependent (Shi et al., 2007). These same researchers showed that S. Montevideo was found to be most adapted to survival on tomatoes compared to S. Enteritidis, S. Typhimurium, and S. Dublin. Salmonella can also survive on the tomato leaf, but poorly if the leaves are allowed to dry (Rathinasabapathi, 2004).

Environment factors can influence the behavior of Salmonella on tomatoes with respect to attachment, survival and growth. The ripening stages of tomato products and temperature and humidity combinations during storage have significant effects on the attachment of Salmonella on tomatoes (Iturriaga et al., 2003). High humidity and storage temperatures can promote the growth of Salmonella on the tomatoes surface (Iturriaga et al., 2007). It was found that the conditions of modified atmosphere, high inoculum levels, high storage temperatures and high oxygen levels supported the survival of Salmonella better than in air, at low inoculum levels or low storage temperatures(Das, Gürakan, & Bayindirli, 2006). These same researchers noted that stem scars are more protective to the bacteria than other sites on the tomatoes. Guo (2002) noted that when tomatoes were contaminated by contact with soil inoculated with Salmonella, increases or decreases in microbial load were dependent on the initial level of inoculum. Beuchat (2008) found that survival and growth of Salmonella inoculated onto the stem scars and pulp tissues of

round and Roma tomatoes were unaffected by exposure to an acidic environment before inoculation and not influenced by variety and stage of ripeness at the time of inoculation

Internalization of Salmonella into fresh fruits and vegetables has drawn a large amount of attention from the research community, and tomato research is no exception. Populations of Salmonella on dip-inoculated tomatoes were found to be largest in the stem scar tissue compared to other sites and other inoculation methods (Zhuang, Beuchat, & Angulo, 1995). It was observed that the distribution of Salmonella Montevideo in tomato pulp after the tomato was cut from the stem end to the blossom end depended on the inoculum level (Lin & Wei, 1997). Colonies tended to cluster in the stem scar region at lower inoculum levels and spread from the stem scar to the center and bottom of cut tomatoes only when a high inoculum level (>106 CFU/tomato) was used (Lin & Wei, 1997). Upon contact with soil highly contaminated with a 250ml culture of 109CFU/ml of Salmonella, the bacteria appear to infiltrate tomatoes through the stem scar into the pulp (Guo et al., 2002).

Disinfection of tomatoes challenged with Salmonella has been widely reported in the literature. Treatment with 100ppm aqueous chlorine for 2 minutes could not kill all Salmonella present at different sites on tomatoes especially when non-selective media were used (Wei et al., 1995). Tomatoes inoculated with 103 CFU/g of S. baildon were still found to contain >102 CFU/g when the produce was immersed for 40s in 120 or 200 µg/ml free chlorine solution, however the pathogen concentration decreased by at least 2 log CFU/g, after storage for 12 days at 4 °C post treatment (Weissinger, Chantarapanont, & Beuchat, 2000). Lactic acid at 2% was shown to be effective in reducing Salmonella on the surface of tomatoes from 103 CFU/m2 to below the detection limit, regardless of

inoculation methods. This study also found that the tomatoes tested negative for internalized pathogens after treatment (IbarraSanchez, AlvaradoCasillas, RodriguezGarcia, MartinezGonzales, & Castillo, 2004). Tap water was not able to significantly reduce Salmonella concentrations on inoculated tomatoes (IbarraSanchez, AlvaradoCasillas, RodriguezGarcia, MartinezGonzales, & Castillo, 2004; Lang, Harris, & Beuchat, 2004a).

A number of studies have been focused on how to develop a standard method to evaluate the disinfection of sanitizers for fresh tomatoes. The primary factors to consider for the protocol include type of produce, pathogen of interest, procedure for inoculation, procedure to evaluate test conditions and retrieval of pathogens (Beuchat et al., 2001). Research has demonstrated that significantly larger populations of Salmonella were recovered from tomatoes that were dip-inoculated than from those whose surface was spot- or spray-inoculated (Lang et al., 2004a). A cocktail of Salmonella strains applied to a tomato surface at an initial population of 6.6 log10 CFU/tomato was reduced by approximately 1 log10 CFU/tomato after drying for 40 min at 22 °C and by 3 log10 CFU/tomato after 3 to 24 hours (Beuchat, Harris, Ward, & Kajs, 2001). This same study recommended a method in which spot-inoculated tomatoes were sprayed with sanitizer and hand rubbed for 30s before being placed in a plastic bag and rinsed with sterile water by agitating for 30s. A second bag with 20 ml 0.1% peptone water was used to rinse the tomato by rubbing it for another 40s and to recover the remaining bacteria. It was found that 200 ppm of chlorine could achieve more than a 3 log10 reduction by using this method (Beuchat et al., 2001).

2.3 Predictive Food Microbiology

Predictive microbiology is a method used to describe microbial response to the food environment by mathematical models(2004; 2004). Predictive modeling was developed as an alternative to more traditional, time-consuming and expensive studies used to enumerate and identify the characteristics of microbes held under test conditions used to estimate shelf life and assess safety. Modeling has also been shown to be a cost-effective means to interpolate between conditions studied in the laboratory, and thereby estimate the effect of environmental conditions not studied by experiments. The growth rates predicted from relatively simple models for the same conditions of pH, temperature and water activity were shown to be similar (Baranyi, Ross, McMeekin, & Roberts, 1996), which demonstrates the importance of these three factors alone in predicting bacterial growth.

The earliest example of predictive microbiology appeared in 1922, when the thermal death of Clostridium botulinum type A spores was described by Esty and Meyer as a log-linear model which revealed that relative death rate is constant with time at a fixed temperature (McMeekin, Olley, Ratkowsky, & Ross, 2002). In 1973, a fundamental discovery recognized that many spoilage processes shared a “universal” spoilage curve (Olley & Ratkowsky, 1973) from which the relative rate concept became crucial in the application of predictive models (Zwietering, de Wit, & Notermans, 1996).

Through the 1980s and 1990s, kinetic modeling methods dominated the field of predictive microbiology (McMeekin et al., 2002). Predictive microbiology typically investigates microbial characteristics like growth, death, survival, and/or toxin formation which are modeled as a function of temperature, water activity, pH, nitrite concentration,

gaseous atmosphere, etc. (McMeekin et al., 2002). The classical modeling approach consists of two steps: The first step is to establish the growth/death model under a single set of constant conditions, which is also known as a primary model development. The second step is to evaluate the effects of environmental factors on the parameters of the primary model, to produce the secondary model (Baranyi & Roberts, 1995). Sigmoid functions are typically used to create the primary model, while secondary models take the form of polynomials (Gibson, Bratchell, & Roberts, 1988). When the secondary model uses temperature alone, the Ratkowsky or square-root equation has been widely used (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983). While temperatures are held constant during experiments, it can be useful to make predictions under changing temperature conditions, which requires the use of dynamic models (Pin, deFernando, Ordonez, & Baranyi, 2001). It has also been recognized that variability in response time estimates has a Gamma or inverse Gaussian distribution, and its variance is proportionally related to the square or cube of the mean response time (Ratkowsky, Ross, Macario, Dommett, & Kamperman, 1996), which can be useful for microbial risk assessment.

Predictive microbiology can be a useful component of food safety risk assessment. According to the National Academy of Sciences, the methodology of microbial risk assessment is made up of four steps: (1) disease characterization/hazard identification; (2) dose response assessment; (3) exposure assessment; and (4) risk characterization (Walls & Scott, 1997). In order to achieve these assessment steps, it is crucial to figure out the number of microorganisms in a food at the time of consumption, which can be achieved through predictive models. At the same time, the empirical distribution of organisms can

be obtained from published data. Once the risk has been evaluated, a decision can be made on whether to accept the levels of risk and what should be done to lower it.

Predictive food microbiology has made impressive progress in recent decades, and several well-known computer programs for the growth and survival of pathogens are in use today. Food MicroModel was initiated by the UK Ministry of Agriculture Fisheries and Food in 1988 and is now known as the Combined Database of Microbial Responses to Food Environment (ComBase), and it's modeling component is called ComBase Predictor (Baranyi & Tamplin, 2004). At the same time US researchers at the Eastern Regional Research Center of the USDA Agricultural Research Service (USDA-ARS) developed a package of predictive models called the Pathogen Modeling Program (PMP). Both programs can predict the growth and inactivation of specific foodborne pathogens under various environmental conditions based on the environmental conditions used to generate the models.

Collaboration between research in the UK, US and Australia have been united under ComBase which includes the PMP models, the ComBase predictor models as well as a database consists of thousands of microbial growth and survival curves collected from unpublished government and food industry research as well as peer reviewed publications. ComBase is free web-based tool for researchers, the food industry and other interested individuals. Through a web-based interface, various criteria, including bacterial species, food type, pH, temperature, water activity, preservative levels, etc. can be used to analyze specific scenarios. The database is constantly being updated, and many EU and other institutions contribute data on a regular basis (Baranyi & Tamplin, 2004).

3. MATERIALS AND METHODS

3.1 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* (*S.* Typhimurium, *S.* Newport, *S.* Javiana, *S.* Braenderup) were generously provided by the Centers for Disease Control and Prevention (CDC, 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 (TSB, Difco Laboratories, Detroit, MI) media (Beuchat et al., 2001). Cultures were mixed in equal proportions to create a strain cocktail and this mixture was diluted 100 times with 0.1% peptone water (Difco Laboratories, Detroit, MI) to reach a bacterial concentration of approximately 10^6 CFU/ml. Whole tomatoes were then dip inoculated by immersion in the diluted culture and then dried overnight in a biosafety cabinet (Lang et al., 2004a; Beuchat et al., 2001; Lang, Harris, & Beuchat, 2004b) to a target final inoculum level of 10^3-10^4CFU/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 (Fisherbrand, sterile stomacher bag, Pittsburgh, PA). Gentle massage was applied to the outside of the bag in order to promote the spreading of *Salmonella* spp. 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.

3.2 Growth Curves

Tomato pieces were incubated at a predetermined range of temperatures (10, 12.5, 15, 18.5, 20, 22.5, 25, 27.5, 30, and 35°C) for a variety of time intervals at each temperature. Experiments were repeated two or three times to obtain suitable growth curves at each temperature. Twenty-five grams of tomato were removed and homogenized in 225 ml 0.1% peptone water at each sampling time. The sample was then serially diluted with the same buffer and inoculated onto XLT4 plates (Difco Laboratories, Detroit, MI). A time point zero was taken immediately after the tomatoes were cut and then at the appropriate time intervals during the incubation period. After 18-24 hours of incubation, the black colonies on the XLT4 plates were counted.

3.3 Modeling

Growth curves corresponding to each temperature were constructed with the software DMFIT 1.0 (Institute of Food Research, Norwich Research Park, Norwich, United Kingdom; http://www.ifr.bbsrc.ac.uk), an Excel (Microsoft, Remind, WA) spreadsheet add-in program, and exponential growth rates of *Salmonella* were calculated. The Ratkowsky or square root model was used to describe *Salmonella* growth rate as a function of temperature as given in the equation below:

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

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

3.4 Model Comparisons

Two computer programs, the Pathogen Modeling Program 7.0 (PMP) and ComBase Modeling toolbox, were also used to predict the growth of *Salmonella* spp. at conditions mimicking those found in fresh cut tomatoes. ComBase allowed predictions at pH (4.27)

and water activity (0.995) values similar to those expected for tomatoes (US Food and Drug Administration, 2007). 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 lab (Dominguez & Schaffner, 2008) was also used for comparison.

4. RESULTS

4.1 Model Development

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 - 10^8 CFU/g (data not shown). In most cases growth commenced with little or no lag time evident. The pH of red round tomatoes used in our experiments ranged from 4.0 to 4.5, with an average pH of 4.3.

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 square root of the growth rate is linear with increasing temperature. The regression line shown in this figure has an R^2 value of 0.9398. The square root or Ratkowsky equation including the parameters corresponding to the regression line shown in Figure 1 is:

$$\sqrt{Growth\ Rate} = = 0.026T - 0.1065$$

Figure 1 also shows the growth rates of *Salmonella* spp. on beef steak and Roma tomatoes, cut or blended, at 22.2°C as obtained by FDA (US Food and Drug Administration, 2007) for comparison. These data closely match predictions made by our model.

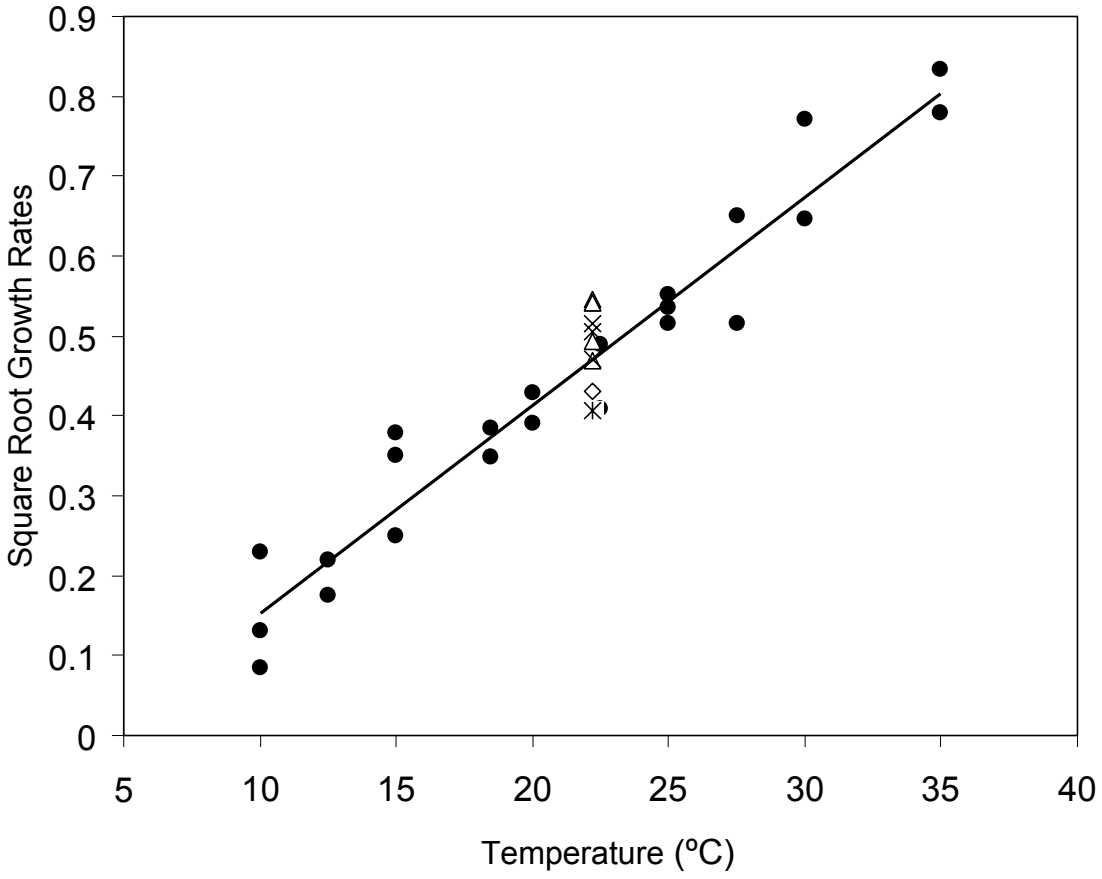

FIGURE 1. Mathematical model for the growth of *Salmonella* spp. on cut red round tomatoes as a function of temperature, $\sqrt{Growth\ Rate} = 0.026\text{T}-0.1065$, $R^2 = 0.9398$, including the data used to create the model (•) and published growth rates of *Salmonella* spp. on cut beefsteak tomatoes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

4.2 Model Comparison

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

$$\sqrt{Growth\ Rate} = 0.0382\text{T}- 0.2783$$

while the model from ComBase was:

$$\sqrt{Growth\ Rate} = 0.0247\text{T} - 0.1423$$

Figure 2 also shows the mathematical model for the growth of *Salmonella* spp. on raw poultry recently published by our lab (Dominguez & Schaffner, 2008), where this model is:

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

A marked difference between the PMP derived model and all the other models is 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. The model derived from ComBase and chicken model more closely match the trend seen in the tomato data (Figure 2). The tomato data show a generally higher growth rate at most temperatures than the ComBase model. The close match between the chicken-based model and the tomato data is somewhat surprising, since poultry has a pH ~ 6, while the pH of tomatoes is significantly less (~4.2).

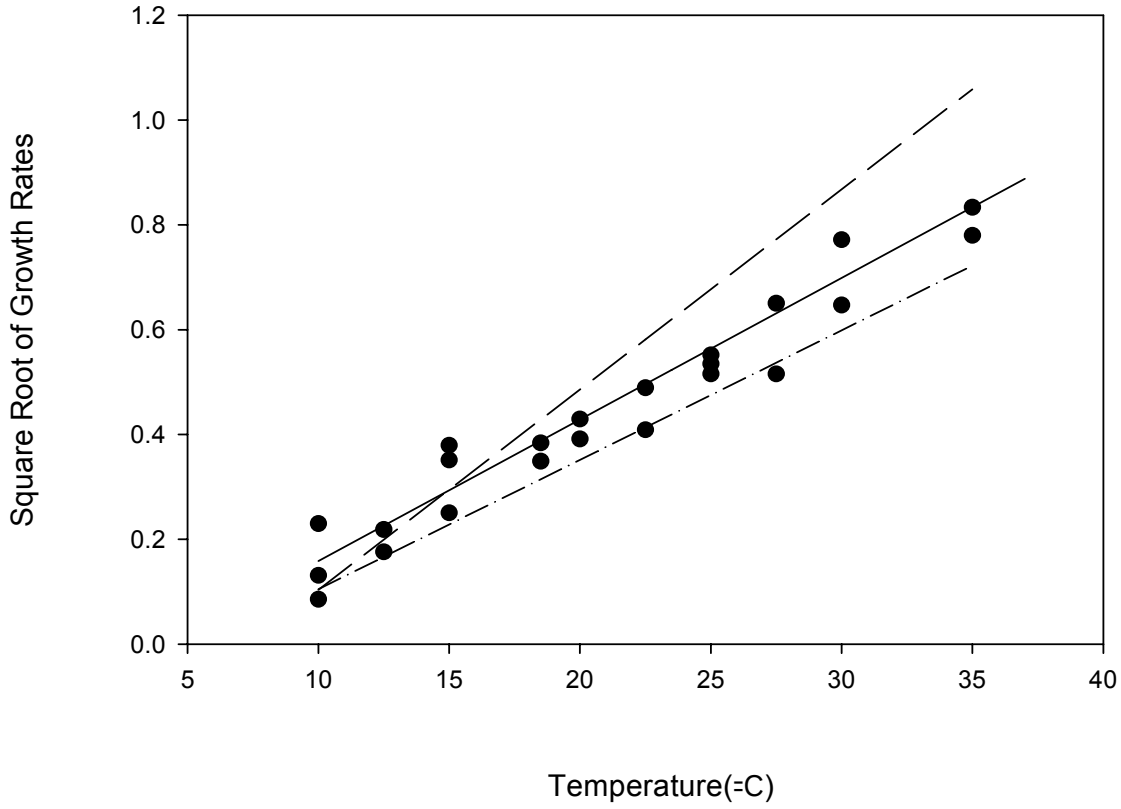

FIGURE 2. A comparison of the regression models from Combase (-.-.-), PMP (---) and a predictive model for raw poultry (-) vs. growth rates of *Salmonella* spp. on cut tomatoes (●).

5. DISCUSSION

The finding of no evident lag time in growth curves is consistent with our observations for the growth of *Salmonella* in raw poultry (Dominguez & Schaffner, 2008) and is similar to the findings of Juneja *et al* who reported that *Salmonella* serotypes growing in brain-heart infusion have short lag times (1-2 h) when cultured at room temperature and above (Juneja & Marks, 2006). This is in marked contrast to FDA research which documents lag times of 3-4 h for *Salmonella* in cut Roma tomatoes and 5-7 h in cut beefsteak tomatoes at 22.2°C (US Food and Drug Administration, 2007). 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 cultivars types may have lead to the differences observed. It should also be noted that despite differences in observed lag times, the observed growth rates were similar. 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 red round tomatoes used in the experiments have an average pH of 4.3. The two predominant acids found in fresh tomatoes are citric and malic acids. The minimum pH at which salmonellae can initiate growth in acidified broth with citric acid or malic acid are 4.05 and 4.30, respectively (Chung & Goepfert, 1970). Beuchat recently reported that ripe tomatoes, which are thought to have a higher pH than green tomatoes, do not in fact differ in their ability to support the growth of *Salmonella*. (Beuchat, 2008) The same study also observed similar growth rates in cut grape tomatoes, red round and Roma tomatoes, despite the fact that when grape tomatoes have a higher pH than the other two (Beuchat, 2008).

The fact that PMP model has the greatest slope 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 (~4.3), and also due to differences in the strains used in the model or differences in the statistical method applied to create the model. The difference between our model and ComBase model may be due to the 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 vs. *Salmonella* strains not obtained in this manner. However, the high similarity between our model and the growth model for *Salmonella* on poultry is surprising because poultry has a pH ~ 6, significantly higher than that of tomatoes. It suggests 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.

6. CONCLUSION

A mathematical model that describes the growth rates of *Salmonella* spp. on cut tomatoes as a function of various temperatures from 10ºC to 35ºC was developed through experimental approaches. When compared with other prediction models, it agreed most closely with a model from our laboratory for *Salmonella* growth in raw chicken. The growth rates were slightly faster than those predicted by ComBase at pH of cut tomatoes (4.3), but slower than those predicted by PMP at higher pH (5.6). Although salmonellosis associated with tomatoes will remain a concern until the ultimate source of Salmonella bacteria can be identified and eliminated, this mathematical model provides a useful tool for estimating the risk posed by cut tomato storage as different temperatures. The model will also be useful in the development of quantitative risk assessment for *Salmonella* in tomatoes.

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