# PREDICTIVE MODEL FOR THE COMBINED EFFECT OF TEMPERATURE, pH, SODIUM CHLORIDE, AND SODIUM PYROPHOSPHATE ON THE HEAT RESISTANCE OF *ESCHERICHIA COLI* O157:H7[1]

VIJAY K. JUNEJA[2], BENNE S. MARMER and BRIAN S. EBLEN

*U.S. Department of Agriculture*
*Agricultural Research Service*
*Eastern Regional Research Center*
*600 East Mermaid Lane*
*Wyndmoor, PA 19038*

Accepted for Publication June 3, 1999

## ABSTRACT

*The effects and interactions of heating temperature (55 - 62.5C), pH (4 - 8), NaCl (0 - 6%, w/v), and sodium pyrophosphate (0 - 0.3%, w/v) on the heat resistance of a four strain mixture of* Escherichia coli *O157:H7 in beef gravy were examined. Thermal death times were determined using a submerged coil heating apparatus. The recovery medium was plate count agar supplemented with 1% sodium pyruvate. Decimal reduction times (D-values) were calculated by fitting a survival model to the data with a curve fitting program. The D-values were analyzed by second order response surface regression for temperature, pH, NaCl and sodium pyrophosphate levels. The four variables interacted to affect the inactivation of the pathogen. Thermal resistance of* E. coli *O157:H7 can be lowered by combining these intrinsic factors. A mathematical model describing the combined effect of temperature, pH, NaCl and sodium pyrophosphate levels on the thermal inactivation of* E. coli *O157:H7 was developed. The model can predict D-values for any combinations of temperature, pH, NaCl and sodium pyrophosphate that are within the range of those tested.*

[1]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]Corresponding author: Telephone: (215) 233-6500; FAX: (215) 233-6406; E-mail: vjuneja@arserrc.gov.

## INTRODUCTION

The use of heat to inactivate foodborne pathogens is a critical control point and the most common means of assuring the microbiological safety of cooked foods. A key to optimization of the heating step is defining the target pathogen's heat resistance. While over-estimating the heat resistance negatively impacts the product quality, under-estimating increases the likelihood that the contaminating pathogen persists after heat treatment or cooking. Inadequate heat treatment or undercooking is one of the most important contributing factors in food poisoning outbreaks (Roberts 1991).

The heat resistance of any given microorganism is known to be affected not only by inherent genetic factors, but also by many environmental factors during heating such as the composition and pH of the heating menstruum (Tomlins and Ordal 1976; Hansen and Riemann 1963). The effectiveness of the individual effects of heat treatment, pH, salt, etc., with regard to pathogen inactivation is maximized by conducting multiple factorial experiments in which the effects and interactions of these parameters in foods are assessed in lowering the heat resistance of foodborne pathogens. Subsequently, inactivation kinetics or thermal death models are developed which enable food processors to design reduced thermal processes for the production of safe food with extended shelf-life without substantially adversely affecting the quality of the product.

*E. coli* O157:H7 is recognized as an important human pathogen of concern that causes variety of clinical manifestations including hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Karmali 1989). This pathogen was first recognized in the U.S. in 1982 when outbreaks of bloody diarrhea in conjunction with slight or no fever occurred in Oregon and Michigan (Riley *et al.* 1983). The organism was isolated from the patients and the meat patties of the implicated lot. Since then, *E. coli* O157:H7 has been implicated with food poisoning caused by consumption of inadequately cooked contaminated beef (Belongia *et al.* 1991; Doyle 1991; Riley 1987), unpasteurized apple juice (McCarthy 1996), lettuce (Anon. 1996), turkey roll (Carter *et al.* 1987; Ryan *et al.* 1986), apple cider (Besser *et al.* 1993), mayonnaise (Weagant *et al.* 1994; Raghubeer *et al.* 1995), raw milk (Borczyk *et al.* 1987), yoghurt (Morgan *et al.* 1993) and unconfirmed food vehicles (Watanabe *et al.* 1996). The heat resistance of the organism has been studied extensively in meat (Ahmed *et al.* 1995; Ahmed *et al.* 1997; Doyle and Schoeni 1984; Kotrola and Conner 1997; Line *et al.* 1991; Jackson *et al.* 1996; Juneja *et al.* 1997 ), milk (D'Aoust *et al.* 1988) and apple juice (Splittstoesser *et al.* 1996). These reports pertaining to the heat resistance of *E coli* O157:H7 provide sufficient evidence that the pathogen does not have an unusually high heat resistance. Since these studies have focused on the effect of temperature alone, Blackburn *et al.* (1997) developed a thermal inactivation model for *E. coli* O157:H7 with temperature, pH and NaCl (salt) as controlling factors. While the study by Blackburn *et al.* (1997) provided some characterization on the heat resistance of the organism, there appears to be a lack of information on the effects of increasing concentrations of sodium pyrophosphate

(SPP) in combination with various salt and pH levels on the heat resistance of the organism. Accordingly, the present study was carried out to quantitatively assess the relative effects and interactions of SPP, NaCl, pH and temperature on the inactivation kinetics of *E. coli* O157:H7. The data were then used to develop a 4-factor thermal death model that could be used by food processors in the design of processing times and temperatures that ensure safety against *E. coli* O157:H7 in cooked food products.

## MATERIALS AND METHODS

### Organisms

The four strains of *E. coli* O157:H7 used throughout this study included 45753-35, 933, A9218-C1 and ent C9490 (Jack-in-the-Box). Strains 45753-35 and 933 are meat and kidney isolates, respectively, and were originally obtained from the Food Safety and Inspection Service, Beltsville, MD. Strains A9218-C1 and ent C9490 are the clinical isolates and were originally obtained from CDC, Atlanta, GA. These strains, maintained as frozen (-70C) stocks in brain heart infusion broth (BHI; Difco) supplemented with 10% glycerol, were obtained from our in-house culture collection. During the course of the study, individual stock cultures were maintained on BHI slants at 4C with monthly transfers to maintain their viability.

### Beef Gravy Formulation

The model beef gravy used in the present study consisted of 1.5% proteose peptone, 5.0% beef extract, 0.5% yeast extract and 1.7% soluble starch. All ingredients were obtained from Difco Laboratories (Detroit, MI). Salt (0.0 - 6.0%, w/v) and/or sodium pyrophosphate (0.0 - 0.3%, w/v) was added in the beef gravy which was again vortexed for 2 min to ensure even distribution. The pH of the gravy was adjusted to 4.0 - 8.0 using 85% (w/w) lactic acid (Sigma) and determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, PA) attached to an Orion model 601A pH meter. The gravy was sterilized by autoclaving prior to use. The pH of the gravy was checked again, following sterilization and equilibration to room temperature, by taking an aliquot. If necessary, pH adjustments to the gravy were made with sterile solutions of the acid under a laminar flow biological safety cabinet.

### Experimental Design

A fractional factorial design was employed to assess the effects and interactions of heating temperature (55, 57.5 60 and 62.5C), salt (0.0. 1.5, 3.0, 4.5, 6.0%, w/v), sodium pyrophosphate (0.0. 0.1, 0.15, 0.2, 0.3%, w/v), and pH (4.0, 5.0, 6.0, 7.0, 8.0). All 40 variable combinations were replicated twice.

### Preparation of Inoculum

To prepare the cell suspensions, a 10 μL loop of stock culture was transferred to 50 mL BHI in 250 mL flasks and incubated at 37C for 24 h. After 2 consecutive transfers using 0.1 mL inocula, final cultures were harvested by centrifugation (5,000 × g, 15 min) at 4C and washed twice in 0.1% peptone water (w/v). The cell pellets were resuspended in 10 mL peptone water. The population density in each inoculum suspension was enumerated by spiral plating (Spiral Biotech, Bethesda, MD; Model D) appropriate dilutions (in 0.1% peptone water), in duplicate, on tryptic soy agar (TSA) plates which were then incubated at 37C for 24 h. Thereafter, 2 mL cultures of each strain were combined in a sterile test tube, mixed throughly, and this cocktail of strains was used for inoculation of beef gravy. Serial dilutions were made in peptone water to obtain the desired cell density before inoculation.

### Beef Gravy Inoculation and Thermal Inactivation

In sterile test tubes, beef gravy (10 mL) containing salt (0.0-6.0%, w/v), sodium pyrophosphate (0.0.-0.3%, w/v) at various pH levels (4.0-8.0) was inoculated with 0.1 mL of the bacterial inoculum to obtain an initial concentration of approximately 7-8 $\log_{10}$ cfu/mL. Inoculated gravy samples were vortexed to ensure even distribution of the organisms. Thermal inactivation was carried out at 55-62.5C using a submerged coil heating apparatus (Cole and Jones 1990). It comprises a stainless steel coil fully submerged in a thermostatically-controlled water bath which allows microbial suspensions to be heated between 20-90C with a short time to temperature equilibrium. During the heating procedure, samples (0.2 mL) were removed at predetermined time intervals. Where low cell numbers were expected 0.6 mL aliquots were removed. Samples were cooled rapidly to room temperature in peptone (0.1% w/v) water.

### Enumeration of Survivors

To determine the number of surviving cfu/mL after heat treatment, the beef gravy was serially diluted in 0.1% peptone water (wt/vol). Thereafter, selected dilutions were surface-plated onto plate count agar (Difco) supplemented with 1% sodium pyruvate using a spiral plater. When necessary, 0.1 mL of the undiluted suspension was also surface plated. Plates were incubated at 30C for 2 - 3 days before counting colony-forming units as the number of survivors. For each replicate experiment, an average surviving cfu of two platings of each sampling point was used to determine the D-values.

### Survivor Curves

Survivor curves were generated by fitting the data to the linear function that allows for the presence of a lag period before initiation of an exponential decline in population density (Buchanan *et al.* 1993; Buchanan *et al.* 1994).

$Y = Y_0$ For $T \leq T_L$
$Y = Y_0 + m(T - T_L)$ For $T \geq T_L$

Where:

$Y$ = $Log_{10}$ count of bacteria at time T. [$Log_{10}$ (CFU/mL)]
$Y_0$ = $Log_{10}$ count of bacteria at time T = 0. [$Log_{10}$ (CFU/mL)]
m = Slope of the survivor curve. [$Log_{10}$ (CFU/mL))/min]
T = Time. (Min)
$T_L$ = Duration of lag period to initiation of inactivation. (min)

The curves were fitted using ABACUS, a nonlinear curve fitting program that employs a Gauss-Newton iteration procedure (Damert 1994). D-values (time to inactivate 90% of the population) were calculated as the negative reciprocal of m.

The z-values were estimated from the absolute value of the inverse slope by computing the linear regression (Ostle and Mensing 1975) of $log_{10}$ D-values versus heating temperatures using Lotus 1-2-3 Software (Lotus Development Corporation, Cambridge, MA).

**Statistical Modeling**

The D-values were transformed to the natural logarithm form and analyzed by second order response surface regression to develop a regression model for temperature, pH, salt and SPP levels.

## RESULTS AND DISCUSSION

The present study assessed the effects and interactions of temperature, pH, salt and sodium pyrophosphate levels to quantify in beef gravy the inactivation of a four strain *E. coli* O157:H7 cocktail. Plate count agar supplemented with 1% sodium pyruvate was used for recovery because prior research, including preliminary studies in our laboratory, indicated that it gave the maximum heat-injured *E. coli* O157:H7 recovery (Czechowicz *et al.* 1996). Based on a minimal root mean square value, the thermal inactivation data could be fitted well to generate survivor curves.

The multiple regression equation for the $log_e$ D-values yielded an $R^2$ value of 0.953. This equation, given below, based on 40 unique combinations, can predict D-values/the pathogen survival for changes in the parameter values in the range tested from any combination of four environmental factors.

$$Log_e \text{ D-value} = -43.0646 + 1.4868(temp) + 3.5737(pH) - 0.1341(salt) - 8.6391(phos) - 0.0419(temp)(pH) + 0.0103(temp)(salt) + 0.1512(temp)(phos) - 0.0544(pH)(salt) + 0.2253(pH)(phos) - 0.2682(salt)(phos) - 0.0137(temp)^2 - 0.0799(pH)^2 - 0.0101(salt)^2 - 6.4356(phos)^2$$

For the environmental variables temperature, pH, salt and SPP levels, the D-values of *E. coli* O157:H7 based on survivor curves generated using the linear model are given in Table 1. The fit between D-values of *E. coli* O157:H7 in beef gravy as predicted by the model and those observed experimentally is given in Fig. 1. Predicted D-values compared well with observed D-values. Thus, the model provides a valid description of the data used to generate it.

Results showed that all variables, i.e., pH, NaCl and SPP affect D-values of *E. coli* O157:H7, at all heating temperatures studied. Figure 2 depicts the effects and interaction of 6% salt, 0.3% SPP in gravy at 4 and 8 pH levels on the observed D-values at 55C. Lower pH of the gravy tended to increase *E. coli* O157:H7 sensitivity to heat at 55C, and this effect was substantially more when SPP was present in gravy. The observed D-values at 55C decreased (76.7%) from 12.0 to 2.8 as the pH of the gravy decreased from 8 to 4. When gravy contained 0.3% SPP, the observed D-values in pH 4 gravy was 80.8% of the value at pH 8 (1.9 min vs 9.9 min). In contrast, Blackburn *et al.* (1997) reported an optimum pH (5.2 - 5.9), dependent on temperature and NaCl, for survival of *E. coli* O157:H7, and increasing acidity or alkalinity increased the rate of inactivation. It must be noted that the pH effect on heat resistance of *E. coli* O157:H7 depends upon the interaction of other variables (SPP, NaCl, etc.) in the heating menstruum.

The lethality of heat to *E. coli* O157:H7 increased when gravy (pH 8) contained 1 to 6% salt. For example, the observed D-values decreased by 37.5% (7.5 min; 6% salt vs 12.0 min; no salt); the D-values decreased by 6.1 min when gravy (pH 8) contained 0.3% SPP in addition to 6% salt (Fig. 2). It was interesting to note that the addition of salt in gravy exhibited a reverse trend at low pH, i.e., salt effect was protective to *E. coli* O157:H7 against the lethal effect of heat in pH 4 gravy. Supplementing salt in gravy (pH 4) increased observed D-values by 66.7% (2.8 min; no salt vs 8.4 min; 6% salt). However, a combination of salt and SPP in gravy increased sensitivity of the pathogen to heat. For example, addition of 0.3% SPP in gravy at pH 4 with 6% NaCl resulted in decreasing the observed D-value by 58.3%; the decrease in gravy at pH 8 was 49.3% (Fig. 2). Thus, SPP interacted with salt, thereby reducing the protective effect of salt. The observations, in this study, with respect to the salt effect enhancing the survival of *E. coli* O157:H7 in pH 4 gravy and the addition of SPP in the presence of salt reducing the protective effect of salt were consistent with those reported in the literature. In a study by Kotrola and Conner (1997), when the heat resistance of *E. coli* O157:H7 inoculated in ground turkey breast meat (3% fat) with 8% NaCl was assessed, the D-values at 55C, obtained by linear regression, increased from 12.5 min (turkey with no salt) to 26.1 min; the D-values increased from 11.0 min (11% fat turkey with no salt) to 20.4 min. In the same study, the authors reported D-values at 55C of 23.0 and 17.9 min in 3 and 11% ground turkey breast meat containing the additive mix (8% NaCl + 4% sodium lactate + 0.5% polyphosphate), respectively. It is worth noting that the study by Kotrola and Conner (1997) was conducted in turkey and the interactive effect of pH with other factors on heat

TABLE 1.
OBSERVED AND PREDICTED D-VALUES AT 55 - 62.5C OF *ESCHERICHIA COLI* O157:H7 IN BEEF GRAVY, AT VARIOUS PH LEVELS (4 - 8) SUPPLEMENTED WITH NACL (0 - 6%, W/V), AND SODIUM PYROPHOSPHATE (0 - 0.3%, W/V)

| Temperature | pH | % NaCl | % Phosphate | D - value Observed[a] | D - value Predicted (UL)[b] |
|---|---|---|---|---|---|
| 55.0 | 4 | 0.0 | 0.00 | 2.8 (0.03) | 4.1 |
| 55.0 | 4 | 0.0 | 0.30 | 1.9 (0.00) | 2.7 |
| 55.0 | 4 | 6.0 | 0.00 | 8.4 (0.26) | 10.4 |
| 55.0 | 4 | 6.0 | 0.30 | 3.5 (0.03) | 4.3 |
| 55.0 | 6 | 3.0 | 0.15 | 11.6 (0.19) | 11.3 |
| 55.0 | 8 | 0.0 | 0.00 | 12.0 (0.48) | 14.0 |
| 55.0 | 8 | 0.0 | 0.30 | 9.9 (0.32) | 12.2 |
| 55.0 | 8 | 6.0 | 0.00 | 7.5 (0.19) | 9.7 |
| 55.0 | 8 | 6.0 | 0.30 | 3.8 (0.03) | 5.2 |
| 57.5 | 5 | 1.5 | 0.10 | 4.1 (0.06) | 4.1 |
| 57.5 | 5 | 1.5 | 0.20 | 3.5 (0.17) | 3.7 |
| 57.5 | 5 | 4.5 | 0.10 | 3.5 (0.07) | 5.6 |
| 57.5 | 5 | 4.5 | 0.20 | 3.5 (0.06) | 4.6 |
| 57.5 | 6 | 3.0 | 0.15 | 5.6 (0.05) | 5.4 |
| 57.5 | 7 | 1.5 | 0.10 | 3.5 (0.01) | 5.5 |
| 57.5 | 7 | 1.5 | 0.20 | 4.1 (0.00) | 5.1 |
| 57.5 | 7 | 4.5 | 0.10 | 5.1 (0.15) | 5.4 |
| 57.5 | 7 | 4.5 | 0.20 | 4.3 (0.00) | 4.6 |
| 60.0 | 4 | 3.0 | 0.15 | 2.1 (0.02) | 2.2 |
| 60.0 | 5 | 3.0 | 0.15 | 2.6 (0.02) | 2.3 |
| 60.0 | 6 | 0.0 | 0.15 | 1.8 (0.02) | 2.1 |
| 60.0 | 6 | 1.5 | 0.15 | 2.1 (0.01) | 2.2 |
| 60.0 | 6 | 3.0 | 0.00 | 2.3 (0.04) | 2.8 |
| 60.0 | 6 | 3.0 | 0.10 | 2.2 (0.06) | 2.5 |
| 60.0 | 6 | 3.0 | 0.15 | 2.4 (0.04) | 2.4 |
| 60.0 | 6 | 3.0 | 0.20 | 2.6 (0.11) | 2.3 |
| 60.0 | 6 | 3.0 | 0.30 | 1.8 (0.01) | 2.1 |
| 60.0 | 6 | 4.5 | 0.15 | 2.1 (0.13) | 2.6 |
| 60.0 | 6 | 6.0 | 0.15 | 2.9 (0.13) | 3.0 |
| 60.0 | 7 | 3.0 | 0.15 | 1.2 (0.04) | 2.2 |
| 60.0 | 8 | 3.0 | 0.15 | 1.7 (0.01) | 1.9 |
| 62.5 | 5 | 1.5 | 0.10 | 0.8 (0.04) | 0.8 |
| 62.5 | 5 | 1.5 | 0.20 | 0.6 (0.01) | 0.8 |
| 62.5 | 5 | 4.5 | 0.10 | 0.9 (0.03) | 1.3 |
| 62.5 | 5 | 4.5 | 0.20 | 0.8 (0.01) | 1.2 |
| 62.5 | 6 | 3.0 | 0.15 | 0.7 (0.00) | 0.9 |
| 62.5 | 7 | 1.5 | 0.10 | 0.5 (0.00) | 0.7 |
| 62.5 | 7 | 1.5 | 0.20 | 0.7 (0.02) | 0.7 |
| 62.5 | 7 | 4.5 | 0.10 | 0.9 (0.01) | 0.8 |
| 62.5 | 7 | 4.5 | 0.20 | 0.7 (0.02) | 0.8 |

[a]Values represent means (± standard deviations) of 40 variable combinations replicated twice.
[b]The upper limit of confidence interval of predicted D-value.

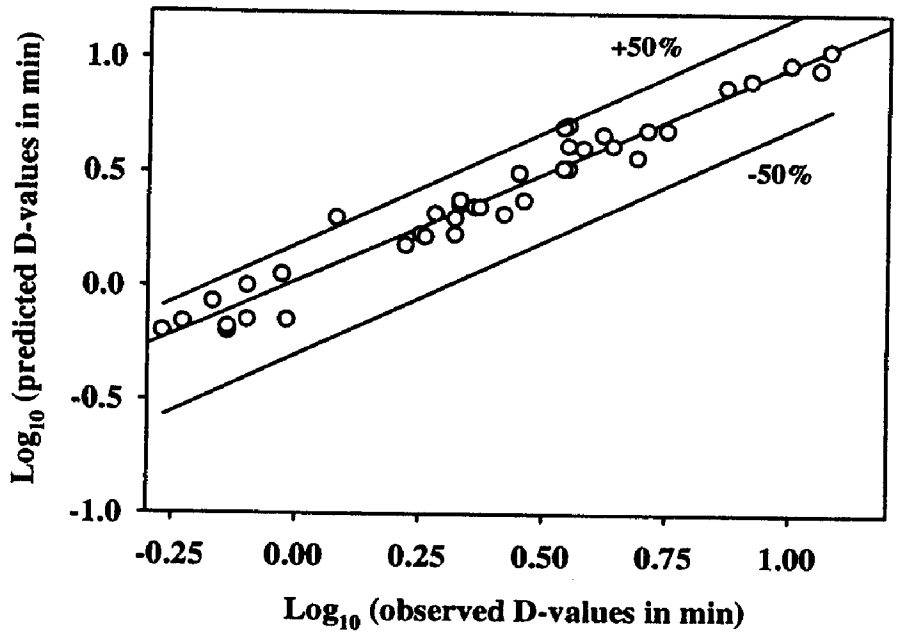


FIG. 1. AGREEMENT BETWEEN PREDICTED AND OBSERVED D-VALUES OF *E. COLI* O157:H7 IN BEEF GRAVY

The center line is the "line of identity" and the others represent ± 50% of the observed value.

resistance was not assessed. It is, therefore, not feasible to quantitatively compare the data with our study in which the heat resistance was assessed in gravy at various pH levels.

In a study by Reichart and Mohacsi-Farkas (1994), when heat destruction of seven foodborne microorganisms as a function of temperature, pH, redox potential and water activity was assessed in synthetic heating media, the heat destruction increased with decreasing pH and increasing water activity. The pH of the heating menstruum is recognized as one of the most important factors influencing the heat resistance of bacteria. Microorganisms usually have their maximum heat resistance at pH values close to neutrality; a decrease in the pH of the heating medium typically results in decreased D-value.

The effects of salt on thermal resistance have mainly been examined by determining the relationships between thermal resistance and either solute concentration or water activity of the heating menstruum. Tuncan and Martin (1990) reported that the heat resistance of *Staphylococcus aureus* MF-31 NaCl increased as the degree of salt-water association increased. Generally speaking, the effect of salts

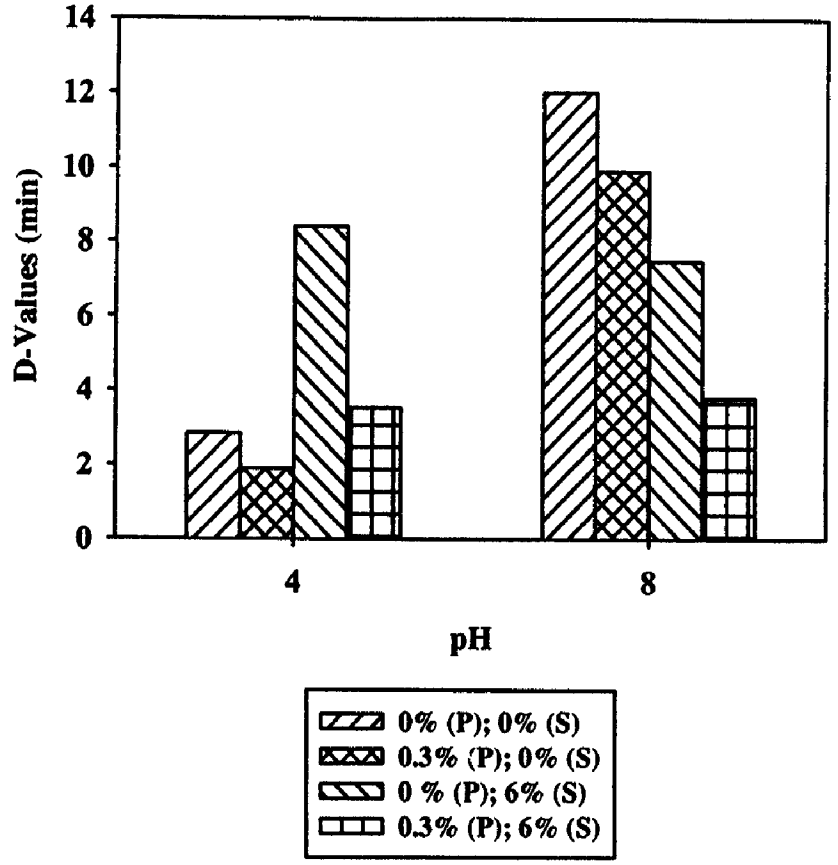


FIG. 2. EFFECTS AND INTERACTIONS OF SALT (S), pH AND SODIUM PYROPHOSPHATE (P) ON THE OBSERVED D-VALUES, AT 55C, OF FOUR STRAINS MIXTURE OF *E. COLI* O157:H7 IN BEEF GRAVY

on thermal inactivation of microorganisms is mainly related to reduced water activity and increased osmotic pressure of the heating menstruum (Tuncan and Martin 1990). Furthermore, for given solutes, a certain concentration of each gives maximum heat protection, whereas levels outside this optimum solute concentration result in increases in the heat sensitivity of the organism (Leistner and Russell 1991). In our study, the protective effect of salt against heat lethality was observed only at pH 4 regardless of the presence or absence of SPP in gravy. However, the effect was less pronounced in the presence of SPP.

The predicted D-values for gravy samples with no SPP or salt present at 55C were 3.2 min and 10.8 min at pH 4 and 8, respectively (Fig. 3). While decreasing pH from 8 to 4 resulted in parallel decrease in predicted D-value by 70.85% at 55C (Fig. 3), the decrease was 55.7% at 57.5C, and 32.6% at 60C; there was no difference in predicted D-values at 62.5C (Data not shown).

Regardless of the presence (up to 6% salt) or absence of salt, SPP addition in gravy at both pH levels (4-8) increased *E. coli* O157:H7 sensitivity to heat. For example, Fig. 3 depicts the effect of SPP on predicted D-values at 55C. At 0.3% SPP content

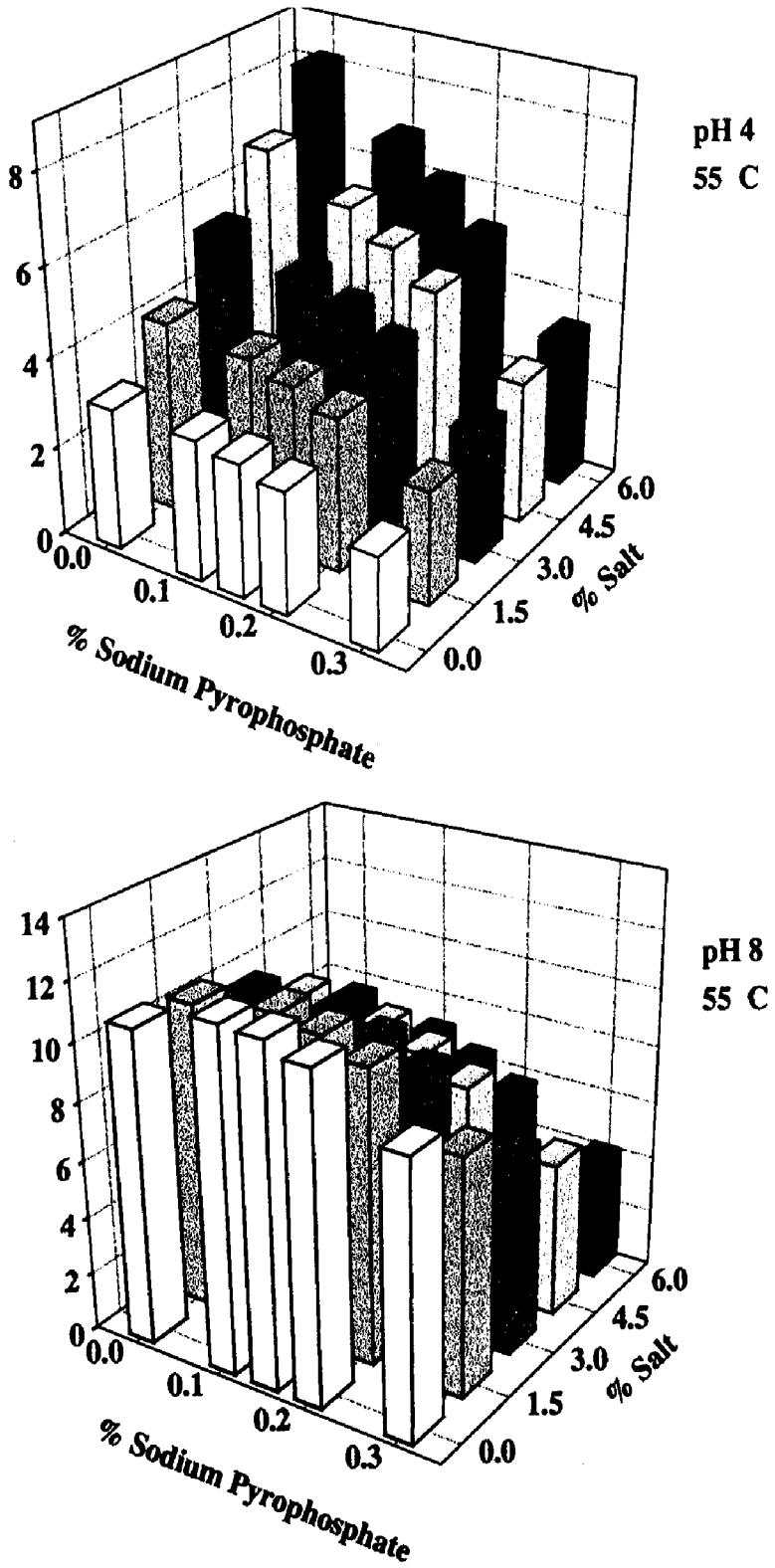


FIG. 3. EFFECTS AND INTERACTIONS OF SALT AND SODIUM PYROPHOSPHATE ON THE PREDICTED D-VALUES, AT 55C, OF FOUR STRAINS MIXTURE OF *E. COLI* O157:H7 IN BEEF GRAVY AT pH 4 AND 8

in gravy, the predicted D-values decreased (32.2 %) from 3.1 min (gravy with 0.1% SPP) to 2.1 min at pH 4 and from 11.8 min to 9.5 min (19.5 % decrease) at pH 8. Increasing salt levels, regardless of the presence (up to 0.3%) or absence of SPP, resulted in parallel decrease in D-values in gravy only at pH 8. At lower salt concentration (1.5%) in gravy, the predicted D-values decreased (29.2%) from 10.6 min to 7.5 min (gravy with 6% salt). A combination of salt and phosphate appeared to be effective in decreasing the predicted D-values in gravy at pH 8. At higher temperatures, the response of the cells to both salt and phosphate levels in gravy (pH 4 or 8) was similar.

Figure 4 depicts the predictive relative impact of various levels of salt and phosphate in increasing the sensitivity of *E. coli* O157:H7 to heat. The z-value calculated from predicted D-values obtained in gravy (pH 4) with no added salt or phosphate was 8.1C. When gravy was supplemented with 1.5% salt and 0.1% sodium pyrophosphate, the z-value was 9.1C (Fig. 4). Predicted D-values obtained in gravy with additional salt and/or phosphate resulted in slightly higher z-values. This effect was more pronounced with increasing NaCl levels. This observation is in agreement

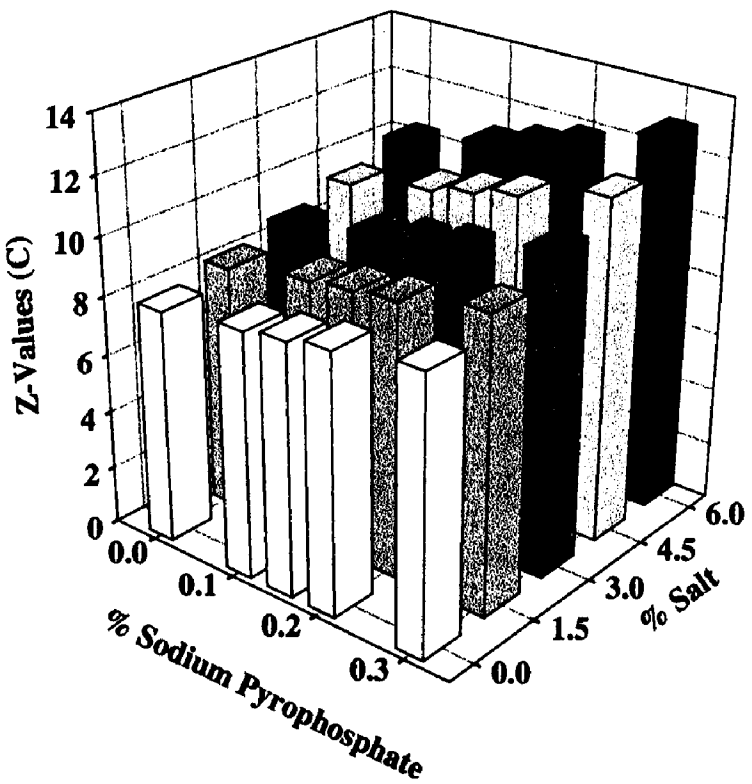


FIG. 4. THE Z-VALUES CALCULATED FROM PREDICTED D-VALUES OBTAINED IN BEEF GRAVY (pH 4) SUPPLEMENTED WITH 0.0-6.0% SALT AND/OR 0.0-0.3% SODIUM PYROPHOSPHATE

with those made by other researchers (Blackburn *et al.* 1997). These authors reported that the z-values increased from 4.6-5.1C at 0.5% w/w NaCl to 5.8 - 7.0C at 8.5% w/w NaCl. According to the present study, higher changes in temperature are required to cause 90% reduction in D-value when a cocktail of *E. coli* O157:H7 strains are evaluated in gravy with increasing levels of salt and/or SPP. It would, therefore, be not advisable to determine z-values under one set of food formulation variables and applying to other set of parameters in foods.

In conclusion, the present study presents an assessment and quantification of the effects and interactions of temperature, pH, salt, and SPP levels and indicates that the thermal inactivation of *E. coli* O157:H7 is dependent on all the four factors. Thermal resistance of *E. coli* O157:H7 can be altered by combining these intrinsic factors. The predictive model, developed in this study, can help either to establish an appropriate heat treatment, or to explain how changes in the formulation of foodstuffs could affect their microbiological safety. The D-values predicted by the model must first be validated with heat resistance data obtained by actual experiments in foods before the predicted values can be used to design thermal processes for the production of a safe food.

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