ABSTRACT

Title of Thesis: The Efficacy of a Boiling Water Blanch on the Inactivation of Listeria monocytogenes in Diced Celery

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The effect of a boiling water blanch on the thermal inactivation of Listeria monocytogenes in raw diced celery was investigated. L. monocytogenes, Scott A was heated in Tryptic Soy Broth with 0.6% yeast extract, pH 5.3, at 52, 54, 56, 58, and 59°C. D values were 13.33, 7.75, 1.29, 0.792, and 0.701 min, respectively. A predicted D value at 60°C = 0.36 min was obtained using a z value of 5. These thermal inactivation characteristics were subsequently used to evaluate the lethal effect of the blanch.

L. monocytogenes was recovered from inoculated (1 X 10^7 cells/g) diced raw celery after heating for 0.25 min in a laboratory boiling water blanch. Viable cells were not recovered after 0.5 min of heating.

Heat penetration data for diced raw celery during a boiling water blanch were obtained in a commercial

setting. These data were analyzed by the general method to determine the cumulative lethality at reference temperatures of 60°C using a z of 5 and, 70 and 71.7°C using a z of 10. It was determined that a recommended process of 2 min at 70°C was reached in 80 sec during the blanch. Based on the findings of this study, a boiling water blanch could be used as part of the hurdles approach to eliminate L. monocytogenes from raw diced celery.

THE EFFICACY OF A BOILING WATER BLANCH ON THE INACTIVATION OF *LISTERIA MONOCYTOGENES* IN DICED CELERY

by

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III

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INTRODUCTION

The development and introduction of new commercially prepared, fresh, non-sterile, food products to be sold in the refrigerator case is a major trend in the U.S. food industry. The number of U.S. companies becoming active in these refrigerated foods is rapidly expanding as a result of perceived opportunities in marketing convenient products with "closer to fresh" characteristics(4). Concomitant with this trend comes the concern for microbiological safety and public health, particularly in view of the emergence of psychrotrophic foodborne pathogens, such as Listeria monocytogenes.

L. monocytogenes, a common environmental organism and the etiologic agent of listeriosis has recently been shown to be a foodborne pathogen. A wide variety of clinical syndromes(66) is caused by L. monocytogenes ranging from a mild influenza-like illness to neonatal listeriosis. Mortality rates are reported to be as high as 54 to 90 percent. In the adult, the major infections are meningitis(55 percent), primary bacteremia(25 percent), endocarditis(7 percent) and nonmeningitic central nervous system infection(6 percent). More than half of these patients have underlying disorders such as cancer, alcoholism, cirrhosis, or diabetes; or are

receiving immunosuppressive drugs(25). The incidence of human listeriosis in the U.S. appears to be increasing. This may be due to the increased awareness and improved methods for identification, and/or to the growing number of patients with immunosuppressive disorders who are exposed to infectious complications(66). Age distribution of listeriosis is uneven with most cases occurring among neonates and the elderly. There is no vaccine for the prevention of listeriosis. Prevention of the foodborne illness centers on the elimination of Listeria from the product.

L. monocytogenes is a gram positive, beta-hemolytic, facultatively anaerobic, non sporeforming rod that is capable of growth at refrigeration temperatures. Therefore, commercially refrigerated food products found to contain L. monocytogenes must be considered unsafe, especially for those individuals considered to be at greatest risk of infection.

Outbreaks of listeriosis associated with coleslaw and salad demonstrate that vegetables may be a potential source of infection(57). In addition, L. monocytogenes has been isolated from salads sold at retail. For example, in one study(59), 60 salad samples were tested and four were found to contain L. monocytogenes. The organism increased two-fold in positive samples when left at 4^{o}C for 4 days, indicating that it can survive

and multiply during storage of the product(59). L. monocytogenes is considered a saprophytic organism, isolated frequently from old, faded, or moldy plants(64,65). Commercially prepared, fresh vegetables are more susceptible to spoilage than whole vegetables since processing disrupts the plant tissues(1). The overall microbial population of vegetable products has been shown to increase with the length of storage time and with an increase in moisture(30).

Federal guidelines that apply to the manufacture of prepacked, refrigerated salads, ready for consumption without further processing, are provided in the Code of Federal Regulation: 21 CFR Part 110 - "Current Good Manufacturing Practices(GMP) in Manufacturing, Packing or Holding Human Food", 9 CFR Part 318 - Meat Inspection Regulations and 9 CFR Part 381 - Poultry Products Inspection Regulations(52). GMPs provide general plant sanitation guidelines and are considered essential for the manufacture and distribution of refrigerated foods(56). In addition, to evaluate potential microbiological hazards in refrigerated food, industry and government are recommending Hazard Analysis Critical Control Point (HACCP) guidelines(56). This concept is used to identify potential microbial hazards, and their associated risks, in manufacturing and distribution. Critical Control Points are specified and appropriate

monitoring programs are established. In this program, key ingredients that may pose problems are identified and steps are taken to eliminate dangers of the pathogen being in a food(52,58). To further eliminate hazards, a "barrier" or "hurdle" approach has been suggested(58). Combinations of controls such as pH, water activity, thermal processing, preservatives, and storage temperature, in conjunction with the intrinsic properties of the food, are applied to the problem ingredient or product to prevent bacterial growth.

L. monocytogenes has been isolated from vegetable products. Furthermore, vegetables have been implicated in two outbreaks of listeriosis. It is important for the food processor to recognize raw vegetables as a potential source of contamination when incorporated into a product such as a prepacked, composed salad ready for consumption with no further process. An example of such a product is a salad composed of tuna fish, mayonnaise and celery. Commercially sterile tuna and mayonnaise would not be considered sources of listerial contamination if properly processed. The celery, however, added as a raw ingredient, may be a potential hazard.

The proper washing of a raw vegetable to remove dirt is essential. The next step, as part of the barrier approach, could be to apply a bacteriocidal thermal

process to the vegetable. The purpose of this study was to evaluate the use of a boiling water blanch to eliminate L. monocytogenes from raw diced celery. This study examines the heat resistance of L. monocytogenes in a broth medium. A boiling water blanch is used to eliminate high numbers of L. monocytogenes from inoculated celery. The heating profile of celery during a blanch is used to determine the lethality achieved at applicable reference temperatures. In addition, this study provides recommendations for the use of blanching to eliminate L. monocytogenes from raw celery destined for use in refrigerated composed salads.

LITERATURE REVIEW

The organism

L. monocytogenes has only recently emerged as a significant pathogen that can be found in a variety of foods(25,32). Surveys suggest that the incidence of listeriosis, considered rare a decade ago, has increased from one to seven cases per million population(2). This rate is low compared to *Salmonella* and *Campylobacter spp.*, however, listeriosis is characterized by a high mortality rate; reported to range from 20-50%(2) and 54-90%(66).

L. monocytogenes is carried by 5% of the general population. Fecal carriage is reported in 29% of poultry workers and in 77% of public health laboratory workers involved in *L. monocytogenes* isolation. The public health importance of human fecal carriage is not known(25).

L. monocytogenes is a gram-positive, microaerophilic, non sporeforming rod that has a characteristic tumbling motility between 20 and 25^{o}C(25). Beta hemolysis distinguishes *L. monocytogenes* from nonpathogenic *Listeria spp.*(32). There are 16 recognized serotypes identified by at least 14 different O (somatic) antigens and 5 H (flagellar) antigens.

Serotypes 1/2a, 1/2b and 4b account for more than 90% of the cases reported worldwide(25,32).

The tests for pathogenicity include: the ability to cause keratoconjunctivitis in guinea pigs(Anton's test); toxicity in chick embryos; and death in mice(25). A hemolysin is thought to be a virulence factor, however, hemolytic activity varies among strains and does not correlate with the severity of infection(32).

The optimum pH for growth of L. monocytogenes is 7.0(53) and growth has been reported as low as pH 4.4(61). The shortest generation times are observed over the temperature range of 30-37^{o}C. The growth range of L. monocytogenes is 2.5-45^{o}C(53) with growth reported as low as 0-1^{o}C(40,55). Generation times in Trypticase Soy Broth(TSB) at pH 7.0 were 44.7 min at 30^{o}C and 33.5 hrs at 4^{o}C(53).

L. monocytogenes has been isolated(25,32) from dust, poor-quality silage(64), vegetation(65), soil(64), sewage, stream water, slaughter-house waste, feces of healthy humans, milk of normal and mastitic cows and raw vegetables. In addition, the organism has been isolated from at least 37 species of mammals and 17 species of fowl, ticks, fish and crustaceans(25). Since L. monocytogenes is an environmental organism, found in soil, water, and silage, raw vegetable products may easily become contaminated(7,8,17,59). In food plants,

Listeria spp. have been isolated from drains, floors, standing water, residues, and food-contact surfaces(18).

Listeriosis in humans

Listeriosis was established as a reportable disease in the United States in 1986. It is estimated to cause at least 1700 serious infections and contribute to approximately 450 deaths and 100 stillbirths annually(32). People predisposed to listerial infection include pregnant women and their fetuses, newborns, patients undergoing immunosuppressive or corticosteroid therapy, and those with underlying diseases such as cancer, diabetes, hepatitis and alcoholism(25,32). Neonatal listeriosis is the largest recognized group of infections due to *L. monocytogenes*(25). Up to 30% of adults and 54% of children and young adults who have listeriosis have no apparent immunocompromising condition(32).

Symptoms including meningitis, spontaneous abortion, septicemia, peritonitis, local abscess formation, endocarditis, urethritis, conjunctivitis, hepatitis, arthritis and cutaneous lesions have been reported(25,66). In adults, more than one half of the cases involve central nervous system infection with meningitis and occasionally parenchymal cerebritis,

which may mimic a stroke syndrome(47). The incubation period may be as long as several weeks(32). Gastrointestinal symptoms occur in approximately one third of patients with listeriosis(32). Pregnant women with listeriosis exhibit a mild illness that resembles influenza, resulting in abortion, stillbirth or an acutely ill newborn with septicemia or meningitis(25). The likelihood that a systemic infection will occur following ingestion of food contaminated with L. monocytogenes depends on host susceptibility, inoculum size and an as yet unidentified virulence factor(32). The fact that the organism can be isolated from the gastrointestinal tract of asymptomatic, high risk individuals, coupled with the ubiquity of the organism in the food chain support the premise that exposure does not constitute disease(32). Ampicillin is recommended for treatment(25), sometimes in combination with gentamicin(32,47). The infectious dose is not known, therefore commercial food products found to contain L. monocytogenes must be considered unsafe especially for those at the greatest risk of infection(32).

Outbreaks of foodborne infection

From 1979 to 1985, four food-associated outbreaks were reported in North America(25). Two outbreaks

involved vegetable products(32). One of the implicated products was a salad of lettuce, celery and tomatoes. Of 23 people involved, two died from listeriosis(25). The use of antacids by some of the afflicted individuals was considered a risk factor and the neutralization of gastric acids was thought to have a possible role in the initial survival of the organism after ingestion(25).

The other implicated vegetable product was cabbage in coleslaw which affected 41 people resulting in 18 deaths. The cabbage had been grown in fields fertilized with compost and raw manure from sheep known to have had listeriosis(25,57). The practice of subjecting cabbage to prolonged cold storage before distributing it to wholesalers was identified as a mechanism to allow a small initial inoculum of L. monocytogenes to proliferate or cause a dying off of competitive organisms(57).

Microbiology of salad vegetables

There is a growing market in the sale of pre-packed ready to eat salad vegetables which are stored and sold under refrigerated conditions(50). During storage of pre-packed salad vegetables at 7°C or 10°C, the major types of microorganisms that multiply are Gram-negative bacteria(50). Studies of celery and cauliflower show

that the bacterial counts on fresh vegetables at harvest are in the range of 10^5 - 10^6/g and that the number of bacteria can be greatly affected by the amount of moisture present(10). Despite these high numbers the products are of acceptable quality and do not exceed the recommended microbiological limits for Escherichia coli(10). Contamination of vegetables can occur in the field, or between harvest and retail sale. Processes such as cutting may introduce additional contamination and increase the availability of moisture and nutrients on the cut surfaces which creates conditions that favor microbial growth(1,10,30,62).

Potential for Listeria monocytogenes in vegetables

Vegetables are less often mentioned as sources of listeriosis than dairy foods(8,17,41). L. monocytogenes has been reported to grow in cabbage juice and raw shredded cabbage at 5°C(37,62). Thus, L. monocytogenes has the potential to survive and grow at normal refrigeration temperatures for raw vegetables. L. monocytogenes has been reported to grow on lettuce stored at 5°C and 12°C resulting in 10^5 cells/g after 7 days incubation and 10^6 cells/g after 14 days at both temperatures(62). L. monocytogenes has been found on uninoculated lettuce and can multiply under proper

refrigeration, mishandling and ambient serving conditions(62).

A wide range of prepacked salads that are now being produced for consumption without further preparation are a potential source of listeria infection. Sizmer and Walker(59) examined 60 samples of salads of 10 different varieties. Of the 60 samples tested, four, representing two salad varieties were found to contain L. monocytogenes serotype 1/2. One of these samples also contained a strain of serotype 4b. The composition of one salad was cabbage, celery, sultanas, onion, and carrots. The other salad consisted of lettuce, cucumber, radish, fennel, watercress, and leeks. The survival of L. monocytogenes in these products was also studied. The samples were left at 4^{o}C for four days. The numbers of Listeria increased two-fold, indicating again that the organism can survive and multiply during storage of the product. These findings suggest that raw vegetables are a source of L. monocytogenes.

In contrast, two studies on the incidence of L. monocytogenes in a variety of raw vegetables purchased at the retail level showed that L. monocytogenes was not isolated, using cold enrichment techniques. One study examined 20 samples representing 10 vegetables(54). Another study examined 110 samples of raw vegetables including lettuce, radishes, tomatoes, and celery(26).

L. monocytogenes was also not detected on three uninoculated vegetables - asparagus, broccoli and cauliflower used in a controlled atmosphere storage(CAS) study(5). Under conditions of air storage and CAS at 4°C, for one week, initial populations of inoculated L. monocytogenes remained constant or decreased in the three vegetables. However, in asparagus and broccoli, populations increased from day 7 to day 21(5). CAS extended the length of time vegetables remained acceptable for consumption to 21 days, 7 days longer than the vegetables in air storage(5).

Heat resistance

Data concerning the heat resistance of L. monocytogenes are conflicting(3,21,23,24,35) with some researchers reporting that in milk, L. monocytogenes can survive the minimum high temperature short time(71.7°C, 15sec) pasteurization process(24). Others state that reports of extraordinary heat resistance are attributable to the methods used to determine heat resistance and that the D-value in milk is 0.1-0.4 min at 62°C when determined in sealed, submerged tubes(20,23). D values at 65°C in phosphate buffer and Tryptic Soy Broth with 0.6% yeast extract are reported to be 0.29 min and 10 min respectively(6). In cabbage

juice, pH 5.6, the D value at 56°C was 6.8 min(7), however the method used was not sealed tubes. Knabel et al.(42), reported that aerobic recovery of L. monocytogenes was inadequate and that by utilizing strict anaerobic conditions, D values are increased sixfold over those previously obtained using cells grown at 37°C and aerobic plating.

Recently, Mackey and Bratchell(49) stated that cooking food to an internal temperature of 70°C(158F) for 2 min is adequate to ensure destruction of L. monocytogenes. This recommendation is based on a review of 25 papers from which D values were plotted. Despite differences in heating media methods and test strains the conformed to a straight line plot of log D vs temperature. The equation for the fitted line was

$$\log D = 10.888-0.14519t$$

where D is the decimal reduction time in seconds and t is the temperature(°C). The range of z values from the plotted data was 4.3-9.9°C.

L. monocytogenes Scott A has been reported to be somewhat more heat resistant than other strains (7,13,14,53). Therefore, it was selected for use in this study.

MATERIALS AND METHODS

Organism

L. monocytogenes, Scott A, serotype 4b, (obtained from the National Food Processors Association) was isolated from a 1983 outbreak of listeriosis in Massachusetts(53). The culture was maintained on sterile Tryptic Soy Agar(Difco) with 0.6% yeast extract(TSAYE) at 4°C and transferred monthly. Inoculum was prepared by transferring a loopful of growth from TSAYE to 10 ml of sterile Tryptic Soy Broth(Difco) with 0.6% yeast extract (TSBYE) and incubating at 30°C for 24 hrs. Another transfer was then made to a fresh tube of TSBYE and incubated at 30°C for 18 hrs(14).

Raw celery

Raw celery, diced to approximately 0.25"(6.35mm), for inclusion in a refrigerated food product was obtained from a food manufacturer in 5 lb quantities. The celery, pH 6.0 was stored at 4°C in the laboratory and subjected to testing immediately, or within 24 hrs.

Media used in testing

All media used throughout this study were sterilized at 121°C according to the manufacturers or authors instructions. Cells heated in broth were recovered by direct plating to TSAYE(14). Unheated celery was subcultured to Enrichment Broth(EB)(27) and samples of heated celery were subcultured to TSBYE. Broth subcultures were streaked on Day 0, 2 and 7 to Modified McBride agar(MMA), and Lithium Chloride Phenylethanol Moxalactam agar(LPM)(27). In addition, Modified Vogel Johnson agar(MVJ)(12,45) and Modified Oxford agar(MOX)(44) were streaked as above. Plates and broth were incubated at 30°C. Isolated black colonies on MOX were tested for catalase production, tumbling motility on wet mount, gram stain(27) and beta hemolysis on thin layer horse blood agar(HL)(51). Selected colonies were confirmed to be L. monocytogenes by the Vitek system(43), a computerized microbial identification system.

Heat treatment monitor

Heat treatments were monitored using thermocouples attached to a Cole Parmer Multichannel Process Signal Scanner, model 02161-42, referenced to a mercury in

glass thermometer. Thermocouples were placed to monitor the temperature of the celery and water or, a reference thermal death time(TDT) tube. Temperatures were recorded manually at 5 sec intervals, alternating between the celery and water or, consistently in the case of the TDT tube. A temperature printout was provided every minute by the scanner.

Thermal inactivation in Tryptic Soy Broth with 0.6% yeast extract

To determine D values(22), 1.5 ml aliquots of an 18 hr TSBYE culture of L. monocytogenes, pH 5.3, were placed in two ml glass ampules and flame sealed. Sealed ampules were completely submerged and heated from 52 to 59^{o}C in a constant temperature circulating oil bath (HAAKE R20, type NBE, HAAKE, Inc., Saddlebrook, N. J). One ml of heated sample was serially diluted in 0.1% peptone water. Counts were performed on TSAYE(14) after 48 hrs of incubation at 30^{o}C(48). Survivor curves indicating the rates of thermal inactivation were plotted using linear regression on the log10 counts/ml vs. heating times. An estimate of D value was obtained by taking the inverse of the slope of the plot. A linear regression was computed from log10 D value vs temperature to plot the phantom TDT curve. The inverse

of the slope of this line provided the z value which was used in the lethality determinations(9).

Thermal inactivation in celery

Six ml of an 18 hr culture of L. monocytogenes were transferred to 594 ml of sterile distilled water and poured over a 400 g sample of celery in a sterile beaker. The sample was covered with sterile foil and incubated overnight at 4°C. The sample was then drained in a 4" square screen basket for 10 min. The drained sample and basket were placed in a 4000 ml beaker containing 2500 ml of boiling water. At 0.25, 0.50, 1.0 and 1.5 min intervals, a sample of celery weighing approximately 25 g was removed using a sterile plastic spoon and immediately placed into a sterile blender jar containing 225 ml of refrigerated TSBYE. Samples were blended for 2 min at low speed to minimize foaming and poured into sterile 500 ml flasks. An unheated sample was blended in the same manner and 1.0 ml was removed for serial dilution and plate count to determine inoculum size. To determine if L. monocytogenes was present in the water, a 25 ml sample was taken at 0.25 min of heating. Test samples were incubated at 30°C for 7 days. At 0, 2, and 7 days, two MOX and two MVJ plates were streaked from each flask.

Freeze dried celery

A 2000 g sample of celery was freeze dried to a 100 g sample in a UNITOP 6005L-Freezemobile 12 freeze dryer (Virtis Company, Guardian, NY, 12525). A 20 g sample of this celery was reconstituted overnight at 4°C in 380 ml of sterile water containing 4 x 10^7 L. monocytogenes/ml. Procedures for heating were the same as described for raw celery.

Temperatures of blanch water and celery under processing conditions

Temperatures of blanch water and celery during processing conditions were monitored in a steam jacketed kettle (model FT 40 SP, Groen MFG Co., Elk Grove Village, IL) in a local manufacturing plant producing refrigerated foods. Six thermocouple leads were used to monitor temperatures. Pieces of diced celery were speared onto the entire length of each of three leads. Leads to monitor water temperature in corresponding positions were held in place next to the celery leads using twist-ties. These double leads were centrally placed in three positions to represent the top, middle, and bottom portions of the celery in the blanching basket. The basket was loaded with 50 lbs (22.7 kg) of

celery. The loaded basket was lowered into 40 gal (151.4 l)of boiling water in the steam kettle and, without agitation, the temperature was recorded every 5 sec, as described, during a 2 min blanch and cooldown. The celery was cooled in running cold water to a temperature of 55°F (12.8°C).

The heat penetration data were analyzed to determine the cold spot in the kettle. Data were analyzed by regression analysis using a non linear asymptotic growth model with 212°F (100°C) as the upper limit and 40°F (4.4°C) as the lower limit. The line which represented the area of celery receiving the least amount of heat was used to determine lethality at reference temperatures of 140, 158 and 161°F (60, 70, and 71.7°C). Lethality was determined by the General Method(46) using the GenGen computer program - copyright, National Food Processors Association.

RESULTS

Thermal inactivation in TSBYE

In order to establish D values (time in minutes at a constant temperature to destroy 90% of the cells present), sealed tube thermal inactivation studies of L. monocytogenes in Tryptic Soy Broth with 0.6% yeast extract, pH 5.3, were conducted at 52, 54, 56, 58, and 59°C (125.6, 129.2, 132.8, 136.4, and 138.2°F). Counts/ml were determined by direct plating(34) to Tryptic Soy Agar with 0.6% yeast extract. This is a nonselective medium which was chosen to detect heat stressed cells(15,19,36,60,63). Plate counts and their respective log10 counts/ml are listed in Table 1. Death rate (survivor) curves were obtained for each temperature and are shown in Figures 1-5. D values obtained from these curves were 13.33, 7.75, 1.29, 0.792 and 0.701 min respectively (Table 1 and Figures 1-5). A phantom TDT curve (Figure 6) was constructed using the D values. The z value of 9°F obtained from this curve was used in subsequent lethality determinations using heat penetration data. The z value is the slope of the phantom TDT curve and represents the number of degrees Fahrenheit required to cause a tenfold change in lethal effect(46).

TABLE 1. Number of survivors of Listeria monocytogenes following heating in Tryptic soy Broth with 0.6% yeast extract and recovered on Tryptic Soy Agar with 0.6% yeast extract.

Temp. (°C)	Time (min)	Ave. Plate Count	Count/ml	Log10 count/ml[a]
52	20	300	3.0×10^8	8.477
	25	59	5.9×10^7	7.771
	30	39	3.9×10^7	7.591
	35	19	1.9×10^7	7.279
54	10	53	5.3×10^8	8.724
	12	34	3.4×10^8	8.531
	16	133	1.3×10^8	8.114
	20	26	2.6×10^7	7.415
56	5	32	3.2×10^5	5.505
	6	66	6.6×10^4	4.819
	8	160	1.6×10^3	3.204
58	5	65	6.5×10^6	6.813
	6	41.5	4.2×10^5	5.618
	8	110	1.1×10^3	3.041
59	4	271	2.7×10^6	6.431
	5	104	1.0×10^5	5.000
	6	38	3.8×10^3	3.580

[a] Log10 count/ml plotted in Figures 1-5 to determine D values.

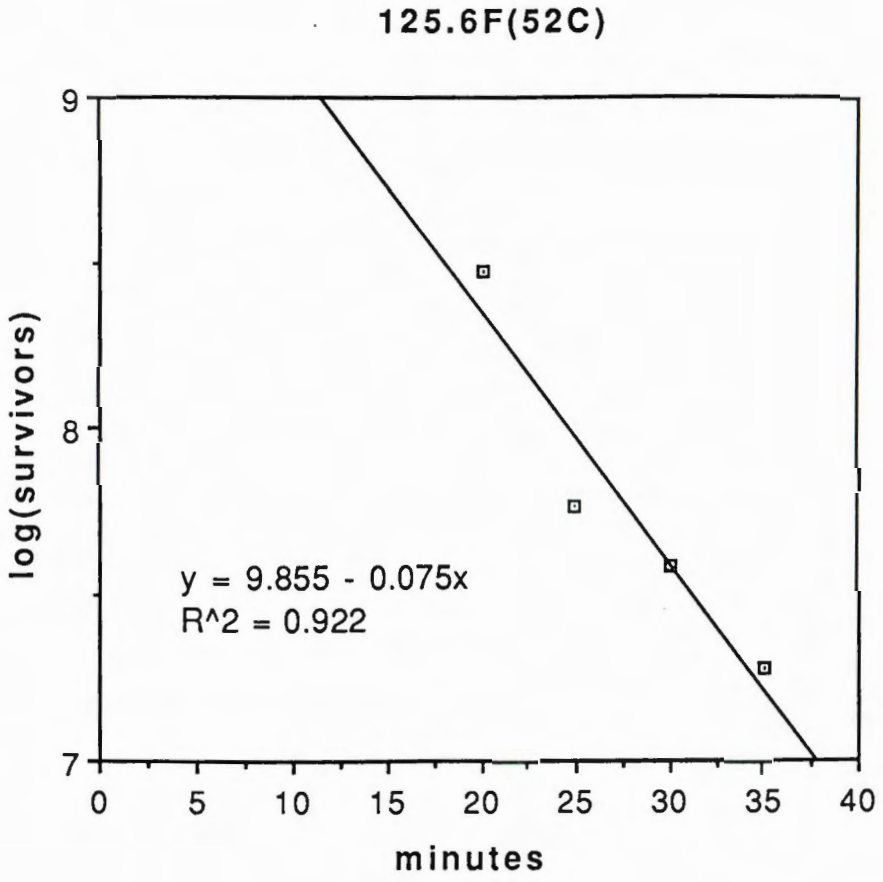


FIGURE 1. Survivor Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract at 52°C (125.6°F). D value = 13.33 min.

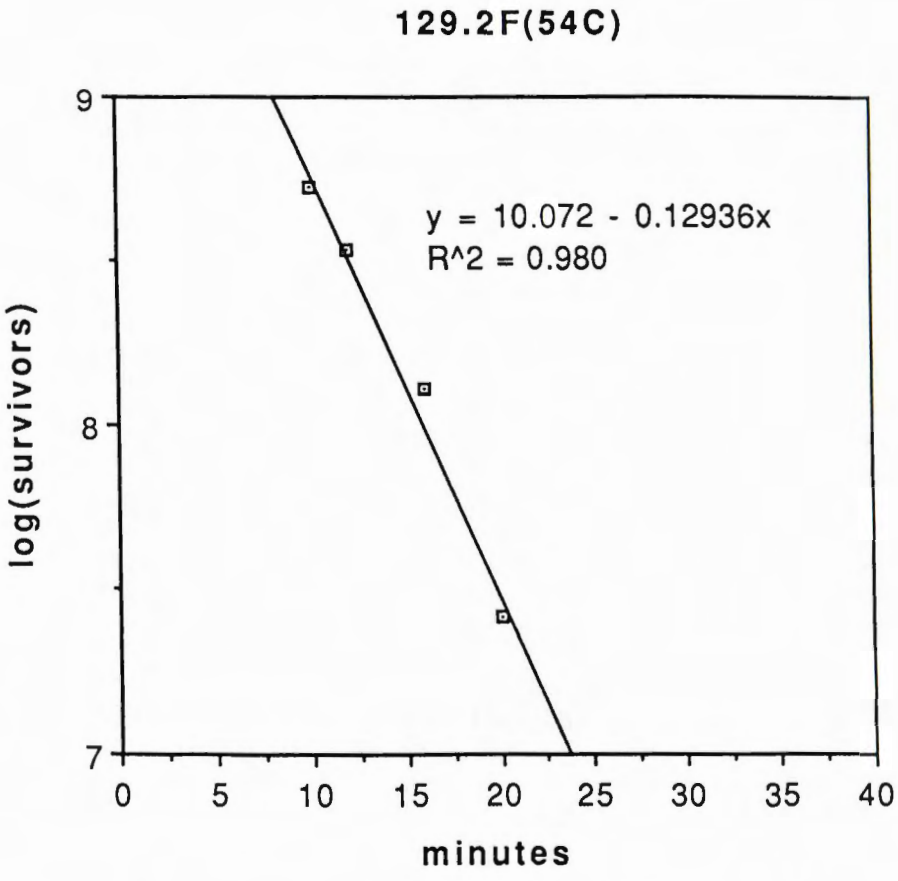


FIGURE 2. Survivor Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract at 54°C (129.2°F). D value = 7.75 min.

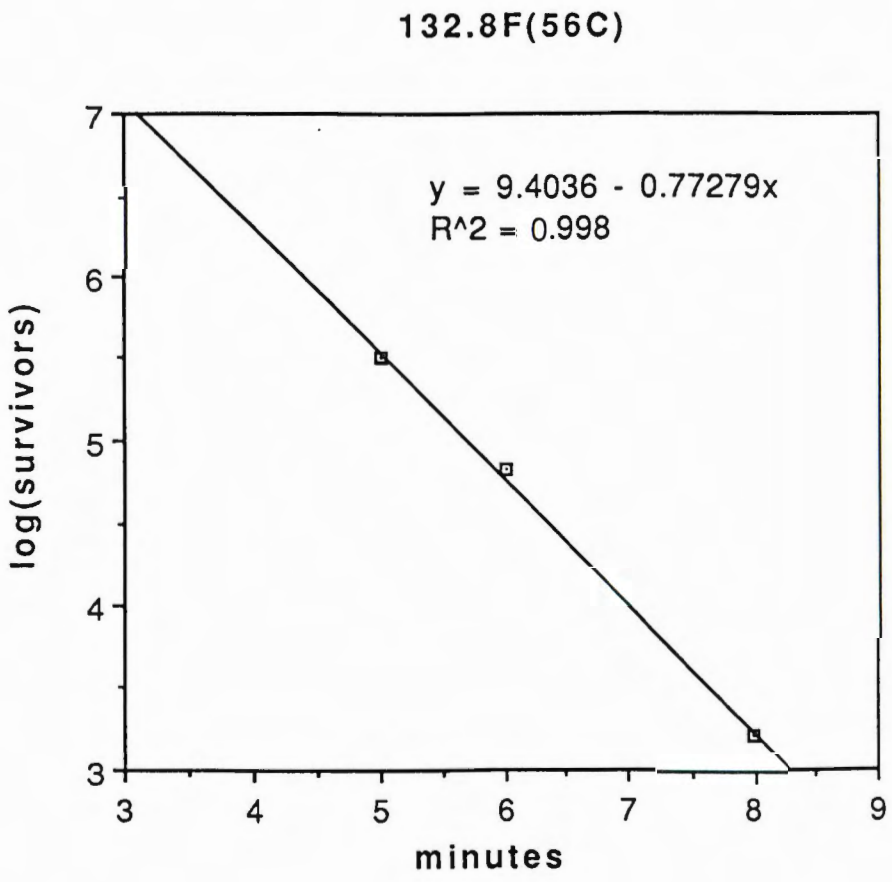


FIGURE 3. Survivor Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract at 56°C (132.8°F). D value = 1.29 min.

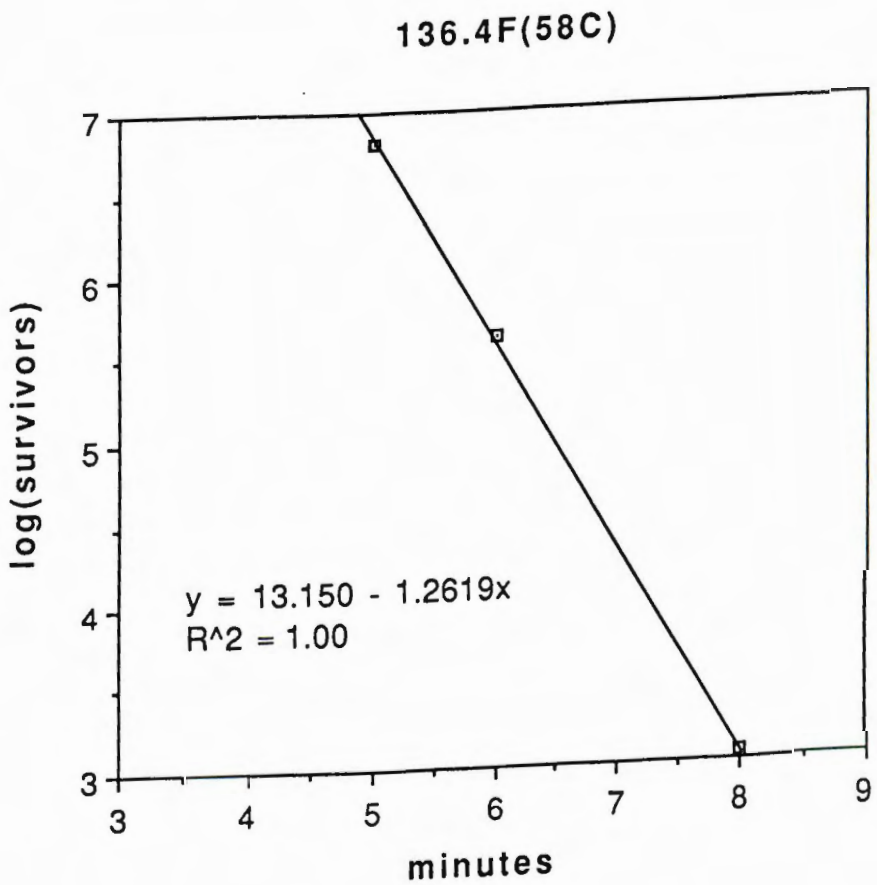


FIGURE 4. Survivor Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract at 58°C (136.4°F). D value = 0.792 min.

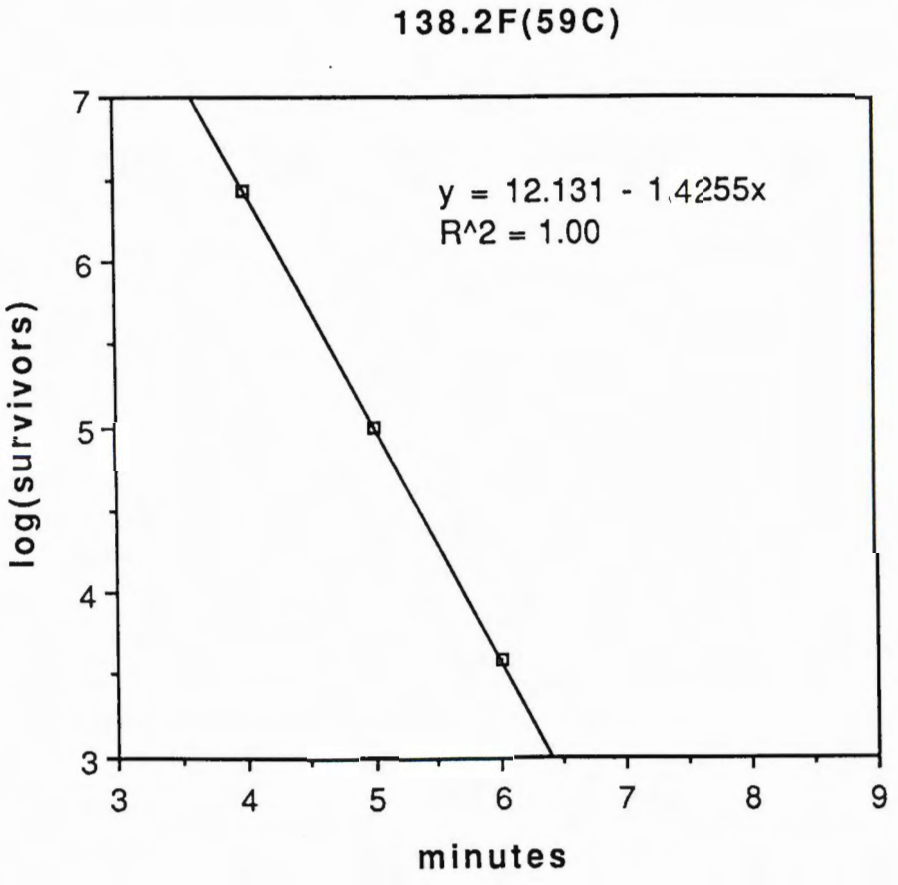


FIGURE 5. Survivor Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract at 59°C (138.2°F). D value = 0.701 min.

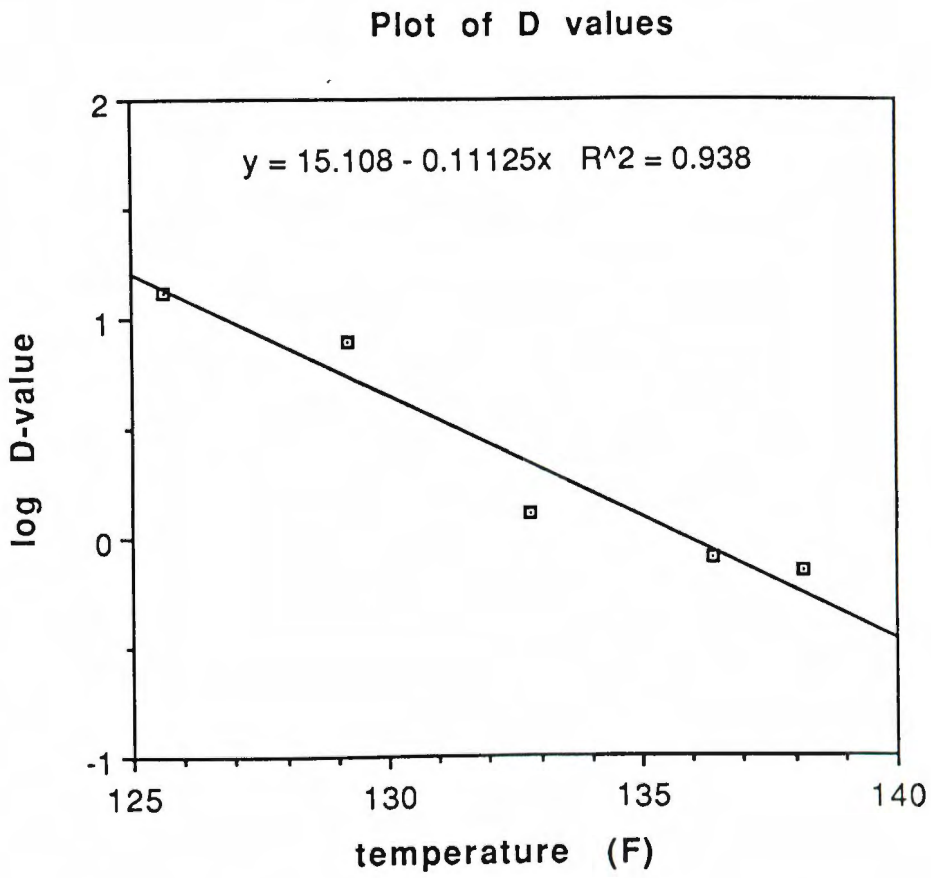


FIGURE 6. Phantom Thermal Death Time Curve: Listeria monocytogenes Scott A heated in Tryptic Soy Broth with 0.6% yeast extract over the temperature range 125.6 - 138.2°F (52 - 59°C). z = 9°F (5°C).

Predicted D values are listed in Table 2. Using a z of 9 and the predicted D, an F value of 4.32 min at 140°F(60°C) was calculated based on the 12D concept (12 x 0.36 = 4.32). Therefore, heating at 60°C for 4.32 min should reduce the population of L. monocytogenes by 12 log cycles.

Table 2. Predicted D values using the equation from the linear regression of the phantom TDT curve (Figure 6).

Temp (°C)	D value minutes	Predicted D minutes
52.0	13.33	14.66
54.0	7.75	5.84
56.0	1.29	2.32
58.0	0.792	0.93
59.0	0.701	0.43
60.0		0.36
71.7		0.0017

Recovery media used in testing

Modified McBride agar(MMA) and Lithium Chloride Phenylethanol Moxalactam agar(LPM) are two of the most widely used plating media for the detection of Listeria spp.(11). Initial studies using these media (the FDA procedure) to detect L. monocytogenes from inoculated celery were difficult and time consuming(28). As

reported in the literature these media lack a differential indicator and the observation of typical, blue colored colonies under oblique lighting was subjective(12,16,37).

Modified Oxford Agar(MOX)(44) and Modified Vogel Johnson(MVJ) Agar(12,45) were included in the positive control study and both were found to give a clear indication of L. monocytogenes. MOX contains ferric ammonium citrate which allows detection of esculin hydrolysis by the formation of a black precipitate(28) and MVJ uses the ability of L. monocytogenes to reduce potassium tellurite. Colonies of L. monocytogenes were black on both media. These media were incorporated into the remainder of the study.

Isolation of Listeria monocytogenes from control celery

To determine whether the celery received from the food manufacturer was naturally contaminated with L. monocytogenes, the celery was blended in Enrichment Broth as described in the FDA procedure and incubated at 30°C for 7 days. On day 0, 2, and 7, subcultures were streaked to MOX and MVJ and incubated at 30°C for 48 hrs. Characteristic black colonies as determined from positive control plates were not observed from the control celery.

Heating of inoculated celery

Raw celery

To determine whether a blanch was sufficient to eliminate a large inoculum of L. monocytogenes from diced celery, the celery was held overnight in a "bath" of inoculum. After draining, a plate count, using MOX agar (30°C for 48 hrs), was performed on the inoculated celery and it was found to contain 1 x 10^7 L. monocytogenes/g. The drained, inoculated celery was heated in a boiling water blanch for various times and subcultured to TSBYE for incubation at 30°C or 4°C for 7 days. This medium was chosen because it is the same as the FDA Enrichment Broth(EB)(39) without the selective agents(33). A number of studies have shown that the selective agents in EB are inhibitory to heat stressed organisms. On day 0, 2, and 7, L. monocytogenes was not recovered on streaked plates of MOX or MVJ when inoculated celery was subjected to blanch times of 0.50, 1.0, 1.5, and 2.0 min. When the blanch time was reduced to 0.25 min, black colonies were observed on MOX Agar but not on MVJ. These colonies were subsequently confirmed to be L. monocytogenes by the VITEK system(43), a computerized microbial identification system.

To determine the number of surviving cells of L. monocytogenes in the celery subjected to the 0.25 min blanch, a count was performed on MOX agar. The result was 5 x 10^2 cells/g.

It was suspected that the L. monocytogenes was free in the water rather than associated with the celery. Therefore, while the inoculated celery was subjected to the blanch, a 25 ml sample of blanch water heated for 0.25 min was withdrawn and tested in the same manner as the heated celery. The results of this test were negative.

Dehydrated celery

To simulate a condition where the L. monocytogenes was thoroughly incorporated into the product and not merely a surface contaminant, the celery was freeze-dried and allowed to rehydrate in water containing the inoculum. The resulting rehydrated celery contained an inoculum of 4 x 10^7 cells/g. Manipulation of the rehydrated celery was more difficult than that of the raw celery. The rehydrated celery was softer and floated more than the raw celery during the blanch. After the boiling water blanch of 0.25 and 0.50 min, the rehydrated celery was found to contain 4 x 10^3 and 2.1 x 10^2 cells/g respectively. Recoveries from the 1.0 and 1.5 min blanch were negative.

Counts on raw celery

Total plate counts using PCA on 5 batches of raw celery ranged from 10^5 to 10^7 cells/g.

Blanch in plant

Heat penetration data obtained from the celery leads are represented graphically in Figure 7. The slowest heating data were obtained from probe 3 which was located in the middle of the kettle. Specific times and temperatures obtained during the blanch from probe 3 are listed in Table 3, columns 1 and 2.

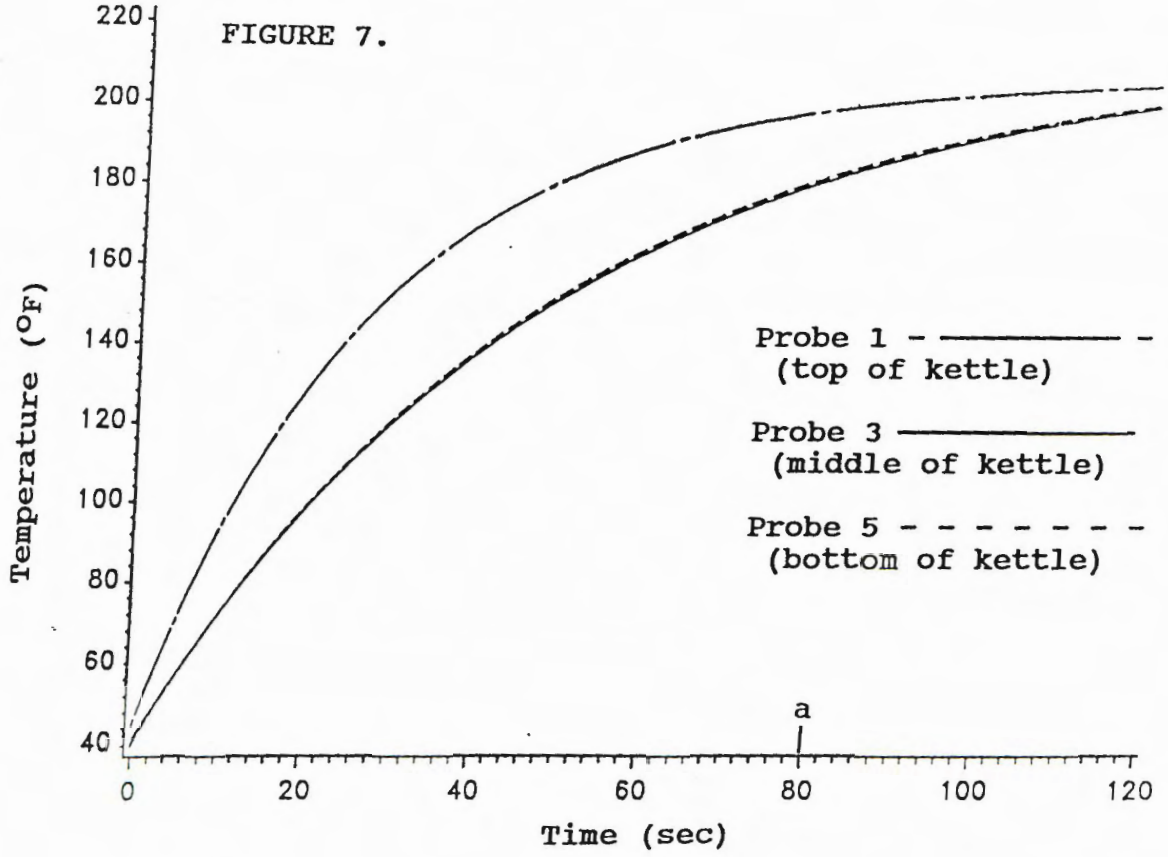
To evaluate a process(46), an F value, defined as the number of minutes required to destroy a given number of organisms at a given reference temperature, must be established. The reference temperature chosen was 140°F and the D value of 0.36 was multiplied by 12 to obtain an F value at 140°F = 4.32 min.

To determine at what point the established F value was attained during the boiling water blanch, the data from the slowest heating point in the product were analyzed by the general method. This method measures the amount of lethal heat equivalent to a reference temperature (in this case, 140°F) that was delivered at each time interval. During the blanch there is a gradual

FIGURE 7. Heating profile of diced raw celery in a commercial boiling water blanch (40 gal of water at 212°F and 50 lbs of celery at 40°F) without agitation

(figure on following page)

FIGURE 7.



a 80 sec - Recommended 2.0 min at 158°F achieved by Probe 3 at the cold spot

TABLE 3. Heat penetration data and cumulative lethality in diced celery during 65 sec of a boiling water blanch in a commercial setting related to the number of D values delivered. (Reference temperature = 140°F, z = 9).

Time (sec)	Temp. (°F)	lethal rate (5 sec)	lethality at the interval	equivalent cumulative lethality	# D values obtained (D = 0.36 min)
0	40.0				
5	57.5				
10	72.8				
15	86.6				
20	99.0				
25	110.0	.000038			
30	120.3	.000537	.00028		
35	129.3	.005372	.00295	.0032	
40	137.5	.043782	.02457	.0278	
45	144.9	.290751	.16726	.1951	.54
50	151.5	1.573446	.93210	1.1271	3.13
55	157.5	7.303357	4.43840	5.5656	15.46[a]
60	162.9	29.075188	18.18927	23.7548	65.98
65	167.7	99.278651	64.17691	87.9317	244.25

[a]15D obtained - 5.56 min of heat received at 55 sec exceeds predicted 12D process at 140°F = 4.32 min determined in this study.

rise in product temperature, the rate being dependent on the physical characteristics of the product(46). Once temperatures exceed the growth range for the organism in question, there is some lethal effect during each time interval of the blanch. Using the z = 9, it can be determined that the equivalent lethal heat for one minute of heating at 140°F is 10 minutes at 131°F, 0.1 min at 149°F and 0.01 min at 158°F. For each 9°F drop in temperature, the time necessary to obtain the equivalent bacterial destruction increases 10 times and for each 9°F increase in temperature, it decreases 10 times. To determine the lethal rate or lethality effective in one minute at other temperatures, the reciprocal of the equivalent time is determined, e.g. one minute of heat at 131°F is equivalent to 0.1 min of heat at 140°F. This equivalent lethality can also be expressed by the formula:

$$\text{lethal rate (L)} = \frac{1}{\log^{-1} \frac{(RT - T)}{z}}$$

where RT is the reference temperature, and T is the temperature achieved at the interval.

The GenGen program determined the lethal rate for 5 sec at each temperature of the blanch (Table 3, column 3). The lethality during each 5 sec interval was then

determined (Table 3, column 4) using the following summation technique:

$$\frac{L_1 + L_2}{2} + \frac{L_2 + L_3}{2} \cdots\cdots \frac{L_{n-1} + L_n}{2}$$

The values at each interval were then summed to provide the cumulative lethality (Table 3, column 5). The established F value of 4.32 min at 140°F determined in this study was exceeded during 55 sec of blanch (cumulative lethality = 5.566 min).

In addition to using 140°F as a reference temperature, two other reference temperatures were evaluated. Lethality was calculated (Table 4) at the HTST temperature of 71.7°C(161°F) because the literature contains many references to D values for L. monocytogenes in milk and it is of interest to determine a comparable heat treatment in a boiling water blanch. Also, in response to the recommendation in two papers(31,49) that products be heated to an internal temperature of 70°C(158°F) for two minutes, lethality was also calculated at 158°F (Table 5). In both cases a z of 18°F was used because it exceeds published z values for L. monocytogenes. Table 5 shows that at 80 sec of blanch the diced celery had been exposed to 2.5 min of

heat at 158°F which exceeds the 2.0 min recommendation by 0.5 min.

TABLE 4. Heat penetration data and cumulative lethality in diced celery during a 2 min boiling water blanch in a commercial setting. (Reference temperature = 161°F, z = 18°F).

Time (sec)	Temp. (°F)	F value (equivalent cumulative lethality)
0	40.0	
5	57.5	
10	72.8	
15	86.6	
20	99.0	
25	110.0	
30	120.3	
35	129.3	
40	137.5	
45	144.9	
50	151.5	
55	157.5	
60	162.9	.15
65	167.7	.29[a]
70	172.0	.56
75	176.0	1.01
80	179.0	1.71
85	182.8	2.81
90	185.7	4.46
95	188.3	6.79
100	190.6	9.99
105	192.3	14.10
110	194.6	19.43
115	196.6	26.42
120	197.9	35.02

[a] 0.29 min exceeds HTST process of 0.25 min at 161°F

TABLE 5. Heat penetration data and cumulative lethality in diced celery during a 2 min boiling water blanch in a commercial setting. (Reference temperature = 158°F, z = 18°F).

Time (sec)	Temp. (°F)	F value (equivalent cumulative lethality)
0	40.0	
5	57.5	
10	72.8	
15	86.6	
20	99.0	
25	110.0	
30	120.3	
35	129.3	
40	137.5	
45	144.9	.01
50	151.5	.04
55	157.5	.09
60	162.9	.21
65	167.7	.43
70	172.0	.82
75	176.0	1.49
80	179.0	2.51[a]
85	182.8	4.11
90	185.7	6.54
95	188.3	9.98
100	190.6	14.46
105	192.3	21.20
110	194.6	28.74
115	196.6	38.50
120	197.9	50.52

[a] Recommended 2.0 min at 158°F exceeded by 0.51 min.

DISCUSSION

In this study, D values for L. monocytogenes in Tryptic Soy Broth with 0.6% yeast extract, pH 5.3, were determined aerobically using a sealed, submerged tube technique, and a nonselective recovery medium with incubation at 30°C. There are comparatively few data on the heat resistance of L. monocytogenes and most of the published values are obtained in milk(31). One heat resistance study(7) was conducted using L. monocytogenes Scott A in cabbage juice at pH 4.6 and 5.6. By withdrawing aliquots of juice from a flask heated in a waterbath at 56°C, the D values obtained at 56°C were 3.64 and 6.80 with z values of 7.01 and 5.63°C respectively. These values are higher than the value obtained in this current study at 56°C which was D = 1.29, z = 5°C. This could be attributable to the method used to determine heat resistance - methods other than the sealed, submerged tube method used in this study are reported to provide inaccurate D values(23). In addition, a number of factors(29) affect heat resistance and contribute to the variability in D values including the composition of the substrate in which the cells are heated; the pH of the heating substrate - an increase in acidity hastens killing by heat; and differences in the

history of the cells used such as culture medium in which the cells are grown and incubation temperature.

Blanching in hot water, ranging from 190-212°F, has been used by food processors to stop enzymatic action in raw vegetables and has been shown to reduce bacterial loads(38). In this study, diced raw celery was inoculated at a level of 10^7 L. monocytogenes/g. In the laboratory, after 0.5 min of exposure to a boiling water blanch, L. monocytogenes was not recovered. The organism was recovered after 0.25 min of heating. This is consistent with the D value at 140°F of 0.36 min in that temperatures exceeding 140°F were not recorded in the laboratory simulated blanch until after 0.25 min of heating.

To determine the heating profile of a large quantity (50 lbs) of refrigerated diced celery during a boiling water blanch under actual processing conditions, heat penetration studies were conducted in a steam kettle in a food processing plant. The cold spot was found to be in the middle of the kettle. The times and temperatures obtained in this cold area were converted to lethal values at a reference temperature of 140°F in order to evaluate the behavior of L. monocytogenes that could be a potential contaminant in the celery. The D value of 0.36 min at 140°F in TSBYE was predicted using a z value of 9°F. This D value and z value were

determined for use as a reference in predicting the lethality in a boiling water blanch. It was found that a 12D process, based on a D_{140} = .36 min, was delivered to the celery 55 sec into the blanch. Once the celery temperature exceeded the reference temperature of 140°F, lethality was accumulated rapidly.

The lethality values in this study were determined at 140°F because the reference D value for the strain used was predicted at this temperature. Two reports(31,49) recommend that the slowest heating point in a product should be held for 2 min at 70°C(158°F) to ensure inactivation of L. monocytogenes. In this study, using a z of 18°F it was found that the recommended heat treatment was achieved 80 sec into the blanch. In addition, the times and temperatures were also evaluated to determine the equivalent lethality for reference temperature of 161°F, a temperature higher than recommended. A z of 18 was chosen as a reference because it exceeds published z values and would predict that target organisms, such as L. monocytogenes, would be reduced less quickly at temperatures exceeding the reference temperature, ie. a tenfold reduction would occur with every 18°F increase in temperature rather than every 9°F as determined in this study. Using this conservative approach in evaluating the lethality, it was determined that an F value, or 12D process = 35 min

at 161°F was achieved in the two minute boiling water blanch (Table 4). In 95 sec, an F value of 6 min was achieved which would provide a 6D reduction for an organism having a D value of 1.0 min at 161°F. D values this high have not been reported for L. monocytogenes.

CONCLUSIONS

Contamination of fresh raw vegetables with L. monocytogenes should be anticipated. In this study, a boiling water blanch, applied to diced celery, provided sufficient lethality at 140°F to inactivate L. monocytogenes at a level of 1 x 10^7cells/g. Based on the heating profile of the diced celery, the recommended heating of 2 min at 70°C (158°F) was accomplished in 80 sec.

In view of these results, a boiling water blanch could be used as part of the hurdles approach to eliminate L. monocytogenes from raw diced celery. Problems could arise due to variations in heating, employee error or heavily contaminated product. Therefore, if a blanch is chosen as a barrier in the hurdle approach to eliminating L. monocytogenes in raw vegetables destined for use in non sterile, refrigerated composed salads, the following recommendations are made:

a) maintain proper sanitation, in particular, the raw product should be thoroughly washed before blanching,
b) determine the times and temperatures applied during the blanch in a particular plant situation to ensure adequate lethality is provided to the cold spot,

c) educate employees as to the purpose and importance of the blanch, and

d) apply additional hurdles such as chlorination, acidification, or preservatives.

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