

Reduction of Microflora of Whole Pickling Cucumbers by Blanching

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ABSTRACT: There is increasing interest in developing methods to control the presence of pathogenic and spoilage microorganisms on fresh fruits and vegetables. Blanching whole pickling cucumbers for 15 s at 80 °C reduced microbial cell counts by 2 to 3 log cycles from an initial population of typically 10^6 CFU/g. Vegetative microorganisms survived this blanching process (10-fold greater in number than the spore count), presumably because they were located beneath the surface of the cucumber. The sensitivity to heat of selected populations was measured by determining D values for pooled microorganisms (termed D_p values) isolated from fresh cucumbers. The *Enterobacteriaceae* population and the total aerobic microflora had similar D_p values to each other and to the D value for a selected lactic acid bacterium.

Key Words: blanching, decimal reduction time, vegetable microflora

Introduction

MINIMALLY PROCESSED FRUIT AND vegetable products are nutritious, convenient, and growing in popularity (Hurst and Schuler 1992). As the fresh-cut produce industry has expanded, sporadic outbreaks of infectious disease have occurred, prompting questions about the general safety of these products. Microbial populations of fresh fruits and vegetables may range from 10^2 to 10^8 or greater (Lund 1992; Nguyen-the and Carlin 1994). Pathogenic microorganisms have been associated with fresh fruits and vegetables, and, in some cases, have been directly linked to disease outbreaks (Hwang and Beuchat 1995; Nguyen-the and Carlin 1994). Researchers have investigated a variety of washing and sanitizing treatments to remove bacteria from fruits and vegetables. In general, these methods result in only a 1- or 2-log reduction in cell counts (Adams and others 1989; Hwang and Beuchat 1995). Examples of washing treatments for fruits and vegetables include the use of chlorine compounds (Beuchat and others 1998), hydrogen peroxide, and trisodium phosphate, singly and in various combinations. In our laboratory we have found that chlorine dioxide is effective at reducing the numbers of bacteria in recycled cucumber hydrocooling wash water, but it does not effectively aid in reducing the numbers of microorganisms on/in the cucumbers themselves (Reina and others 1995).

The ineffectiveness of washing treatments in removing bacteria from fresh fruits and vegetables has been attributed to a variety of factors. Microbial biofilms, hydrophobic pockets, waxy cuticles, narrow grooves, and hollows or tissue damage may all contribute to the inability of sani-

tizing agents to contact and reduce the number of bacteria of fruits and vegetables (Adams and others 1989; Nguyen-the and Carlin 1994; Smith and others 1979; Zhang and Farber 1996). Inactivation of sanitizing agents by organic material and plant tissue components prior to contact with bacteria of plant surfaces may also be involved (Lundstedt 1983). In addition, there has been evidence for the presence of microorganisms located within healthy tissue of cucumbers and other fruits. Bacteria may gain entrance to the interior of cucumbers through stomata (Daeschel and Fleming 1981). The presence of enteric bacteria has been described in the interior of healthy cucumbers (Meneley and Stanghellini 1974). We, therefore, chose to investigate the use of heat (blanching procedures), which, unlike chemical washes that only affect the surface of the fruit, can penetrate the cucumber tissue and kill bacteria.

The goal of our research was to determine if blanching can be used as a practical means to reduce the microflora on fresh pickling cucumbers. This implies that sensory properties of the fresh cucumbers should be maintained with a reduction in microflora equal to or greater than that achievable with chemical washes. Previous attempts to reduce the numbers of microorganisms of fresh pickling cucumbers in our laboratory by washing or using chlorine-based sanitizing agents typically resulted in only a 1- or 2-log cycle reduction in total aerobic microflora. Reduction in the microflora of pickling cucumbers is desired for improving the safety of non-acidified refrigerated pickles (Breidt and Fleming 1997), including "half sour" pickles, which typically have a brine pH above 5. There-

fore, we determined how blanching conditions affected the reduction in various microbial populations of the cucumbers (*Enterobacteriaceae*, lactic acid bacteria, and aerobic and anaerobic spores) which may affect the safety and quality of these non-acidified brined cucumber products. We also considered that blanching may be used to reduce the aerobic microflora prior to controlled cucumber fermentations. This may be necessary to allow the predominance of a starter culture over the naturally present lactic acid bacteria for controlled cucumber fermentation processes currently under development (Fleming and others 1995).

To determine the effects of blanching on the cucumber microflora, we measured decimal reduction times for populations of microorganisms recovered on agar media from fresh cucumbers. To distinguish these studies from pure culture experiments, we used the term D_p and Z_{D_p} in place of D and Z_D to refer to D and Z values for a population. While use of these methods on mixed populations is unconventional, we found D_p or D value data to be useful in determining how heat was affecting the total aerobic microflora, the *Enterobacteriaceae* population, and selected lactic acid bacteria commonly found on fresh cucumbers. This methodology may have application for studying the effects of other antimicrobial agents or treatments for fresh or minimally processed foods.

Materials and Methods

Bacterial strains and growth media

Lactobacillus plantarum LA70 (ATCC 14917) and *Escherichia coli* B178 (ATCC 11775) were obtained from the USDA

Food Fermentation Laboratory Culture Collection (Raleigh, N.C., U.S.A.) PCA (Difco Laboratories, Detroit, Mich., U.S.A.) plates were used for enumerating total aerobic microflora from cucumber samples and for enumerating all bacterial samples after heat treatments. Lactic acid bacteria were grown on MRS agar (MRS broth, Difco, supplemented with 1.5% agar). The MRS agar plates were supplemented with 0.02% sodium azide (Fisher Scientific Co., Fairlawn, N.J., U.S.A.), to prevent the growth of yeasts and molds when plating cucumber samples (Fleming and others 1992). *Enterobacteriaceae* were plated on VRBG agar (VRB agar, Difco, supplemented with 0.5% dextrose, Sigma Chemical Co., St. Louis, Mo., U.S.A.).

Blanching procedures

Size 2B pickling cucumbers (3.5 to 3.8 cm in dia) obtained from both retail and commercial sources and free of obvious mechanical damage or microbial spoilage were washed to remove dirt and other debris. The cucumbers were then placed in a wire basket and submerged in water for the indicated time-temperature conditions. Immediately upon removal from the blanch water, samples of approximately 300 g cucumbers were placed in blender jars containing an equal weight of ice-cold sterile saline. The cucumbers were then homogenized and processed as described below.

General microbiology

Bacterial cell counts were determined using a spiral plating machine (Autoplate 4000, Spiral Biotech, Bethesda, Md., U.S.A.) and an automated colony counter (Protos Plus, Microbiology International, Frederick, Md., U.S.A.). For low cell numbers (less than 5×10^2 CFU/ml), spread plates were used with 0.1 ml spread on the agar surface, or pour plating was used with 1 ml added to molten agar at 45 °C immediately prior to plating. To prepare cells for thermal decimal reduction time experiments, 0.1 ml of the appropriate culture dilution was spread on the surface of the agar plates to give plates containing 200 to 500 colonies per plate. The plates were incubated at 30 °C for 20 h (PCA and VRBG agar) or 48 h at 30 °C (MRS agar). Cells were then harvested from the agar plates by scraping the colonies from the surface with 5 ml of sterile saline and a bent glass spreader rod. One ml of the cell suspension was vortexed, harvested in a microcentrifuge, and washed twice with sterile saline. The cell concentration was adjusted to 1×10^9 CFU/ml by determining the optical density of the diluted suspension with a spectrophotometer (Nova Spec II, Pharmacia Biotech, Inc., Piscataway, N.J.,

U.S.A.) and using a previously prepared standard curve for optical density in contrast to CFU/ml (data not shown). Fifty ml of the cell suspension was loaded into glass capillary tubes (1.5 by 90 mm, Kimax #34505, Fisher) with a syringe for D or D_p value determinations.

To plate cucumber samples, approximately 300 g of whole cucumbers (usually 3 size 2B cucumbers) were homogenized in a Waring blender (Waring Blender, Dynamic Products Corp., New Hartford, Conn., U.S.A.) with an equal volume of sterile saline. Approximately 100 ml of the slurry was then transferred to a stomacher bag (Filter bag, SFB 0410, Spiral Biotech). The stomacher bag was placed in a stomacher (Model 400, Spiral Biotech) and processed on the high speed setting for 1 min. One-ml samples for plating were removed from the filter side of the stomacher bag, transferred to a microfuge tube, and diluted appropriately (serial 10-fold dilutions) for plating. All dilutions were carried out using sterile saline (0.85% NaCl) solution. After heat treatment, all cell counts were determined on PCA plates and incubated at 30 °C for 24 h.

Spore counts were determined by a modification of the method of Thompson and Stevenson (1984); cucumber samples (300 g) were homogenized with an equal volume of 0.1% peptone water (Bacto-Peptone, Difco) and processed in a stomacher as described above. A 10-ml aliquot of the stomacher bag filtrate was heated in an 80 °C water bath for 30 min in 15 ml plastic screw cap tubes (Costar #3318, Corning Costar Corp., Cambridge, Mass., U.S.A.). Aliquots of 1 ml, 0.1 ml, or 10 μ l were mixed with 25 ml of sterile, molten tryptone glucose extract agar (TGE agar, Difco) in 50-ml plastic tubes (#403829, Corning Costar) at 45 °C and immediately poured into sterile petri plates (Falcon #1001, Becton Dickinson and Co., Lincoln Park, N.J., U.S.A.). The pour plates were incubated at 35 °C for 48 h and colonies enumerated with an automated counter as described above. For anaerobic spore counts, 10 ml of the stomacher filtrate were incubated at 100 °C in a boiling water bath for 20 min, and then samples were immediately transferred to an anaerobic hood (Coy Laboratory Products Inc., Grass Lake, Mich., U.S.A.), and 1.0 ml of a series of 10-fold dilutions was transferred to triplicate sets of tubes containing 10 ml of thioglycolate broth. The tubes were incubated in the anaerobic hood at 30 °C for 7 d and scored for growth, as determined by visible turbidity. The anaerobic spore counts were determined by the most probable number technique (Lake and Lynt 1984).

Thermal reduction time measurements and statistical analysis

All decimal reduction time measurements (D values) were carried out using triplicate samples for each of five or more time points at a given temperature. For D values (or D_p , see below), cells were heated over a time course, giving a reduction in cell numbers of at least 4 log cycles from a starting population of 10^9 to 10^{10} CFU/ml. The results were plotted and analyzed using Sigma Plot software (Version 4 or 5, SPSS, Inc., Richmond, Calif., U.S.A.). The lack of fit test was carried out using the General Linear Models (GLM) procedure of the SAS program (SAS ver. 6.12, SAS Institute Inc., Cary, N.C., U.S.A.). The decimal reduction time was calculated as the inverse of the slope of the regression line for a plot of the log of surviving CFU/ml against time. The Z_D and Z_{D_p} values were similarly determined by plotting the log of the decimal reduction time values against temperature; D or D_p values showing approximately 1 log or greater decrease over the temperature range tested were used for these calculations.

To determine D or D_p values, the glass capillary tubes (prepared with cells as described above) were heat-sealed by flaming the top with a Bunsen burner. The sealed capillary tubes were placed in a test tube rack covered with a fine wire mesh to support the tubes. The rack was placed in a heating/cooling circulating water bath (MGW Lauda model B-2, Brinkman Instrument Co., Westbury, N.Y., U.S.A.) at the appropriate temperature. In all cases, triplicate sets of capillary tubes were removed from the water bath at each timepoint. On removal from the water bath, the capillary tubes were immediately placed in an ice water bath and held for 5 min. Tubes were then submerged in sodium hypochlorite solution (5.25% commercial bleach) for 3 min. The tubes were then transferred aseptically to three successive sterile saline baths. Each tube was then placed in a glass test tube (Kimax 16 \times 150 mm screw cap, Fisher) containing 5 ml of sterile saline, and the capillary tubes were crushed aseptically with a solid glass rod (6 \times 250 mm). The glass particles were allowed to settle for 60 s, the supernatant was decanted into a microcentrifuge tube, and the contents diluted for plating, as described above.

Results and Discussion

THE EFFECTS OF BLANCHING ON THE MICROFLORA of size 2B pickling cucumbers were determined. The blanched cucumbers retained fresh color and flavor properties with no obvious loss of chlorophyll or adverse effect on texture. A controlled

study of the effects of blanching on sensory properties was not reported in this study, but is the subject of other research of our laboratory. For our initial experiments, a range of temperatures between 55 and 95 °C was chosen with a blanching time of 15 s (Figure 1). Longer blanching times were not investigated to maintain desired sensory properties of the fresh fruit (color, flavor), as determined in preliminary tests. A blanching treatment of 80 °C for 15 s was chosen for further experiments. In 20 or more independent trials with cucumbers from both retail and commercial sources, the total aerobic plate count was reduced from an initial value of log 6.28 (fresh cucumbers) to 3.85 (blanched cucumbers). The blanching treatment, therefore, resulted in a 2.43 log CFU/g reduction in cell count (a 2.48 log CFU/g reduction was predicted by the regression equation in Figure 1). To determine if the aerobic cell counts were due to heat resistant bacterial spores, we enumerated spore-forming bacteria before and after blanching (Figure 2). The total aerobic spore counts on fresh cucumbers ranged from 280 CFU/g to undetectable levels (less than 1/g), depending on the source of the cucumbers. Anaerobic spore counts could be measured only with MPN (most probable number) determinations, and were typically less than 1/g (data not shown). The cell counts for the total aerobic microflora surviving blanching at 80 °C for 15 s were at least 10-fold higher than the spore counts after blanching, which were log CFU/g of 1.7 (Figure 2). These data demonstrated that the surviving microorganisms at 80 °C (with 15 s blanching) were not simply due to the presence of heat-resistant bacterial spores, although bacterial spores were present.

To investigate the components of the total microbial population surviving

blanching at 80 °C, we selectively plated various populations of surviving bacteria after blanching for up to 2 min (Figure 3). The cell counts for lactic acid bacteria were less than 200 CFU/ml on MMRS agar, and were not enumerated. The reduction in cell counts for *Enterobacteriaceae* due to blanching was similar to the reduction seen with the total aerobic microflora (enumerated on PCA). With blanching times between 30 s and 2 min, the populations of surviving microorganisms remained unchanged between 5×10^3 and 1×10^4 for both the aerobic microflora as well as the *Enterobacteriaceae*. Because *Enterobacteriaceae* species (which do not form spores) survived up to 2 min of blanching at 80 °C, we concluded these organisms must have been protected from the heat by being located in the interior of the fruit. The ratio of total aerobic microflora to *Enterobacteriaceae* in heated cucumbers was similar to that for the microorganisms of unheated cucumbers (Figure 3).

To characterize the heat resistance of selected populations of microorganisms from fresh cucumbers, we determined the decimal reduction times for cucumber microflora grown on agar media. To distinguish these studies from pure culture experiments, we used the term D_p and Z_{Dp} in place of D and Z_D to refer to a D -value and Z -values for a population. The application of D value data to mixed populations is unconventional, but we considered it appropriate to use a mixed population for these experiments because we wanted to determine the effects of heating on groups of microorganisms instead of a single microorganism, or the most heat-resistant microorganism. Because of the possible formation of spores during growth of the microbial colonies on the agar plates used for the source of cells in

our D_p value determinations, we determined total aerobic spore counts for the cell suspension washed from the PCA plates. We found, however, that the cell suspensions obtained from the agar plates contained undetectable (less than 1 per ml of cell suspension) levels of bacterial spores (data not shown). It is possible that spore-forming organisms were present in the cell suspension, but these had not formed spores on the PCA during the time (20 h) that the plates were incubated. We investigated the effects of heat on both the total aerobic microflora and the *Enterobacteriaceae* (Figure 4). The D_p values for the total aerobic microflora and the *Enterobacteriaceae* (for cells recovered from PCA and VRBG media, respectively) over a range of temperatures between 48 and 56 °C are presented in Figure 4. To test the appropriateness of using a linear model with these data, we conducted a lack of fit test. We found that the results were not significant ($P > 0.05$). For the aerobic microflora data, the probability values for the lack of fit test were 0.603, 0.636, 0.128, and 0.380, for the D_{p48} , D_{p50} , D_{p52} , and D_{p56} data, respectively, justifying our use of a linear model. The D_p data were used to determine the corresponding Z_{Dp} values (Figure 5). The lactic acid bacteria naturally present on fresh cucumbers were too low in numbers to determine D_p values. Therefore, we determined D values for the ATCC type strains of *L. plantarum* (LA70), as well as *Escherichia coli* (B0178) for comparison with the cucumber microflora D_p values. The D values for *L. plantarum* (LA70) were 7.1 (D_{50}), 3.7 (D_{52}), 1.9 (D_{54}), and 0.92 (D_{56}), similar to the D_p values obtained for the total aerobes and the *Enterobacteriaceae* populations from the cucumbers. We noted that higher temperatures were required to obtain D values for *E. coli* (B0178) that were in the same

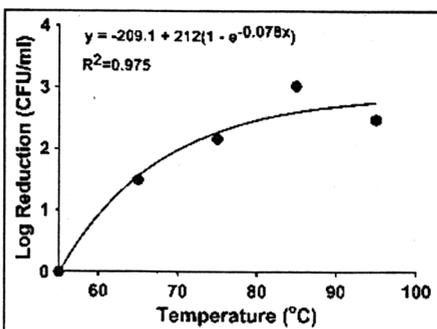


Fig. 1—A 1-Log reduction at various blanching temperatures. ● = mean values for the log reduction from four independent trials, blanching size 2B cucumbers for 15 s at the indicated temperatures. The regression line was computed from the mean values, and the standard error of the regression coefficient was 0.93.

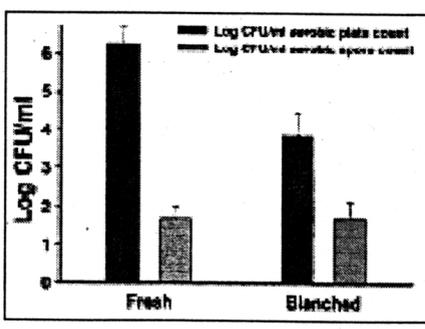


Fig. 2—Aerobic spore counts. The black bars represent the total aerobic plate count (PCA) microflora and the gray bars represent the aerobic spore counts before and after blanching for 15 s at 80 °C. The error bars indicate one standard deviation for three or more independent trials.

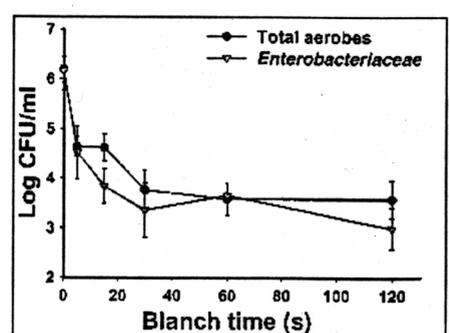


Fig. 3—Microflora surviving 80 °C blanching treatment. ▽ = surviving *Enterobacteriaceae*; ● = surviving total aerobic microflora after blanching for the indicated times. The error bars represent one standard deviation for 3 independent trials.

range as the D_p values determined for the *Enterobacteriaceae* from cucumber populations. The D values for *E. coli* (B0178) were 7.8 (D_{55}), 4.2 (D_{56}), 1.7 (D_{58}), and 0.8 (D_{60}). The aerobic microflora and the *Enterobacteriaceae* population from the fresh cucumbers, as well as *L. plantarum* LA70, all had similar D or D_p values.

Previous studies from our laboratory have shown that bacteria can gain entrance to the interior of cucumber fruit through stomata (Daeschel and Fleming 1981; Mattos and others unpublished). This can occur during the process of gas exchange (Daeschel and Fleming 1981), or during hydrocooling where a significant temperature differential exists between the initial temperature of the fruit and the colder hydrocooling water (Reina and others 1995). It is also possible that bacteria are harbored in the seeds, or otherwise gain entrance to the interior of the cucumber fruit during growth (Meneley and Stanghellini 1974). The role interior microorganisms play in fermentation or spoilage of the cucumber fruit (or the presence of potential human pathogens at this location) remains unclear.

A heat transfer model for fresh cucumbers (Fasina and Fleming unpublished), using the D_p and Z_{Dp} values obtained in this study, has allowed us to estimate that a temperature of 54 °C is not reached at 2 mm depth below the surface of fresh cucumbers (size 2B) with our 15 s blanching treatment at 80 °C. The D_{p54} value (Figure 4) for the total aerobic microflora is 60 s, 4 times the 15-s blanching time, so less than 1 log decrease in microbial cell counts would be predicted to occur at a depth of 2 mm beneath the cucumber surface. Because we saw a 3-log drop in the cell counts during blanching, we concluded that approximately 99.9% of the microorganisms were located within 2 mm or less of the cucumber surface. The data in Figure 3 show, however, that vegetative bacterial cells did survive during blanching treatments. Approximately 10^3 CFU/g of the total aerobic microflora and also *Enterobacteriaceae* (which do not form spores) were present after 2 min of blanching at 80 °C, indicating the surviving organisms were located in the interior of the fruit.

Based on the D_p and Z_{Dp} values for the total aerobic microflora, we determined that a 5-log decrease (which has recently been proposed for FDA regulation of fresh fruits and vegetables) in the initial cell count of 10^6 CFU/g of these microorganisms should require only about 0.4 s at 80 °C. This assumes, however, that the microorganisms were directly exposed to the heat. These data support the conclusion that vegetative cells of bacteria were protected from the heat treatment because

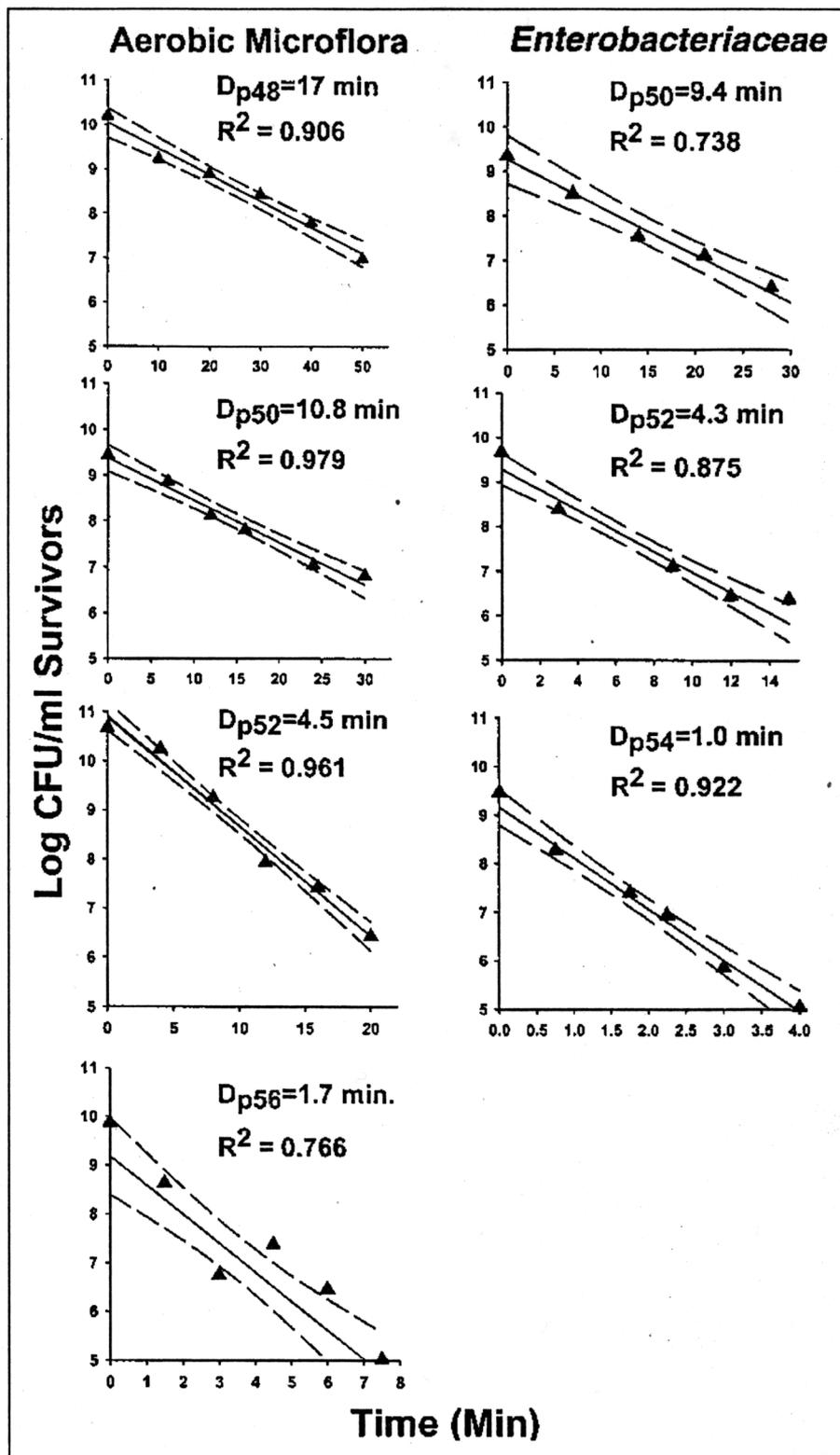


Fig. 4— D_p value determinations. D_p values were determined for the total aerobic microflora and *Enterobacteriaceae* populations isolated from fresh cucumbers at the indicated temperatures. The triangles represent mean values for triplicate determinations of the surviving microorganisms. The regression lines (solid lines) and 95% confidence limits (dashed lines) were determined using the entire data set for each graph. The x axis values vary to reflect the time required to generate a 3 or 4 log kill.

they were located in the interior of the fruit and point out the difficulty of achieving greater than a 3-log reduction in cell numbers. We recognize, however, that many

factors such as cell injury, growth phase, and others may also influence thermal inactivation of bacteria on cucumber fruit.

Blanching pickling cucumbers for 15 s

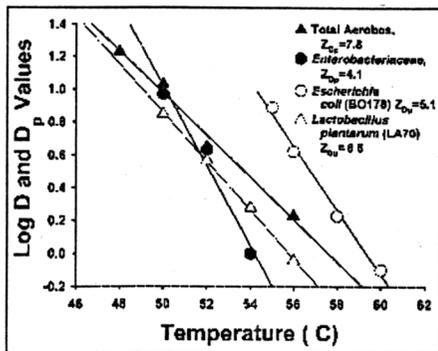


Fig. 5— Z_{D_p} and Z_D Data. The symbols represent D or D_p values for total aerobes (\blacktriangle), Enterobacteriaceae (\bullet), *Escherichia coli* B0178 (\circ), *Lactobacillus plantarum* LA70 (\triangle). The lines were generated by linear regression. The Z_D and Z_{D_p} values (Fig. 4) are the inverse of the absolute value of the slope for each regression line.

at 80 °C has been shown to be an effective means of reducing the total aerobic microflora cell counts. The development of brief blanching treatments for pickling cucumbers may, therefore, avoid problems noted above with various aqueous chemical sanitizing agents. This blanching treatment did not have obvious effects on the sensory properties of the cucumbers, or affect the ability of the cucumber tissue to gen-

erate 2,6-nonadienal or 2-nonenal (Palma and McFeeters unpublished data), the principal flavor impact components of cucumbers (Schieberle and others 1990). It is possible that similar blanching treatments may be developed to reduce bacteria cell counts on other fruits or vegetables without greatly affecting sensory quality.

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