Reduction of Microflora of Whole Pickling Cucumbers by Blanching

F. BREIDT, J.S. HAYES, AND H.P. FLEMING

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 sanitizing 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. Therefore, 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 cu http://www.journaloffoodsafety.com/content/65/8/1998.full.pdf
Reduction of Microflora.

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, Frederick, Md., U.S.A.). For low cell numbers, 0.1 ml of a series of 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 0.1 ml 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 aerobic 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 aerobic spore counts were determined by the most probable number technique (Lake and Lyt 1984).

Blanching procedures

Size 2B pickling cucumbers (3.5 to 3.8 cm in dia) obtained from both retail and commercial sources and for enumerating all aerobic microflora from cucumber 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, Frederick, Md., U.S.A.). For low cell numbers, 0.1 ml of a series of 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 0.1 ml 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 aerobic 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 aerobic spore counts were determined by the most probable number technique (Lake and Lyt 1984).

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 × 10^5 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 × 10^7 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 Dp 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.

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 Dp, 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 10^3 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 ZD and ZDp values were similarly determined by plotting the log of the decimal reduction time values against temperature; D or Dp values showing approximately 1 log or greater decrease over the temperature range tested were used for these calculations.

To determine D or Dp, 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 submersed 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 × 150 mm screw cap, Fisher) containing 5 ml of sterile saline, and the capillary tubes were crushed aseptically with a solid glass rod (6 × 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 senso- 
ry properties was not reported in this 
study, but is the subject of other research 
of our laboratory. For our initial experi-
ments, 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 pre-
liminary tests. A blanching treatment of 80 
°C for 15 s was chosen for further experi-
ments. In 20 or more independent trials 
with cucumbers from both retail and com-
mercial sources, the total aerobic plate 
count was reduced from an initial value of 
log 6.28 (fresh cucumbers) to 3.85 
(blanchined 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 re-
gression equation in Figure 1). To deter-
mine if the aerobic cell counts were due to 
heat resistant bacterial spores, we enu-
merated 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 aero-
bic 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 (data not shown). The 
spore survival of a single microorganism, or the most 
heat-resistant microorganism species (which do not 
form spores) survived up to 2 min of 
blanching at 80 °C, we concluded these or-
ganisms must have been protected from 
the heat by being located in the interior of 
the fruit. The ratio of total aerobic microf-
lora to Enterobacteriaceae in heated cu-
cumbers was similar to that for the micro-
organisms 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 mi-
icroflora grown on agar media. To distin-
guish these studies from pure culture ex-
periments, we used the term D p and Z Dp to refer 
to a D-values and Z-values for a population. The 
application of D value data to mixed popula-
tions is unconventional, but we consid-
ered it appropriate to use a mixed popula-
tion for these experiments because we 
noticed the effects of heat 

ing on groups of microorganisms instead of 
the 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 deter-
mined 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 bacte-
rial spores (data not shown). It is possible 
that spore-forming organisms were 

iven in the cell suspension, but these 

had not formed spores on the PCA during 
the time (20 h) that the plates were incu-
batated. 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 mod-
el 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 
and 56 °C data, respectively, justifying our use 
of a linear model. The D p data were used 
to determine the corresponding Z Dp val-
ues (Figure 5). The lactic acid bacteria nat-
urally 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 microf-
ora 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 val-
ues obtained for the total aerobes and the 

Enterobacteriaceae populations from the 
cucumbers. We noted that higher tem-
peratures 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 blanch-
ing for 15 s at 80 °C. The error bars indicate 
one standard deviation for three or more in-
dependent 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 
inddependent trials.
Reduction of Microflora . . .

range as the Dp values determined for the Enterobacteriaceae from cucumber populations. The D values for E. coli (B0178) were 7.8 (D53), 4.2 (D56), 1.7 (D58), and 0.8 (D60). The aerobic microflora and the Enterobacteriaceae population from the fresh cucumbers, as well as L. plantarum LA70, all had similar D or Dp 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 Dp and ZDp 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 Dp54 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 10³ 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 Dp and ZDp 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 10⁶ CFU/g of these microorganisms should require only about 0.4 s at 80 °C, indicating the surviving organisms were located in the interior of the fruit.

Blanching pickling cucumbers for 15 s . . .
Reduction of Microflora . . .

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 generate 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.

References


Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

We acknowledge Mr. R. L. Thompson for statistical advice, Dr. R. F. McFeeters for helpful discussions, and Ms. Dora Toler for excellent secretarial assistance. This investigation was supported in part by a research grant from Pickle Packers International, Inc. (St. Charles, Ill., U.S.A.).

Authors are with the U.S. Dept. of Agriculture, Agricultural Research Service, and North Carolina Agricultural Research Service, Department of Food Science, North Carolina State University, Raleigh, NC 27695-7624, USA. Direct inquiries to author Breidt (E-mail: breidt@ncsu.edu)