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Effects of a brief blanching process on quality, safety, and shelf life of refrigerated cucumber pickles

Lisa J. LaFountain[1,2] | **Suzanne D. Johanningsmeier**[1] | **Frederick Breidt Jr.**[1] | **George N. Stoforos**[2] | **Robert E. Price**[1]

[1]U.S. Department of Agriculture, Agricultural Research Service, SEA, Food Science and Market Quality and Handling Research Unit, North Carolina State University, Raleigh, North Carolina, USA

[2]Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina, USA

Correspondence
Suzanne D. Johanningsmeier, U.S. Department of Agriculture, Agricultural Research Service, SEA, Food Science and Market Quality and Handling Research Unit, North Carolina State University, 322 Schaub Hall, Box 7624, Raleigh, NC 27695-7624, USA.
Email: suzanne.johanningsmeier@usda.gov

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Abstract: Refrigerated pickles are characterized by crisp, crunchy texture, opaque flesh, and fresh flavor. Typically produced without a thermal process, microbial safety relies on preventive controls, brine composition, and sufficient hold time prior to consumption. We hypothesized that brief blanching of whole cucumbers prior to pickling could provide an additional hurdle for pathogenic microbes without negatively impacting finished product quality. Blanch treatments (15, 90, or 180 s) in 80°C water were conducted in duplicate on two lots of cucumbers prior to cutting into spears, acidifying, and storing at 4°C. Enumeration of total aerobes, lactic acid bacteria, and glucose-fermenting coliforms was conducted for fresh and blanched cucumber. Texture, color, cured appearance development, and volatile compound profiles were analyzed for fresh and blanched cucumber and corresponding pickle products during refrigerated storage. The 90 s blanch consistently achieved a minimum 2-log reduction in cucumber microbiota and a predicted 5-log reduction of *Escherichia coli* O157:H7 up to 1.1 mm into the cucumber fruit. Blanching had no impact on tissue firmness during refrigerated storage for 1 year ($p > 0.098$). There were no differences in flavor-active lipid oxidation products (E,Z)-2,6-nonadienal and (E)-2-nonenal, and consumers ($n = 110$) were unable to differentiate between control and 90 s blanched cucumber pickles stored for 62 days. Exocarp color and mesocarp opacity were preserved by the blanching treatment, potentially extending product shelf life. This method offers processors an option for reducing the risk of microbial contamination while maintaining the quality attributes associated with refrigerated cucumber pickles.

KEYWORDS
acidified foods, color, cure, *E. coli* O157:H7, GCXGC-ToF-MS, texture, two-dimensional chromatography, vegetable preservation, volatile compounds

Practical Application: Refrigerated pickles do not undergo thermal processing, which can leave them vulnerable to microbial contamination. This study illustrates that adding a brief blanching step in refrigerated pickle processing can reduce indigenous microbiota without negatively impacting quality attributes.

This blanching process could assist pickled vegetable manufacturers in providing additional safeguards for consumers while maintaining a high-quality product.

1 | INTRODUCTION

Refrigerated pickles are typically produced by acidification but are held under refrigeration, so processors need not comply with the requirements for shelf-stable acidified foods under the Code of Federal regulations 21 CFR 114.3b. However, with the introduction of the Food Safety Modernization Act (FSMA) and its focus on preventive controls, refrigerated pickle manufacturers must demonstrate that these products pose no risk to human health. Although outbreaks of disease are not commonly associated with refrigerated pickles, bacterial pathogens can survive under certain conditions. Lu et al. (2013) found that one of the most acid-resistant pathogens, *Escherichia coli* O157:H7, survived for >30 days in refrigerated pickle brines. Furthermore, a 5-log reduction in *Listeria monocytogenes* strains in a fermented refrigerated pickle required about 50 days (Kim et al., 2005). Therefore, refrigerated pickles may not meet FSMA standards within a typical consumption timeframe. Supplementation of acidified product formulations with an additional preservative (Lu et al., 2013; Putnam et al., 2018) and/or high acid content at low pH (Breidt et al., 2007, 2013) achieved 5-log reductions in pathogenic microbiota within a reasonable timeline. However, formulations containing preservatives may not meet growing consumer expectations for 'preservative-free' products, and lowering the pH significantly alters product quality characteristics. Since the typical shelf life of refrigerated pickles is short (~4–6 months), storing product for extended periods prior to shipping to ensure a 5-log reduction of potential pathogens is not practical, so alternative methods to reduce the risk of bacterial pathogen survival are needed. Employing a rapid heat treatment to whole cucumbers prior to acidification may provide an additional hurdle by reducing microbial contamination on and below the cucumber surface (Mattos et al., 2005; Reina et al., 2002) and increasing microbial susceptibility to acid or salt conditions through heat-induced injury (Busta, 1976; Ray, 1979).

Acidified, refrigerated pickles have crisp texture, opaque flesh, and fresh flavor that appeals to consumers seeking fresh-like, plant-based foods. The lack of thermal process during manufacture is key to retaining these quality characteristics. Consumers associate loss of opaqueness with loss of texture or freshness, important determinants of shelf life in refrigerated pickles (Fellers & Pflug, 1967). Interestingly, mild thermal treatment of cucumbers prior to pickling preserves the mesocarp opacity and crisp texture of refrigerated pickles (Buescher et al., 2013). Similarly, hot water dip treatments preserve the texture of raw produce such as strawberries, broccoli, and potatoes (Lurie, 2006). However, such treatments rely on the entire mesocarp reaching the desired temperature and are typically conducted at low temperature (50°C) for long durations (45–60 min), which are not compatible with most large-scale producers' operations. Mattos et al. (2005) found that even at a blanch temperature of 80°C, the center of grade 3B cucumbers (~50 mm diameter) did not reach 50°C until ~15 min into the treatment. It is therefore unlikely that brief heat treatment to reduce surface microbiota will significantly affect the cucumber mesocarp, the portion of the cucumber that largely drives the perception of crispness and crunchiness in pickles (Buescher et al., 2011; Jeon et al., 1973; Thompson et al., 1982).

Processing can significantly impact the flavor quality of pickles, by either producing or limiting production of aroma active secondary oxidation products. One such product, E-Z-(2,6)-nonadienal, is generated by enzymatic oxidation of linolenic acid by lipoxygenase upon injury of cucumber cell walls (Tressl et al., 1981). This volatile compound along with 2-nonenal is largely responsible for distinctive fresh cucumber flavor (Schieberle et al., 1990). Both fermentation and pasteurization processes prevent the formation of E-Z-(2,6)-nonadienal (Buescher & Buescher, 2001; Palma-Harris et al., 2002), which influences the flavor profiles of those types of pickles. Isolated cucumber fruit lipoxygenase was previously inactivated after 2 min at 70°C and shown to be more active in the exocarp (Wardale & Lambert, 1980). However, it is not known how a brief thermal treatment of whole cucumber would influence the production of aroma-active volatile compounds in refrigerated pickles and whether these differences would significantly impact the overall flavor of the product.

The objectives of this study were to (1) determine the reduction in native cucumber microbiota that could be achieved with a brief blanching of whole cucumber in 80°C water at pilot plant scale; (2) model the predicted reduction in *E. coli* O157:H7 with whole cucumber blanching time; and (3) assess the impact of cucumber blanching treatments on refrigerated pickle quality.

2 | MATERIALS AND METHODS

2.1 | Cucumber procurement and preparation

Two lots of grade 3B (50 ± 5 mm diameter) pickling cucumbers (n = 400) were procured on two separate dates from a commercial pickle processor and transported to a pilot scale processing facility in Raleigh, NC, USA. Cucumbers were stored at 3.9 ± 1.5°C overnight. Refrigerated cucumbers were rinsed in water with mild manual abrasion to remove debris and equilibrated to room temperature. Each lot was separated into eight subgroups of 50 cucumbers. The subgroups were randomly assigned to the following four treatments, in duplicate: no blanch (control), blanch at 80°C for 15, 90, or 180 s, respectively. Cover brine was formulated based on typical commercial refrigerated pickle composition (Buescher et al., 2013; Lu et al., 2013) such that cucumber pickles equilibrated to 74 mM (0.44% wt/vol) acetic acid, 376 mM (2.2% wt/vol) sodium chloride, 6.9 mM (0.1% wt/vol) sodium benzoate to inhibit yeasts and molds and prevent fermentation, and 11.0 mM (0.12% wt/vol) calcium chloride with an equilibrated pH ~3.8.

2.2 | Blanching and pickling

Blanching was conducted using a steam-jacketed kettle filled with 132.5 L of distilled water maintained at 80°C. Cucumbers were placed in a 5-gallon, uniformly perforated (1-in. diameter holes) container that allowed unimpeded flow of water through the vessel, which was submerged completely in the hot water. The blanching vessel was removed from the water at the designated blanch time and cucumbers removed from the vessel. Randomly selected cucumbers (n = 3) from each treatment were aseptically collected for microbiological analysis. The remaining cucumbers were cut into spears using a 6-Section Vegetable Wedger (Choice, WebstaurantStore, Lititz, PA, USA), packed into 32-ounce glass jars, and covered with cold brine at a 55:45 cucumber-to-brine volumetric ratio. The jars were sealed and held at 3.9 ± 1.5°C. Spears without brine were collected for quality analysis of raw and blanched cucumber.

2.3 | Microbiological analysis

Cucumbers were aseptically removed from the sterile collection bag, cut into small pieces, and blended into a slurry. The slurry was decanted into a homogenization bag with a 250 μm side filter (Interscience Laboratories Inc.) and placed in a Stomacher® unit (Stomacher 400, Tekmar, Cincinnati, OH, USA) for 120 s on high. Filtered cucumber homogenate was diluted 1:10 using sterile 0.85% saline. Cucumber homogenates were plated on Plate Count (PCA), Violet Red Bile Glucose (VRBG), and de Man, Rogosa and Sharpe (MRS) agars (Difco, BD, Sparks, MD, USA) using a spiral plater (AP5000, Advanced Instruments, Norwood, MA, USA) to enumerate total aerobic bacteria, glucose-fermenting coliforms, and lactic acid bacteria, respectively. PCA and VRBG plates were incubated at 37°C for 24 h. MRS plates were incubated at 30°C for 48 h. Colony-forming units (CFU) were determined using an automated plate reader (Q-Count, Advanced Instruments). Non-linear reduction curves were modeled using a Weibull function (Breidt et al., 2005). Missing values (plate counts of zero) were replaced with log transformed random values between 1 and 82.

2.4 | Quality analyses

Texture, color, and cure appearance development (CAD) analyses were conducted on fresh cucumbers (control), blanched cucumber samples post-blanching, and refrigerated pickles during storage (approximately 3, 30, 60, 120, 180, 240, 300, and 365 days). At each time point, jars of pickles were removed from refrigerated storage and equilibrated to room temperature. Samples were photographed and analyzed in a randomized order for texture and color.

2.4.1 | Cure appearance development

Pickle spears (n = 15) were removed from each jar and placed on black corrugated plastic. Photographs were taken on an iPhone 6s, using an overhead mount to control distance between camera and spears. Each photograph was blind-coded using a three-digit numerical code. Three assessors were trained using a reference scale (Figure S1) and several randomly selected photographs to ensure correct usage of the scale. The order of presentation of each photograph (n = 128) was randomized for analysis using a random number generator, into five sets of 25 or 26 photographs. Panelists assessed one set of photographs per day for 5 days, taking a 5-min break after every seven photographs to reduce fatigue. An average CAD score (%) was calculated for each individual spear based on scores given by the three assessors.

2.4.2 | Exocarp color

Exocarp color measurements were taken using a calibrated CM-700d Spectrophotometer fitted with a 3-mm aperture (Konica Minolta Sensing Americas, Ramsey, NJ, USA). The handheld spectrophotometer was placed on a lab bench with the aperture facing up. L^*, a^*, b^*, C^*, and h^* measurements were taken by placing the center of each spear exocarp onto the aperture, ensuring that the sample covered the entire aperture.

2.4.3 | Texture

A 6-mm slice from the center of each of 15 spears per sample was taken using a twin blade sample preparation tool (Stable Micro Systems, Godalming, UK). A TA.XTPlus Texture Analyzer (Stable Micro Systems) fitted with a 3-mm diameter blunt probe was used to puncture the mesocarp of each slice (Thompson et al., 1982; Yoshioka et al., 2009), and peak force (N) was recorded using Texture Exponent software (Stable Micro Systems).

2.4.4 | Statistical analysis

Texture and appearance (color and cure appearance) data were analyzed using JMP Software (SAS, Cary, NC, USA). Prior to statistical analysis, data for individual spears (subsamples) were averaged to provide a sample mean for each independently replicated blanch treatment and storage time. A general linear model two-way analysis of variance (ANOVA) was used. Statistical significance was indicated at $p < 0.05$.

2.5 | Sample preparation for volatile compound profiling

Cucumber and refrigerated pickle samples (~1-inch sections of 15 individual spears per jar) were collected, vacuum sealed in polyethylene bags (FoodSaver® FM5000, Atlanta, GA, USA), and stored at −80°C. Uncapped headspace vials (10 ml) were placed in an anaerobic chamber 24 h prior to sample preparation. Prior to sample preparation, headspace vials were prepared with the addition of 0.4 g NaCl, 886 µl deionized H_2O, and 4 µl 3N H_2SO_4. Samples were removed from −80°C storage and held in a temperature monitored ice water bath maintained between 0 and 1°C. The water bath was placed in an anaerobic chamber, where the remaining sample preparation occurred. Once thawed, the contents of each bag were added to a 250 ml stainless steel blender jar and blended for 60 s using a 1-speed, 700G Waring blender base (Model WF2212112, Conair Corporation, Stamford, CT, USA). An aliquot of the blended slurry (100 µg) was added to a sample vial, immediately capped, and placed on ice. Internal standard (d-11 hexanoic acid) was added to each sample. Prepared samples were removed from the anaerobic chamber, vortexed for 20 s, and placed in the CombiPal autosampler (Model CTC Analytics (Switzerland), LEAP Technologies, Carrboro, NC, USA) refrigerated sample tray (~4°C).

2.6 | Volatile compound profiling

Volatile compound analysis was conducted for samples, water blanks, and alkane standards, using Solid Phase Microextraction (SPME) two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS), as described in Johanningsmeier and McFeeters (2011) with the modified sample preparation and dilutions described above, and a 3 s second-dimension separation time with 0.6-s hot pulse. Data were processed using ChromaTOF® software (LECO Corporation, St. Joseph, MI, USA) as described by Johanningsmeier and McFeeters (2011) and exported to Excel 2010 for peak area log transformation and missing values replacement. Data were modeled using ANOVA and Principal Component Analysis of log transformed peak areas in JMP Genomics 9.4 (SAS Institute). Differentiating compounds were identified using an FDR adjusted p-value of 0.05.

2.7 | Consumer sensory testing

Subjects were recruited under approval from the NC State University Institutional Review Board (IRB #14038), and informed consent was obtained from all participants. A Tetrad discrimination test (ASTM, 2015) was conducted with 110 consumers (based on alpha and beta values of 0.05 and a delta value of 1) to compare cucumber pickles (control) with blanched cucumber pickles (90 s) after 62 days of refrigerated storage, which is a typical consumption period (Personal communication with pickle manufacturers). Participants received two blind-coded samples of cucumber pickles produced without blanching (control) and two blind-coded samples of cucumber pickles produced with a 90 s blanch step. Samples were coded with 3-digit random numbers and presented in one of the six possible sample orders, randomized among participants. Participants were directed to consume a portion of each sample and group the four blind-coded samples into two groups of similar samples. The number of participants who had correctly grouped the samples by their corresponding treatment was counted and compared to the

chance value of guessing the correct grouping combinations (ASTM, 2015). For this study, the minimum number of correct responses was 45.

2.8 | Thermal inactivation study

A thermal inactivation study was conducted to determine the D & z values of a pathogenic *E. coli* cocktail in fresh cucumber. Five strains (B200, B201, B202, B203, and B204) of *E. coli* O157:H7 (USDA-ARS Food Science and Market Quality and Handling Unit Culture Collection, Raleigh, NC, USA) were each inoculated in triplicate, into separate 15 ml conical centrifuge tubes containing 5 ml Luria-Bertani (LB) broth, and allowed to grow statically overnight at 37°C. After incubation, the five strains of *E. coli* were combined in a 50 ml conical centrifuge tube, in triplicate, to produce three independent bacterial cocktails. The 50 ml tubes were centrifuged at 2988 *g* for 15 min. The supernatant was poured off, and the pellet re-suspended in 5 mL sterile filtered, 100% cucumber homogenate (pH 6.4, adjusted using 3 M sodium hydroxide) prepared as described previously (Dupree et al., 2019), without the final dilution step. Three sterilized, water-jacketed flasks were connected in series to a hot water bath (50°C). A magnetic stir bar was added to each of the three flasks, which were then placed on a stir plate, and filled with 99 ml of 100% cucumber homogenate, adjusted to pH 6.4 to represent the average pH of fresh pickling cucumber. A sterilized thermocouple was inserted into each of the flasks and connected to a recording device to allow monitoring of cucumber homogenate temperature. Once the cucumber homogenate reached 50°C, it was held at temperature for 30 min prior to continuing. Each independent *E. coli* cocktail (1 ml) was added to a unique flask, resulting in 10^7 to 10^8 CFU/ml initial cell counts. A 1 ml sample of the cucumber homogenate was removed from each flask immediately following inoculation and pipetted into a microcentrifuge tube held on ice. Subsequent 1 ml samples of the inoculated cucumber homogenate were removed at 60-, 120-, 180-, and 240-min post-inoculation. Each 1 ml sample was immediately diluted 10-fold into 0.85% room temperature saline. Serial 10-fold dilutions were prepared and plated as described above for enumeration on Luria Agar (LA) medium. This study was repeated with temperatures of 52, 54, 56, and 58°C. Plating times were adjusted as needed for each temperature to allow four to five sampling times before counts were below limit of detection (Table S1). *D* and *z*-values were determined by regression as previously described (Breidt et al., 2005, 2010).

2.9 | Thermal modeling

Predictive thermal modeling of temperatures within a grade 3B cucumber during 80°C blanch treatments was performed with Multiphysics Software COMSOL 5.2a (Comsol Inc., Burlington, MA, USA). COMSOL 5.2a computational fluid dynamics (CFD) module was used to simulate and numerically evaluate the heat transfer of the 3B cucumber during blanching. A time-dependent, 3D CFD model was developed, using cylindrical coordinates (Table S2) for the examined 3B cucumber. The model comprised differential equations of heat transfer in solids, described by Fourier's law (Equation (1)), and thermo-physical properties of cucumbers set forth by Fasina and Fleming (2001) (Table S3), to obtain the temperature data of an average 3B cucumber during blanching, with a preset convergence limit of 1×10^{-3}. Finer mesh grid was used for the discretization of 3B cucumber geometry, with a total of 10,350 mesh cells.

The boundary conditions at the external surface of the 3B cucumber were modeled based on the heat flux, q (W m^{-2}), across the external surface of the examined cucumber (Equation (2)). Natural convection was applied as the boundary conditions of the model, using a heat transfer coefficient (h) of 1168 W/(m^2·°C), and assuming a constant temperature for the surrounding water at 80°C, while the initial temperature of the cucumber was at room temperature (20°C). Library database of COMSOL 5.2 was used as the source for the temperature-dependent properties used for water.

$$\rho \cdot C_P \cdot \frac{\partial T}{\partial t} = \nabla \cdot (k \cdot \nabla T) \tag{1}$$

where ρ is the density (kg m^{-3}), C_p is the specific heat capacity at constant pressure (J kg^{-1} K^{-1}) of 3B cucumber, k is the thermal conductivity (W m^{-1} K^{-1}), and T is the time-dependent temperature function (°C) of 3B cucumber.

$$q = h \cdot (T_{ext} - T) \tag{2}$$

where T_{ext} is the temperature (°C) of the surrounding water, constant at 80°C, and T is the time-dependent temperature function of 3B cucumber surface.

Using a time step of 0.5 s, the time-temperature data were obtained from the surface of the cucumber to the geometric center at 0.1-mm intervals, and were used along with D and z values, determined during the thermal inactivation study, to calculate a predicted log reduction (CFU/ml) of pathogenic *E. coli* during blanch treatments.

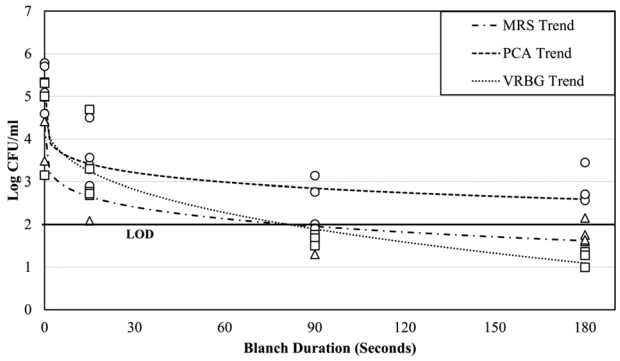

FIGURE 1 Reduction in natural microbiota of cucumbers following blanch treatments at 80°C. Curves for lactic acid bacteria (Δ), total aerobes (○), and glucose fermenting coliforms (□) were fitted with a Weibull model. Values below the limit of detection (LOD) were imputed

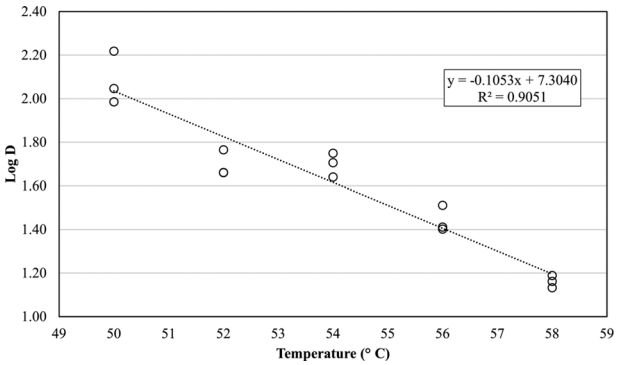

FIGURE 2 Log D values of *Escherichia coli* O157:H7 using three independent samples at five temperatures (50, 52, 54, 56, and 58°C). Calculated z-value = 9.49°C

3 | RESULTS AND DISCUSSION

3.1 | Cucumber microbiota

Cucumber microbiota decreased non-linearly with increasing time of blanch treatments (Figure 1). The estimated duration of an 80°C blanch treatment required for a 2-log reduction of total aerobes, lactic acid bacteria (LAB), and glucose-fermenting coliforms was 5 ± 6.5 s, 28 ± 10.2 s, and 34 ± 5.6 s, respectively. The time to obtain a 2-log reduction in aerobes was consistent with the 15 s reported by Mattos et al. (2005). However, the reduction in this population was not indicative of LAB and glucose-fermenting coliforms, which required longer blanching times to achieve a 2-log reduction. Nonetheless, the 90 s blanch treatment delivered at least a 2-log reduction in total aerobes, LAB, and glucose-fermenting coliforms consistently across cucumber lots and blanching batches.

3.2 | Thermal modeling of pathogen reduction

Thermal modeling of pathogen reduction assists researchers by predicting the effect of a thermal treatment on a product. The z value of *E. coli* O157:H7 in a model cucumber homogenate system was calculated based on five different temperatures (50, 52, 54, 56, and 58°C) and found to be 9.49°C (Figure 2). This value is similar to the 9.66°C z-value of *E. coli* O157:H7 in cucumber juice with a pH of 4.6 (Breidt et al., 2014). The predicted log reduction of *E. coli* O157:H7 for a given depth up to 6 mm below the surface of the fruit for each of the three blanch treatments

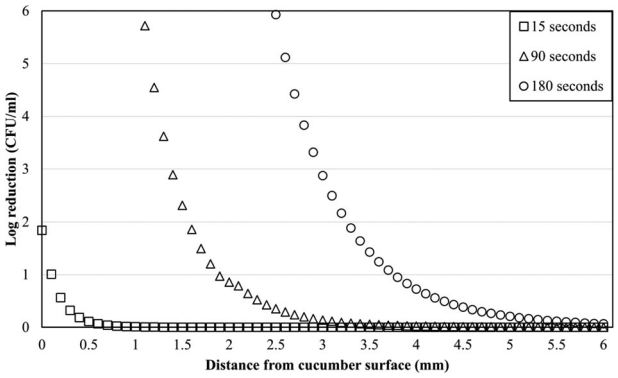

FIGURE 3 Computed log reduction of *Escherichia coli* O157:H7 on the surface and up to 6 mm deep into a size 3B pickling cucumber blanched at 80°C for 15 s (□), 90 s (Δ) and 180 s (○) using z value = 9.49°C and D_{50} = 125.22 s

was computed in COMSOL (Figure 3), using z = 9.49°C and D_{50} = 125.22 s. Mattos et al. (2005) determined that the critical depth, where most microorganisms are found within a cucumber, was between the surface and 0.65 mm deep into the fruit. Based on the thermal model for whole cucumber and the heat sensitivity of *E. coli* O157:H7, both the 90 s and 180 s blanch treatments should be able to achieve at least a 6-log reduction for *E. coli* O157:H7 within this depth range. This thermal model indicates that a 90 s blanch could achieve a 5-log reduction in these microorganisms up to 1.1 mm deep into the cucumber tissue. Moreover, a 5-log reduction could be achieved at the critical depth of 0.65 mm with a blanch duration of approximately 59 s. These results demonstrate that a whole cucumber blanch treatment could assist processors in reducing the potential for pathogenic survival in refrigerated pickles.

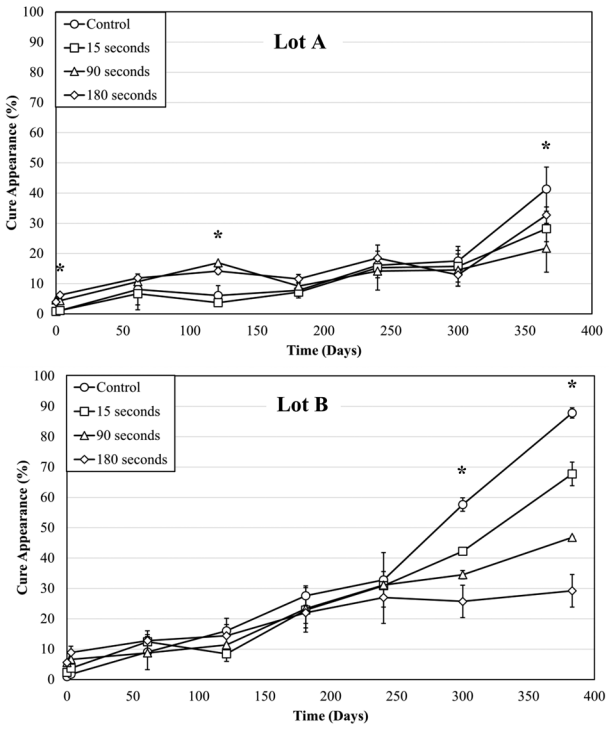

FIGURE 4 Cure appearance development (CAD) in refrigerated cucumber pickles during storage at 4°C for two lots of size 3B cucumbers (A & B) subjected to various whole cucumber blanching times in 80°C water. Time points with an asterisk denote significant differences between treatments at an alpha value of 0.05

3.3 | Cure appearance development

As expected, CAD was one of the major quality changes during refrigerated pickle storage, consistent with previous studies (Buescher et al., 2013; Howard & Buescher, 1993) and commercial products (Personal communication with pickle manufacturers). CAD results from proteolytic changes in cucumber cell wall constituents (Buescher et al., 2013; Howard & Buescher, 1993; Mok & Buescher, 2012) that are perceived as a shift from an opaque to translucent appearance due to movement of liquid into gas-filled intercellular spaces (Corey et al., 1983a, 1983b). The onset and severity of CAD differed greatly between lots of cucumbers (Figure 4). Lot-to-lot variation in cucumber protein content, proteolytic activity, and/or redox potential would likely explain these differences in the rate of CAD (Howard & Buescher, 1993; Mok & Buescher, 2012). Despite these differences, significant interactions between treatment and storage time were found for both lots ($p = 0.0015$ and $p < 0.0001$, respectively). Cucumbers in lot A exhibited less than 20% cure for most of the storage period, but significant differences in cure were apparent between treatments after a year of refrigerated storage ($p = 0.0190$). Control pickles exhibited a higher average CAD (~42%) than pickles prepared with cucumbers that were first blanched for 90 s at 80°C (~22% CAD). Cucumbers in lot B underwent a much more rapid cure, and differences in cured appearance between treatments were more prominent, especially between the control and 90 s treatment (Figure S2). Despite both the control and 90 s treatment exhibiting an increasing level of cure throughout the shelf life of the refrigerated pickles, the rate of cure onset for control samples was much higher than 90 s treatment samples during extended storage (240–383 days). Interestingly, for cucumbers in lot B, the 180 s treatment appeared to maintain a similar level of cure throughout the shelf life (Figure 4). Buescher et al. (2013) found that heat conditioning whole cucumbers for 45 min aided in maintaining a minimally cured appearance (<20% CAD over a 12-month shelf life) by production of small peptides and stabilization of the cucumber cell wall polysaccharides and proteins during refrigerated storage. During the heat conditioning in Buescher's study, mesocarp and endocarp tissue reached ~48–49°C. The 180 s blanch in this study is predicted to achieve similar temperatures up to 5.7 mm deep into the cucumber (Figure 5), which is approximately one-third of the mesocarp depth, suggesting that some heat conditioning could be taking place around the periphery of the cucumber. Based on an approximate mesocarp depth of 16 mm, the predicted temperature range of the entire mesocarp tissue is between 21 and 71°C after a 180 s blanch treatment. This study shows that a 90 s or longer blanch of whole cucumbers may extend the shelf life of refrigerated pickles by delaying development of a cured appearance.

3.4 | Exocarp color

Exocarp color of refrigerated cucumber pickles is immediately noticeable to consumers due to the transparent glass packaging. Differences in product appearance, including color, can have a significant impact on consumer perception of a product (Wilson et al., 2015). The hue of the exocarp is especially important, as it determines what we perceive as 'color' such as green or yellow. Hue angle of cucumber exocarp blanched at 90 and 180 s was found to be significantly lower (more yellow) than the exocarp of the control cucumber or 15 s blanched cucumbers prior to direct acidification ($p = 0.0021$). The same trend was noticed between the control and pickles made with 90 s blanched cucumbers ($p = 0.0069$) and 180 s blanched cucumbers ($p = 0.0001$) at day 3 of refrigerated storage (4°C). However, these differences were no longer observed when measurements were taken at 30 days, nor at any time point thereafter (Figure S3). The rate of hue angle change

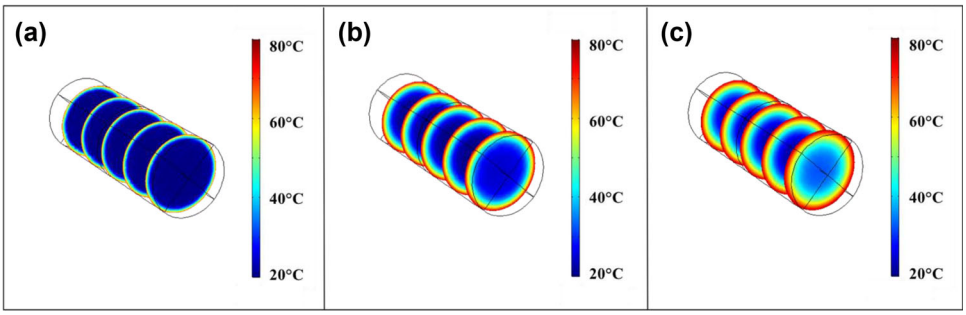

FIGURE 5 Thermal model diagram of 3B pickling cucumbers subjected to various whole cucumber blanching times (15 s (a), 90 s (b) and 180 s (c)) at 80°C. The 90 s blanch achieves significant thermal treatment of the exocarp, while most of the mesocarp remains at ambient temperature. The 180 s blanch raises the temperature of a significant portion of the mesocarp to 40°C and higher

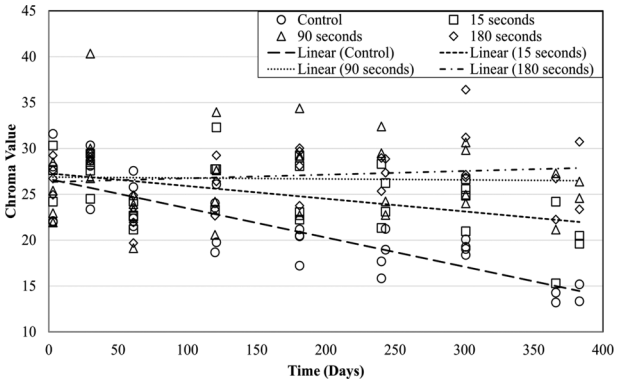

FIGURE 6 Chroma value of refrigerated cucumber pickle exocarp during storage at 4°C for two lots of size 3B cucumbers subjected to various whole cucumber blanching times in 80°C water

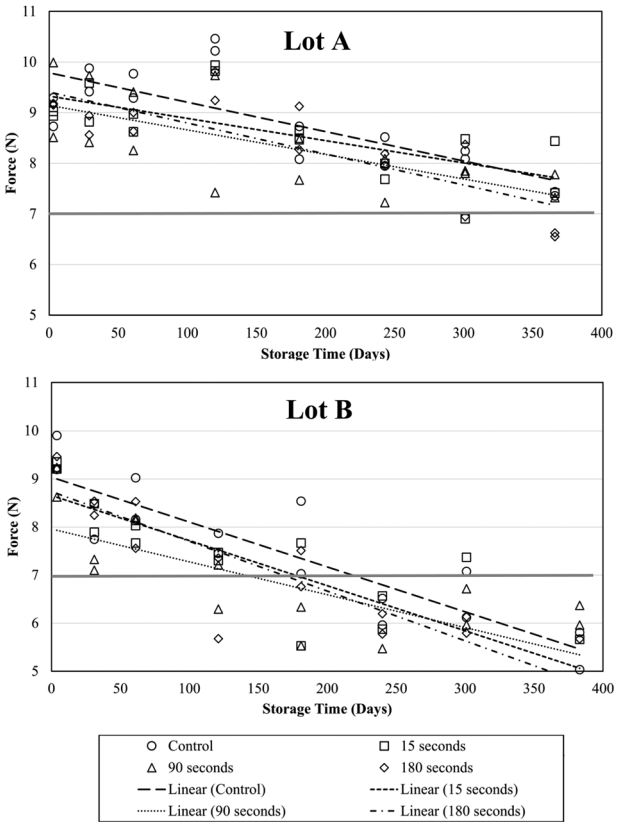

FIGURE 7 Peak force (*N*) required to puncture 6 mm cross-section of cucumber pickle mesocarp during shelf life of refrigerated pickles for two lots of size 3B cucumbers (A & B) subjected to various whole cucumber blanching times in 80°C water

was not significantly different between blanched and control cucumber pickles across the entire shelf life of ~365 days. The rapid reduction in hue angle during the first 60 days of storage was consistent with previous research, suggesting chlorophyll was converted to pheophorbides and pheophytins as expected under the acidic conditions (White et al., 1963). No significant difference was observed between the control and three blanch treatments (15, 90, and 180 s) in terms of exocarp chroma, up to and including the 120 day time point during shelf life. However, a significant difference was observed between the control and three blanch treatments at all sampling test points thereafter. Exocarp chroma values of the cucumber pickles decreased between 180 and ~365 days of refrigerated storage (Figure 6). This is consistent with a perceived loss of color (aka 'bleaching') that occurs due to oxidation of cucumber pickles (Buescher & Hamilton, 2000). In contrast, the chroma remained significantly higher in the blanched cucumber pickles ($p < 0.05$), showing that blanching preserved saturation of exocarp color during refrigerated storage.

3.5 | Mesocarp texture

Blanching cucumbers prior to acidification had minimal impact on texture quality of cucumber pickles over the course of a one-year shelf life (Figure 7, p (lot A) = 0.2388 p (lot B) = 0.1635). Despite the very similar initial firmness of the fresh pickling cucumbers, the rate of pickle softening differed greatly between the two lots of cucumbers (Figure 7). Differences in texture due to cucumber cultivar

and growing environment have previously been reported by Yoshioka et al. (2009). Since the two lots of cucumbers used in the current study were grown in different locations at different times of the year, they could vary in several attributes that are known to influence texture retention in cucumber pickles, such as cultivar, maturity, natural level of calcium, and endogenous or exogenous polygalacturonases (reviewed by Franco et al., 2016).

Interestingly, despite lot B pickles softening more rapidly and developing cure appearance at a higher rate than lot A, there was no direct correlation between softening and CAD in the 180 s treatment, which maintained a low cure level throughout shelf life. This may be explained by the different mechanisms for texture degradation and CAD: softening is related to changes in pectic substances, whereas CAD is linked with changes to proteins affecting membrane permeability (Howard & Buescher, 1993; Mok & Buescher, 2012). This suggests that the blanching may have affected proteins but not pectic substances in refrigerated pickles. The significant influence of lot-to-lot variability on rate of softening in this study demonstrates that further research should be conducted to investigate potential methods to minimize the magnitude of this variability.

3.6 | Volatile compound profiles

Nine hundred and forty-six peaks were detected during volatile compound analysis by SPME-GCxGC-TOFMS. Among those peaks, 212 tentatively identified compounds were consistently detected in cucumber pickle homogenate and subjected to statistical analysis. Of these compounds, 34 were classified as aldehydes, along with 35 ketones, 35 alcohols, 27 alkanes, 21 carboxylic acids, 13 alkenes, 13 furans, and 34 others (including, but not limited to, phenols, thiols, esters, and ethers). The largest changes in the overall volatile compound profile occurred between raw cucumber, freshly packed (3 days) pickles that had just reached equilibrium pH, and after 2 months of refrigerated storage, illustrated by the clear grouping of these samples based on the overall volatile compound profiles (Figure 8). There were no clear groupings between cucumbers and blanched cucumbers or the respective pickles (Figure 8), showing that the 90 s blanching treatment did not significantly alter the composition of the majority of these 212 volatile components.

3.6.1 | Volatile compounds in cucumber and pickles subjected to blanching

Differences in volatile compounds between cucumber and blanched cucumbers, as well as the respective pickles at day 3 of storage were determined by ANOVA. The 10 compounds displayed in Figure 9 had the largest peak areas among the 75 significantly different compounds (Table S4) and p values < 0.001. These compounds can be produced as secondary lipid oxidation products of linoleic or linolenic acids and were more abundant in freshly prepared pickles than in the raw pickling cucumbers. Two of these compounds, pentenal and (E)−2-hexenal, were previously identified in pasteurized (fresh-pack) dill pickle samples that had been prepared both anaerobically and aerobically (Cleary & McFeeters, 2006) and in fermented cucumber pickles (Wolter, 2013), indicating that these are present in freshly prepared cucumber pickles regardless of processing method. All 10 compounds are known to be odor active with characteristic aromas ranging from fruity and green to sweaty, earthy, and metallic (Table 1). No significant differences in these compounds were found between blanch treatments for either cucumbers or cucumber pickles. These findings are consistent with Zhou et al. (2000) who determined that (E)-2-hexenal, (E)-2-heptenal and (E)-2-pentenal are capable of being formed non-enzymatically and could therefore be produced in the absence of lipoxygenase. Interestingly, (E,Z)-2,6-nonadienal did not differ among the treatments, indicating that fresh cucumber flavor is likely to be present in refrigerated pickles very early in the shelf life, with or without a 90 s, 80°C blanch of whole cucumber prior to pickling (Figure 10).

3.6.2 | Changes in volatile compounds during refrigerated storage of pickles

Fifty-seven compounds varied in abundance during the shelf life of pickles produced using either cucumbers or blanched cucumbers during a year of refrigerated storage (Table S5). Of these 57 compounds, 18 were classified as aldehydes, 12 as ketones, while the remainder were alcohols, alkanes, alkenes, carboxylic acids, esters, ethers, furans, and sulfur-containing compounds.

Compounds related to fresh cucumber flavor

Cucumbers contain approximately 103 mg of total lipid per 100 g of tissue (Kinsella, 1971) composed of approximately 27.5, 22.7, and 45.8 mg/100 g fresh tissue of palmitic, linoleic, and linolenic acids, respectively (Peng & Geisman, 1976). While cucumbers contain a relatively small amount of total lipids compared to other foods, oxidation of these lipids has a significant impact on product quality. Secondary products of lipid oxidation such as ketones and aldehydes may be aroma-active and have low detection thresholds (Kochhar, 1993). Notably, E-Z-(2,6)-nonadienal and 2-nonenal are largely responsible for fresh cucumber flavor (Schieberle et al., 1990; Tressl et al., 1981) and demonstrated antimicrobial activity against *E. coli* O157:H7

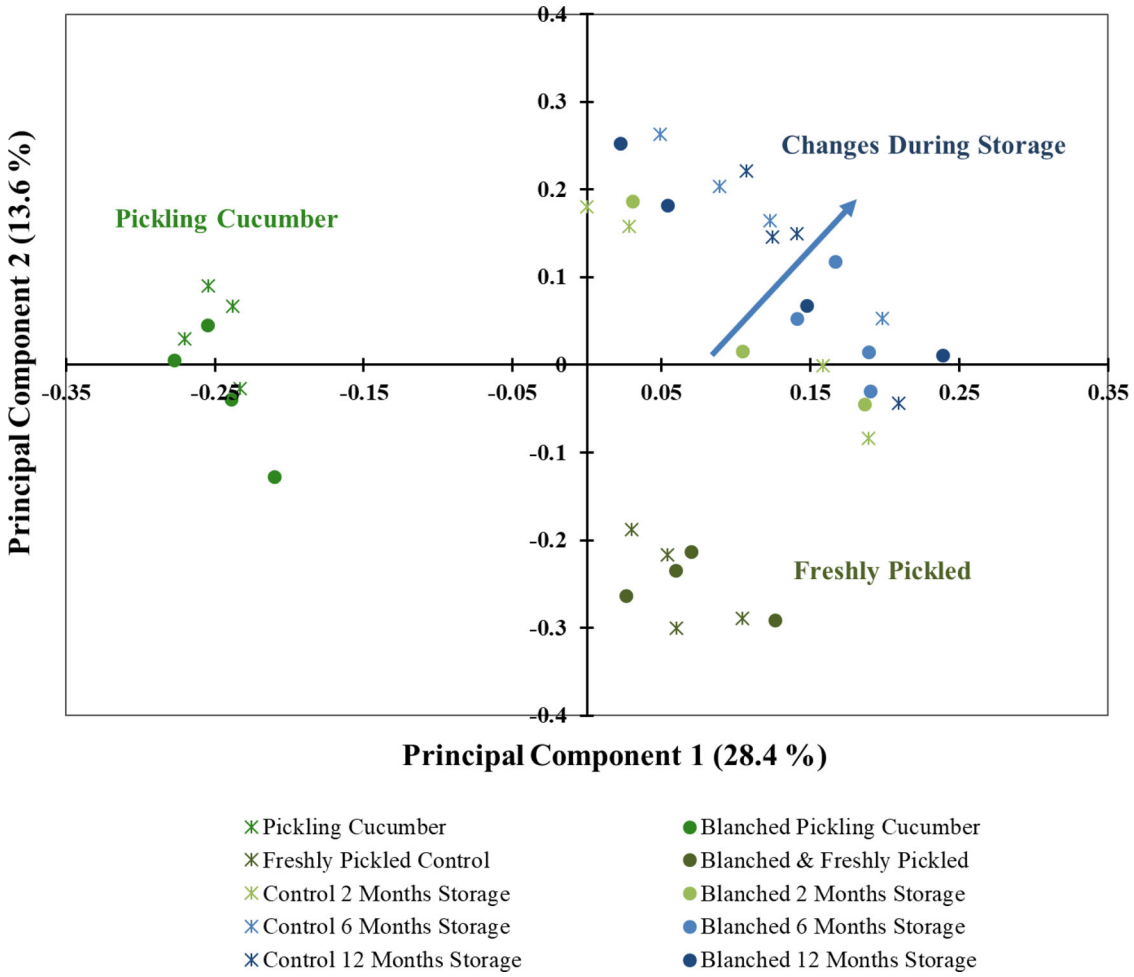

FIGURE 8 Principal component plot showing the grouping of cucumbers and pickles with (•) and without (∗) a 90 s whole cucumber blanch in 80°C water based on the overall volatile compound profile

TABLE 1 Characteristic odors of compounds found to be more abundant in refrigerated cucumber pickles after 3 days of refrigerated storage than fresh cucumbers

Compound name	Characteristic odors[a]
Pentanal	Fermented, bready, fruity, nutty, berry
1-Penten-3-one	Pungent, peppery, mustard, garlic, onion
2-Pentyl-furan	Fruity, green, earthy, beany, vegetable, metallic
1-Pentanol	Fusel, oily, sweet, balsamic
(E)-2-hexenal	Green, leafy
1-Octen-3-ol	Mushroom, earthy, fungal, green, oily, vegetable, savory, brothy
(E,E)-2,4-heptadienal	Fatty, green, oily, aldehydic, vegetable, cinnamon
(Z)-2-heptenal	Green, fatty
2-Ethyl-furan	Dirty, musty, brown, earthy, beany, malty
(E)-2-pentenal	Pungent, green, fruity, apple, waxy

[a]Odor notes adapted from http://www.thegoodscentscompany.com

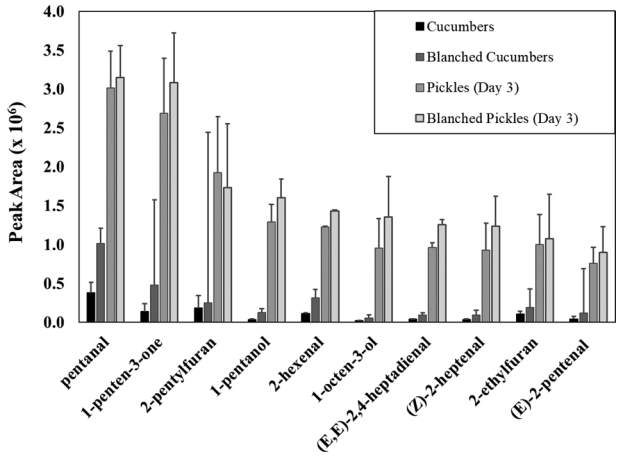

FIGURE 9 Differences in the most abundant volatile compounds in cucumber pickles prepared with or without whole cucumber blanching for 90 s in 80°C water. Pickles were sampled on day 3 of refrigerated storage following equilibration with the brine

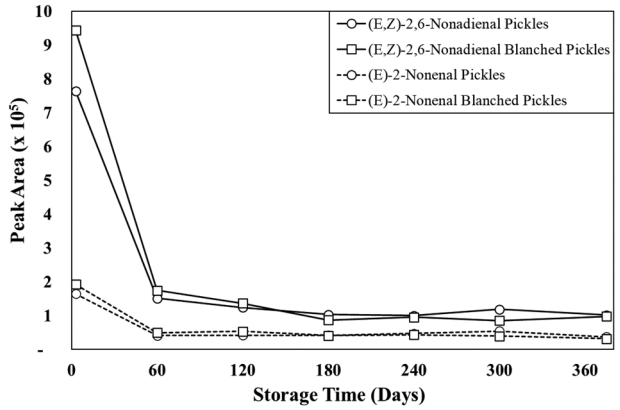

FIGURE 10 Changes in fresh cucumber flavor compounds, (E,Z)-2,6-nonadienal and (E)-2-nonenal, during shelf life of refrigerated pickles produced with and without blanching at 80°C for 90 s. Average peak area of (E,Z)-2,6-nonadienal was approximately 25×10^5 in fresh pickling cucumbers compared with 36×10^5 in blanched cucumbers

in a model system (Cho et al., 2004). These compounds are produced by fresh pickling cucumbers (~10–12 ppm, Buescher & Buescher, 2001) at levels well below the antimicrobial concentration (500 ppm), and the acidic pH of pickles reduces production further. Therefore, the primary role of these compounds in pickles is the influence on flavor. In this study, a decline in (E,Z)-2,6-nonadienal and (E)-2-nonenal production during shelf life was observed in refrigerated pickles regardless of blanching treatment (Figure 10). These data are consistent with previous findings (Palma-Harris et al., 2002), despite having an equilibrated pH much lower than the initial pH of pickles in the previous study. Buescher and Buescher (2001) determined that refrigerated pickles lost the ability to produce (E-Z)-2,6-nonadienal within 6 days of acidification or if cucumbers were frozen prior to pickling. Furthermore, both Buescher and Buescher (2001) and Palma-Harris et al. (2002) found that production of (E-Z)-2,6-nonadienal was dramatically reduced at pH levels below 5. Interestingly, we found that (E-Z)-2,6-nonadienal was present up to and including 375 days post-pack in a pickle with an equilibrated pH ~3.8 that was stored at −80°C prior to analysis. This finding indicates the stability of the compound during extended refrigerated storage or the cucumber's ability to produce it at around 20% of the amount produced 3 days after packing and 3% of the amount produced in fresh cucumbers. The low detection threshold of this compound (0.0001 mg kg^{-1}; Forss, 1972) makes it likely that some of the fresh cucumber flavor would still be evident after extended storage of refrigerated pickles, provided that there were no high levels of off-flavors, capable of flavor masking. Levels of (E-Z)-2,6-nonadienal were not significantly influenced by the blanching treatment for samples from any of the time points used in this study. Several other lipid oxidation products exhibited similar trends to (E-Z)-2,6-nonadienal, starting at high levels at day 3, decreasing significantly by day 60 of storage, and remaining relatively consistent thereafter for pickles prepared with and without blanching. This trend indicates that such compounds are produced by the same method of enzymatic oxidation and that blanching cucumbers prior to processing refrigerated pickles did not change the levels of these characteristic lipid oxidation products. Although cucumber exocarp has been shown to have twice as much lipoxygenase activity as the flesh (Wardale & Lambert, 1980), formation of (E-Z)-2,6-nonadienal was 6- to 7-fold higher in the mesocarp and endocarp tissues (Buescher & Buescher, 2001). Therefore, it is plausible that lipoxygenase and associated enzyme activities in the mesocarp and endocarp are driving the generation of these flavor-active volatile compounds.

Changes in other volatile compounds during shelf life

Eighteen volatile compounds increased during refrigerated storage. 3-Hexen-1-ol, an alcohol linked with odors such as green, foliage, vegetable, and herbal (www.goodscentscompany.com), was found in low levels at 3 days and increased by approximately 300% (Figure S4A). Diphenyl ether, a compound with a characteristic geranium, leafy, green, phenolic, metallic, or medicinal odors (www.goodscentscompany.com), was not detected in pickles up to and including 60 days of storage but was present at timepoints thereafter (Figure S4B), suggesting that it may play a role in shelf life of refrigerated pickles. Additionally, (E) 3,7-dimethyl-2,6-octadienoic acid (geranic acid), a compound associated with woody, green, sweet notes (www.goodscentscompany.com), was

detected in low levels in samples at 3 days and increased in a somewhat linear fashion thereafter (Figure S4C). Interestingly, there was a trend for this compound to be lower in blanched cucumber pickles at the later storage times, warranting further research to determine the threshold levels of this compound in cucumber pickles.

3.6.3 | Effect of blanching on volatile compound composition

Among the 212 compounds profiled, very few were altered in response to whole cucumber blanching. One compound of note, methyl benzoate, was substantially affected by blanching (Figure S5). Methyl benzoate was significantly lower in pickles made with blanched cucumbers. Methyl benzoate is not a product of lipid oxidation and is produced from methanol and benzoic acid during a condensation reaction that can be catalyzed enzymatically or chemically. Methyl benzoate is odor active and has a phenolic, wintergreen, and camphorous aroma at certain concentrations (www.goodscentscompany.com). However, a consumer sensory panel ($n = 110$) could not differentiate the pickles prepared from fresh or blanched cucumbers, suggesting that this differences in methyl benzoate content had minimal to no impact on the flavor of these products.

3.7 | Sensory consumer testing

Discrimination testing can unearth significant differences in products perceivable by consumers, which may not be evident from instrumental testing alone. We conducted a consumer tetrad test to determine whether pickles produced with a 90 s whole cucumber blanch could be distinguished from fresh cucumber pickles at a typical consumption shelf life of 63 days. Most participants (78.2%) consumed pickles at least once every 2 weeks, and 41.8% were regular consumers of refrigerated pickles. Only 39 of the 110 participants correctly grouped the samples, which is less than the critical value (45), so it can be concluded that no difference could be detected between samples. These results suggest that manufacturers could implement the proposed process in a commercial setting without significantly altering the perception of the product.

4 | CONCLUSION

A 90 s blanching process at 80°C applied to whole cucumbers consistently reduced background microbiota without significantly deteriorating the quality of refrigerated cucumber pickles. A 5-log reduction in *E. coli* O157:H7 could be achieved under these blanching conditions within the region of cucumber containing the majority of the microorganisms. This blanch treatment had minimal impact on the overall volatile compound profile, suggesting that there is negligible risk of altering the flavor characteristics of refrigerated pickles produced with this added hurdle for safety. Furthermore, findings related to exocarp chroma and cure appearance development indicate that this blanch treatment could also aid in the preservation of refrigerated cucumber pickle appearance, extending the shelf life of these products.

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AUTHOR CONTRIBUTIONS

Lisa J. LaFountain: conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review & editing. **Suzanne D. Johanningsmeier**: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – review & editing. **Fred Breidt Jr.**: conceptualization; formal analysis; funding acquisition; methodology; resources; writing – review & editing. **George N. Stoforos**: formal analysis; methodology; writing – original draft; writing – review & editing. **Robert E. Price**: investigation; methodology; writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available upon request.

ORCID

Suzanne D. Johanningsmeier https://orcid.org/0000-0002-9084-4666

Frederick Breidt Jr. https://orcid.org/0000-0002-5298-2216

George N. Stoforos https://orcid.org/0000-0001-7317-8676

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