Microbiological Preservation of Cucumbers for Bulk Storage Using Acetic Acid and Food Preservatives

I.M. PÉREZ-DÍAZ AND R.F. MCFEETERS

ABSTRACT: Microbial growth did not occur when cucumbers were preserved without a thermal process by storage in solutions containing acetic acid, sodium benzoate, and calcium chloride to maintain tissue firmness. The concentrations of acetic acid and sodium benzoate required to ensure preservation were low enough so that stored cucumbers could be converted to the finished product without the need to wash out and discard excess acid or preservative. Since no thermal process was required, this method of preservation would be applicable for storing cucumbers in bulk containers. Acid tolerant pathogens died off in less than 24 h with the pH, acetic acid, and sodium benzoate concentrations required to assure the microbial stability of cucumbers stored at 30 °C. Potassium sorbate as a preservative in this application was not effective. Yeast growth was observed when sulfite was used as a preservative.

Keywords: acidification, ethanol, organic acids, stabilization, vegetables

Introduction

Several vegetables, particularly cucumbers, cabbage, and peppers, are preserved and stored in large quantities in tanks prior to conversion to consumer products. Cabbage is preserved by fermentation to sauerkraut. Peppers are preserved by addition of acid, salt, and preservatives without fermentation. Cucumbers are preserved by both fermentation and nonfermentation bulk storage. Currently, small cucumbers are grown and preserved in vinegar in India and shipped to the United States and other countries. Preservation without fermentation is achieved by equilibrating the fresh cucumbers with 3.5% acetic acid from vinegar, 4% NaCl, 0.5% calcium chloride, and 150 ppm of sodium metabisulfite, calculated as SO_2. The acetic acid concentration used for preservation is equal to that required to achieve preservation without addition of preservative based upon the equation given by Binsted and others (1971) and close to the acetic acid concentration required for products made from fermented cucumbers based upon the preservation prediction model of Bell and Etchells (1952).

The acid concentration in these bulk-stored cucumbers is greater than that needed in products made from them. Therefore, some of the acid and salt must be washed out of the cucumbers. As the quantities of the preserved cucumbers imported to the United States have increased, disposal of both the brine solution in the barrels and the more dilute solution generated from washing acid and salt from the cucumbers has become a difficult and expensive problem for the processors who use these cucumbers. The ability to reduce acid concentration during bulk storage to a level that did not require washing would reduce the volume of water use, disposal of nonbiodegradable salt, and the biological oxygen demand (BOD) that must be treated.

McFeeters (1998) found that cucumbers were microbiologically stable when they were acidified with HCl to reduce pH to 3.5 and 300 ppm sulfite were added to prevent the growth of microorganisms. However, use of only HCl as an acidulant results in preserved cucumbers with low buffer capacity so that pH could rise rapidly if errors were made in brining and handling the preserved cucumbers. Also, it was observed that cucumbers rapidly lose the opaque white appearance of fresh cucumbers and develop a translucent "cured" appearance in the presence of sulfite, like that which develops when cucumbers are fermented and stored.

Sodium benzoate and potassium sorbate have a long history of use in acidified and fermented foods. Potassium sorbate is commonly used in cucumber fermentation tanks at about 0.03% (2 mM) to prevent growth of yeasts and other fungi during air purging because it is relatively noninhibitory to the lactic acid bacteria that carry out the fermentation (Gates and Costilow 1981). Benzoate prevents the growth of yeasts at low concentrations (Senses-Ergul and others 2007) and has also been considered to be more inhibitory to lactic acid bacteria than potassium sorbate, though this effect was not observed when sodium benzoate was added to olive fermentations (Turantas and others 1999). This study was conducted to define conditions of pH and acidity that would be required to microbiologically preserve fresh cucumbers with either sodium benzoate or potassium sorbate as a preservative in combination with acetic acid as the primary acidulant. These preservatives do not cause the rapid development of cured appearance in the cucumber tissue that is observed with sulfite.

Materials and Methods

Evaluation of the ability of preservatives to prevent microbial growth

Brining and storage of cucumbers. Size 2A cucumbers (25.4- to 31.8-mm dia) were obtained from a local processor and packed without washing into 46 oz. jars. The cucumber:brine ratio in the

MS 20070935 Submitted 12/17/2007, Accepted 4/6/2008. *Authors are with USDA-ARS, SAA Food Science Research Unit, North Carolina State Univ., Raleigh, NC 27695-7624, U.S.A. Direct inquiries to author Pérez-Díaz (E-mail: ilenys.pérez-díaz@ars.usda.gov).*

Article nr. FSR07-31 of the Journal Series of the Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27695-7624, U.S.A. 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 or imply approval to the exclusion of other products that may be suitable.

doi: 10.1111/j.1750-3841.2008.00795.x

jars was 55:45 (w/v). Cover solutions contained acetic acid from commercial vinegar and calcium chloride such that after equilibration with the cucumbers, the acetic acid concentration was 150 mM and the calcium chloride, added to maintain cucumber firmness, was between 20 and 100 mM. Sodium metabisulfite, potassium sorbate, and sodium benzoate were prepared as concentrated solutions in water so that less than 10 mL were added to a 1.36-L jar to obtain the target preservative concentration after equilibration with the cucumbers. Any required pH adjustments, so that the initial equilibrated pH of the brined cucumbers was 3.5 to 3.6, were done by addition of small volumes of 3 N HCl or 5 N NaOH solution. The amount of HCl or NaOH required was determined by mixing cucumber slurry and cover solution 55:45 (w/v) and then titrating the mixture with acid or base to the target pH. Triplicate jars of each treatment were prepared. The jars were closed with commercial lug caps fitted with a rubber septum to allow for inoculation and sampling of the jars with sterile syringes. The lids were heated in boiling water for at least 30 s to soften the sealing compound and immediately applied to the filled jars. The jars were incubated at 30 °C, except for control jars that were stored at 4 °C to prevent microbial growth. After equilibration of the jars (4 d), initial samples of cover brine solutions were collected for chemical and microbiological analyses. Samples were then collected during the storage period as required to evaluate whether microbial growth occurred.

Evaluation of microbial growth. The jars were visually monitored for the development of turbidity and/or pressure on the lids. After turbidity developed in a container, brine samples were taken using aseptic techniques for microbiological analysis. Samples were plated on deMan Rogosa and Sharpe agar (MRS), plate count agar (PCA), yeast morphology agar supplemented with 1 mM Chlortetracycline and 1.5 mM Chloramphenicol (YM/A), and violet bile red agar supplemented with 1% glucose (VRBG). The MRS and PCA plates were incubated at 30 °C for 72 h. The MRS plates were incubated under anaerobic conditions. The YM/A plates were incubated at 25 °C, while the VRBG plates were incubated at 37 °C for 72 h.

The occurrence of microbial growth was also evaluated by determination of changes in sugars, organic acids, and ethanol during incubation of samples at 30 °C. Analyses were done by HPLC on a 30-cm HPX-87H column (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) (McFeeters and Barish 2003). The column was heated to 65 °C and eluted with 0.03 N sulfuric acid at a flow rate of 1.0 mL/min. A Thermo Separations UV6000 diode array detector (Spectra System Thermo Scientific, Waltham, Mass., U.S.A.) set to collect data at 210 nm was used to detect organic acids, sorbic acid, benzoic acid, and sulfite. A Waters model 410 refractive index detector (Waters Corp., Millipore Corp., Billerica, Mass., U.S.A.) connected in series was used to measure glucose, fructose, and ethanol.

Identification of microorganisms resistant to the treatments. Colonies of the microorganisms that were able to survive the preservative treatments were isolated on MRS and classified using partial 16S rRNA gene sequences. The partial gene sequences were obtained from the products of polymerase chain reaction (PCR) amplification using chromosomal DNA as a template, which was extracted using a commercial genomic DNA purification kit (Promega Corp., Madison, Wis., U.S.A.). The PCR mixtures contained Platinum PCR SuperMix (Invitrogen Corp., Carlsbad, Calif., U.S.A.), chromosomal DNA, and the Rd1 (5′-GTC TCA GTC CCA ATG TGG CC-3′), and Ru2 (5′-AGA GTT TGA TCC TGG CTC AG-3′) primers (Sigma-Genosys, St. Louis, Mo., U.S.A.; Barrangou-Poueys and others 2002). The PCR cycle consisted of 4 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 61 °C, and 30 s at 72 °C, with a final extension step of 7 min at 72 °C, and stored at 4 °C until used. The PCR products were purified (Qiagen PCR purification kit, Valencia, Calif., U.S.A.) and sequenced by Davis Sequencing (Davis, Calif., U.S.A.) using an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, Calif, U.S.A.). The sequences obtained were analyzed by BLAST (Basic Local Alignment Search Tool; Altschul and others 1990) in the GenBank database (Benson and others 2006) to determine the identities of the isolates.

Addition of aciduric microorganisms. Preservation treatments that had no detectable microbial growth after 3 wk of incubation were challenged by inoculating *Lactobacillus plantarum* LA0219 (Table 1), and a cocktail of 5 *Zygosaccharomyces globiformis* isolates (Table 1). Preparation of cultures was done by streaking the bacterium and fungi from frozen stock cultures onto MRS and YM/A, respectively. Plates were incubated at 30 °C for 48 h. Single colonies were transferred to 10 mL of the appropriate broths and incubated at 30 °C for 18 and 72 h for the *L. plantarum* and *Z. globiformis* strains, respectively. Cultures were centrifuged, washed twice, and resuspended with saline solution prior to the inoculation of the jars. The bacterium and yeasts were individually inoculated through the lid septum of the sample jars to give initial populations of 10^6 and 10^3 CFU/mL in each jar, respectively. The control jars were not inoculated. The development of turbidity and pressure on the lids was monitored daily. On the inoculation day and at the end of the storage period, brine samples from each jar were analyzed for sugars, organic acids, and ethanol by HPLC. Samples from those jars that developed turbidity and/or pressure were plated on the MRS and YM/A agar plates. Serial dilutions were performed on oxygen-free saline solution. The plates were incubated under aerobic conditions at 30 and 25 °C, respectively, for 72 h.

Survival of acid tolerant pathogenic bacteria. Aliquots of 1 mL of the brine from jars containing 7 mM sulfite, 12 mM potassium sorbate, and 10 mM sodium benzoate were transferred to a microcentrifuge tube inside an anaerobic hood and inoculated with outbreak isolates of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* (Table 1). These pathogenic strains were grown in oxygen-free tryptic soy agar containing 1% glucose (TSAG) under anaerobic conditions and incubated at 37 °C. Cells were collected by centrifugation at 6000 rpm for 10 min, resuspended, and washed twice with 10-mL saline solution. The final pellet was resuspended with 3-mL saline solution. Cocktails of 5 strains of each of the 3 pathogens were prepared by mixing the

Table 1—Strains of acid-tolerant bacterial pathogens used to inoculate equilibrated cover solutions from preserved cucumbers.

Microorganisms	Strains	Source
Listeria monocytogenes	SRCC529	Pepperoni
	SRCC1791	Yogurt
	SRCC1506	Ice cream
	SRCC1838	Cabbage
	SRCC2075	Diced coleslaw
Escherichia coli O157:H7	ATCC43888	Human feces
	SRCC1675	Apple cider outbreak
	SRCC1486	Salami outbreak
	SRCC2061	Ground beef
	SRCC1941	Pork
Salmonella belem	SRCC549	Sour cream and chives
Salmonella branderup	SRCC1093	10% salted yolk
Salmonella cerro	SRCC400	Cheese powder
Salmonella enteritidis	SRCC1434	Ice cream
Salmonella newport	SRCC551	Broccoli and cheese
Salmonella typhimurium	SRCC1846	Liquid eggs

SRCC = silliker Research Center Collection; ATCC = American Type Culture Collection.

resuspended cells of the 5 strains. Separate brine aliquots were inoculated with the 5-strain cocktails of the 3 acid-tolerant pathogens, individually, to give initial populations of 10^9 CFU/mL. Inoculated brine samples were incubated at 30 °C for 24 h under anaerobic conditions. Viable cell numbers after incubation for 6, 18, and 24 h were determined by plating onto TSAG using a Spiral Plater (Spiral Biotech) and a Q-count (Spiral Biotech). Serial dilutions were performed with oxygen-free saline solution under anaerobic conditions.

Results and Discussion

Assurance that microbial growth will not occur, either by food fermentation organisms or by spoilage organisms, is difficult due the potential for the harvested produce to contain a wide range of microorganisms, because production spans wide geographic areas, many climate and soil conditions, and variable harvest and transport conditions over periods of years. The approach to this problem has been to use cucumbers obtained from a local processor. These cucumbers were harvested from different growing areas in the United States and Mexico in multiple growing seasons and moved through normal commercial shipping channels for fresh pickling cucumbers.

Table 2 shows the ability of sulfite, potassium sorbate, and sodium benzoate to prevent microbial growth in cucumbers stored in 150 mM acetic acid with pH adjusted to 3.5. Microbial counts on the fresh cucumbers were 10^4 CFU/g. In this experiment, microbial growth was only observed in cucumbers that were stored without any added preservative. In those containers cell numbers increased to 10^8 CFU/g. After 1 mo, the jars of cucumbers were inoculated with a mixture of a *Lactobacillus plantarum* strain that is used for cucumber fermentations (Fleming and others 1988) and *Zygosaccharomyces globiformis* strains that were isolated from spoiled commercial products (Bell and Etchells 1952). Microbial growth as indicated by development of turbidity in the brine, development of pressure on the jar lid, and plating in PCA, MRS, and YM agars was observed at $\leq$ 2 mM sulfite, $\leq$ 6 mM sodium benzoate, and at all concentrations of potassium sorbate up to 15 mM.

Occurrence of microbial growth was also evaluated based upon the HPLC analysis of sugars, acids, and alcohols present in the brine samples from the jars. The results for selected components are given in Table 2. Malic acid is the major organic acid present in cucumbers (McFeeters and others 1982a). It is degraded, primarily to lactate and CO_2, in cucumber fermentations by the homofermentative lactic acid bacteria that dominate the fermentation (McFeeters and others 1982b, 1984), but it is not degraded by yeasts. Therefore, loss of malic acid and formation of lactic acid indicate growth of lactic acid bacteria. Lactic acid production in excess of that produced from the degradation of malic acid results from fermentation of sugars by lactic acid bacteria. Formation of ethanol and a small amount of glycerol is typical of yeast fermentation. The HPLC analysis and absence of viable microorganisms indicated the absence of microbial growth in all preservative treatments in which visible growth was absent. The only ambiguous result was 15 mM potassium sorbate, where slight turbidity was observed, but no fermentation products were detected by HPLC. Yeast growth, indicated by the formation of ethanol and glycerol, was only observed when sulfite was used as a preservative. It was not surprising that it required lower concentrations of sulfite to prevent microbial growth when acetic acid was used as an acidulant than when acidification was done with HCl alone (McFeeters 1998).

Additionally, these results conclude that potassium sorbate is not an effective preservative for acidified cucumbers in relatively low acid brines. Concentrations approaching the solubility limit (15 mM) did not clearly prevent microbial growth. Even if this concentration prevented microbial growth, it is so far above the legal limit that the preservative would have to be washed out of the cucumbers so that the concentration in the final product would be no greater than the 6.5 mM, which is allowed. Microorganisms resistant to the treatments containing potassium sorbate included *Lb. brevis*, *Lb. plantarum*, and *Lb. buchneri* based upon 16S rRNA sequencing of selected colonies isolated from MRS plates. This

Table 2—Evaluation of microbial growth in cucumber jars stored at 30 °C in 150 mM acetic acid with pH adjusted to 3.5 with HCl and inoculated 28 d postpacking.

Treatment	$CaCl_2$ (mM)	Preservative (mM)	Malic acid[a] (mM)	Lactic acid[a] (mM)	Glycerol[b] (mM)	Ethanol[b] (mM)	Days until turbidity or cap pressure
Control,	20	0	11.6 ± 0.4	0	0	0	>160
refrigerated	20	0	0	96 ± 2	1.2 ± 2.0	1.5 ± 2.6	23
sulfite	20	1	0	53.3 ± 1.5	3.6 ± 0.1	57.7 ± 2.8	34
	20	2	10.4 ± 0.4	0	3.1 ± 0.1	53.9 ± 2.5	44
	20	3	10.8 ± 0.6	0	0.2 ± 0.3	0	>160
	20	4	10.1 ± 0.4	0	0	0	>160
	20	5	11.3 ± 0.1	0	0	0	>160
Potassium	20	0	0	96 ± 2	1.2 ± 2.0	1.5 ± 2.6	23
sorbate	20	6	0	86.3 ± 2.9	0	0	34
	20	9	0	77.9 ± 3.7	0	0	34
	20	12	0	50.0 ± 16.5	0	0	49
	20	15	9.4 ± 0.5	0.7 ± 0.2	0	0	76
Sodium	20	0	0	96 ± 2	1.2 ± 2.0	1.5 ± 2.6	23
benzoate	20	3	0	78.1 ± 3.7	0	0	34
	20	6	11.0 ± 0.3	0	0.6 ± 0.5	7.9 ± 7.0	83
	20	8	10.9 ± 0.6	0	0	0	>160
	20	10	10.4 ± 0.6	0	0	0	>160
	20	12	11.2 ± 0.5	0	0	0	>160
Sodium	100	0	0	79.7 ± 0.3	0	0	30
benzoate	100	3	0	74.6 ± 0.8	0	0	34
	100	6	0	28.8 ± 3.4	0	1.5 ± 1.3	58
	100	9	9.7 ± 0.3	0	0	0	>160
	100	12	8.9 ± 0.2	0	0	0	>160

[a]Disappearance of malic acid and formation of lactic acid indicates growth of lactic acid bacteria.
[b]Formation of ethanol and glycerol indicates growth of yeasts.

preservative is recognized to be relatively noninhibitory to the lactic acid bacteria (Bell and others 1959).

Figure 1 shows the lactic acid production by the natural microflora present on fresh cucumbers that grew in the presence of 150 mM acetic acid with an initial pH adjusted to 3.5. The amount of lactic acid produced after 2-mo storage declined linearly as the concentration of sodium benzoate increased. Extrapolation to zero lactic acid production resulted in an estimate of 9 mM sodium benzoate as the minimum preservative concentration that would prevent growth of bacteria in the cucumbers to the extent that no detectable fermentation products could be produced. In experiments with 10 lots of cucumbers obtained from different growing areas in the United States and Mexico and in different growing seasons, microbial growth has never been observed with a sodium benzoate concentration of > 9 mM and an initial pH ≤ 3.6 based upon visual inspection or by the HPLC analysis for loss of malic acid and production of lactic acid, ethanol, or glycerol as fermentation products. Addition of 12 mM sodium benzoate prevented detectable growth of microorganisms based upon these criteria with a pH ≤ 3.8, but growth was observed when the initial pH was adjusted to 3.9 (data not shown).

Viable organisms (< 200 CFU/mL) have been observed in jars of cucumbers with no detectable turbidity. Identification of selected colonies by 16S rRNA sequencing indicated that the colonies observed were the result of germination of spores belonging to the *Bacillus* and *Paenibacillus* genuses on the MRS agar plates.

Figure 2 shows the time until visible growth was observed in at least one of 3 jars of a preservative treatment as the concentration of sodium benzoate was increased. If microbial growth occurred in any preservative treatment, the growth was always observed within the first 60 d of incubation. Therefore, if microorganisms do not grow within a 60-d period, it can be concluded that preservation has been achieved.

Cucumbers preserved in bulk in tanks or barrels by fermentation in salt brine or by storage in acid solutions with preservatives are not suitable for consumption because the levels of one or more of the brine ingredients, including acid, salt, calcium chloride, or preservatives, are present in excess. This type of bulk preservation is considered to be a part of the postharvest field operation for cucumbers to hold them for a period of weeks or months. The cucumbers must be further processed into products suitable for consumption. Processing will include transfer of the cucumbers in a new cover brine solution containing flavorings, spices, and other ingredients. Equilibration of water soluble components in the bulk-stored cucumbers with the cover brine solution will reduce the concentrations of acid, calcium chloride, and sodium benzoate to the levels that are intended in the final product. Processing may also include cutting and heating steps. This means that the final elimination of any field contamination by acid-tolerant pathogens such as *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 needs to be ensured by the final process rather than during the bulk storage stage of the production system. Nevertheless, if pathogen numbers on the freshly harvested cucumbers were reduced during the intermediate storage period, it would be an additional benefit of the bulk storage stage of production.

Survival of these pathogens in the conditions that ensured preservation of cucumbers without fermentation was evaluated by anaerobic inoculation of brines from preserved cucumbers with cocktails of pathogenic strains of *L. monocytogenes, E. coli* O157:H7, and *Salmonella* spp. isolated from outbreaks in the United States (Table 1) that were grown under conditions to maximize their acid resistance (Buchanan and Edelson 1999). Table 3 shows that with 150 mM acetic acid at pH 3.5, a sodium benzoate concentration of 9 mM, and a temperature of 30 °C, all 3 pathogens were reduced in numbers by at least 5 logs in 18 h, except for *Salmonella* spp. which declined by more than 5 logs within 6 h (Table 3). Among these pathogens, *E. coli* O157:H7 was most resistant to die off. Since cucumbers preserved in acid solutions would typically be held for at least a few weeks in warm weather during the growing season, these results indicate that field contamination of cucumbers by these pathogens would be eliminated during bulk storage.

Table 3—Log reduction of acid-tolerant pathogens in equilibrated cover solutions of nonfermented cucumbers preserved with 150 mM acetic acid, 9 mM sodium benzoate, and 100 mM $CaCl_2$ at pH 3.5 and 30 °C.

Time (h)	Microorganisms		
	Salmonella spp.	*Listeria monocytogenes*	*Escherichia coli* O157:H7
6	7.60 ± 1.4[a]	4.06 ± 2.3	5.92 ± 2.1
18	7.82 ± 0.8	7.51 ± 0.8	7.22 ± 1.1
24	7.82 ± 0.8	7.51 ± 0.8	7.65 ± 0.8

[a]Represents the standard deviations of the logarithmic values of 3 replicas.

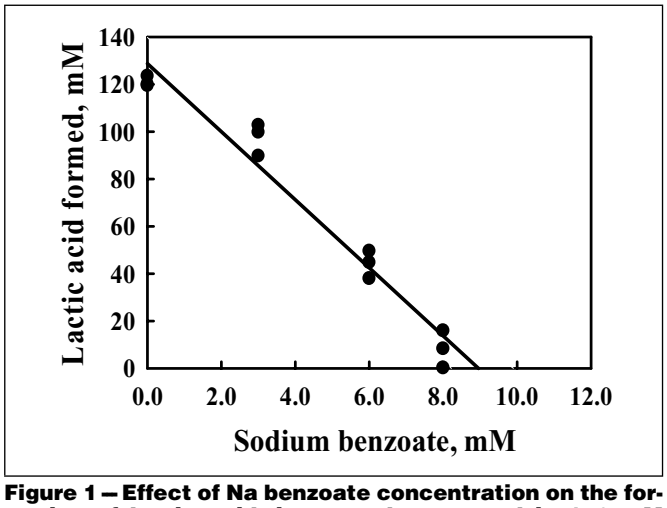

Figure 1—Effect of Na benzoate concentration on the formation of lactic acid, in cucumbers stored in 150 mM acetic acid and 20 mM calcium chloride with pH adjusted with HCl to 3.6. Cucumbers were stored for 126 d at 30 °C.

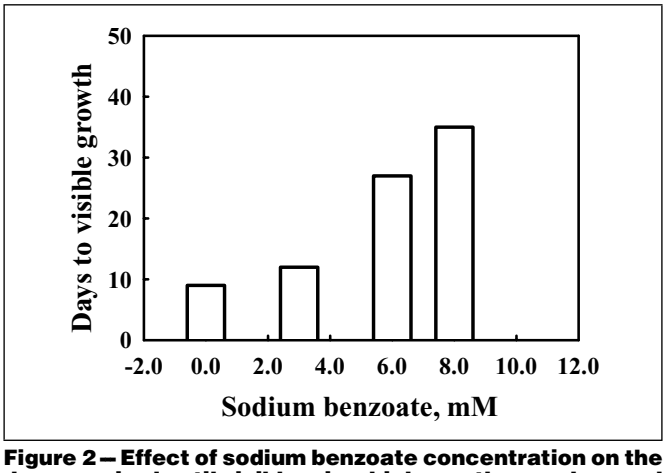

Figure 2—Effect of sodium benzoate concentration on the days required until visible microbial growth was observed in at least one of 3 jars of cucumbers stored in 150 mM acetic acid, and 20 mM calcium chloride with pH adjusted with HCl to 3.6. Cucumbers were stored for 126 d at 30 °C. No growth was observed at sodium benzoate concentrations above 8 mM.

Conclusions

The results of this study provided evidence that microbial growth can be prevented without a thermal process in cucumbers stored in solutions containing acetic acid, sodium benzoate, and calcium chloride to maintain tissue firmness. The concentrations of acetic acid and preservative required to ensure microbial stability of the stored cucumbers were sufficiently low so that the cucumbers could be converted to edible products without the need to wash out and discard excess acid or preservative. Since a thermal process was not required to achieve microbial stability, cucumbers could be stored in bulk containers before further processing to produce edible cucumber pickles. However, scale-up trials under commercial conditions will be needed to confirm these results. In an initial pilot-scale trial, no evidence of microbial growth occurred after 7 mo when cucumbers were stored in 208-L plastic drums at pH 3.6 at a commercial processing plant (unpublished observations). An advantage of sodium benzoate as a preservative in this application was that it did not cause rapid development of a cured appearance of stored cucumbers as occurs when sodium metabisulfite is used to ensure microbial stability (McFeeters 1998). Acid tolerant pathogens died off in less than 24 h with pH, acetic acid, and sodium benzoate concentrations sufficient to ensure microbial stability of cucumbers stored at 30 °C. Potassium sorbate was not effective as a preservative in this application.

Acknowledgments

We thank Ms. Ashlee Webber, Mr. Seth Fornea, and Dr. Sabina Morris of the USDA-ARS at the SAA Food Science Research Unit located in Raleigh, N.C., U.S.A. for their excellent technical assistance.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215(3):403–10.

Barrangou-Poueys R, Yoon SS, Breidt F Jr, Fleming HP, Klaenhammer T. 2002. Identification and characterization of *Leuconostoc fallax* strains isolated from an industrial sauerkraut fermentation. Appl Environ Microbiol 68(6):2877–84.

Bell TA, Etchells JL. 1952. Sugar and acid tolerance of spoilage yeasts from sweet-cucumber pickles. Food Technol 6(12):468–72.

Bell TA, Etchells JL, Borg AF. 1959. The influence of sorbic acid on the growth of certain species of bacteria, yeasts, and filamentous fungi. J Bacteriol 77(5):573–80.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2006. GenBank. Nucleic Acids Res 34(database issue):D16–20.

Binsted R, Devey JD, Dakin JC. 1971. Principles involved in the microbiological keeping quality of pickles and sauces. In: Pickle and sauce making. 3rd ed. London, U.K.: London Food Trade Press Ltd. p 106–18.

Buchanan RL, Edelson SG. 1999. pH-dependent stationary-phase acid resistance response of enterohemorrhagic *Escherichia coli* in the presence of various acidulants. J Food Prot 62:211–8.

Fleming HP, McFeeters RF, Daeschel MA, Humphries EG, Thompson RL. 1988. Fermentation of cucumbers in anaerobic tanks. J Food Sci 53:127–33.

Gates K, Costilow RN. 1981. Factors influencing softening of salt-stock pickles in air-purged fermentations. J Food Sci 46:274–7, 282.

McFeeters RF. 1998. Use and removal of sulfite by conversion to sulfate in the preservation of salt-free cucumbers. J Food Prot 61(7):885–90.

McFeeters RF, Barish AO. 2003. Sulfite analysis of fruits and vegetables by high-performance liquid chromatography (HPLC) with ultraviolet spectrophotometric detection. J Agric Food Chem 51(6):513–7.

McFeeters RF, Fleming HP, Thompson RL. 1982a. Malic and citric acids in pickling cucumbers. J Food Sci 47(6):1859–61, 1865.

McFeeters RF, Fleming HP, Thompson RL. 1982b. Malic acid as a source of carbon dioxide in cucumber fermentations. J Food Sci 47(6):1862–5.

McFeeters RF, Fleming HP, Daeschel MA. 1984. Malic acid degradation and brined cucumber bloating. J Food Sci 49(4):999–1002.

Senses-Ergul S, Karasu-Yalcin S, Ozbas ZY. 2007. The effect of some environmental parameters on the growth of yeasts originating from sugar containing foods. Annals Microbiol 57:191–6.

Turantas F, Göksungur Y, Dincer AH, Ünlütürk A, Güvenc U, Zorlu N. 1999. Effect of potassium sorbate and sodium benzoate on microbial population and fermentation of black olives. J Sci Food Agric 79:1197–202.