Preservation of Acidified Cucumbers with a Combination of Fumaric Acid and Cinnamaldehyde That Target Lactic Acid Bacteria and Yeasts

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Abstract: The naturally occurring compound, fumaric acid, was evaluated as a potential preservative for the long-term storage of cucumbers. Fumaric acid inhibited growth of lactic acid bacteria (LAB) in an acidified cucumber juice medium model system resembling conditions that could allow preservation of cucumbers in the presence of sodium benzoate. Forty millimolars of fumaric acid were required to inhibit growth of an extremely aciduric *Lactobacillus plantarum* LA0445 strain at pH 3.8. Half of this concentration was required to achieve inhibition of *L. plantarum* LA0445 at pH 3.5. As expected growth of the spoilage yeasts *Zygosaccharomyces globiformis* and *Z. bailii* was not inhibited by fumaric acid at near saturation concentrations in the same cucumber juice medium. To usefully apply fumaric acid as a preservative in acidified foods it will be necessary to combine it with a food grade yeast inhibitor. The antimicrobial agent, cinnamaldehyde (3.8 mM) prevented growth of *Z. globiformis* as well as the yeasts that were present on fresh cucumbers. Acidified cucumbers were successfully preserved, as indicated by lack of yeasts or LAB growth and microbial lactic acid or ethanol production by a combination of fumaric acid and cinnamaldehyde during storage at 30 °C for 2 mo. This combination of 2 naturally occurring preservative compounds may serve as an alternative approach to the use of sodium benzoate, potassium sorbate, or sodium metabisulfite for preservation of acidified vegetables without a thermal process.

Keywords: cinnamaldehyde, fumaric acid, lactic acid bacteria, vegetable preservation, *Zygosaccharomyces*

Practical Application: This study evaluates the potential application of alternative preservatives for the long-term storage of cucumbers in a reduced NaCl cover brine solution and without a thermal process.

Introduction

Sodium benzoate, potassium sorbate, and sodium metabisulfite have a long history of use to assist preservation of acid and acidified fruits and vegetables. These preservatives inhibit growth of lactic acid bacteria (LAB) and yeasts, which are the primary spoilage microorganisms in these products. However, many consumers consider use of these traditional preservatives in products to be undesirable. In recent years, there have been efforts to determine the antimicrobial activities of naturally occurring compounds that might substitute for these preservatives (Burt 2004). Fumaric acid, an intermediate on the citric acid cycle, is one of the compounds reported to inhibit growth of some bacteria. Fumaric acid was found to delay bacterial malo-lactic fermentation in wines and to reduce the numbers of viable leuconostoc bacteria (Ough and Kunkee 1974; Pilone and others 1974). It also inhibited the activity of LAB in refrigerated grape juice (Splittstoesser and Stoyla 1989). Fumaric acid was reported to have a bactericidal effect against *Escherichia coli* O157:H7 in commercial apple cider at pH 3.3 (Comes and Beelman 2002) and it reduced the numbers of natural microflora and inoculated pathogens (*E. coli*, *Staphylococcus aureus*, and *Salmonella* spp.) on fresh-cut lettuce (Kondo and others 2006). Fumarate esters have also been shown to be active against *Clostridium botulinum* (Dymicky and others 1987).

Fumaric acid has a long history of use in foods as an acidulent. The Food and Drug Administration granted a generally regarded as safe (GRAS) status and requests the addition of minimal levels of this acid to consumables. This acid has a tart/sour flavor and is often used to substitute citric acid and tartaric acid in foods.

Pérez-Díaz and McFeeters (2008) showed that cucumbers could be preserved in a way that would allow bulk storage without fermentation by storage in a brine solution containing calcium chloride to maintain the firmness of cucumbers. Acetic acid (150 mM) and 12 mM sodium benzoate with pH adjusted to 3.6 or lower prevented microbial growth. This investigation was undertaken to determine if sodium benzoate could be replaced by fumaric acid in this application. A successful preservation method for cucumbers at ambient temperatures will require inhibition of both very acid-tolerant LAB and spoilage yeasts (Bell and Etchells 1952), which cause ethanolic fermentation. Uptake of fumaric acid and

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its conversion to malic acid by yeasts (Corte-Real and Leao 1990; Saayman and others 2000), naturally present in cucumber fermentations such as *Hansenula* spp. and *Candida* spp. (Etchells and Bell 1950; Etchells and others 1952), have been documented. Thus, the ability of combinations of fumaric acid and the yeast inhibitor, cinnamaldehyde (Tampieri and others 2005; Jantan and others 2008), to preserve cucumbers was also evaluated. Cinnamaldehyde constitutes up to 75% of cinnamon oil and it is the main active ingredient (Calsamiglia and others 2007; Jantan and others 2008). Cinnamon is currently added to selected pickle products as a seasoning, primarily for the sweet varieties.

The objective of this investigation was to determine the ability of fumaric acid and combinations of fumaric acid and cinnamaldehyde to inhibit growth of LAB that are capable of carrying out lactic acid fermentations of cucumbers as well as potential spoilage yeasts that cause ethanolic fermentations in cucumber pickle products.

Materials and Methods

Evaluation of the effect of fumaric acid on microbial growth in acidified cucumber juice

The experimental media (EM) consisted of fresh cucumber juice and cover brine solution mixed in a 50:50 ratio (w/w). Fresh cucumber juice was expressed from 2 lots of size 2A pickling cucumbers (25.4 to 31.8 mm dia) using an automatic juice extractor (JM400 Juiceman Jr., Black and Decker, Towson, Md., U.S.A.). The extracted juice was frozen until used, thawed and centrifuged for 1 h at 18660 × *g* (25 °C) using an Eppendorf Centrifuge 5810 R (West Bury, N.Y., U.S.A.) to remove solids. Cover brine solution consisted of 300 mM acetic acid and 60 mM calcium chloride, so that the final acid and calcium chloride concentrations in the EM were 150 and 30 mM, respectively. Dry fumaric acid (Spectrum Laboratory Products, Inc., Gardena, Calif., U.S.A.) was dissolved in the EM at concentrations shown in Table 2 and 3. The EM pH was adjusted to 3.8 or 3.5 as indicated by addition of small volumes (<0.5%) of 3 N HCl or 5 N NaOH solution and filter sterilized.

Microorganisms listed in Table 1 were propagated on deMan Rogosa and Sharpe (MRS) or Yeast and Mold (YM) medium for bacteria and yeasts, respectively. Bacteria and yeast broth cultures were incubated for 16 or 72 h, centrifuged, and then washed twice and resuspended with saline solution prior to the inoculation into the cucumber juice experimental medium. Mixtures of 2 to 5 strains of the individual microbes listed in Table 1 were used to inoculate the EM to 10^5 and 10^4 CFU/mL for bacteria and yeasts, respectively.

Table 1–Strains of aciduric microorganisms used in this study.

Microorganisms	Strains	Source
Zygosaccharomyces globiformis	NRRL SPY 9	Spoiled sweet pickles
	NRRL SPY 15	Spoiled sweet pickles
	NRRL SPY 21	Spoiled sweet pickles
	NRRL SPY 29	Spoiled sweet pickles
	NRRL SPY 32	Spoiled sweet pickles
Zygosaccharomyces bailii	NRRL Y7260	Spoiled salad dressing
	NRRL Y7258	Spoiled salad dressing
Lactobacillus brevis	ATCC8287	Green, fermenting Sevillano variety olives
	ATCC14869	Human feces
Leuconostoc mesenteroides	ATCC19255	Dairy
	ATCC 8293	Fermenting olives
Lactobacillus plantarum	ATCC8014	Corn silage
	ATCC14917	Pickled cabbage
	FSRU LA0445	Fermented cucumbers
Pediococcus pentosaceous	ATCC25745	Plants
	ATCC33316	Dried beer yeast

NRRL = USDA-Agriculture Research Service Culture Collection; ATCC = American Type Culture Collection; FSRU = USDA-ARS Food Science Research Unit Culture collection.

For each treatment, triplicate 15-mL tubes of the EM were inoculated and incubated statically at 30 °C for 1 wk. Preservative effectiveness was evaluated based upon the development of turbidity as determined by measurements of optical density at 600 nm using the Nanodrop ND-1000 UV-Visible Spectrophotometer (Nanodrop, Wilmington, Del., U.S.A.), and followed by an assessment of any reduction or increases in the numbers of inoculated microorganisms by plating on either MRS or YM agar at the end of the incubation period. In addition to microbiological analysis, samples of the inoculated EM were analyzed by high performance liquid chromatography (HPLC) to determine if sugars and malic acid were utilized during the incubation period and if lactic acid and ethanol were formed. Chromatography was done using 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 set to collect data at 210 nm was used to detect organic acids. A Waters model 410 refractive index detector connected in series was used to measure glucose, fructose, and ethanol. External standards were used for calibration and quantification of sugars, ethanol, and organic acids.

Evaluation of the effect of a combination of fumaric acid and cinnamaldehyde on the growth of naturally occurring microorganisms and spoilage yeasts, *Zygosaccharomyces globiformis* and *Z. bailii,* in acidified cucumber jars

Size 2B 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 jars was 55:45 w/v. Cover solutions contained commercial vinegar (20% acetic acid) and calcium chloride such that after equilibration with the cucumbers the concentration of acetic acid was 0 or 150 mM and the concentration of calcium chloride was either 100 mM or 30 mM. Sodium benzoate (Fisher Scientific Co., Pittsburgh, Pa., U.S.A.) and sodium fumarate (Alfa Aesar, Ward Hill, Mass., USA) were added as dry powders to equilibrated concentrations of 12 mM and 30mM, respectively. Cinnamaldehyde (Sigma-Aldrich Co., St. Louis, Mo., U.S.A.) was added to the equilibrated concentrations indicated in the text. The initial equilibrated pH of the brined cucumbers was adjusted to 3.5 ± 0.1 by addition of 3 N HCl solution. The amount of HCl required was determined by mixing cucumber slurry and cover brine solution in a 55:45 (w/v) ratio, and then titrating the mixture with acid or base to the target pH. Triplicate jars of 2 different lots of cucumbers were prepared for each treatment. 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 to soften the plastisol gasket and immediately applied to the filled jars. Jars were incubated at 30 °C.

Preservation treatments in which there was no detectable microbial growth after 3 wk of incubation were challenged by inoculating *Lactobacillus plantarum* LA0445, and a mixture of 5 *Z. globiformis* isolates and 2 *Z. bailii* strains (Table 1). The mixed yeast

isolates and the bacterium were individually inoculated through the lid septum of the sample jars to give initial populations of 10^4 CFU/mL and 10^5 CFU/mL in each jar, respectively.

Selected jars were pasteurized by heating in water in a steam-jacketed kettle to a temperature of 74 °C at the slowest heating point in the jars for 15 min. Subsequently, jars were cooled to 40 °C in cold tap water before removing them from the steam-jacketed kettle. Pasteurization was done to evaluate the ability of inoculated microorganisms to grow without interference from organisms naturally present on the fresh cucumbers.

Microbial and chemical analysis for samples collected from acidified cucumber jars

After equilibration of the acidified cucumber jars (2 d), initial samples of cover brine solutions were aseptically collected

Table 2–Effect of fumaric acid on growth of *Lactobacillus* spp. and *Zygosaccharomyces* spp. in cucumber juice medium at pH 3.8 and 30 °C, 1 wk postinoculation.

Microorganisms tested	Fumaric acid added (mM)	Log of CFU/mL†	Substrate utilization (mM)‡				Product formation (mM)	
			Glucose	Fructose	Fumaric acid	Malic acid	Lactic acid	Ethanol
Lactobacillus plantarum	0	7.54 ± 0.1[a]	26.8 ± 0.8[a]	32.3 ± 7.0[a]	–	12.9 ± 1.0[a]	84.4 ± 2.1[a]	ND*[a]
	5	7.84 ± 0.8[a]	26.5 ± 2.5[a]	34.9 ± 6.4[a]	4.1 ± 0.5[a]	12.8 ± 0.7[a]	88.2 ± 3.1[a]	ND[a]
	10	7.58 ± 0.1[a]	23.3 ± 1.5[a]	29.2 ± 4.0[a]	9.8 ± 2.3[a]	12.9 ± 1.0[a]	76.6 ± 9.1[a]	ND[a]
	20	7.57 ± 0.2[a]	21.1 ± 0.9[a]	23.7 ± 2.6[b]	3.1 ± 1.3[a]	12.4 ± 1.1[a]	51.7 ± 5.1[b]	ND[a]
	30	3.86 ± 0.8[b]	9.8 ± 5.3[b]	11.7 ± 5.3[c]	ND[a]	3.8 ± 2.4[b]	ND[c]	ND[a]
	40	ND[c]	ND[b]	ND[c]	ND[a]	ND[b]	ND[c]	ND[a]
Lactobacillus brevis	0	7.80 ± 0.3[a]	24.2 ± 1.9[a]	32.2 ± 0.2[a]	–	10.9 ± 0.4[a]	35.4 ± 11.2[a]	ND[a]
	5	7.82 ± 0.1[a]	23.1 ± 3.8[a]	32.2 ± 0.2[a]	5.3 ± 1.0[a]	10.5 ± 0.7[a]	39.1 ± 14.9[a]	ND[a]
	10	7.49 ± 0.2[a]	16.1 ± 0.9[b]	32.2 ± 0.2[a]	5.7 ± 0.9[a]	9.7 ± 0.4[a]	29.9 ± 12.5[a]	ND[a]
	20	4.62 ± 2.9[a]	2.1 ± 0.6[b]	4.2 ± 0.8[b]	ND[a]	1.8 ± 0.3[b]	2.27 ± 0.1[b]	ND[a]
	30	3.08 ± 0.6[b]	ND[b]	ND[b]	ND[a]	ND[b]	ND[b]	ND[a]
Pediococcus pentosaceous	0	6.97 ± 0.3[a]	4.6 ± 3.3[a]	10.1 ± 6.7[a]	–	9.1 ± 1.7[a]	35.4 ± 6.8[a]	ND[a]
	5	7.00 ± 0.3[a]	3.7 ± 0.6[a]	9.5 ± 6.2[a]	5.3 ± 1.0[a]	8.3 ± 1.5[a]	34.9 ± 0.4[a]	ND[a]
	10	4.77 ± 1.1[b]	ND[a]	ND[b]	ND[a]	ND[b]	1.64 ± 0.8[b]	ND[a]
	20	ND[c]	ND[a]	ND[b]	ND[a]	ND[b]	ND[b]	ND[a]
Leuconostoc mesenteroides	0	7.79 ± 0.3[a]	5.6 ± 0.2[a]	5.0 ± 0.2[a]	–	4.1 ± 0.8[a]	24.3 ± 6.3[a]	ND[a]
	5	7.52 ± 0.4[b]	1.2 ± 0.9[a]	2.5 ± 0.6[a]	2.5 ± 0.3[a]	3.1 ± 0.8[a]	10.5 ± 3.4[a]	ND[a]
	10	ND[c]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
	20	ND[c]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Zygosaccharomyces globiformis	0	7.56 ± 0.3[a]	22.2 ± 8.6[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	48.8 ± 8.0[a]
	40	6.02 ± 0.1[b]	22.4 ± 6.1[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	48.5 ± 10.5[a]
	50	5.75 ± 0.4[b]	24.4 ± 9.7[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	48.7 ± 14.3[a]
	60	5.91 ± 0.4[b]	22.3 ± 5.4[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	49.9 ± 10.6[a]
Zygosaccharomyces bailii	0	6.52 ± 0.1[a]	19.3 ± 7.5[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	54.6 ± 12.6[a]
	40	5.87 ± 0.5[b]	25.6 ± 9.9[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	21.3 ± 10.1[a]
	50	6.07 ± 0.4[a]	23.1 ± 7.0[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	34.3 ± 7.5[a]
	60	6.26 ±0.1[a]	18.6 ± 2.8[a]	32.3 ± 7.0[a]	ND[a]	ND[a]	ND[a]	49.2 ± 9.7[a]

*ND = not detected.
†Bacterial and yeast cultures were inoculated to 5.0 ± 0.5 and 4.0 ± 0.5 CFU/mL, respectively.
‡Initial glucose, fructose, and malic acid concentrations were 25.2 ± 0.9, 29.9 ± 1.4, and 14.9 ± 3.3, respectively.
Mean values ± standard deviation for two trials. Mean values within columns for each microorganism with similar letters are not significantly different (Duncan's multiple range test, $P > 0.05$). A zero value was assigned to those measurements that were below the detection limit (ND).

Table 3–Effect of fumaric acid on growth of *Lactobacillus* spp. and *Zygosaccharomyces* spp. in a cucumber juice medium at pH 3.5 and 30 °C, 1 wk postinoculation.

Microorganism tested	Fumaric acid added (mM)	Log of CFU /mL†	Substrate utilization (mM)‡				Product formation (mM)	
			Glucose	Fructose	Fumaric acid	Malic acid	Lactic acid	Ethanol
Lactobacillus plantarum	0	6.65 ± 1.9[a]	19.3 ± 9.3[a]	21.5 ± 2.9[a]	–	15.2 ± 7.5[a]	102.3 ± 41.7[a]	ND*[a]
	10	4.89 ± 1.4[a]	14.3 ± 5.5[ab]	19.4 ± 6.2[a]	10.5 ± 1.5[a]	14.9 ± 7.5[a]	86.2 ± 47.7[ab]	ND[a]
	20	ND[b]	0.5 ± 0.1[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[a]
	30	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[a]
Lactobacillus brevis	0	7.86 ± 0.6[a]	25.5 ± 1.5[a]	12.6 ± 2.3[a]	–	15.5 ± 7.5[a]	45.2 ± 10.6[a]	ND[a]
	10	ND[b]	ND[b]	ND[b]	ND[a]	ND[b]	ND[b]	ND[a]
	20	ND[b]	ND[b]	ND[b]	ND[a]	ND[b]	ND[b]	ND[a]
Zygosaccharomyces globiformis	0	5.93 ± 0.5[a]	10.7 ± 1.5[a]	30.1 ± 10.4[a]	–	3.1 ± 0.6[a]	ND[a]	48.8 ± 16.1[a]
	40	6.10 ± 0.6[a]	11.6 ± 1.6[a]	30.1 ± 1.5[a]	ND[a]	7.6 ± 1.1[b]	ND[a]	58.2 ± 18.2[a]
	50	5.90 ± 0.2[a]	12.1 ± 1.4[a]	30.0 ± 1.5[a]	ND[a]	7.3 ± 0.7[b]	ND[a]	57.3 ± 18.9[a]
	60	6.24 ± 0.6[a]	ND[b]	27.2 ± 1.5[a]	ND[a]	7.4 ± 1.1[b]	ND[a]	36.5 ± 5.2[a]
Zygosaccharomyces bailii	0	6.58 ± 0.3[a]	12.5 ± 1.6[a]	34.5 ± 8.6[a]	–	11.5 ± 5.8[a]	ND[a]	72.4 ± 5.3[a]
	40	6.19 ± 0.2[a]	7.3 ± 0.8[b]	29.8 ± 1.5[a]	ND[a]	7.6 ± 1.2[b]	ND[a]	69.4 ± 18.6[a]
	50	5.89 ± 0.4[a]	6.7 ± 1.6[b]	29.6 ± 6.4[a]	ND[a]	7.5 ± 0.6[b]	ND[a]	65.1 ± 16.7[a]
	60	6.09 ± 0.7[a]	ND[c]	28.5 ± 6.4[a]	ND[a]	7.4 ± 1.2[b]	ND[a]	49.8 ± 1.3[a]

*ND = not detected.
†Bacterial and yeast cultures were inoculated to 5.0 ± 0.5 and 4.0 ± 0.5 CFU/mL, respectively.
‡Initial glucose, fructose, and malic acid concentrations were 25.2 ± 0.9, 29.9 ± 1.4, and 14.9 ± 3.3, respectively.
Mean values ± standard deviation for 2 trials. Mean values within columns for each microorganism with similar letters are not significantly different (Duncan's multiple range test, $P > 0.05$). A zero value was assigned to those measurements that were below the detection limit (ND).

for microbiological analysis. Jars were visually monitored for the development of turbidity and/or pressure on the lids during storage. Additional brine samples for microbiological analysis were aseptically collected, after turbidity developed in a jar. Samples were plated on MRS or YM for bacteria and yeasts, respectively. Plates were incubated at 30 °C for 72 h. MRS plates were incubated under anaerobic conditions. Occurrence of microbial growth was also evaluated by determination of changes in sugars, organic acids, and ethanol using HPLC for which samples of brine were taken from jars, centrifuged at 18660 × g for 10 min (Eppendorf Centrifuge 5810R, West Bury, N.Y., U.S.A.), and the supernatants stored at −20 °C until analyzed. Samples were thawed, spun down again to remove particles, and diluted appropriately with water into autosampler vials so that peaks were within the ranges of the standards. HPLC analysis to measure glucose, fructose, ethanol, and organic acids was performed as described above.

Statistical analysis

Experiments were repeated 2 times with 3 jars per replicate. Log numbers and measured concentrations for lactic acid and ethanol were analyzed using the analysis of variance (ANOVA) procedure with the Duncan's multiple range test of the Statistical Analysis Systems version 9.0 (Statistical Analysis System, SAS Inst., Cary, N.C., U.S.A.) using a significance level of 0.05.

Results and Discussion

Table 2 shows viable counts for the inoculated microorganisms and the utilization of glucose, fructose, and malic acid and formation of lactic acid and ethanol in the EM to which different concentrations of fumaric acid were added. With 5 mM fumaric acid added to pH 3.8 EM, all the LAB species evaluated were able to produce some lactic acid (Table 2). Furthermore, some of the added fumaric acid was metabolized. *L. plantarum* has been shown to have both fumarase and fumarate reductase, which can utilize fumaric acid as a substrate (Chen and McFeeters 1986). The other LAB have putative genes for these enzymes. *L. plantarum* and *L. brevis* produced no detectable lactic acid with 30 mM fumaric acid at pH 3.8. Viable organisms survived, but their numbers were lower than the inoculated level. This showed fumaric acid was an effective preservative against these acid-tolerant LAB. Twenty millimolar fumaric acid prevented lactic acid production by *Pediococcus pentosaceous* and reduced viable cell counts below detectable levels. *Leuconostoc mesenteroides* died off to nondetectable numbers and produced no lactic acid in the presence of only 10 mM fumaric acid at pH 3.8.

When the pH of the EM was reduced to pH 3.5, *P. pentosaceous* and *Leu. mesenteroides* were unable to grow and produce lactic acid in the absence of fumaric acid. At pH 3.5 *L. plantarum* produced no detectable lactic acid and viable cell counts were below detectable levels with 20 mM added fumaric acid. *L. brevis* produced no lactic acid and the inoculated cells died off with only 10 mM fumaric acid at pH 3.5. These results showed that LAB, including the highly acid-tolerant *L. plantarum*, were prevented from growing in cucumber juice when fumaric acid was added at concentrations that could reasonably be added to acidified cucumbers.

In contrast to LAB, the spoilage yeasts *Z. globiformis* and *Z. bailii* increased in numbers and produced ethanol at all concentrations of fumaric acid at both pH 3.8 and 3.5 (Table 2 and 3). Under these conditions they utilized primarily fructose from the EM medium and a reduced amount of glucose. The highest concentration of fumaric acid added, 60 mM, is near its solubility limit in water. Therefore, fumaric acid added by itself to acidified cucumbers would not be able to prevent growth of potential spoilage yeasts. Inhibition of *Zygosaccharomyces* spp. in foods has been traditionally a challenge due to the ability of these microorganisms to tolerate high levels of preservatives, sugars, salts, and acidic pH (Martorell and others 2007). Additionally, a variety of organic acids, including fumaric acid, are either actively or passively transported by yeasts (Corte-Real and Leao 1990). Fumaric acid is primarily metabolized to malic acid via fumarase by a number of yeasts (Corte-Real and Leao 1990; Saayman and others 2000).

Table 4 shows the results of an initial survey to determine the effectiveness of cinnamaldehyde to prevent growth of the spoilage yeasts, *Z. globiformis* and *Z. bailli* in pasteurized and acidified cucumber jars. The standard preservative for acidified vegetables, sodium benzoate, prevented sugar utilization and ethanol production by the inoculated yeasts at a concentration of 12 mM in the pasteurized cucumber jars acidified with HCl to pH 3.5 (Table 4). Among the cinnamaldehyde concentrations tested, 3.8 mM was the minimal level found to inhibit carbohydrate utilization and ethanol production by the spoilage yeasts, in the absence of acetic acid and sodium chloride, which are traditionally added to acidified cucumbers for preservation purposes. Jars containing cinnamaldehyde at 3.8 mM or higher did not develop pressure or turbidity during the 60-d incubation period.

Table 5 shows the inhibition of microbial growth as indicated by sugar utilization and formation of lactic acid and ethanol in cucumbers acidified with 150 mM acetic acid, 30 mM $CaCl_2$, and 3.8 mM cinnamaldehyde with an adjusted pH of 3.5. The cucumbers were not pasteurized. Under these conditions, both glucose and fructose were utilized and lactic acid and ethanol were produced in the control jars to which fumaric acid was not added. However, lactic acid was not detected in treatment jars containing 30 mM fumaric acid. Addition of 3.8 mM cinnamaldehyde at the concentrations found to prevent spoilage yeast growth in the previous experiment resulted in successful preservation of the cucumbers in that there was no reduction in the concentration of either sugar and no production of ethanol beyond that expected from metabolism of the cucumbers in the acidified brines (4.0 ± 0.3 mM). In addition, no visible turbidity developed in any of the jars during a 60-d incubation period. Preservation was also successful upon inoculation of both *Z. globiformis* and *Z. bailii* into jars containing 30 mM fumaric acid and 3.8 mM cinnamaldehyde. Although, the ability of cinnamaldehyde to preserve cucumbers

Table 4–Ethanol production and fructose utilization 60 d after inoculation of *Z. globiformis* and *Z. bailii* into pasteurized cucumber jars at pH 3.5 with 0 mM acetic acid, 30 mM fumaric acid, and 100 mM $CaCl_2$ supplemented with the yeast inhibitor, cinnamaldehyde.

Preservative added (mM)	Substrates utilized (mM)		Products formed (mM)	
	Glucose	Fructose	Glycerol	Ethanol
12.0 Sodium benzoate	None[a]	None[a]	ND*[a]	ND[a]
19.0 Cinnamaldehyde	None[a]	None[a]	ND[a]	ND[a]
7.6 Cinnamaldehyde	None[a]	None[a]	ND[a]	ND[a]
3.8 Cinnamaldehyde	None[a]	None[a]	ND[a]	ND[a]
0.8 Cinnamaldehyde	2.15 ± 0.7[a]	11.9 ± 2.1[a]	ND[a]	ND[a]

*ND = not detected.
Mean values ± standard deviation for two trials. Mean values within columns for each microorganism with similar letters are not significantly different (Duncan's multiple range test, $P > 0.05$). A zero value was assigned to those measurements that were below the detection limit (ND).

Table 5–Cucumber preservation without pasteurization by a combination of 30 mM fumaric acid and 3.8 mM cinnamaldehyde in brine solutions containing 150 mM acetic acid and 30 mM calcium chloride at pH 3.5.

Fumaric acid added (mM)	Cinnamaldehyde added (mM)	Inocula *Lactobacillus plantarum*/ *Zygosaccharomyces* spp. (CFU/mL)	Substrates utilized (mM)		Products formed (mM)	
			Glucose	Fructose	Lactic acid	Ethanol
0.0	0.0	None	29.8 ± 1.2^{a}	37.6 ± 3.6^{a}	80.8 ± 2.6^{a}	49.8 ± 11.3^{a}
0.0 (12 mM benzoate)	0.0	None	None^{b}	None^{c}	ND†^{b}	3.6 ± 0.5^{b}
30	0.0	None	None^{b}	25.2 ± 1.8^{b}	ND^{b}	23.9 ± 6.1^{b}
30	3.8	None	None^{b}	None^{c}	ND^{b}	4.0 ± 0.3^{b}
30	3.8	$10^{5}/10^{4}$	None^{b}	None^{c}	ND^{b}	4.3 ± 1.9^{b}

†ND = not detected.
Mean values ± standard deviation for 2 trials. Mean values within columns for each microorganism with similar letters are not significantly different (Duncan's multiple range test, $P > 0.05$). A zero value was assigned to those measurements that were below the detection limit (ND).

in the absence of fumaric acid was not tested, independent experiments suggest that 10 mM fumaric acid in combination with 2.0 mM cinnamaldehyde is not an effective preservation treatment for nonpasteurized cucumber jars brined with 150 mM acetic acid and 100 mM calcium chloride to pH 3.5 in which inoculated *L. plantarum* and *Z. globiformis* proliferated (data not shown).

Based on preliminary observations, the color and texture of the preserves, brined with 150 mM acetic acid, 100 mM $CaCl_2$, 30 mM fumaric acid, and 3.8 mM cinnamaldehyde was not significantly different from that observed in acidified pickles traditionally brined with up to 350 mM acetic acid. An overwhelming chalky flavor was detected due to the addition of 100 mM calcium chloride to maintain firmness. However, $CaCl_2$ concentrations can be wash out the pickles with water before making final products (McFeeters and Pérez-Díaz 2010). Such washing step could potentially reduce the amount of both cinnamaldehyde and fumaric acid that would be carried out into final products. Ultimately, the flavor that would be perceived by consumers in the final products would be primarily dependent on the formulation of the brine cover solutions that would be used for packing the washed-acidified and preserved pickles.

Further studies would be necessary before applying the treatment proposed here at the commercial scale. A variable that could play a significant role in preventing preservation at such scale would be the dissolve oxygen content present in commercial fermentation tanks, which is expected to be higher than that potentially present in the sealed experimental cucumber jars used for this work. Availability of higher oxygen concentrations in the commercial tanks would likely promote a more abundant growth of the oxidative and film yeasts naturally present in cucumbers (Etchells 1941; Bell and Etchells 1952). A more significant loss of the volatile preservative, cinnamaldehyde, from the open-top fermenting tank with time is also expected.

Conclusion

Combination of fumaric acid with compounds that target yeasts, such as cinnamaldehyde, could be a useful preservative system in some applications. Such treatment could make it possible to preserve cucumbers in bulk without sodium benzoate or sodium metabisulfite (Pérez-Díaz and McFeeters 2008), if larger scale trials were to be successful. Sodium benzoate has been reported to be degraded to produce part per billion levels of benzene when low pH sodas and juice drinks are thermally processed (Nyman and others 2008). Some consumers avoid products containing sulfite compounds due to the fact that a small fraction of people are hypersensitive to sulfites (Lester 1995).

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