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Antimicrobial Effects of Mustard Flour and Acetic Acid against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* Serovar Typhimurium

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This study was designed to investigate the individual and combined effects of mustard flour and acetic acid in the inactivation of food-borne pathogenic bacteria stored at 5 and 22°C. Samples were prepared to achieve various concentrations by the addition of acetic acid (0, 0.5, or 1%) along with mustard flour (0, 10, or 20%) and 2% sodium chloride (fixed amount). Acid-adapted three-strain mixtures of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium strains (10^6 to 10^7 CFU/ml) were inoculated separately into prepared mustard samples stored at 5 and 22°C, and samples were assayed periodically. The order of bacterial resistance, assessed by the time required for the nominated populations to be reduced to undetectable levels against prepared mustards at 5°C, was *S. enterica* serovar Typhimurium (1 day) < *E. coli* O157:H7 (3 days) < *L. monocytogenes* (9 days). The food-borne pathogens tested were reduced much more rapidly at 22°C than at 5°C. There was no synergistic effect with regard to the killing of the pathogens tested with the addition of 0.5% acetic acid to the mustard flour (10 or 20%). Mustard in combination with 0.5% acetic acid had less bactericidal activity against the pathogens tested than did mustard alone. The reduction of *E. coli* O157:H7 and *L. monocytogenes* among the combined treatments on the same storage day was generally differentiated as follows: control < mustard in combination with 0.5% acetic acid < mustard alone < mustard in combination with 1% acetic acid < acetic acid alone. Our study indicates that acidic products may limit microbial growth or survival and that the addition of small amounts of acetic acid (0.5%) to mustard can retard the reduction of *E. coli* O157:H7 and *L. monocytogenes*. These antagonistic effects may be changed if mustard is used alone or in combination with >1% acetic acid.

Food-borne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* are found in a wide variety of foods. Enterohemorrhagic *E. coli* O157:H7 has a low infective dose (10, 11) and causes hemorrhagic colitis, which is occasionally complicated by hemolytic uremic syndrome (16, 22, 23, 26). It has been implicated in over 73,500 cases of illness each year in the United States (21). Although most outbreaks have been associated with undercooked ground beef and raw milk (10), a variety of acidic foods traditionally considered of low risk have subsequently been implicated in outbreaks, including unpasteurized apple juice, salami, yogurt, and mayonnaise (2, 5, 6, 36). *L. monocytogenes* has been isolated from various environments. It primarily causes meningitis, encephalitis, or septicemia and is responsible for nearly one-fourth of all estimated food-borne disease-related deaths caused by known pathogens in the United States each year (21). Outbreaks of listeriosis have been also associated with acid foods such as coleslaw (14). *S. enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype, accounting for 23% of laboratory-confirmed *Salmonella* cases that occur in over 1.4 million *Salmonella* infections each year in the United States (7). Also, *S. enterica* serovar Typhimurium has been found in commercial apple cider (4).

The involvement of these pathogens in outbreaks associated with the consumption of acidic foods has drawn attention to the acid adaptation response of these pathogens and the impact on resistance to environmental stresses, especially acid. Acid-adapted cells have increased resistance to inactivation by organic acids, and therefore acid-adapted cells can survive better than nonadapted cells in various low-pH foods (1, 3, 15, 17, 31). Moreover, several workers have found that acid-adapted *E. coli* O157:H7 strains survived longer at refrigeration temperature than at room temperature in mayonnaise (13, 25, 34, 36), ketchup (31), mustard (20), and unpasteurized apple cider (37). Zhao et al. (37) observed that *E. coli* O157:H7 survived for up to 31 days at 8°C in unpasteurized apple cider (pH 3.6 to 4.0). Because of their pathogenicity and their ability to survive in hostile environments, the development of acid resistance by food-borne pathogenic bacteria may have important implications in food production.

Allyl isothiocyanate (AITC), a component of mustard oil and of human food plants such as cabbage, cauliflower, and horseradish (28), has potential for use as an antimicrobial agent in a variety of foods because of its natural origin (8, 18, 19, 24, 33, 35). Acetic acid has also been widely tested as a food preservative for killing of food-borne pathogenic bacteria (1, 9, 12, 27, 29). There is little doubt that both mustard and acetic acid have a bactericidal effect on food-borne pathogenic bacteria. However, we have focused on factors affecting the anti-

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microbial activity of prepared mustards against food-borne pathogens for the better control of food-borne pathogens in acidic foods. Most studies concerning acidic foods have limited their scope to the survival of food-borne pathogens. Moreover, available information on the effectiveness of acetic acids and AITC for the destruction of food-borne pathogens has been generated from several individual factors (acetic acid, AITC, salt, or temperature). For example, when mustard flour is combined with acetic acid, the effectiveness of the combination in retarding growth or killing food-borne pathogenic bacteria may vary in various environments. The stability of AITC may be different in various low-pH environments, and the undissociated form of acetic acid may change because of the additive amount of mustard flour. Therefore, it is necessary to understand the combined effects of several factors in acidic foods in the control of food-borne pathogens.

The present study was designed to investigate the individual and combined effects of mustard flour and acetic acid in the inactivation of food-borne pathogenic bacteria at 5 and 22°C.

MATERIALS AND METHODS

Bacterial strains. Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *L. monocytogenes* (ATCC 7644, ATCC 19113, and ATCC 19114), and *S. enterica* serovar Typhimurium (ATCC 19585, ATCC 363755, and DT104 Killercow) were obtained from the Food Microbiology Culture Collection at Washington State University (Pullman, Wash.). All cultures were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 4°C and subcultured monthly.

Cell suspension and inoculation. Each strain of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium was cultured twice in tryptic soy broth (Difco) overnight at 37°C before use in experiments, and a portion (500 μl) of the overnight culture was inoculated into 50 ml of tryptic soy broth (Difco) supplemented with 1% (wt/vol) glucose for 24 h at 37°C for acid adaptation. The addition of glucose to the broth results in a pH drop overnight, and cells undergo acid adaptation (3, 27). The final pHs of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium cultures after incubation ranged from 4.6 to 4.8, from 4.4 to 4.7, and from 4.6 to 4.8, respectively. Differences among six replicates were less than 0.1 pH unit.

The cultures of each group were combined in plastic 50-ml centrifuge tubes (Corning Inc., Corning, N.Y.), and cells were harvested by centrifugation (Centra-CL2; IEC, Needham Heights, Mass.) at 2,600 × *g* for 20 min. After the supernatant was discarded, the pellet was washed twice with 0.2% sterile peptone water. The final pellet was resuspended in 0.2% sterile peptone water to a concentration calculated to yield 10^6 to 10^7 CFU per milliliter of sample. Prepared culture cocktails of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium were added separately to the samples and then completely mixed with the sample for 30 s for uniform distribution.

Sample treatment. Mustard flour (Baltimore), commonly used by manufacturers of prepared mustards, was obtained from a local company in Washington State, and commercial vinegar (containing 5% acetic acid) and salt were purchased at a grocery store. Samples were prepared to achieve various concentrations of acetic acid (0, 0.5, and 1% [vol/vol]) by adding 0, 10, and 20 ml of vinegar along with 0, 10, and 20 g of mustard flour and 2 g of salt (fixed amount) to a sterilized 250-ml glass bottle with a screw cap (Corning Inc.) and bringing the volume up to 100 ml with sterilized distilled water. Sodium chloride was chosen because of its synergistic antimicrobial effect on food-borne pathogens in combination with acetic acid (12) and because both substances are commonly used in retail prepared mustards. The samples were made 12 h prior to inoculation and were held at 5 ± 1°C or 22 ± 1°C for equilibration. The samples stored at 22°C were examined at 0, 12, and 24 h. The samples stored at 5°C were examined at 0, 1, 2, 3, 5, and 7 days. These experiments were repeated six times.

Bacterial enumeration and enrichment. Sorbitol MacConkey agar (Difco), Oxford agar base (Difco), and xylose lysine desoxycholate agar (Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *L. monocytogenes,* and *S. enterica* serovar Typhimurium, respectively. Aliquots (1 ml) of sample were serially diluted (10^{-1} to 10^{-5}) with 9 ml of 0.2% sterile peptone water. Following 10-fold serial dilution, 0.1 ml of diluent was spread-plated onto sorbitol MacConkey agar, Oxford agar base, and xylose lysine desoxycholate agar in duplicate, and 0.1 ml of nondiluted samples (10^0) were spread-plated on five plates of each selective agar. The plates were incubated at 37°C for 48 h, and then cells were enumerated. The detection limit of the analysis was 0.3 $\log_{10}$ CFU/ml. To investigate the extent of recovery of injured cells, 1 ml of sample was inoculated into 50 ml of tryptic soy broth and incubated at 37°C for 24 h. Enriched samples were streaked with a flamed loop onto sorbitol MacConkey agar, Oxford agar base, or xylose lysine desoxycholate agar in duplicate, and the plates were incubated at 37°C for 48 h. Following incubation, the results were recorded as positive or negative.

pH measurement. The pHs of samples were determined with a flat-surface combination probe (model 430; Corning Inc.) before and after inoculation and during the time course.

Statistical analysis. For a factorial set of treatments, mustard flour at 0, 10, or 20% added and acetic acid at 0, 0.5, or 1.0% added were used in a three by three factorial arrangement in six randomized complete blocks, each block consisting of nine treatments. Before analysis, the average of duplicate plate counts from six replications was converted to $\log_{10}$ CFU per milliliter for analysis of variance. Analyses were performed with the geometric least means procedure of the SAS package (version 8.1; SAS Institute Inc., Cary, N.C.). Least-squares means were generated for the dependent variables (*E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium). When analyses of variance indicated statistical significance ($P < 0.05$), least-squares means were separated by the probability option (PDIFF, a pairwise *t* test).

RESULTS

A significant ($P < 0.01$) three-way interaction (mustard level × acetic acid level × storage time) was found in all pathogenic bacteria tested regardless of storage temperature.

pH. The initial pH of the samples ranged from 2.56 to 6.25 (Tables 1 and 2). There was little change (<0.1 pH unit) in pH after bacterial inoculation and during storage except for the control. When compared, the pHs of the combined treatments at the same concentrations of acetic acid (0.5 or 1%), solutions containing 20% mustard had a higher pH (>0.1 pH unit) than those containing 10% mustard.

Survival of food-borne pathogens stored at 5°C. The survival of *S. enterica* serovar Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* in various solutions containing mustard flour, acetic acid, and salt at 5°C is presented in Table 1. *S. enterica* serovar Typhimurium was strongly reduced in all combined treatments except for the control. After 1 day of storage, *S. enterica* serovar Typhimurium was not detected in all treatments by either direct plating or enrichment. For *E. coli* O157:H7, the numbers were slowly reduced compared to *S. enterica* serovar Typhimurium and were undetectable after 5 days (Table 1). The reduction of *E. coli* O157:H7 was clearly differentiated ($P < 0.05$) among the combined treatments at 1 day as follows: control (a reduction of 0.7 $\log_{10}$ CFU/ml) < 10% mustard–0.5% acetic acid (a reduction of 1.2 $\log_{10}$ CFU/ml) < 20% mustard–0.5% acetic acid (a reduction of 1.5 $\log_{10}$ CFU/ml) < 10% mustard–0% acetic acid (a reduction of 2.5 $\log_{10}$ CFU/ml) = 20% mustard–0% acetic acid (a reduction of 2.75 $\log_{10}$ CFU/ml) < 20% mustard–1% acetic acid (a reduction of 3.3 $\log_{10}$ CFU/ml) < 10% mustard–1% acetic acid (a reduction of 3.6 $\log_{10}$ CFU/ml) < 0% mustard–0.5% acetic acid = 0% mustard–1% acetic acid (a reduction of 6.6 $\log_{10}$ CFU/ml).

During the time course, the slowest antimicrobial action in mustard combined with 0.5% acetic acid (10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid) was also observed and resulted in the survival of *E. coli* O157:H7 for up to 3 days at 5°C. Although statistical differences ($P < 0.05$) in the reduction rate between 10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid or 10% mustard–1% acetic acid

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TABLE 1. Antimicrobial effect of mustard flour combined with acetic acid on strains of three species stored at 5 ± 1°C[a]

Species or pH	Day	Log$_{10}$ CFU/ml (growth)								
		0% mustard plus acetic acid (%)			10% mustard plus acetic acid (%)			20% mustard plus acetic acid (%)		
		0.0	0.5	1.0	0.0	0.5	1.0	0.0	0.5	1.0
S. enterica	0	6.62^{e}	6.61^{e}	6.47^{e}	6.48^{e}	6.56^{e}	6.51^{e}	6.59^{e}	6.51^{e}	6.60^{e}
	1	5.60^{f}	<0.3 (−)[d]	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)
	2	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
E. coli	0	6.58^{e}	6.65^{e}	6.63^{e}	6.69^{e}	6.78^{e}	6.79^{e}	6.75^{e}	6.72^{e}	6.84^{e}
	1	5.83^{f}	<0.3 (−)	<0.3 (−)	4.15^{h}	5.52^{j}	3.20^{ln}	4.00^{h}	5.23^{p}	3.54^{k}
	2	6.01^{f}	<0.3 (−)	<0.3 (−)	1.59^{i}	3.47^{kl}	0.53^{o}	1.39^{i}	3.16^{n}	<0.3 (−)
	3	5.91^{f}	<0.3 (−)	<0.3 (−)	<0.3 (−)	2.31^{m}	<0.3 (−)	<0.3 (−)	1.97^{q}	<0.3 (−)
	5	4.83^{g}	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)
	7	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
L. monocytogenes	0	6.45^{e}	6.75^{e}	6.68^{e}	6.75^{e}	6.67^{e}	6.68^{e}	6.65^{e}	6.65^{e}	6.72^{e}
	1	6.64^{e}	<0.3 (−)	<0.3 (−)	5.49^{fg}	6.23^{e}	5.06^{fghi}	5.40^{fgh}	6.15^{e}	4.61^{ij}
	2	6.64^{e}	<0.3 (−)	<0.3 (−)	4.81^{hij}	5.61^{f}	2.19^{l}	4.37^{j}	5.58^{fg}	1.98^{ln}
	3	6.60^{e}	<0.3 (−)	<0.3 (−)	3.35^{k}	5.04^{fghi}	0.55^{m}	2.98^{k}	4.92^{ghij}	<0.3 (+)
	5	6.33^{e}	<0.3 (−)	<0.3 (−)	<0.3 (+)	2.21^{l}	<0.3 (+)	<0.3 (+)	1.37^{n}	<0.3 (−)
	7	6.28^{e}	<0.3 (−)	<0.3 (−)	<0.3 (+)	<0.3 (+)	<0.3 (−)	<0.3 (+)	<0.3 (+)	<0.3 (−)
	9	(+)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
	11	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
pH		6.25	2.75	2.56	5.12	3.84	3.60	5.04	3.96	3.80

[a] Three-strain mixtures of all speeres were used. All treatments contained 2% salt (fixed). Day 0 values are bacterial inocula. Recovery of cells was done by enrichment of samples. Least-squares means lacking a common superscript within each microorganism differed significantly ($P < 0.05$).

and 20% mustard–1% acetic acid were detected, the differences were <0.35 log$_{10}$ CFU/ml on the same storage day. These results indicate that the amount of mustard flour may not dramatically affect the effectiveness of the combination with acetic acid in killing *E. coli* O157:H7. For *L. monocytogenes*, trends similar to those found with *E. coli* O157:H7 were observed (Table 1). However, *L. monocytogenes* was more resistant than the other pathogens tested in the same environmental conditions. *L. monocytogenes* was recovered from the 10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid cultures for up to 5 days at 5°C by direct plating, and small numbers of survivors were recovered in 10% mustard–0.5% acetic acid for up to 9 days at 5°C. The 20% mustard–0.5% acetic acid combination reduced *L. monocytogenes* populations more rapidly than did 10% mustard–0.5% acetic acid ($P < 0.05$) after 5 days, whereas the reduction rate of *L. monocytogenes* was not affected ($P > 0.05$) by the amount of mustard added (10 or 20%), as shown in *E. coli* O157:H7 reductions.

Survival of food-borne pathogens stored at 22°C. Survival of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium in solutions containing various concentrations of mustard flour, acetic acid, and salt at 22°C is shown in Table 2. Room-temperature environments led to strong reductions in food-borne pathogens. As shown at 5°C, *S. enterica* serovar Typhimurium was very sensitive to prepared mustards. Likewise, *S. enterica* serovar Typhimurium strains were not found in any treatments tested at 12 h. Also, the numbers of *E. coli* O157:H7 and *L. monocytogenes* were reduced much more rapidly at 22°C than at 5°C. *E. coli* O157:H7 strains were effectively eliminated within 12 h in mustard combined with 1% acetic acid. In contrast, *E. coli* O157:H7 was detected in 10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid

TABLE 2. Antimicrobial effect of mustard flour combined with acetic acid on strains of three species stored at 22 ± 1°C[a]

Species	Time (h)	Log$_{10}$ CFU/ml (growth)								
		0% mustard plus % acetic acid (pH)			10% mustard plus % acetic acid (pH)			20% mustard plus % acetic acid (pH)		
		0.0 (6.24)	0.5 (2.76)	1.0 (2.57)	0.0 (5.09)	0.5 (3.83)	1.0 (3.61)	0.0 (5.02)	0.5 (3.96)	1.0 (3.81)
S. enterica	0	6.86^{e}	6.72^{e}	6.79^{e}	6.67^{e}	6.75^{e}	6.63^{e}	6.68^{e}	6.81^{e}	6.67^{e}
	12	6.78^{e}	<0.3 (−)[d]	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)
	24	5.78^{f}	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)
E. coli	0	6.96^{e}	6.79^{e}	6.80^{e}	6.84^{e}	6.76^{e}	6.93^{e}	6.78^{e}	6.99^{e}	6.77^{e}
	12	6.43^{f}	<0.3 (−)	<0.3 (−)	<0.3 (+)	1.66^{h}	<0.3 (−)	<0.3 (+)	1.30^{i}	<0.3 (−)
	24	6.11^{g}	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (−)
	48	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
L. monocytogenes	0	6.94^{e}	6.84^{e}	6.85^{e}	6.85^{e}	6.75^{e}	6.74^{e}	6.89^{e}	6.84^{e}	6.79^{e}
	12	6.97^{e}	<0.3 (−)	<0.3 (−)	2.30^{g}	5.65^{h}	<0.3 (+)	2.19^{g}	3.83^{i}	<0.3 (+)
	24	6.57^{f}	<0.3 (−)	<0.3 (−)	<0.3 (−)	<0.3 (+)	<0.3 (−)	<0.3 (−)	<0.3 (+)	<0.3 (−)
	48	(+)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
	72	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)

[a] See Table 1, footnote *a*.

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by direct plating, and small numbers of *E. coli* O157:H7 were recovered in 10% mustard–0% acetic acid and 20% mustard–0% acetic acid by enrichment at 12 h. *L. monocytogenes* also survived longer in the combined treatments at 22°C than did *E. coli* O157:H7. Small numbers of *L. monocytogenes* were recovered even in 12-h samples treated with 10% mustard–1% acetic acid and 20% mustard–1% acetic acid. Clear differences ($P < 0.05$) in the reduction rate of *L. monocytogenes* between 10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid were observed during the time course. The 10% mustard–0.5% acetic acid and 20% mustard–0.5% acetic acid solutions reduced the *L. monocytogenes* count by 1.1 and 3.0 $\log_{10}$ CFU/ml within 12 h, respectively, and recovered *L. monocytogenes* cells were found in 10% mustard–0.5% acetic acid for up to 48 h.

DISCUSSION

The order of bacterial resistance against prepared mustards was *S. enterica* serovar Typhimurium < *E. coli* O157:H7 < *L. monocytogenes*. Previous studies have shown that gram-negative bacteria were more sensitive than gram-positive bacteria to similar environments. Lin et al. (19) demonstrated that *L. monocytogenes* was more resistant to AITC than *E. coli* O157:H7 and *Salmonella enterica* serovar Montevideo. Tsai and Ingham (31) reported that *E. coli* O157:H7 strains generally survived longer than *Salmonella* strains and generic *E. coli* strains in acidic conditions. Also, some researchers have examined the survival of food-borne pathogens in mustard-related products and retail mustards. Tsai and Ingham (31) found that all of the *E. coli* O157:H7 (10^5 CFU/g) and *Salmonella* (10^6 CFU/g) strains died within 1 h in mustard (pH 3.1) regardless of storage temperature (5 and 23°C). They (31) reported that adaptation to acid and low temperature enhanced the survival of *E. coli* O157:H7 and *Salmonella* strains in ketchup (pH 3.6) but not in mustard or sweet pickle relish (pH 2.8).

Mayerhauser (20) reported that *E. coli* O157:H7 strains (10^6 CFU/g) were effectively eliminated within hours to days in retail mustards (pH 3.17 to 3.63). Weagant et al. (34) observed that *E. coli* O157:H7 (10^7 CFU/g) was not detected in mayonnaise-mustard sauce (pH 3.68) after 4 days, whereas *E. coli* O157:H7 survived for up to 35 days in several mayonnaise-based sauces. Although *E. coli* O157:H7 was not examined, in a study of specialty mustards, Nelson et al. (J. Nelson, J. J. Churey, R. W. Worobo, and O. J. Padilla-Zakour, Abstr. 2001 Inst. Food Technol. Annu. Meet., p. 206, 2001) reported rapid reduction of generic *E. coli* in both acetic acid at 0.5% and 4% mustard. They suggested that specialty mustards prepared with at least 4% mustard flour and 0.5% acetic acid at 20°C can be safely prepared without heating, even when protective ingredients like eggs are added. Our results also show that the sensitivity of *E. coli* O157:H7 and *L. monocytogenes* is enhanced in prepared mustards held at 22°C versus 5°C.

However, our findings indicate that mustard (10 or 20%) in combination with 0.5% acetic acid is less bactericidally active against *E. coli* O157:H7 and *L. monocytogenes* than is mustard alone. The order of lethality among treatments on the same storage day was generally control < mustard in combination with 0.5% acetic acid < mustard alone < mustard in combination with 1% acetic acid < acetic acid alone. Moreover, in another experiment, we found that the combination of small amounts of acetic acid (0.25, 0.50, and 0.75%) with 10% mustard exhibited no synergistic or additive effect in the killing of *E. coli* O157:H7 (data not shown). However, it is difficult to understand why a mustard-only product with the higher pH (>5.0) had better antimicrobial effects than mustard plus acetic acid products with a lower pH (<4.0).

The first major assumption for the lack of a synergistic or additive effect of the combination of mustard and small amounts of acetic acid in the killing of *E. coli* O157:H7 and *L. monocytogenes* concerns the stability of AITC in aqueous media. Tsao et al. (32) reported that pure sinigrin and AITC were relatively stable in buffered water at pHs of 5.0 to 7.0. When combined with acetic acid, AITC may be less stable in a low-pH environment. The stabilization of AITC may contribute to the more effective destruction of food-borne pathogenic bacteria. A second assumption concerns unknown ingredients in commercial vinegar. Commercial vinegar contains some ethyl esters, which give it some of its aroma, so the vinegar may have some inhibitory compounds other than acetic acid. The aqueous medium-related stability of AITC could differ in different treatments containing various amounts of the non-acetic acid phase of vinegar. Even though these variable volumes of the non-acetic acid phase of vinegar can affect the results, this possibility is not of sufficient importance.

A third assumption concerns the diminished antibacterial action of the product containing small amount of acetic acid, which could be caused by increasing pH associated with the addition of mustard flour. Acetic acid's antimicrobial activity is based primarily on its pH-lowering effect, and it exhibits this antimicrobial activity only in an undissociated form (29). Acetic acid has a pK_a of 4.75 and undissociated acetic acid percentages of 95 and 63% at pHs of 3.5 and 4.5, respectively (30). Thus, its effectiveness in retarding growth or killing food-borne pathogenic bacteria may vary among treatments, depending on the percentage of undissociated acid at a given pH. Nevertheless, we cannot explain the facts that no difference in antimicrobial action between 10% mustard–0.5% acetic acid (pH 3.84) and 20% mustard–0.5% acetic acid (pH 3.96) or better antimicrobial action for 20% mustard–1% acetic acid (pH 3.80) than for 10% mustard–1% acetic acid (pH 3.60) against *L. monocytogenes*.

Overall, acidified products may limit microbial growth or survival, and the extent of this survival depends on the types of microorganisms harbored in the food and the type and amount of acid, especially its buffering capacity. For example, Zhao and Doyle (36) found that *E. coli* O157:H7 survived slightly longer in real mayonnaise (pH 3.9) than in the reduced-calorie formulation made with less acid (pH 3.8). They supposed that the reduced-calorie mayonnaise dressing contained an ingredient with anti-*E. coli* O157:H7 properties that was not present in real mayonnaise. Our study indicates that the addition of small amounts of acetic acid (0.5%) to mustard retards the reduction of *E. coli* O157:H7 and *L. monocytogenes*. These antagonistic effects may be changed if mustard is used alone or in combination with >1% acetic acid. However, the scope of this study was quantitative for assessing at what concentrations added mustard flour and acetic acid can affect the survival of food-borne pathogenic bacteria. Due to the general relevance of the pathogen to food safety and the fact that mustard is

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normally consumed without preconsumption heat treatment, the results of this study may be potentially useful for the food industry.

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