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Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including *Escherichia coli* O157:H7

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ABSTRACT

The bacteriostatic and bactericidal actions of vinegar on food-borne pathogenic bacteria including enterohemorrhagic *E. coli* (EHEC) O157:H7 were examined. The growth of all strains evaluated was inhibited with a 0.1% concentration of acetic acid in the vinegar. This inhibition was generally increased in the presence of sodium chloride or glucose. There was almost no difference in sensitivity to the bacteriostatic action of vinegar among the strains of pathogenic *E. coli*. Vinegar had a bactericidal effect on food-borne pathogenic bacteria including EHEC O157:H7. This action against EHEC O157:H7 was synergically enhanced by sodium chloride but was attenuated with glucose. For EHEC strains (O157:H7, O26:H11, O111:HNM) the difference in the inactivation rate due to vinegar among strains used was small, although an enteropathogenic *E. coli* (EPEC) O111:K58:H$^-$ strain was more sensitive, being more quickly killed compared with EHEC strains. The inactivation rate due to vinegar was constant irrespective of inoculum size. However, it differed greatly depending on growth phase of the cells, where logarithmic growth phase cells were more sensitive and easily killed than stationary phase cells. The bactericidal activity of vinegar increased with the temperature. Various conditions for bactericidal effects on EHEC O157:H7 were examined by the multiparametric analysis of five factors: acetic acid concentration in the vinegar, sodium chloride concentration, temperature, incubation time, and viable cell number. The combined use of vinegar and sodium chloride, with use of an appropriate treatment temperature, was found to be markedly effective for the prevention of bacterial food poisoning.

Although the antibacterial action of vinegar (acetic acid) on various kinds of microorganisms has long been known *(11)*, few studies have examined its antibacterial action on many kinds of food-borne pathogenic bacteria *(8)* including enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 *(3, 7)*, except for studies of the decontamination of beef with a solution of acetic acid (a major constituent of vinegar) on beef contaminated with EHEC O157:H7 *(1, 2, 4, 5, 6, 9, 10, 12, 13)*.

In this study, we examined the bacteriostatic action of vinegar on food-borne pathogenic bacteria including EHEC O157:H7, which caused serious problems in Japan in 1996. In addition, we investigated the combined effects of sodium chloride or glucose to clarify their impact on the use of vinegar in the prevention of bacterial food poisoning. We also studied the changes in the bactericidal action of vinegar solutions against EHEC O157:H7 with additions of sodium chloride or glucose, as well as the effects of the size of the bacterial inoculum, the growth phase, and temperature on the bactericidal action.

MATERIALS AND METHODS

Bacterial strains. As shown in Table 1, 17 strains of food-borne pathogenic bacteria belonging to seven species were used. They included eight strains of EHEC O157:H7 of different origins (NGY-10: Kasugai-shi, Aichi-ken, 1996; NGY-11: Kasugai-shi, Aichi-ken, 1996; RIMD 0509861: Oku-cho, Okayama-ken, 1996; RIMD 0509881: Niimi-shi, Okayama-ken, 1996; RIMD 0509890: Sakai-shi, Osaka-fu, 1996; RIMD 0509891: Sakai-shi, Osaka-fu, 1996; RIMD 0509894: Sakai-shi, Osaka-fu, 1996; and RIMD 0509865: Sakai-shi, Osaka-fu, 1996; of these, RIMD 0509865 was isolated from a sporadic case of food poisoning and the other strains from general outbreaks), one strain of EHEC O26:H11 (NGY-9688: Nagoya-shi, Aichi-ken, 1996), one strain of EHEC O111:HNM (NGY-42: Nagoya-shi, Aichi-ken, 1996), and one strain of enteropathogenic *E. coli* (EPEC) O111:K58:H$^-$ (provided by Ulskov).

Reagents. Spirit vinegar produced by Nakano Vinegar Co., Ltd. was used (10% concentration of acetic acid; hereinafter, concentrations will be expressed in %, wt/vol). Commercial sodium chloride and glucose were used.

The acid in the vinegar (spirit vinegar) used in this experiment was 100% acetic acid. The percentage of vinegar constituents other than acetic acid was less than 0.2%.

Measurement of bacteriostatic activity. On the surface of nutrient agar, which originally contained no sodium chloride (containing 0.5% meat extract, 1% peptone, and 1.5% agar at pH 7.0) and was supplemented with vinegar in an appropriate amount to achieve the specified concentration of acetic acid and with sodium chloride or glucose at the specified concentration, 0.01 ml of cell suspension of the bacterial strain was applied. The bacterial strains were cultured for 4 days at 30°C, and their growth was observed. The bacteriostatic activity of vinegar on the strains was evaluated in terms of the number of elapsed days until bacteria could be observed with the naked eye. The agar prepared to provide a 0.1% concentration of acetic acid had a pH of 5.1. The water activity (a_w) values of the agars containing 10% sodium chloride and 30% glucose were approximately 0.94.

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TABLE 1. *Effect of sodium chloride on the bacteriostatic activity of vinegar*

Strain	Bacterial growth[a]																			
	Concentration of sodium chloride (%, wt/vol)																			
	0					2					5					10				
	Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar				
	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1
Escherichia coli O157:H7 NGY-10	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	+++	−	−	−	−	−	−	−
E. coli O157:H7 NGY-11	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509861	++++	++++	++++	++	−	++++	++++	++++	++	−	++++	+++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509865	++++	++++	++++	++	−	++++	++++	++++	++	−	++++	+++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509881	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	+++	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509890	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	+++	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509891	++++	++++	++++	++	−	++++	++++	++++	+	−	++++	+++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509894	++++	++++	++++	++++	−	++++	++++	++++	+++	−	++++	+++	+++	−	−	−	−	−	−	−
E. coli O26:H11 NGY-9688	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	+++	−	−	−	−	−	−	−
E. coli O111:HNM NGY-42	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	++++	+	−	−	−	−	−	−	−
E. coli O111:K58:H$^-$	++++	++++	++++	+++	−	++++	++++	++++	++	−	++++	++++	+++	−	−	−	−	−	−	−
Salmonella enteritidis IID 604	++++	++++	+++	+	−	++++	++++	+++	+	−	++++	+++	+++	−	−	−	−	−	−	−
Salmonella typhimurium NCI 17024	++++	++++	++++	+++	−	++++	++++	++++	+++	−	++++	+++	+++	−	−	−	−	−	−	−
Vibrio parahaemolyticus IFO 12711	−	−	−	−	−	++++	+++	−	−	−	++++	++	−	−	−	−	−	−	−	−
Aeromonas hydrophila IFO 3820	++++	++++	+++	−	−	++++	+++	−	−	−	−	−	−	−	−	−	−	−	−	−
Staphylococcus aureus IFO 3060	++++	++++	++++	++++	−	++++	++++	++++	+	−	++++	++++	+++	−	−	++	++	+	−	−
Bacillus cereus MK	++++	++++	++++	++++	−	++++	+++	++	−	−	+++	++	−	−	−	−	−	−	−	−

[a] ++++, growth was observed within 1 day; +++, growth was observed within 2 days; ++, growth was observed within 3 days; +, growth was observed within 4 days; −, no growth was observed within 4 days. Inoculated media were incubated at 30°C.

Preparation of inactivation test solutions for measurement of bactericidal activity. Vinegar was diluted with distilled water to attain various concentrations of acetic acid. The pH values of the concentrations of acetic acid were 2.2 for the 10% acetic acid (stock solution of vinegar) and 2.5 for the 2.5% acetic acid (fourfold dilution of the spirit vinegar). In addition test vinegar solutions containing 2.5% acetic acid were prepared with sodium chloride or glucose added at the indicated concentrations. The combined effects of vinegar and sodium chloride were examined using a solution with a 2% concentration of acetic acid and one with a 2% concentration of sodium chloride solution mixed together in specified ratios. To examined the influence of temperature on the combined effects of vinegar and sodium chloride, test vinegar solutions were prepared by mixing 0.5 to 2.5% concentrations of acetic acid and 0 to 5% concentrations of sodium chloride.

Preparation of bacterial cell suspension. Bacterial cells were cultured on nutrient agar (for *Vibrio parahaemolyticus* IFO 12711, 3% sodium chloride was added) at 37°C for 24 h, then suspended in sterilized water (for *V. parahaemolyticus* IFO 12711, sterilized saline solution with 3% sodium chloride was used).

Measurement of time required for inactivation. A cell suspension of EHEC O157:H7 NGY-10 (0.1 ml) at a concentration of 2.0×10^8 CFU/ml was added in 10 ml of inactivation test solution incubated at 30°C. Then, 0.05 ml of inactivation test solution was inoculated in the nutrient broth at the specified time intervals. This medium was cultured at 30°C for three days to observe whether bacterial growth could be detected with the naked eye. The time until the bacterial growth was not found after culturing was considered the time necessary for inactivation (the time required for achieving inactivation from 2.0×10^6 CFU/ml to $<2.0 \times 10^1$ CFU/ml) to compare the degree of inactivation of each inactivation test solution.

Determination of survival in inactivation test solutions. Bacterial cells were incubated in inactivation test solutions to measure colony forming units (CFU) with time by the dilution plating method using nutrient agar, and the survival curve was obtained by the least squares method.

Effect of size of bacterial inoculum. A cell suspension of EHEC O157:H7 NGY-10 was added to diluted vinegar solution containing 2.5% acetic acid so that the specified size of bacterial inoculum could be obtained, and the survival curve at 30°C was obtained.

Effect of growth phase. EHEC O157:H7 NGY-10 was incubated in the nutrient broth with shaking at 37°C. The culture solution, after having been cultured for the specified time, was inoculated into the diluted vinegar solution containing 2.5% acetic acid, and the percent survival was calculated after 10 min at 30°C. The sensitivities of the bacterial cells to vinegar in the respective growth phases were compared.

Effect of temperature. A cell suspension of EHEC O157:H7 NGY-10 was inoculated into the diluted vinegar solution containing 2.5% acetic acid at 10 to 50°C. An Arrhenius plot was obtained from the survival curves to calculate, by extrapolation when necessary, the time required for the inactivation of a given number of cells at each temperature.

Influence of temperature on the combined effects of vinegar and sodium chloride. For preparing a survival curve, a cell suspension of EHEC O157:H7 NGY-10 was incubated at 10 to 50°C in inactivation test solutions of various compositions prepared by the above-mentioned method containing acetic acid and sodium chloride at the specified concentrations. From the slope of this survival curve, the inactivation rate (k) was calculated.

RESULTS

Bacteriostatic activity of vinegar. The growth of all strains examined was inhibited by 0.1% acetic acid (Table 1). This effect was enhanced when sodium chloride (Table 1) or glucose (Table 2) was added except for *V. parahaemolyticus* IFO 12711. In agar containing 0.1% acetic acid the pH was 5.1. In culture medium at the same pH prepared using hydrochloric acid, the growth of the tested strains was not inhibited.

Bactericidal activity of vinegar. Among three kinds of vinegar solutions, vinegar stock solution (acetic acid concentration 10%), a twofold dilution (acetic acid concentration 5%) and a fourfold dilution (acetic acid concentration 2.5%), the time necessary for inactivation of EHEC O157:H7 NGY-10 at 30°C was 1 min, 25 min, and 150 min, respectively (measured as the time required to decrease colony forming units from 2.0×10^6 CFU/ml to $<2.0 \times 10^1$ CFU/ml). The results obtained when logarithm of the time necessary for inactivation was plotted against concentration of acetic acid (2.5 to 10%) showed linearity.

Difference in sensitivity to vinegar among strains of pathogenic *E. coli*. Figure 1 shows the survival curves of six strains of EHEC O157:H7 of different origins, one strain of EHEC O26:H11, one strain of EHEC O111:HNM, and one strain enteropathogenic *E. coli* (EPEC) O111:K58:H^- in diluted vinegar solutions containing 2.5% acetic acid. The inactivation rates of the eight strains of EHEC O157:H7, O111:HNM, and O26:H11 were similar. However, the EPEC O111:K58:H^- strain exhibited higher sensitivity to vinegar than the other strains.

Effect of size of the bacterial inoculum on bactericidal action of vinegar. For EHEC O157:H7 NGY-10 in a diluted vinegar solution containing 2.5% acetic acid, the inactivation rate due to the vinegar was similar regardless of the size of the inoculum. Especially for inocula of approximately 10^7 CFU/ml or less, the inactivation rate ($k = 0.07$ min^{-1}) was identical.

Effect of growth phase on bactericidal action of vinegar. Cells of EHEC O157:H7 NGY-10 in different growth phases were incubated in diluted vinegar solution (2.5% acetic acid). Cells in the logarithmic growth phase had high sensitivity to vinegar, while those in stationary phase showed lower sensitivity (Fig. 2).

Effects of sodium chloride or glucose. The effects of sodium chloride or glucose on the bactericidal activity of vinegar against EHEC O157:H7 NGY-10 were investigated using an inactivation test solution in which sodium chloride or glucose was added to the diluted vinegar solution with 2.5% acetic acid. For the diluted vinegar solution itself, the time required for inactivation was 150 minutes. When sodium chloride was added to this solution at concentrations of 0.01, 0.03, 0.1, 0.3, 1, 5, and 15%, the time necessary for inactivation was decreased to 120, 90, 50, 20, 15, 6, and 4 min, respectively. On the other hand, when glucose was

TABLE 2. *Effect of glucose on the bacteriostatic activity of vinegar*

Strain	Bacterial growth[a]																			
	Concentration of glucose (%, wt/vol)																			
	0					10					20					30				
	Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar					Concentration of acetic acid (%, wt/vol) from vinegar				
	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1	0	0.025	0.05	0.075	0.1
Escherichia coli O157:H7 NGY-10	++++	++++	++++	+++	−	++++	++++	+	−	−	+	−	−	−	−	−	−	−	−	−
E. coli O157:H7 NGY-11	++++	++++	++++	+++	−	++++	++++	++	−	−	+++	++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509861	++++	++++	++++	++	−	++++	++++	+	−	−	+++	−	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509865	++++	++++	++++	++	−	++++	++++	+	−	−	+	−	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509881	++++	++++	++++	+++	−	++++	++++	+	−	−	+++	++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509890	++++	++++	++++	+++	−	++++	++++	+	−	−	+++	++	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509891	++++	++++	++++	++	−	++++	++++	+	−	−	+	−	−	−	−	−	−	−	−	−
E. coli O157:H7 RIMD 0509894	++++	++++	++++	++++	−	++++	++++	++	−	−	+++	++	−	−	−	−	−	−	−	−
E. coli O26:H11 NGY-9688	++++	++++	++++	+++	−	++++	++++	++	−	−	+	−	−	−	−	−	−	−	−	−
E. coli O111:HNM NGY-42	++++	++++	++++	+++	−	++++	++++	++	−	−	+	−	−	−	−	−	−	−	−	−
E. coli O111:K58:H$^-$	++++	++++	++++	+++	−	++++	++++	+	−	−	+	−	−	−	−	−	−	−	−	−
Salmonella enteritidis IID 604	++++	++++	+++	+	−	++++	+++	+	−	−	+	−	−	−	−	−	−	−	−	−
Salmonella typhimurium NCI 17024	++++	++++	++++	+++	−	++++	+++	+	−	−	−	−	−	−	−	−	−	−	−	−
Vibrio parahaemolyticus IFO 12711	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Aeromonas hydrophila IFO 3820	++++	++++	+++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Staphylococcus aureus IFO 3060	++++	++++	++++	++++	−	++	++	+	−	−	+	+	−	−	−	−	−	−	−	−
Bacillus cereus MK	++++	++++	++++	++++	−	+++	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] +++, growth was observed within 1 day; ++, growth was observed within 2 days; +, growth was observed within 3 days; −, growth was observed within 4 days; −, no growth was observed within 4 days. Inoculated media were incubated at 30°C.

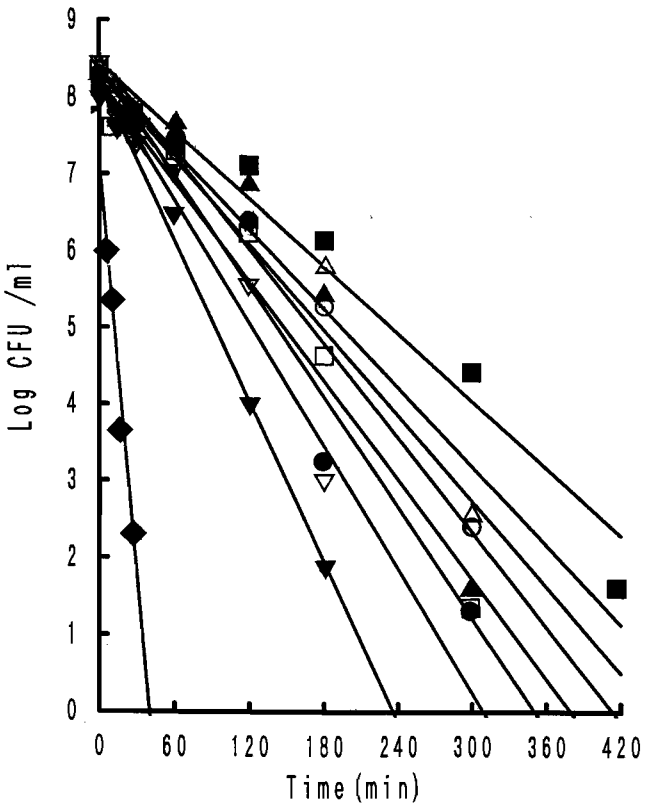

FIGURE 1. *Bactericidal activity of diluted vinegar solution containing 2.5% acetic acid against* Escherichia coli *O157:H7, O26:H11, O111:HNM, and O111:K58:H⁻ at 30°C.* ●, E. coli *O157:H7 NGY-10;* ○, E. coli *O157:H7 NGY-11;* ▲, E. coli *O157:H7 RIMD 0509861;* △, E. coli *O157:H7 RIMD 0509881;* ■, E. coli *O157:H7 RIMD 0509890;* □, E. coli *O157:H7 RIMD 0509891;* ▼, E. coli *O26:H11;* ▽, E. coli *O111:HNM NGY-42;* ◆, E. coli *O111:K58:H⁻*.

added at concentrations of 5, 20, and 45%, the time increased to 240, 300, and 300 min, respectively. These vinegar solutions showed almost the same pH value of about 2.5.

Combined effects of vinegar and sodium chloride. A cell suspension of EHEC O157:H7 NGY-10 was inoculated into an inactivation test solution comprised of diluted vinegar solution containing 2% acetic acid and sodium chloride solution at the same concentration mixed at a specified ratio. The results of measuring CFU/ml after 20 and 25 min are shown in Figure 3. When diluted vinegar solution and sodium chloride solution were mixed, despite the decreased concentrations of each component, bactericidal activity was increased. The mixing ratio of acetic acid (in diluted vinegar) and sodium chloride for maximum bactericidal activity was 4:1.

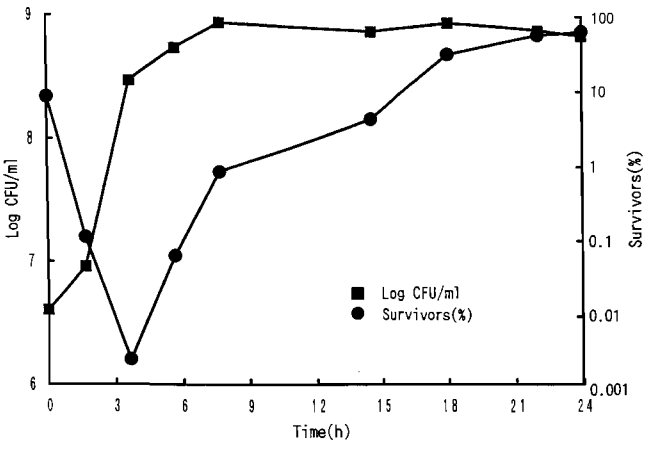

FIGURE 2. *Effect of the growth phase on bactericidal activity of a diluted vinegar solution containing 2.5% acetic acid against* Escherichia coli *O157:H7 NGY-10 at 30°C.*

Effect of temperature on bactericidal activity of vinegar. The survival EHEC O157:H7 NGY-10 in the diluted vinegar solution with a 2.5% concentration of acetic acid was examined with changes in temperature (Fig. 4A). Arrhenius plots were obtained from the slopes of these survival curves (Fig. 4B) to calculate, by extrapolation when necessary, the time required for inactivation of a given number of viable cells at each temperature. The following example is one of the results. When the temperature was increased, the time necessary for the inactivation decreased. The time required for a 3-log decrease in viable cell numbers of EHEC O157:H7 NGY-10 was 0.84 min at 50°C, 14.4 min at 40°C, 137 min at 30°C, 739 min at 20°C, and 4,516 min at 10°C.

Influence of temperature on the combined effects of vinegar and sodium chloride. Inactivation test solutions containing 0.5 to 2.5% acetic acid and 0 to 5% sodium chloride in various combinations were prepared to measure the bactericidal activity (inactivation rate) to EHEC O157:H7 NGY-10. The following example is one of the results: the plot results obtained when logarithm of the inactivation rate was plotted against acetic acid concentration were linear at each temperature (Fig. 5A). The plot results obtained when logarithm of inactivation rate was plotted against sodium chloride concentration were also linear for each concentration of acetic acid (Fig. 5B).

DISCUSSION

The antibacterial action of vinegar can be classified into the following two types: bacteriostatic action, namely, inhibition of bacterial growth, and bactericidal action, that is, reduction of viable cell numbers. For food antisepsis, both actions are important. In the present study, growth of all 17 bacterial strains was inhibited by acetic acid at a concentration as low as 0.1%, demonstrating its strong bacteriostatic activity (Table 1). This effect was enhanced by sodium chloride (Table 1) or glucose (Table 2), except in the case of *V. parahaemolyticus* IFO 12711. In food cooking, salt and sugar are both essential; therefore, these results are important in discussing the bacteriostatic action of vinegar.

On the other hand, to prevent bacterial food poisoning, bactericidal action is more important than bacteriostatic action. Vinegar had bactericidal effects on EHEC O157:H7 NGY-10, with the time necessary for inactivation of the bacteria (time for a decrease CFU from 2.0×10^6 CFU/ml to $<2.0 \times 10^1$ CFU/ml) in the stock solution of vinegar (10% acetic acid) being 1 min. The correlation of the logarithm of time necessary for inactivation with acetic acid concentration was linear. This suggests the time necessary for

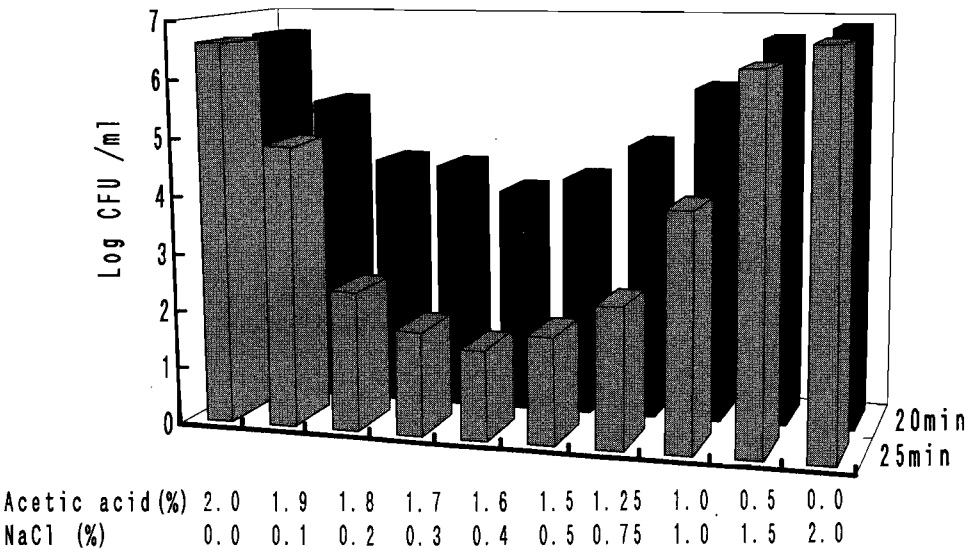

FIGURE 3. *Synergism of vinegar and sodium chloride in bactericidal activity against* Escherichia coli *O157:H7 NGY-10 at 30°C.*

inactivation can be extrapolated for any acetic acid concentration within the range of 2.5 to 10%.

Because about 98% of the material other than water in the vinegar in this study was acetic acid, which is known to be antibacterial *(11),* the bacteriostatic and bactericidal actions of vinegar in this study were considered to be due to acetic acid.

The sensitivity to vinegar among eight verotoxin-producing strains of EHEC O157:H7, O26:HII, and O111:HNM from various origins was not significantly different, while the sensitivity of enteropathogenic *E. coli* (EPEC) O111:K58:H^- not producing verotoxin was markedly higher (Fig. 1). Further studies are need to clarify the relationship between sensitivity to vinegar and verotoxin production.

We found that the number of cells in the inoculum of EHEC O157:H7 NGY-10 had almost no effect on the bactericidal activity of vinegar. Especially, when the size of the bacterial inoculum was about 10^7 CFU/ml or less, the inactivation rate was constant. This indicates that the time necessary for inactivation can be determined, by extrapolation if necessary, for any inoculum size of 10^7 CFU/ml or less.

Cells of EHEC O157:H7 NGY-10 in the logarithmic growth phase were demonstrated to have high sensitivity to vinegar, while those in the stationary phase had lower sensitivity (Fig. 2). These findings are important for evaluating the bactericidal activity of vinegar and the application of vinegar as bacteriocide in food preparation and disinfection of cooking utensils, and they suggest that cells used in experiments should be those in the stationary phase, not in the logarithmic growth phase.

The effect of adding sodium chloride or glucose on the bactericidal activity of vinegar was investigated by changing the concentrations of these solutes. With pH remaining unchanged, the bactericidal activity of vinegar against EHEC O157:H7 NGY-10 was increased by sodium chloride and was decreased by glucose.

The bactericidal activity of vinegar against EHEC O157:H7 NGY-10 was enhanced by adding sodium chloride. Therefore, the combined effects of vinegar and sodium chloride were investigated using EHEC O157:H7 NGY-10.

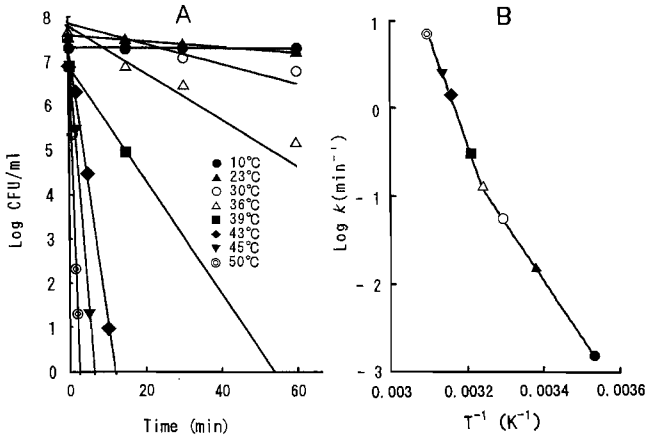

FIGURE 4. *(A) Effect of temperature on bactericidal activity of a diluted vinegar solution containing 2.5% acetic acid against* Escherichia coli *O157:H7 NGY-10. (B) Arrhenius plots derived from the data plotted in panel A.*

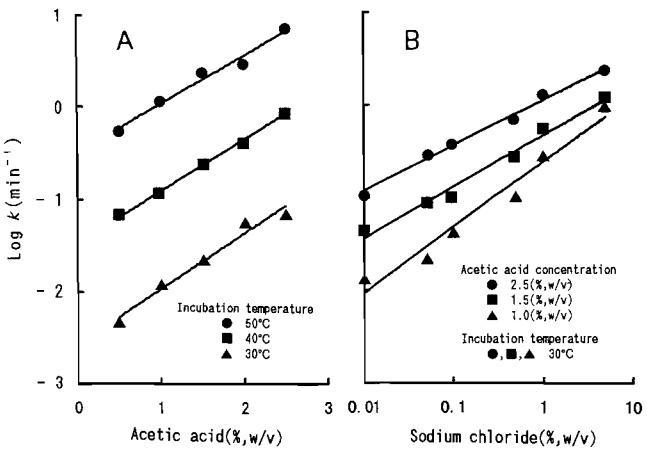

FIGURE 5. *Effects of acetic acid (A) and sodium chloride (B) concentrations on the rate of inactivation of* Escherichia coli *O157:H7 NGY-10.*

It was found that in combination vinegar and sodium chloride had a highly synergic effect on inactivation (Fig. 3).

When the effects of temperature on the bactericidal activity of vinegar against EHEC O157:H7 NGY-10 were examined, linearity was observed both in the high temperature range (36 to 50°C) and in the intermediate temperature range (10 to 36°C) (Fig. 4A and 4B). This indicated that the time necessary for inactivation of viable cells less than about 10^7 CFU/ml could be obtained by extrapolation. For example, when the time required to decrease the viable cell number of EHEC O157:H7 NGY-10 to 1/1,000 of the original number in a diluted vinegar solution of 2.5% acetic acid was calculated, the time was only 3.4 min at 45°C, which was about 1/40 of the time needed at 30°C. This finding suggested that vinegar treatment at 40 to 50°C can be an effective method of preventing bacterial food poisoning. Although heating inactivation at 75°C for 1 min or more has been reported to be the most effective method for sterilizing EHEC O157:H7, the above-mentioned inactivation treatment using vinegar at 40 to 50°C would appear to be more practical since alteration of food taste and loss of nutritional value would be small.

To obtain a more effective method, we examined the influence of both sodium chloride and temperature factors. Linearity was observed when logarithm of the inactivation rate was plotted against logarithm of sodium chloride concentration at a given acetic acid concentration and when logarithm of the inactivation rate was plotted against acetic acid concentration at various temperatures (Fig. 5A and 5B). In addition, these individual values could be expressed in a formula. Thus, the time necessary for the inactivation of a predetermined viable cell number could be predicted by calculating the inactivation rate at any acetic acid concentration (0.5 to 2.5%), any sodium chloride concentration (0.01 to 5%) and any temperature (10 to 50°C) within the experimental range.

From this prediction system, suitable inactivation conditions were determined. The following example shows one case: the time required for a 3-log decrease in viable cell number of EHEC O157:H7 NGY-10 using 2.5% acetic acid at 20°C (739 min) could be shortened to 1/140 (5.27 min) by adding 5% sodium chloride, to 1/51 (14.4 min) at 40°C, and to 1/830 (0.89 min) under both conditions. These results indicated that the combined use of vinegar and sodium chloride at a suitable into temperature provided effective inactivation conditions for the prevention of bacterial food poisoning.

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