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Inhibition of *Citrobacter freundii* by lactic acid, ascorbic acid, citric acid, *Thymus vulgaris* extract and NaCl at 31 ºC and 5 ºC

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Abstract - *Citrobacter freundii* has been implicated in food spoilage and food poisoning outbreaks. This study examines the effects of some compounds (e.g. citric acid, ascorbic acid, lactic acid, sodium chloride, and *Thymus vulgaris* extract) on growth of two strains of *Citrobacter freundii* at 31 ºC and 5 ºC. At 31 ºC, lactic acid (0.2%) or ascorbic acid (0.2%) alone completely inhibited growth of the tested strains, as there was 100% reduction in growth of the strains after 24 h incubation in nutrient broth containing these compounds. *Thymus vulgaris* extract (0.3%) reduced the growth rate ($p < 0.05$), the percentages of inhibition after 24 h incubation were about 60% for both strains. NaCl (5%) greatly reduced growth, the percentages of inhibition were about 84% for both strains. Combination of *T. vulgaris* extract (0.3%) and NaCl (4%) together completely inhibited growth of *C. freundii* species tested. Ascorbic acid (0.1%) or citric acid (0.03%) did not affect growth of the strains ($p > 0.05$), but a lag occurred before increase in number could be observed. In chicken and fish homogenates, combination of NaCl (4%) and ascorbic acid (0.1%) reduced the growth ($p < 0.05$) (growth inhibition was 40%). At 5 ºC, lactic acid (0.1%) alone greatly reduced the growth ($p < 0.05$). The activity of NaCl, or ascorbic acid alone against the tested strains was greatly increased ($p < 0.05$). For *C. freundii* 4, the percentage of growth inhibition after 6 days incubation in broth containing 3% NaCl or 0.1% ascorbic acid were 88% and 72%, respectively. For *C. freundii* 38, the percentage of growth inhibition after 6 days incubation in broth containing these compounds were 60% and 54%, respectively.

Key words: *Citrobacter freundii*, inhibition, chemicals.

INTRODUCTION

Citrobacter species are found in water, soil and decaying matter, and can be isolated from the faeces of man and animals (Joklik *et al.*, 1992). They are small, Gram-negative, non-spore forming rods, and belong to the family Enterobacteriaceae. *Citrobacter* spp. grow best at moderate temperatures and can grow at low temperatures (7 ºC). *Citrobacter* is one of the major genera of bacteria that are found on fresh meat, minced meat, poultry, plants and plant products (Jay, 2000; Kleeberger and Busse, 1975). Sources of these microorganisms to food may be the original environment of meats and vegetables (such as water and soil). Treated wastewater, for example, is reused for irrigation and other purposes in many countries (WHO, 1989). *Citrobacter* is one of the prevalent species in the influent and effluent of wastewater treatment plants (Abu-Ghazaleh, 2001); therefore, vegetables, fishes and other foods in contact with this water may be contaminated. Also, food utensils, the cutting block, knives and grinders may introduce these microorganisms to food during processing. Contaminated tools (from initial food samples harboring the microorganisms) enhance the spread of microorganisms to other food that was originally free from bacteria.

Citrobacter has been implicated in food poisoning outbreaks, and enterotoxigenic *C. freundii* strains have been isolated from patients with diarrhea. Joklick *et al*. (1992) reported that 46 of 328 patients with diarrhea had *C. freundii* isolated from their stools, and that some of the 46 isolates had enterotoxin similar to the Sta of *Escherichia coli*. Also, *Citrobacter* can cause spoilage of fish and other food products. Gonzalez- Rodriguez *et al*. (2001) reported that number of bacterial cells belonging to the Enterobacteriaceae increased from initial 10^3 CFU/g (immediately after packaging) to 10^6 CFU/g after storage of trout fillets and salmon slices for 7 days at 3 ºC. Various genera were recovered from the spoiled fish; *C. freundii* and *Hafnia alvei* accounted for 45% of the isolated genera. Similarly, Lindberg *et al*. (1998) reported high numbers of bacteria belonging to the Enterobacteriaceae (including *Citrobacter*) in retailed whole freshwater fish after 3 days of storage at 7 ºC. Application of compounds to eliminate these organisms before storage may extend the shelf life of food and improve their quality.

Information on the effect of food preservatives on *Citrobacter* spp. is scarce. Therefore, this study was undertaken to examine the effects of sodium chloride, lactic acid, ascorbic acid, citric acid, *Thymus vulgaris* extract, and combinations of these compounds on strains of *C. freundii* at 31 ºC at 5 ºC.

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MATERIALS AND METHODS

Bacterial strains. Two *Citrobacter freundii* strains were used in this study. *Citrobacter freundii* 4 was isolated from the infleunt of large urban wastewater treatment plant, and *C. freundii* 38 was isolated from the effluent of large rural wastewater treatment plant. The strains had been previously isolated and were identified at the genus and species level as described in Abu-Ghazaleh (2001). In brief, samples were cultured in lauryl tryptose broth (Oxoid, UK) at 37 ºC for 48 h, then loopfuls of the above cultures were streaked on eosin methylene blue agar (EMB agar, Oxoid) and incubated at 37 ºC for 24 h. Colonies appearing on the EMB agar plates were identified to the genus and species level using the API-20E system (Biomerieux, Marcy I'Etoile, France).

The organisms were maintained on nutrient agar slants at 4 ºC. To prepare the inoculum, nutrient broth (20 ml) was inoculated with appropriate cultures and incubated at 31 ºC for 20 h.

Media and chemicals. Nutrient broth was obtained from Scharlau Chemie (Barcelona, Spain). Citric acid, ascorbic acid, sodium chloride and lactic acid were obtained from Gainland Chemical Co. (Hampshire, UK). Crude extract of *Thymus vulgaris* (extracted by hydrodistillation) was obtained from Systema Co. Ltd. (Amman, Jordan). Stock solutions of citric acid and ascorbic acid were freshly prepared before each use and sterilized by filtration through filters membrane (0.45 um, Micron Separation Inc., Philadelphia, Pa., USA).

Growth conditions.
Growth in nutrient broth. Flasks containing 30 ml of nutrient broth (pH 7.4) alone, or nutrient broth plus lactic acid (0.1% or 0.2% v/v), citric acid (0.03% w/v), ascorbic acid (0.1% or 0.2% w/v), crude extract of *Thymus vulgaris* (0.3% v/v), NaCl (3%, 4% or 5% w/v), or nutrient broth plus various combinations of these compounds were inoculated with 0.6 ml of overnight grown bacterial culture. (For combination of the tested compounds, the lowest possible concentrations of the compounds were examined to test the effect on bacterial growth). The flasks were incubated static at 31 ºC for 24 h or at 5 ºC for 6 days. Samples were withdrawn from each flask at suitable intervals and growth was monitored by measuring optical density at 560 nm spectrophotometrically (Spectronic, Cheshire, UK). During incubation, at least five readings were obtained from each flask.

Growth in chicken or fish homogenates. Skin and fat tissue (40 g) were aseptically obtained from fresh chicken, and chicken homogenates were prepared by blending with 300 ml distilled water in a sterile blender. The homogenate was sterilized by heating at 70 ºC for 10 min, then cooled. A 30 ml portion of the homogenate was dispensed into screw cap test tubes, and sodium chloride, citric acid, ascorbic acid, lactic acid, or combinations of these compounds were added per tube, aseptically. For growth in fish homogenates, skin and fat tissue (40 g) were aseptically obtained from fresh fish, and fish homogenates were prepared, sterilized, dispensed into tubes and treated as mentioned above. Then, overnight grown bacterial culture (0.6 ml) was added to each test tube, and the tubes were incubated static at 31 ºC for 2 d or 5 ºC for 6 days. Samples were withdrawn from each tube at suitable intervals and growth was monitored spectrophotometrically.

All experiments in this study were performed five times, and the optical density readings presented are the mean values. Student's t- test was used to determine the significant differences ($p < 0.05$) among the different compounds tested (Schefler, 1980).

RESULTS

Ascorbic acid, citric acid, lactic acid and sodium chloride effect

The optical density readings of *C. freundii* 4 and *C. freundii* 38 subjected to ascorbic acid alone at 31 ºC are presented in Tables 1 and 3. The presence of ascorbic acid (0.1%) in the growth medium did not inhibit growth of the tested strains, but the strains grew in this medium after a lag of

TABLE 1 – Inhibition of growth of *Citrobacter freundii* 4 in nutrient broth by combination of various compounds at 31 ºC

Ascorbic acid (%)	Citric acid (%)	NaCl (%)	Lactic acid (%)	Growth after			Reduction in growth (%)[a]
				2 h	3 h	24 h	
0	0	0	0	0.016	0.056	0.59	-
0.1	0	0	0	0	0.02	0.66	0
0.2	0	0	0	0	0	0*	100
0	0.03	0	0	0	0.02	0.58	2
0	0	3	0	0	0.01	0.38*	36
0.1	0	3	0	0	0	0.27*	54
0	0	4	0	0	0.002	0.37*	37
0	0	5	0	0	0	0.1*	83
0	0.03	4	0	0	0	0.25*	58
0.1	0	4	0	0	0	0.2*	66
0.1	0.03	0	0	0	0	0.4*	32
0	0	0	0.2	0	0	0*	100

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.
* Means are significantly different ($p < 0.05$) from control (compound(s) is not added to growth medium).

TABLE 2 – Inhibition of growth of *Citrobacter freundii* 4 in nutrient broth by various compounds at 5 ºC

Ascorbic acid (%)	Citric acid (%)	NaCl (%)	Lactic acid (%)	Growth after			Reduction in growth (%)[a]
				1 d	4 d	6 d	
0	0	0	0	0	0.16	0.32	-
0.1	0	0	0	0	0	0.09*	72
0	0.03	0	0	0	0.07	0.24*	25
0	0	3	0	0	0.01	0.04*	88
0	0	0	0.1	0	0	0*	100

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.
* Means are significantly different ($p < 0.05$) from control (compound(s) is not added to growth medium).

TABLE 3 – Inhibition of growth of *Citrobacter freundii* 38 in nutrient broth by combination of various compounds at 31 ºC

Ascorbic acid (%)	Citric acid (%)	NaCl (%)	Lactic acid (%)	Growth after			Reduction in growth (%)[a]
				2 h	3 h	24 h	
0	0	0	0	0.014	0.05	0.52	-
0.1	0	0	0	0	0.01	0.59	0
0.2	0	0	0	0	0	0.1*	81
0	0.03	0	0	0.01	0.02	0.49	6
0	0	3	0	0	0.01	0.33*	37
0.1	0	3	0	0	0.01	0.31*	40
0	0	4	0	0	0	0.26*	50
0	0	5	0	0	0	0.08*	85
0	0.03	4	0	0	0	0.25*	52
0.1	0	4	0	0	0	0.13*	75
0.1	0.03	0	0	0	0	0.22*	58
0	0	0	0.2	0	0	0*	100

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.
* Means are significantly different ($p < 0.05$) from control (compound(s) is not added to growth medium).

about 3 h. However, 0.2% ascorbic acid completely inhibited growth of *C. freundii* 4 and greatly reduced growth of *C. freundii* 38 ($p < 0.05$). Addition of citric acid (0.03%) alone to the growth medium only slightly inhibited growth of the tested strains ($p > 0.05$); OD_{560} readings after 24 h incubation at 31 ºC in nutrient broth with or without citric acid were nearly the same (Tables 1 and 3).

Lowering growth temperature to 5 ºC enhanced the activity of ascorbic acid (0.1%) or citric acid (0.03%) against both strains ($p < 0.05$) (Tables 2 and 4). The percentages of growth inhibition of *C. freundii 4* and *C. freundii* 38 after 6 days incubation in broth containing 0.1% ascorbic acid alone were 72% and 54%, respectively. For citric acid (0.03%), the percentages of inhibition of *C. freundii* 4 and

TABLE 4 – Inhibition of growth of *Citrobacter freundii* 38 in nutrient broth by various compounds at 5 ºC

Ascorbic acid (%)	Citric acid (%)	NaCl (%)	Lactic acid (%)	Growth after			Reduction in growth (%)[a]
				1 d	4 d	6 d	
0	0	0	0	0.01	0.22	0.35	-
0.1	0	0	0	0	0.05	0.16*	54
0	0.03	0	0	0	0.07	0.17*	51
0	0	3	0	0	0	0.14*	60
0	0	0	0.1	0	0	0*	100

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.
* Means are significantly different ($p < 0.05$) from control (compound(s) is not added to growth medium).

C. freundii 38 after 6 days incubation in broth containing this compound alone were 25% and 51%, respectively. Also, there was a lag of about 4d before growth of the strains could be detected (Tables 2 and 4).

Addition of lactic acid (0.2%) alone to the growth medium completely inhibited growth of the tested strains at 31 ºC (Tables 1 and 3). At 5 ºC, the presence of 0.1% lactic acid alone in the growth medium was effective against both strains; growth of cells was not detected after 6 days incubation (Tables 2 and 4).

Optical density readings of *C. freundii* strains subjected to sodium chloride (3-5%) alone at 31 ºC and 5 ºC are presented in Tables 1-4. The presence of sodium chloride (3%) in the growth medium slightly inhibited growth of the tested strains at 31 ºC ($p < 0.05$). The percentage of inhibition after 24 h incubation was about 36% for both strains. Exposure of the strains to 4% NaCl caused enhanced inhibition of growth, where the percentages of inhibition of *C. freundii* 4 and *C. freundii* 38 were 37% and 50%, respectively. However, when the broth contained 3% or 4% sodium chloride, a lag of about 3h occurred before increase in number could be observed (Tables 1 and 3). Sodium chloride (5%) greatly inhibited growth of both strains at 31 ºC. The percentages of inhibition after 24 h incubation were 83% and 85% for *C. freundii* 4 and *C. freundii* 38, respectively.

Reduction of growth temperature to 5 ºC enhanced the activity of sodium chloride against both strains ($p < 0.05$), where the percentages of growth inhibition after 6 d incubation in broth containing 3% NaCl were 88% and 60% for *C. freundii* 4 and *C. freundii* 38, respectively. Also, there was a lag of about 4 days before growth of the strains could be detected (Tables 2 and 4).

Interaction between ascorbic acid or citric acid and sodium chloride

At 31 ºC, exposure to ascorbic acid (0.1%) and NaCl (3%) together, ascorbic acid (0.1%) and citric acid (0.03%) together, or citric acid (0.03%) and NaCl (4%) together reduced the growth rate of *C. freundii* 4 ($p < 0.05$); the percentages of inhibition after 24 h incubation were 54%, 32% and 58%, respectively. Also, a lag was observed before increase in growth could be detected (Table 1). For *C. freundii* 38, nearly similar results were obtained for growth in broth containing these compounds (Table 3).

In addition, presence of ascorbic acid (0.1%) and NaCl (4%) together in the growth medium caused enhanced inhibition of the tested strains at 31 ºC. The percentages of inhibition after 24 h incubation were 66% and 75% for *C. freundii* 4 and *C. freundii* 38, respectively (Tables 1 and 3).

***Thymus vulgaris* extract effect**

The results of exposure of *C. freundii* 4 and *C. freundii* 38 to thyme extract at 31 ºC and 5 ºC are presented in Tables 5 and 6. At 31 ºC, the presence of thyme extract (0.3%) alone in the growth medium reduced the growth rate ($p < 0.05$), where the percentages of inhibition after 24 h incubation were 60% and 55% for *C. freundii* 4 and *C. freundii* 38, respectively (Tables 5A and 6A). Also, the strains grew in broth containing thyme extract after a long lag (about 3 h).

Lowering growth temperature to 5 ºC did not increase the activity of thyme extract (0.3%) alone against *C. freundii* 4 (Table 5B) and slightly increased the activity of this compound against *C. freundii* 38 (Table 6B).

However, for both strains a lag of about 4 days occurred before growth could be observed.

Addition of thyme extract (0.3%) and ascorbic acid (0.1%) together to the growth medium at 31ºC caused enhanced inhibition of *C. freundii* 4, but only slightly inhibited growth of *C. freundii* 38. The percentages of inhibition after 24 h incubation were 74% and 35% for *C. freundii* 4 and *C. freundii* 38, respectively (Tables 5A and 6A).

Lowering growth temperature to 5 ºC did not further increase the activity of thyme extract (0.3%) and ascorbic acid (0.1%) together against *C. freundii* 4 (Table 5B) but enhanced these compounds together against *C. freundii* 38 (Table 6B).

Exposure of the tested strains to thyme extract (0.3%) and NaCl (4%) together completely inhibited growth at 31 ºC. Increase in optical density was not observed after 24 h

TABLE 5 – Inhibition of *Citrobacter freundii* 4 by *Thymus vulgaris* extract at 31 ºC (A) and 5 ºC (B)

(A)

Thyme extract (%)	Ascorbic acid (%)	NaCl (%)	Growth after			Reduction in growth (%)[a]
			2 h	3 h	24 h	
0	0	0	0.015	0.55	0.57	-
0.3	0	0	0	0.02	0.23*	60
0.3	0.1	0	0	0	0.15*	74
0.3	0	4	0	0	0*	100

(B)

Thyme extract (%)	Ascorbic acid (%)	Growth after			Reduction in growth (%)[a]
		1 d	4 d	6 d	
0	0	0	0.15	0.31	-
0.3	0	0	0.04	0.14*	55
0.3	0.1	0	0	0.086*	72

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.

TABLE 6 – Inhibition of *Citrobacter freundii* 38 by *Thymus vulgaris* extract at 31 ºC (A) and 5 ºC (B)

(A)

Thyme extract (%)	Ascorbic acid (%)	NaCl (%)	Growth after			Reduction in growth (%)[a]
			2 h	3 h	24 h	
0	0	0	0.013	0.05	0.51	-
0.3	0	0	0	0.02	0.23*	55
0.3	0.1	0	0	0.03	0.33*	35
0.3	0	4	0	0	0*	100

(B)

Thyme extract (%)	Ascorbic acid (%)	Growth after			Reduction in growth (%)[a]
		1 d	4 d	6 d	
0	0	0.01	0.2	0.34	-
0.3	0	0	0	0.13*	62
0.3	0.1	0	0	0.07*	79

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.

TABLE 7 – Inhibition of growth of *Citrobacter freundii* 4 in chicken homogenates by combination of various compounds at 31 ºC (A) and 5 ºC (B)

(A)

Ascorbic acid (%)	Citric acid (%)	NaCl (%)	Growth after		Reduction in growth (%)[a]
			1 d	2 d	
0	0	0	0.21	0.4	-
0.1	0	4	0.1	0.24*	40
0	0.03	4	0.09	0.26*	35

(B)

Ascorbic acid (%)	NaCl (%)	Lactic acid (%)	Growth after		Reduction in growth (%)[a]
			2 d	6 d	
0	0	0	0.12	0.3	-
0.1	0	0	0.06	0.14*	53
0	3	0	0.08	0.09*	70
0	0	0.1	0.04	0.06*	80

[a] Reduction in growth (%) = 100 x Growth in broth without chemicals – Growth in broth with chemical (s) / Growth in broth without chemicals.

incubation in nutrient broth containing these compounds (Tables 5A and 6A).

Effects of sodium chloride, ascorbic acid, citric acid, lactic acid, and combination of these compounds in chicken and fish homogenates

The growth of *C. freundii* 4 at 31 ºC and 5 ºC in chicken homogenates containing these compounds is presented in Table 7. At 31 ºC, addition of citric acid (0.03%) and NaCl (4%) together or ascorbic acid (0.1%) and NaCl (4%) together to chicken homogenates decreased the growth rate ($p < 0.05$); the percentages of growth inhibition after 2 d incubation were 35% and 40%, respectively (Table 7A).

At 5 ºC, the presence of ascorbic acid (0.1%), NaCl (3%) or lactic acid (0.1%) alone in chicken homogenates greatly reduced the growth of the tested strain ($p < 0.05$). The percentages of growth inhibition after 6 d incubation were 53%, 70%, and 80%, respectively (Table 7B). Similar results were obtained for growth of this strain at 31 ºC and 5 ºC in fish homogenates containing these compounds (data not shown).

DISCUSSION

Citrobacter freundii can cause food poisoning and /or food spoilage of fish and other food products (Kleeberger and Busse, 1975). Therefore, application of compounds to control growth of this bacterium is important to extend the

shelf life of food. In this study, the effects of lactic acid, citric acid, ascorbic acid, sodium chloride, *T. vulgaris* extract, and combinations of these compounds on the growth of *C. freundii* were investigated. At 31 ºC, ascorbic acid (0.1%) did not show antimicrobial activity against the tested strains. It is possible that the tested strains utilized this compund. Other studies have reported that ascorbic acid at low concentrations is not as effective as other preservatives. Fletcher *et al*. (1983) observed that ascorbic acid had some inhibitory properties toward *Campylobacter jejuni*. However, at higher concentrations (e.g. 0.2%), this study showed that ascorbic acid completely inhibited growth of *C. freundii* 4 and greatly reduced growth of *C. freundii* 38. Citric acid (0.03%) showed a slight but not significant ($p > 0.05$) inhibitory effect on the tested *C. freundii* strains at 31 ºC. Similar results were obtained by Blaszyk and Holley (1998), who reported that growth of *E. coli* and *Listeria monocytogenes* was slightly reduced by 0.2% sodium citrate at 7 ºC and 18 ºC. Also, it is possible that the strains utilized this compound; *Citrobacter* spp. have been shown to use citrate as the sole carbon source (Jay, 2000; Kleeberger and Busse, 1975).

Sodium chloride reduced the growth of *C. freundii* strains tested; and the effect was proportional to the concentration of NaCl. This is consistent with results obtained by other studies. Glass *et al*. (1992) reported that *E. coli* O157:H7 could grow in tryptone soy broth containing < 6.5% NaCl, but the organism was inactivated at 8.5% NaCl. Also, Abu-Ghazaleh (2000) showed that growth of *Aeromonas caviae* and *Aeromonas sobria* was decreased by sodium chloride.

Lactic acid (0.2%) completely inhibited growth of the tested strains. Similar results were obtained by Ismail *et al*. (2001), who reported that immersion of chicken wings in 2% lactic acid caused a significant reduction in numbers of aerobic bacteria. Also, Kolsarici and Candogan (1995) observed that number of total psychrotrophic aerobic bacteria, staphylococci and coliform bacteria on chicken leg and breast meats was decreased after treatment with lactic acid and storage at 4 ± 1 ºC. Also, lactic acid (0.2% and 0.1%) lowers the pH of the medium to 3.61 and 4.03, respectively; and *C. freundii* is not able to grow at this pH.

Lowering growth temperature to 5 ºC significantly increased the activity of ascorbic acid, citric acid, lactic acid or NaCl against *C. freundii* strains tested. This is consistent with results obtained by other studies. Jay and Rivers (1984) showed that < 10 ppm of diacetyl (a flavoring agent) inhibited *Pseudomonas fluorescens* and *Pseudomonas geniculata* at 5 ºC, whereas higher amounts of diacetyl (240 ppm) were required to inhibit these microorganisms at 30 ºC.

Interactions between the tested compounds were investigated in this study for possible synergism against *C. freundii*. The antimicrobial effect of ascorbic acid, citric acid, or thyme extract was greatly enhanced in the presence of NaCl. Also, ascorbic acid activity against *C. freundii* was greatly increased in the presence of citric acid. Similar results were obtained by other investigators. Combination of NaCl and sorbate has been reported to be synergistic against *Staphylococcus aureus* (Liewen and Marth, 1985). Unda *et al*. (1991) reported that best destruction of *L. monocytogenes* in beef roasts was obtained with brines containing phosphate and sodium lactate or glycerol monolaurin in combination with one, or two, cookings. Blom *et al*. (1997) showed that addition of lactate and acetate together inhibited growth of *L. monocytogenes* in processed meat products. Other studies have reported that presence of 0.1% potassium sorbate and 0.1% sodium benzoate together in apple cider inactivated enterohemorrhagic *E. coli* O157: H7 (Zhao *et al*. (1993).

In this study, the effects of chemicals on *C. freundii* in chicken or fish homogenates were examined. In general, the strains were more resistant to the effect of the tested compounds when treated in homogenates than in broth. Organic molecules, fats and proteins in the homogenates may react with the chemicals added, causing a decrease in their activity. It has been reported that the composition of food is one of the factors that affect the activity of chemicals against organisms in it (Banwart, 1989).

In conclusion, this study showed that lactic acid, ascorbic acid, or sodium chloride alone inhibited or greatly reduced the growth rate of *C. freundii* strains tested. Also, various combinations of the tested compounds prevented growth of the strains at moderate and low temperatures.

REFERENCES

Abu-Ghazaleh B.M. (2000). Inhibition of *Aeromonas caviae* and *A. sobria* by sodium chloride, citric acid, ascorbic acid, potassium sorbate and extracts of *Thymus vulgaris*. Jpn. J. Infect. Dis., 53: 111-115.

Abu-Ghazaleh B.M. (2001). Fecal coliforms of wastewater treatment plants: antibiotic resistance, survival on surfaces and inhibition by sodium chloride and ascorbic acid. Microbiologica, 24: 379-387.

Banwart G.J. (1989). Basic Food Microbiology. 2nd edn., Chapman and Hall, New York, USA.

Blaszyk M., Holley, R.A. (1998). Interaction of monolaurin, eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. Int. J. Food Microbiol., 39: 175-183.

Blom H., Nerbrink E., Dainty R., Hagtvedt T., Borch E., Nissen H., Nesbakken T. (1997). Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory-acceptable servelat sausage and cooked ham stored at 4 ºC. Int. J. Food Microbiol., 38: 71-76.

Fletcher R.D., Albers A.C., Chen A.K., Albertson J.N. (1983). Ascorbic acid inhibition of *Campylobacter jejuni* growth. Appl. Enviro. Microbiol., 45: 792-795.

Glass K.A., Loeffelholz J.M., Ford J.P,. Doyle, M.P. (1992). Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol., 58: 2513-2516.

Gonzalez-Rodriguez M.-N.G., Sanz J.J., Santos J.A., Otero A., Lopez M.-L.G. (2001). Bacteriological quality of aquacultured freshwater fish portions in prepackaged trays stored at 3 ºC. J. Food Prot., 64: 1399-1404.

Ismail S.A., Deak T., Abd el-Rahman H.A., Yassien M.A., Beuchat L.R. (2001). Effectiveness of immersion treatments with acids, trisodium phosphate, and herb decoctions in reducing populations of *Yarrowia lipolytica* and naturally occuring aerobic microorganisms on raw chicken. Int. J. Food Microbiol., 64: 13-19.

Jay J.M., Rivers G.M. (1984). Antimicrobial activity of some food flavoring compounds. J. Food Safety, 6: 129-139.

Jay J.M. (2000). Modern Food Microbiology. 6th edn., Chapman and Hall, New York, USA.

Joklik W.K., Willett H.P., Amos D.B., Wilfert C.M. (1992). Zinsser Microbiology. 20th edn., Appelton and Lange, Norwalk, Connecticut, USA.

Kleeberger A., Busse M. (1975). Total count and bacterial flora of minced meat with special reference to enterobacteria and pseudomonads. Z. Lebensm. Unters. For., 158: 321-331.

Kolsarici N., Candogan K. (1995). The effects of potassium sor-

bate and lactic acid on the shelf- life of vacuum- packed chicken meats. Poultry Sci., 74: 1884-1893.

Liewen M.B., Marth E.H. (1985). Growth and inhibition of microorganisms in the presence of sorbic acid: a review. J. Food Prot., 48: 364-375.

Lindberg A.-M., Ljungh A., Ahrne S., Lofdahl S., Molin G. (1998). Enterobacteriaceae found in high numbers in fish, minced meat and pasteurised milk or cream and the presence of toxin encoding genes. Int. J. Food Microbiol., 39: 11-17.

Schefler W.C. (1980). Statistics for the Biological Sciences. 2nd edn., Addison-Wesley, Massachusetts, USA.

Unda J.R., Molins R.A., Walker H.W. (1991). *Clostridium sporogenes* and *Listeria monocytogenes*: survival and inhibition in microwave-ready beef roasts containing selected antimicrobials. J. Food Sci., 56: 198-205.

WHO Technical Report Series 778 (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. World Health Organization, Geneva, Switzerland.

Zhao T., Doyle M.P., Besser R.E. (1993). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with or without preservatives. Appl. Environ. Microbiol., 59: 2526-2530.