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Effects of organic acids to prolong the shelf-life and improve the microbial quality of fresh cut broccoli florets

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RESEARCH ARTICLE

Abstract

This study was conducted to evaluate the effectiveness of a combination of specific organic acids for disinfecting fresh cut broccoli floret samples contaminated with *Listeria monocytogenes* serotype 1/2b and *Salmonella enterica* serovar Typhimurium. In addition, the effects of organic acids on the microbial load of broccoli samples during storage at +4 °C were determined for 7 days. The organic acids tested were acetic, benzoic, sorbic, fumaric, malic, tartaric, lactic and succinic acids and the concentrations used were 1 and 2% (w/v and v/v). *L. monocytogenes* and *S.* Typhimurium were grown on the selective media before the contamination. The broccoli samples were dipped first into the pathogen solutions containing *L. monocytogenes* and *S.* Typhimurium, then into the organic acids and subsequently counted on Oxford agar and bismuth sulphite agar, respectively. The broccoli samples dipped into the organic acids without pathogen culture were also investigated in terms of total mesophilic aerobic microorganisms, psychrotrophic microorganisms, *Pseudomonas* spp., total lactic acid bacteria, *Enterobacteriaceae* spp. and total yeast-moulds for 7 days stored at 4 °C. *S.* Typhimurium and *L. monocytogenes* log reductions were determined at between 3 to 3.28 and 2.63 to 2.84 log colony-forming units/g with 1 and 2% of malic acid and between 3.68 to 4.17 and 2.68 to 2.9 log with 1 and 2% of tartaric acid, respectively. The malic, tartaric, lactic, succinic acids and acetic acid in the tested concentrations could be used to inhibit *S.* Typhimurium and *L. monocytogenes* and prolong the shelf-life of fresh-cut broccoli.

Keywords: food safety, microbiology, pathogens

1. Introduction

Fresh-cut fruits and vegetables are no longer considered low risk in terms of food safety. Recently, several foodborne outbreaks have been traced on fresh-cut fruits and vegetables that were processed under poor sanitary conditions (Gil *et al.*, 2009). In the USA, foodborne illnesses affect 6-8 million people every year and cause approximately 9,000 deaths (Rayner *et al.*, 2004). *Salmonella* spp. and other pathogen contaminants in food products are a significant risk to public health and a serious economic risk for the food industry (Doran *et al.*, 2005). *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* are very important microorganisms among the foodborne pathogens (Theron and Lues, 2007). *S.* Typhimurium is one of the major concerns to public health, and it is one of the most important serovars implicated in the occurrence of gastroenteritis throughout the world (Nazer *et al.*, 2005). *L. monocytogenes* has been isolated from a variety of foods and has been implicated in a number of foodborne outbreaks (Irkin and Korukluoglu, 2009; King *et al.*, 2003; Marcos *et al.*, 2007; Mytle *et al.*, 2006). It was reported that 1,097 disease outbreaks occurred in the USA during 2007, resulting in 21,244 cases of foodborne illness and 18 deaths. Among the 18 reported deaths, three cases were attributed to *L. monocytogenes*. It can be said that leafy vegetables caused 14% of the illnesses (Crous, 2011). *L. monocytogenes* is a gram-positive, food-borne pathogen that is widely distributed in the environment and occurs naturally in many raw foods. It is psychrotrophic and halotolerant, and can

grow in the temperature range of 1 to 45 °C. It is able to grow in 10% NaCl solution concentration. As a result, it may grow in many food products that have extended shelf lives (Neetoo *et al.*, 2008). On the other hand, *L. monocytogenes* can survive and grow in ready-to-eat food products.

A number of important human pathogens can contaminate fresh-cut produce, and there has been an increase in the number of produce-linked, foodborne outbreaks in recent years (Francis *et al.*, 2012). Different chemical washing agents have been studied to determine their efficacy in the deactivation or inactivation of pathogenic bacteria on vegetables. Sanitisers, including chlorinated water, have been studied for their effectiveness in removing pathogens from fruits and vegetables (Sengun and Karapinar, 2005). Unfortunately, chlorine vapour from chlorinated water can irritate the skin and respiratory tract. According to numerous studies, some trihalomethanes, such as chloroform ($CHCl_3$) and bromodichloromethane ($CHBrCl_2$) occurring in chlorinated water, may be carcinogenic, mutagenic, teratogenic, or toxic (Alvaro *et al.*, 2009; Rico *et al.*, 2007). Generally, solutions of 50-200 mg/kg chlorine have been used extensively on a commercial scale for washing fruits, vegetables, and fresh-cut produce. However, since chlorine compounds have some toxic effects, alternative sanitisers are being evaluated for their ability to reduce bacterial pathogens in food (Carmen-Velazquez *et al.*, 2009). Consumers prefer additive-free, fresher and more natural tasting food products, while expecting microbiological safety to be maintained (Nazer *et al.*, 2005).

Organic acids are natural antimicrobials that are used extensively in the food industry to inhibit the growth of important microbial pathogens, such as *L. monocytogenes* and *Escherichia coli* O157:H7. Organic acids are easily applied by wash, spray, or dip techniques to decontaminate the surfaces of fresh products, and the salts of organic acids are included in product formulations to prevent the growth of pathogens in a variety of ready-to-eat foods (Carpenter and Broadbent, 2009; Nazer *et al.*, 2005; Over *et al.*, 2009; Theron and Lues, 2007).

Broccoli is marketed either as a fresh product (e.g. frozen or chopped). The USA per capita consumption of fresh broccoli has increased steadily over the last two decades, mainly because of its popularity in salad bars. Broccoli has a high vitamin C, vitamin A, fibre, and mineral content, as well as being reported to have several anti-cancer compounds. These attributes make it a popular food among health-conscious consumers. Recent outbreaks of foodborne illness related to leafy vegetables, including spinach and lettuce, indicate that the increased consumption of fresh produce may present new challenges with regard to food safety (Gomes *et al.*, 2008; Padaga *et al.*, 2000). The rough surfaces of these vegetables and the presence of crevices that help organisms to attach to the crop make vegetables vulnerable to the presence of microorganisms (Crous, 2011). The pertinent literature indicates that some recalls of broccoli have occurred due to concerns about *Listeria*. Over recent years the U.S. Food and Drug Administration has tested 1000 samples of imported celery, green onions, lettuce, strawberries and broccoli for the presence of *Salmonella*, *Shigella*, and *E. coli* O157:H7 to prevent foodborne diseases (Liang *et al.*, 2001). Quiroz-Santiago *et al.* (2009) reported *Salmonella* spp. contaminations of 9% in broccoli samples. Gilbert *et al.* (2003) identified *Salmonella* spp. on the broccoli as well as in other food products.

Published data are quite sparse on the application of sanitisers that may be useful for broccoli. Broccoli is grown close to the ground, and even though it can be contaminated by pathogens depending on irrigation water and other conditions, it undergoes minimal processing before consumption. Therefore the aim of this research was to evaluate the antimicrobial effects of some organic acids and commercial sanitisers on the microbial load (total aerobic mesophilic microorganism, psychrotrophic microorganisms, *Enterobacteriaceae* spp., *Pseudomonas* spp., total yeast and mould, lactic acid bacteria numbers) of broccoli florets during 7 days' storage at 4 °C. The antibacterial effects were also determined in the samples contaminated with *L. monocytogenes* and *S.* Typhimurium.

2. Materials and methods

Vegetable materials

Broccoli samples were bought from a supermarket in Balikesir, Turkey. The outer contaminated parts of 3,000 g of broccoli florets were removed aseptically and discarded. All samples were washed with de-chlorinated tap water, then drained and placed on filter paper. They were then placed in sterile stomacher bags.

Preparation of sanitisers

The fresh chemical solutions of about 1 l from each concentration prepared in sterile distilled water were used on the same experimental day. The pH values of the solutions were determined with a Sartorious PT15 device (Sartorious, Göttingen, Germany). The following washing solutions were assayed, and average pH values were determined, respectively: sterile distilled water (control, pH=6.32), 1 and 2% lactic acid (v/v, Merck 1.00466.2500, pH=2.30 and 2.16; Merck, Darmstadt, Germany), 1 and 2% acetic acid (v/v, Merck 1.00063.2511, pH=2.86 and 2.68), 1 and 2% malic acid (w/v, Merck 8.00384.0050, pH=2.39 and 2.23), 1 and 2% tartaric acid (w/v, Merck 1.00804.1000, pH=2.40 and 2.02), 1 and 2% benzoic acid (w/v, Sigma Aldrich 33047, pH=3.91 and 3.85; Sigma Aldrich, St. Louis, MO, USA), 1 and 2% sorbic acid (w/v, Pancreac

141055.1209, pH=4.31 and 4.15; Pancreac, Barcelona, Spain), 1 and 2% succinic acid (w/v, Sigma Aldrich 14079-250, pH=3.50 and 3.26), 1 and 2% fumaric acid (w/v, Sigma Aldrich 240745-100, pH=2.81 and 2.77).

Preparation of broccoli florets

After washing and draining, one group of broccoli florets was placed directly in sterile stomacher bags. For the other groups of broccoli florets, 1 l of sterilised peptone water (0.1%, w/v) was prepared for each treatment. In this group, the broccoli florets were inoculated with the culture suspensions of *S*. Typhimurium and the *L. monocytogenes*. The broccoli samples were dipped into the culture suspension (contained 10^7-10^8 colony-forming unit (cfu)/ml) at room temperature for 1 min; then they were drained to remove excess liquid, and the inoculated samples were placed in sterilised stomacher bags (Sengun and Karapinar, 2005).

Treatment with the organic acid and disinfectant solutions

The microorganism inoculated samples were immersed in either 1 l of 1 or 2% (w/v) of each organic acid washing solution for 5 min. Distilled water was included as a control treatment. All experiments were performed at room temperature (23±2 °C) (Park *et al.*, 2011).

Microbial analysis

Ten grams of each sample was diluted in sterile 90 ml 0.1% peptone + 0.85% NaCl and homogenised in a stomacher bag for 90 s at room temperature. A serial 10-fold dilution series was prepared in sterile dilution water. Total mesophilic aerobic microorganisms, psychrotrophic microorganisms, *Pseudomonas* spp., total lactic acid bacteria, *Enterobacteriaceae* spp. and total yeast-mould counts were enumerated on days 1, 4 and 7.

Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/g). For the total mesophilic aerobic microorganisms (TMAM) counts, plate count agar (PCA) (Merck 1.05463) was used and incubated at 35 °C for 2 days (Halkman, 2005). Psychrotrophic counts were determined on PCA (Merck 1.05463) at 7 °C for 7 days (Soldatou *et al.*, 2009). *Enterobacteriaceae* members were determined on double-layer violet-red-bile-dextrose agar (Merck 1.10275) at 37 °C for 24 h (Govaris *et al.*, 2007). *Pseudomonas* spp. were counted on *Pseudomonas* agar supplemented with fucidin cephaloridine selective agar (Merck 1.05284) incubated at 25 °C for 44 h (Mead and Adams, 1977). To determine the total lactic acid bacteria counts, *Lactobacillus* spp. were counted on double-layer at pH=5.6 and incubated in de Man-Rogosa-Sharpe agar (Merck 1.10660) at 28 °C for 72 h (Russo *et al.*, 2006). For the total yeast and mould counts, yeast and moulds were enumerated on chloramphenicol added rose bengal agar (Merck 1.00467) plates following the surface plate method and incubated at 25 °C for 5-7 days (Skandamis and Nychas, 2001).

Pathogen bacterial strains

S. Typhimurium and *L. monocytogenes* serotype 1/2b were obtained from Erciyes University's Veterinary Faculty Microbiology Department, Kayseri, Turkey. Each isolate was grown in tryptic soy agar (Merck 1.05458) slants for 18 h at 37 °C and then kept at 4 °C as stock cultures. Tryptic soy broth (Merck 1.00550) was used to activate the stock cultures, and the activation took place at 37 °C for 24 h (the activated cultures included 10^7-10^8 cfu/ml-McFarland standard 0.5, *S. Typhimurium*, *L. monocytogenes*). Then the activated cultures were inoculated onto the broccoli samples (10^7-10^8 cfu/g broccoli samples). The limits of detection were calculated by the International Union of Pure and Applied Chemistry method for each microbiological analysis and were approximately 10 cfu/g for the media used (Mocak *et al.*, 1997).

Preparation of inoculums and inoculation of broccoli florets

1 l of sterilised peptone water (0.1%, w/v) was prepared for each treatment of the vegetables. Each of the broccoli florets was inoculated with the *S*. Typhimurium and *L. monocytogenes* culture suspensions. The broccoli samples were dipped into the culture suspension (contains 10^7-10^8 cfu/ml) at room temperature for 1 min; then they were drained to remove excess liquid, and the inoculated samples were placed in sterilised stomacher bags (Sengun and Karapinar, 2005).

Treatment with the organic acid and sanitiser solutions

The inoculated samples were immersed in either 1 l of 1 or 2% of each organic acid and sanitiser solutions for 5 min. Distilled water was included as a control treatment. All experiments were performed at room temperature (23±2 °C) (Park *et al.*, 2011).

Bacterial enumeration

For the determination of *S*. Typhimurium, 25 g of treated samples were weighed and put in 225 ml sterilised pepton water, then homogenised in a stomacher (BagMixer; Interscience, Saint-Nom-la-Bretêche, France) for 60 s. Then the homogenates were diluted serially in sterile 0.1% peptone water and surface plated (0.1 ml in duplicate) on bismuth sulphite agar (Merck 1.05418). The plates were incubated at 37 °C for 48 h (Halkman, 2005; Karapinar and Sengun, 2007). Presumptive *Salmonella* colonies were then subjected to some biochemical tests using triple sugar iron

agar, urea broth and lysdine decarboxylase broth. All tests were performed at 37 °C for 18-24 h.

The broccoli samples treated with the organic acids and the control were homogenised separately in the stomacher mixer (Interscience) in 0.1% peptone water for 60 s. Serial dilutions were prepared and *L. monocytogenes* was determined on nalidixic acid added (40 mg/l agar media at 50 °C) Oxford agar and incubated at 37 °C for 24-48 h. Typical *Listeria* colonies were subjected to catalase, gram staining tests and urea broth tests at 37 °C for 18-24 h.

Statistical analysis of data

Three replicate trials were conducted for each experiment. Data were subjected to analysis of variance and Duncan's multiple tests, as well as general multivariate analysis tests SPSS 16.0 (SPSS Inc., Chicago, IL, USA) to determine if there were significant differences ($P<0.05$) in the populations.

3. Results and discussion

pH values of washing solutions

Average pH values of the organic acids and the disinfectant washing solutions (1 and 2%) belonged to the broccoli samples are given in Table 1. The samples contained lactic acid and malic acids (1%) and lactic and tartaric acids (%) had the lowest pH values at 2.30, 2.39 and 2.16, 2.02, while the samples contained sorbic acid (1 and 2%) showed the highest pH values at 4.31 and 4.15, respectively.

The effects of organic acids or disinfectants on the shelf-life of broccoli samples

Organic acids (e.g. lactic, citric, acetic and tartaric acid) have been described as strong antimicrobial agents against psychrophilic and mesophilic microorganisms in fresh-cut fruit and vegetables. The antimicrobial activity of organic acids is aroused by the low pH value in the medium, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid (Rico *et al.*, 2007; Theron and Lues, 2007). Broccoli harvested from different geographical regions showed notable variations in total microbial flora and associated species (Padaga *et al.*, 2000).

In this research total aerobic microorganism, psychrotrophic microorganism, *Enterobacteriaceae* spp., *Pseudomonas* spp., total yeast and mould and lactic acid bacteria numbers are investigated at 4 °C during 7 days' storage in the samples dipped into different organic acid solutions (1 and 2% (v/v) concentrations) (Table 2). In line with our research, several studies have reported that organic acids may improve the shelf-life of products. Sabah *et al.* (2004), Cliffe-Byrnes and O'Beirne (2008) showed that organic acids treatments, when used in combination with other systems, may extend the shelf-life of products. In a study by Eswaranandam *et al.* (2006) whole apples and diced apples were coated in soy protein + glycerol + lactic acid and malic acid edible films and the product's shelf-life was shown to be extended. Mesophilic bacteria, yeast and mould counts of harvested mushrooms were decreased by immersing them in 1% acetic, citric and lactic acid solutions in the Park *et al.* (2005) research. Singla *et al.* (2012) determined that the decontamination of harvested mushrooms with malic acid solutions increased the shelf-life by about 5 days at 15 °C. Bin Jasass (2011) found that 1% lactic acid and 0.5% acetic acid concentration solutions decreased psychrotrophic and total viable counts and prolonged the shelf-life of the products by about 8 more days than the control groups.

Total mesophilic aerobic microorganism numbers

On the first day, the TMAM numbers of the samples dipped in the solutions containing 1% lactic acid (v/v), 2% tartaric acid (w/v), fumaric (w/v), acetic (v/v) and malic acids (w/v) were showed a statistically significant difference from the control groups ($P<0.05$). The samples washed with 1 and 2%

Table 1. Average pH values of broccoli washing solutions.

Organic acids and sanitiser washing solutions	1% concentration (m/v or v/v)	2% concentration (m/v or v/v)
Control (water)	6.32±1.2	6.32±1.2
Lactic acid	2.30±0.7	2.16±0.3
Acetic acid	2.86±0.5	2.68±1.3
Malic acid	2.39±1.3	2.23±0.7
Tartaric acid	2.40±0.4	2.02±1.1
Benzoic acid	3.91±0.2	3.85±1.2
Sorbic acid	4.31±0.8	4.15±0.6
Succinic acid	3.50±0.6	3.26±0.3
Fumaric acid	2.81±0.2	2.77±0.5

Table 2. Effects of organic acids on broccoli's microbial load (log cfu/g ± standard deviation) during storage at 4 °C.[1,2]

	Day	Total mesophilic aerobic microorganisms (TMAM)	Psychrotrophic microorganisms	*Enterobacteriaceae* spp.	*Pseudomonas* spp.	Total yeast-moulds	Total lactic acid bacteria (LAB)
Control	1	6.29±1.11^{a*}	6.17±0.44^{a}	4.81±0.23^{a}	6.31±0.63^{a}	5.70±0.08^{a}	5.09±1.46^{a}
	4	7.12±0.09^{k*}	7.53±0.25^{k}	5.46±0.41^{k}	6.99±0.18^{k}	6.57±1.46^{k}	6.50±0.10^{k}
	7	7.40±0.17^{A*}	7.54±0.37^{A}	6.07±0.24^{A}	7.39±0.33^{A}	7.35±0.30^{A}	7.44±0.18^{A}
1% AA	1	3.55±1.10^{c}	2.50±0.12^{h}	2.70±0.66^{de}	4.94±0.22^{ce}	3.64±0.12^{ef}	4.25±0.13^{abcd}
	4	2.32±0.06^{r}	4.09±0.19st	4.60±0.20^{t}	2.22±0.22^{s}	2.49±0.17^{s}	6.28±0.14^{k}
	7	6.55±0.57^{ABCD}	6.54±0.08^{BCD}	5.36±0.13^{BC}	4.87±0.19^{E}	6.33±0.34^{DE}	6.71±0.53^{BC}
2% AA	1	3.10±0.00^{f}	2.83±0.70^{gh}	1.68±0.15^{g}	4.60±1.00^{i}	4.38±1.00^{j}	3.65±0.24^{def}
	4	3.97±0.25^{s}	3.21±0.29^{ü}	1.54±0.80^{t}	4.30±0.70^{t}	4.21±0.00^{t}	4.22±0.08^{n}
	7	5.21±0.64^{F}	6.19±0.05^{D}	3.10±0.10^{G}	4.17±0.47^{F}	3.98±0.14^{G}	4.21±0.36^{F}
1% LA	1	2.22±0.14^{d}	3.23±0.90^{fgh}	2.91±0.34^{ef}	6.00±0.20^{i}	3.09±0.12^{fghi}	4.72±0.17^{ab}
	4	5.13±0.25^{o}	5.15±0.06^{r}	4.54±0.07^{lm}	5.35±0.14^{o}	4.50±0.90^{nopr}	5.81±0.16^{l}
	7	6.37±0.85^{BCDE}	6.34±0.51^{CD}	4.87±0.24^{CD}	5.03±0.36^{E}	5.63±0.08^{F}	6.03±0.64^{D}
2% LA	1	1.22±0.13^{e}	2.14±0.47^{h}	2.45±0.31^{cd}	5.13±0.30^{i}	2.68±0.31^{hi}	2.26±0.18^{h}
	4	3.65±0.14^{p}	3.89±0.15^{tu}	2.06±0.64^{s}	4.53±0.22^{p}	3.80±0.52^{r}	2.47±0.44^{p}
	7	5.56±0.10^{DEF}	4.56±1.24^{E}	3.48±0.08^{FG}	3.41±0.49^{G}	3.67±0.23^{G}	3.41±0.27^{H}
1% BA	1	4.43±0.24^{bc}	3.87±1.02^{efg}	2.41±0.17^{ef}	4.26±0.05^{defg}	3.57±0.41^{efg}	4.57±0.80^{abc}
	4	5.54±1.01^{no}	6.37±0.13^{n}	3.72±0.11^{op}	6.53±0.22^{l}	4.58±0.16^{nopr}	6.44±0.14^{r}
	7	6.47±0.21^{ABCD}	6.93±0.15^{BCD}	4.33±0.09^{E}	7.31±0.18^{A}	5.66±0.10^{F}	6.91±0.20^{AB}
2% BA	1	4.15±0.14^{c}	2.62±0.69^{h}	2.25±0.20^{f}	4.04±0.07^{efgh}	2.61±0.34^{i}	4.42±0.79^{abcd}
	4	6.25±0.19^{lmn}	6.32±0.22^{no}	3.18±0.06^{pr}	6.60±0.17^{kl}	4.41±0.17^{nopr}	6.29±0.13^{r}
	7	6.40±0.29^{BCDE}	6.78±0.12^{ABCD}	3.66±0.09^{F}	5.61±0.15^{DE}	5.46±0.22^{F}	5.26±0.13^{E}
1% SA	1	6.08±0.94^{ab}	5.73±0.42^{ab}	4.43±0.15^{ab}	5.61±0.27^{abc}	3.17±0.16^{fghi}	3.87±0.71^{bcde}
	4	5.65±1.12^{mno}	6.43±0.18^{n}	4.39±0.43^{mn}	6.62±0.07^{kl}	5.34±0.98^{mno}	6.43±0.23^{k}
	7	6.61±0.48^{ABC}	7.11±0.15^{ABC}	5.49±0.31^{B}	7.38±0.26^{A}	6.27±0.72^{DE}	7.32±0.11^{AB}
2% SA	1	5.40±0.05^{a}	4.83±0.70^{bcde}	4.35±0.30^{b}	6.11±0.39^{ab}	3.14±0.12^{fghi}	3.16±0.07^{efg}
	4	6.60±0.19^{kl}	6.76±0.04^{mno}	4.16±0.07^{mno}	5.87±0.59^{mn}	4.35±0.17^{opr}	4.67±0.27^{m}
	7	5.46±0.30^{EF}	6.78±0.18^{ABCD}	5.42±0.19^{B}	6.74±0.31^{AB}	5.84±0.07^{EF}	7.25±0.08^{AB}
1% FA	1	5.28±0.14^{ab}	4.39±1.05^{cdef}	3.21±0.25^{c}	3.00±0.54^{h}	4.59±0.48^{bcd}	2.73±0.41^{gh}
	4	6.33±0.13^{lm}	7.09±0.02^{klm}	3.83±0.36^{no}	6.58±0.16^{kl}	5.49±0.30^{lmn}	5.62±0.31^{l}
	7	7.17±0.14^{ABC}	7.33±0.08^{AB}	4.50±0.27^{DE}	7.17±0.06^{AB}	7.16±0.24^{AB}	6.90±0.53^{AB}
2% FA	1	1.09±0.43^{ef}	2.91±0.25^{gh}	0.00±0.00^{h}	6.12±0.28^{i}	4.09±0.20^{de}	2.16±0.19^{h}
	4	6.47±0.07^{kl}	6.47±0.05^{n}	0.00±0.00^{t}	6.20±0.20^{lmn}	6.17±0.11^{klm}	6.31±0.16^{k}
	7	6.46±0.24^{ABCD}	6.85±0.12^{ABCD}	0.00±0.00^{H}	7.13±0.10^{AB}	6.28±0.15^{DE}	6.26±0.35^{CD}
1% TA	1	3.40±0.60^{f}	5.38±0.16^{abcd}	4.18±0.30^{h}	3.68±1.04^{fgh}	3.57±0.14^{efg}	3.71±0.15^{cdef}
	4	2.75±0.56^{r}	4.31±0.16st	3.05±0.77^{r}	5.26±0.05^{o}	4.01±1.18^{r}	4.35±0.19^{i}
	7	6.00±0.66^{CDEF}	6.76±0.65^{ABCD}	0.00±0.00^{H}	6.39±0.08^{BC}	6.61±0.25^{CD}	5.28±0.83^{E}
2% TA	1	3.30±0.20^{f}	2.79±1.04^{gh}	0.00±0.00^{h}	4.60±0.20^{i}	3.50±0.07^{efgh}	3.00±0.19^{efgh}
	4	2.27±0.16^{r}	3.47±0.18^{uü}	0.00±0.00^{t}	3.45±0.32^{r}	4.21±0.83^{pr}	3.43±0.20^{o}
	7	5.14±0.62^{F}	3.54±0.34^{F}	0.00±0.00^{H}	5.15±0.11^{E}	6.54±0.40^{D}	3.51±0.23^{GH}
1% SUC	1	5.20±0.37^{ab}	5.47±0.64^{abc}	0.00±0.00^{h}	4.52±0.91^{cdef}	5.22±1.46^{abc}	4.92±0.35^{i}
	4	6.86±0.07^{kl}	6.46±0.07^{n}	0.00±0.00^{t}	6.55±0.07^{kl}	6.11±0.30^{klm}	6.46±0.11^{r}
	7	7.16±0.20^{AB}	7.38±0.15^{A}	0.00±0.00^{H}	6.99±0.61^{AB}	6.41±0.40^{D}	7.37±0.25^{A}
2% SUC	1	3.91±0.38^{c}	4.24±0.76^{def}	0.00±0.00^{h}	4.48±0.64^{def}	4.48±0.55^{cd}	4.80±0.23^{i}
	4	6.15±0.39^{lmn}	5.65±0.95^{p}	0.00±0.00^{t}	5.99±0.6^{1mn}	5.18±0.16^{mno}	6.49±0.17^{k}
	7	7.04±0.06^{AB}	7.29±0.53^{AB}	0.00±0.00^{H}	5.95±0.69^{CD}	6.36±0.33^{DE}	7.23±0.10^{AB}
1% MA	1	5.24±0.41^{c}	5.68±0.68^{ab}	2.67±0.32^{def}	5.41±0.26^{ab}	5.41±0.26^{ab}	3.22±0.12^{efg}
	4	6.86±0.30^{kl}	7.25±0.06^{kl}	4.44±0.12^{mn}	6.36±0.59^{lm}	6.49±0.27^{kl}	3.47±0.41^{o}
	7	5.93±1.40^{CDEF}	6.83±0.38^{ABCD}	4.29±0.39^{E}	5.47±0.18^{DE}	6.80±0.12^{BCD}	4.02±0.14^{FG}
2% MA	1	3.92±0.10^{f}	5.62±0.21^{ab}	3.88±0.41^{h}	5.10±0.20^{i}	4.29±0.26^{de}	2.85±0.08^{fgh}
	4	4.32±0.15^{p}	4.41±0.14^{s}	2.71±0.90^{r}	4.39±0.22^{p}	3.66±0.69^{r}	3.16±0.13^{o}
	7	3.05±0.49^{G}	6.35±0.24^{CD}	3.27±0.99^{FG}	5.22±0.46^{DE}	6.51±0.17^{D}	3.45±0.10^{GH}

[1] Mean values within the same column with different superscript small and capital letters are statistically different (a-j, k-u, A-H) (P<0.05).

[2] AA = acetic acid; BA = benzoic acid; FA = fumaric acid; LA = lactic acid; MA = malic acid; SA = sorbic acid; SUC = succinic acid; TA = tartaric acid.

acetic acid, tartaric acid, 2% lactic and malic acid exhibited significantly greater bacterial reduction than the control groups after 4 days' storage. After 7 days, the most effective washing treatment was 2% (w/v) malic acid solution and when compared to control no significant differences from the control were found among the other washing solutions. Our results are in agreement with Padaga *et al.* (2005) who determined 10^6 cfu/g total bacteria at the time of harvest on broccoli.

Psychrotrophic microorganism numbers

Considering the number of psychrotrophic groups of bacteria which are found generally in stored cold foods, 1 and 2% fumaric, acetic, lactic, benzoic acids, 2% sorbic, tartaric and succinic acids had a statistically significant effect compared to control groups on the 1st day ($P<0.05$). on day 4, with the exception of 1% fumaric and malic acids, all washing solutions showed more effective results than the control groups. After 1 week storage, washing with 1 and 2% acetic and lactic acids, 2% tartaric and malic acids caused a significant limitation in the numbers of psychrotrophic microorganisms ($P<0.05$).

Uyttendaele *et al.* (2004) investigated psychrotrophic flora on carrot, lettuce and peppers and found that this was reduced by 1 log unit after 2% lactic acid treatment for 1 min. The reduction obtained from our samples in psychrotrophic microorganisms was 4.03 log cfu/g. This was probably caused by the longer washing time of 5 min.

Enterobacteriaceae spp. numbers

Food hygiene indicator group of microorganisms *Enterobacteriaceae* spp. are often found in broccoli (Padaga *et al.*, 2000). With the exception of 1 and 2% sorbic acid solution, all solutions were found to be effective on the first day. When considering all the washing solutions, the numbers of *Enterobacteriaceae* spp. were significantly lower than the control groups after 7 days of storage ($P<0.05$).

Akbas and Olmez (2007a) determined a 2.2 log cfu/g decrease in the number of *Enterobacteriaceae* spp. in iceberg lettuce samples immersed in disinfectant solution containing 5 ml/l lactic acid at 6 days of storage. Compatible with our results, it was determined that the application with 2% lactic acid solution caused a 2.59 log cfu/g decrease in the number of *Enterobacteriaceae* spp. in broccoli samples after one week storage.

Pseudomonas spp. numbers

Pseudomonas spp. microorganisms are an important group that causes soft rot in vegetables during cold storage (Padaga *et al.*, 2000). *Pseudomonas* groups were significantly controlled by all the washing solutions except 1 and 2% sorbic acid, and 1% malic acid on the first day ($P<0.05$). However, no statistically significant differences were determined between the control group and 1 and 2% benzoic, 1% sorbic, fumaric and succinic acids on the 4th day ($P>0.05$). At the end of the storage period, 1 and 2% acetic, lactic, tartaric, malic acids and 2% benzoic, succinic acids were found to be more effective at inhibiting *Pseudomonas* growth.

Total yeast-mould numbers

On the 1st day all the washing solutions showed statistically significant inhibitory effects against yeast and moulds except 1% succinic and malic acids ($P<0.05$). On the 4th day, all the washing applications were effective except 1% succinic and malic acids. After 7 days' storage, 1% fumaric acid was not found to be effective. Romanazzi *et al.* (2012) determined the inhibitory effects of 1% acetic acid on the yeast and moulds detected on table grape samples during 2-6 days of storage at 22 °C.

Total lactic acid bacteria numbers

No statistically significant differences were found between the 1% acetic and lactic acids, 1 and 2% of benzoic acids treatments and control groups on the first day ($P>0.05$). All the washing solutions, except 1% acetic and sorbic acids, 2% fumaric and succinic acids, were found to be effective against the lactic acid bacteria on day 4. On day 7, all the washing solutions were still effective, except 1% benzoic acid, 1 and 2% sorbic acid, and 1% fumaric acid compared to the control samples ($P<0.05$).

Inhibitory effects of different washing solutions on *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated on broccoli

The antimicrobial effects of the washing solutions on the two pathogens tested on broccoli are given in Table 3. Limits of detection were calculated as 1.090875 and 0.821794 log cfu/g for *S.* Typhimurium and *L. monocytogenes*, respectively. The highest inhibitory effects on *S.* Typhimurium were obtained from lactic, malic and tartaric acids ($P<0.05$), while the lowest inhibition values were obtained from 1% acetic acid, and 1 and 2% ascorbic, fumaric, sorbic, benzoic and succinic acids ($P>0.05$).

Lactic acid, tartaric acid, and malic acids were effective on *S.* Typhimurium. It was reduced by both concentrations (1 and 2%) of these solutions by 4.11, 4.48, 3.68, 4.17, 3.00 and 3.28 log cfu/g, respectively ($P<0.05$). Acetic acid (2%) caused 3.18 log reductions on *L. monocytogenes* whereas sorbic acid, benzoic acid, fumaric acid and ascorbic acids had no significant inhibitory effect on *L. monocytogenes* on the broccoli samples. In our study, lactic acid (1 and 2%), and acetic acid (2%) can be considered as an effective

Table 3. Average populations of *Salmonella* Typhimurium and *Listeria monocytogenes* on broccoli treatment with organic acids (log cfu/g ± standard deviation).[1]

Organic acids	*S.* Typhimurium		*L. monocytogenes*	
Concentration (m/v, v/v)	1%	2%	1%	2%
Initial microorganism load	7.98±0.3		7.86±1.2	
Control (water)	7.67±0.8^{a}		7.70±1.3^{a}	
Benzoic acid	6.43±0.6^{a}	6.40±0.9^{a}	7.60±0.8^{a}	6.65±0.4^{a}
Sorbic acid	6.54±1.1^{a}	6.50±0.5^{a}	6.87±0.7^{a}	6.58±0.3^{a}
Fumaric acid	6.60±0.5^{a}	6.49±0.4^{a}	6.82±0.5^{a}	6.38±0.8^{a}
Malic acid	4.98±0.8^{b}	4.70±0.7^{b}	5.23±0.8^{b}	5.02±0.7^{b}
Tartaric acid	4.30±1.2^{b}	3.81±1.1^{c}	5.18±0.6^{b}	4.96±0.5^{b}
Lactic acid	3.87±0.6^{c}	3.50±0.3^{c}	4.74±1.2^{b}	4.35±0.7^{c}
Acetic acid	7.12±0.9^{a}	4.45±0.8^{b}	6.16±0.8^{ab}	4.68±0.7^{b}
Succinic acid	6.23±0.5^{ab}	6.14±0.9^{ab}	5.07±0.9^{b}	4.93±0.9^{b}

[1] Values (± standard deviation) in the same column with different superscripts (a-c) are significantly different (P<0.05).

sanitiser for controlling *L. monocytogenes* to extend the reduction by more than 3 log cfu/g.

Park *et al.* (2011) investigated the antimicrobial effects of propionic, acetic, lactic, malic, and citric acids on *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* found on whole red organic apples and lettuce. In this research, compared to control, the antibacterial effects on three pathogens on the apple samples treated with 1 and 2% of organic acids for 10 min were as follows: propionic acid (0.92-2.75 log cfu/g reduction), acetic acid (0.52-2.78 log reduction), lactic acid (1.69 to >3.42 log reduction), malic acid (1.48 to >3.42 log reduction) and citric (1.52 to >3.42 log reduction). The antibacterial effects regarding lettuce samples were also found to be as follows: propionic acid (0.93-1.52 log reduction), acetic acid (1.13-1.74 log reduction), lactic acid (1.87-2.54 log reduction), malic acid (2.32-2.98 log reduction), and citric acid (1.85-2.86 log reduction). In our study, the inhibitory effects on *S.* Typhimurium and *L. monocytogenes* compared to the control were as follows: 2% malic acid (2.84-3.28 log reduction), 2% acetic acid (3.18-3.53 log reduction), and 2% lactic acid (3.51-4.48 log reduction), respectively.

Akbas and Olmez (2007b), used the dipping treatment for fresh-cut iceberg lettuce by treating samples with 0.5% citric acid and 0.5% lactic acid for 2 min. They reported 1.5 and 0.8 log reductions of *L. monocytogenes*, respectively. In addition, they concluded that the results were not affected by increasing the solution concentration to 1% and the dipping time to 5 min.

In our study, the treatments with 1 and 2% lactic acid solutions inhibited *L. monocytogenes* by 3.12 and 3.51 log units, whereas the treatments with 1 and 2% acetic acid solutions caused 1.70 and 3.18 log unit reductions, respectively.

The results of the research showed that the antimicrobial effect of organic acids was not dependent on the pH of the washing solutions. In fact, it was evident that there are many factors affecting the antimicrobial activities of the solutions, including chain length, degree of branching, and the ratio of undissociated forms of the organic acids (Park *et al.*, 2011). Several publications have addressed this issue. Sengun and Karapinar (2004) used lemon juice and vinegar (1:1) as a washing mixture for inhibiting *S.* Typhimurium on carrots. They reported that the initial population of their inoculums was between 5.64 and 2.68 log cfu/ml. After treatment with their experimental washing solutions, the initial population of the microorganism was reduced to 4.14 and 3.52 log cfu/g. If the dipping time for this mixture was increased to 15 min., 4.11 and 5.00 log cfu/g reductions were obtained. In another study, the counts of *S.* Typhimurium on rocket leaves treated with vinegar showed maximum reductions of 2.81 and 3.12 log cfu/g at high and low inoculums levels, respectively (Sengun and Karapinar 2005). Also, our study indicated that *S.* Typhimurium on broccoli can be inhibited effectively with 2% acetic acid solution with a 3.46 log cfu/g reduction.

In the present study, organic acids showed were very effective at inactivating *S.* Typhimurium and *L. monocytogenes*. Park *et al.* (2011) have stated that bacterial cells in the damaged tissue are less accessible to sanitisers, and the effectiveness of organic acids also depends on the target pathogen. Plant pathogens are generally settled in protected areas on produce, such as the stomata, broken trichomes,

and wounds or cracks in the cuticle layer. Also, several reports have shown that bacterial biofilms on plant surfaces exhibit an increased resistance to sanitisers and detergents (Carmen-Velazquez *et al.*, 2009). However, pathogens may initially be found on naturally contaminated vegetables, and sufficient time and proper environmental conditions may allow pathogens to grow to populations exceeding 10^7 cfu/g of vegetable, which was similar to the initial flora in this research.

4. Conclusions

Some decontamination procedures should be followed to ensure that broccoli is pathogen-free before consumption, an important factor from the perspective of public safety. It can be concluded that organic acids have a potential application as alternative sanitisers to chemical decontamination and improve the shelf-life of some fresh vegetables. In addition, treatment with combinations of organic acids, along with appropriate packaging and other methods, e.g. ozone treatment and ultraviolet treatments, can produce a greater antimicrobial effect for some vegetables. In this study, no differences in visual quality of the vegetables were observed after the washing treatment with the various sanitising agents. However, their efficacy in bacterial reduction can be developed, and the sensory quality of treated products should also be evaluated in storage conditions. It is also significant that the organic acids investigated in our research are natural substances and can be effectively used for disinfection and decontamination purposes in the production of organic foods which have become increasingly common in recent years.

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Conflict of interest

No conflict of interest declared.

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