

## Full Length Research Paper

# Effect of antimicrobial activity of sodium hypochlorite and organic acids on various foodborne pathogens in Korean ginseng root

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Ginseng is a widely used therapeutic medicinal agent for a number of diseases. It is generally distributed in both processed and unprocessed whole roots and is the most liable to be contaminated by microorganisms as harvest progresses and during manufacturing process. Thus, decontamination process is necessary for combating the microbiological quality of the products. Here, we examined the effect of sodium hypochlorite and organic acids on bacteria growing on ginseng roots. Sodium hypochlorite showed a higher antibacterial activity against the tested bacteria, except *Escherichia coli*. Its strong inhibitory effect appeared to be due to its higher alkaline pH value. However, sodium hypochlorite has less sensitivity to the Gram-negative bacteria strains. We also examined the effect of three different organic acids such as acetic, citric and lactic acids on bacterial growth of ginseng roots. These organic acids showed anti-bacterial activities against Gram-negative strains. Especially, lactic acid inhibited bacteria growth from slice of ginseng than whole ginseng roots. Combination of sodium hypochlorite and organic acid treatment might be improved to fight against both Gram-negative and positive bacteria and would increase storage periods until the second product is made.

**Key words:** Korean ginseng, sodium hypochlorite, organic acids, anti-bacterial, food borne pathogen.

## INTRODUCTION

Ginseng is a medicinal food of the *Araliaceae* family; genus, *Panax*, and a highly valued plant in the East Asia Pacific Region. It has gained popularity in the West during the past decade (Barnes et al., 2004; Greer and Dilts 1995). Ginseng is widely used for prevention of and as therapeutic intervention for many diseases (Attele et al., 1999; Siegel, 1979; Sotaniemi et al., 1995). It is generally distributed as both unprocessed and processed

whole root (raw ginseng), such as white and red ginseng (Sievenpiper et al., 2006; Sun et al., 2009). Of these, ginseng products are most liable to be contaminated by microorganisms during harvest and manufacturing. Thus, decontamination processes are necessary to ensure microbiological quality of the products.

The most frequently used material for the anti-bacterial processing of surfaces is sodium hypochlorite (NaOCl), a

broad-spectrum anti-microbial agent that has demonstrated effectiveness against bacteria, bacteriophages, spores, yeasts and viruses (Krug et al., 2012; Mentz 1982). Sodium hypochlorite is a highly active cytotoxic oxidant recognized to be among the most potent antiseptic and disinfectant agents against bacteria, fungi and viruses (Lu et al., 2007; Siqueira et al., 2007). Organic acids also have anti-microbial activities that vary in potency depending on the compound (el-Shenawy and Marth, 1989; Ismail et al., 2011). However, the observed resistance of pathogens to organic acids indicates the ability of food-borne pathogens to survive under acidic conditions and highlights the need to quantify the antibacterial activity of food-associated organic acids against bacteria.

The goal of this study is to determine the survival of representative pathogens on ginseng root being affected by the types of organic acid and pH. We evaluated sodium hypochlorite and three organic acids (citric acid, acetic acid and lactic acid) against three different Gram-negative and Gram-positive bacteria.

## MATERIALS AND METHODS

### Preparation of Korean ginseng samples

Korean ginseng (GS, 2009 product) was purchased in Jinan, South Korea. GS was washed four times with deionized water prior to storage at 4°C until it was used.

### Bacterial strains

*Escherichia coli* KFRI836, *Listeria monocytogenes* KFRI799, *Salmonella* Typhimurium KFRI191, *Bacillus cereus* KFRI181, *Staphylococcus aureus* KFRI240 and *Pseudomonas aeruginosa* KFRI252 were obtained from the Korea Food Research Institute (Kyonggi, South Korea). Each isolate was grown in nutrient broth (Difco, Franklin Lakes, NJ, USA) for 24 h at 37°C. Each culture was washed three times by centrifugation (1,800 ×g, 10 min, 21°C) with 0.1 M sterile phosphate buffered saline and pH 7.0. Cell pellets were re-suspended in the same buffer. All microorganisms were stored at 4°C immediately prior to use.

### Preparation of fresh ginseng root inoculation

Colonies suspended in sterile water were produced at a cell density of about  $10^7$ – $10^{10}$  colony forming units/mL. Two milliliters of each suspension was cultured on 300 g of ginseng root and incubated for 12 h at 4°C.

### Treatment with Sterilizers

Commercial 10% sodium hypochlorite was purchased at a local market in Siheung City, South Korea. Citric (glacial), acetic (glacial) and lactic acids (DL, syrup) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sterilizers were washed in raw ginseng root of 0.1, 0.5, 1.0 and 2.0% with distilled water. Each treated sample was held at 20°C for 10 min and then microbiological analysis was carried out immediately.

## Microbiological analysis

Twenty-five grams of triplicate samples were acquired aseptically from each treatment, transferred to sterile plastic pouches, and homogenized for 2 min at room temperature with 225 ml sterile 0.1% Bacto Peptone water (Difco) using a Lab-Blender 78860 stomacher (ST-Nom, Interscience, St. Nom, France). Serial dilutions were prepared in buffered peptone water, and 1 ml sample of appropriate dilutions was poured onto total count and selective agar plates. Total plate counts were conducted on Plate Count Agar (Difco) and aerobically incubated at 35°C for 48 h. Lactic acid bacteria were enumerated on MRS Agar (Difco) and anaerobically incubated at 35°C for 24 h. *E. coli* counts were obtained on *coli/Coliform* Count Plate Petrifilm (3M Health Care, St. Paul, MN, USA) and aerobically incubated at 35° for 48 h. Gram-positive catalase-positive cocci counts were obtained on Mannitol Salt Agar (Difco) and aerobically incubated at 35°C for 72 h. *Salmonella* Typhimurium was enumerated on *Salmonella* Typhimurium selective agar (Difco) and aerobically incubated at 35°C for 72 h. The counts for each sample time were calculated as the average value of two determinations per replicate.

## Statistical analysis

Samples were analyzed in triplicate, and results are presented as a mean ± standard deviation. An analysis of variance was performed on all variables measured using the general linear model procedure of the SAS statistical package (SAS, Cary, NC, USA). Duncan's multiple range test was used to determine differences between treatment means. A  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

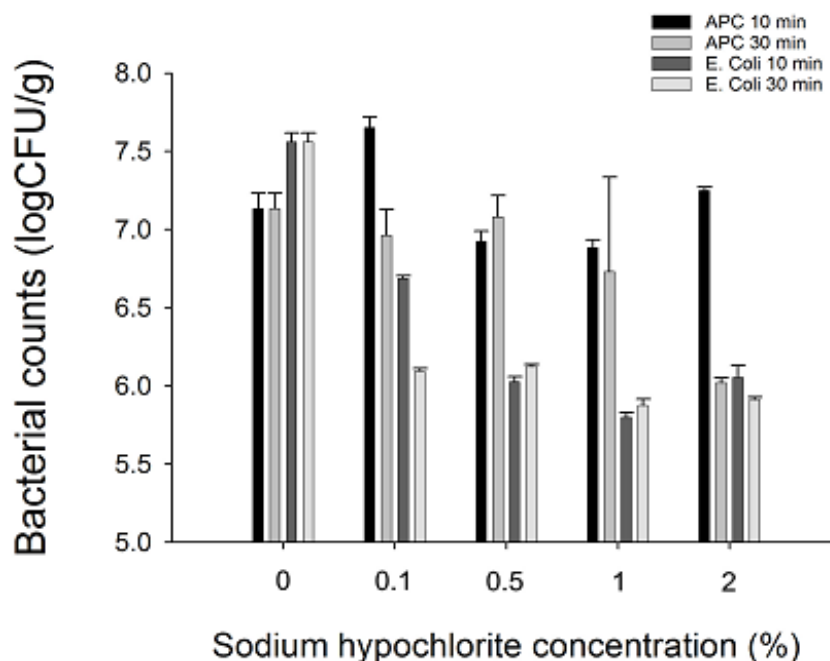
### Anti-bacterial effect of sodium hypochlorite against Gram-negative and positive microorganisms

The viability of a variety of Gram-positive and Gram-negative microorganisms on ginseng root subjected to sodium hypochlorite exposure was examined after 10 min of treatment (Table 1). Both *E. coli* and *S. Typhimurium* were slightly affected by 0.5% sodium hypochlorite. In contrast, *P. aeruginosa*, *B. cereus*, *S. aureus*, and *L. monocytogenes* were significantly affected by 0.05% sodium hypochlorite (Sagripanti et al., 1997; Wu and Kim 2007). All Gram-positive bacteria were sensitive to sodium hypochlorite, whereas Gram-negative bacteria displayed less sensitivity to sodium hypochlorite. To determine the effect of sodium hypochlorite on bacterial growth, we increased the sodium hypochlorite incubation time on ginseng root from 10 to 30 min and found that *E. coli* was the most resistant to sodium hypochlorite. As shown in Figure 1, no significant difference was observed between the 10 and 30 min incubations. It is known that Gram-negative bacteria are more resistant than Gram-positive bacteria to agents such as hydrochloric acid, ethyl alcohol and sodium hypochlorite (Mazzola et al., 2006) and that they are resistant to alkaline conditions (Table 2). To test bacterial viability under acidic conditions, we examined the influence of low pH organic acids in both Gram-negative and Gram-positive bacteria.

**Table 1.** Effect of sodium hypochlorite on gram-negative and gram-positive bacterial strains.

	Sodium hypochlorite concentration (%)					
	0	0.05	0.1	0.5	1	2
Gram negative						
ECO	8.55±0.16	8.30±0.83	7.65±0.07	7.50±0.14	6.94±0.05	6.94±0.02
SAL	7.77±0.01	7.55±0.10	6.71±0.57	6.39±0.01	ND	ND
PSE	7.77±0.01	ND	ND	ND	ND	ND
Gram positive						
BAC	7.55±0.08	4.66±0.26	4.44±0.20	3.09±0.12	ND	ND
STA	8.11±0.09	2.75±0.21	ND	ND	ND	ND
LIS	7.72±0.03	7.54±0.09	ND	ND	ND	ND

Values are mean ± standard deviation of duplicates. ND, not detected. BAC, *Bacillus cereus* KFRI 181; STA, *Staphylococcus aureus* KFRI 240; LIS, *Listeria monocytogenes* KFRI 799; PSE, *Pseudomonas aeruginosa* KFRI 252; ECO, *Escherichia coli* KFRI 836; SAL, *Salmonella Typhimurium* KFRI 191.

**Figure 1.** Effect of sodium hypochlorite on survival of bacteria on ginseng root-mediated medium. Data are representative of three experiments.

As shown in Table 2, citric, acetic and lactic acids were potent at low pH at solution strengths of 0.1 to 2%. Therefore, we used up to 2% of the organic acids to evaluate the viability of both Gram-negative and Gram-positive bacteria.

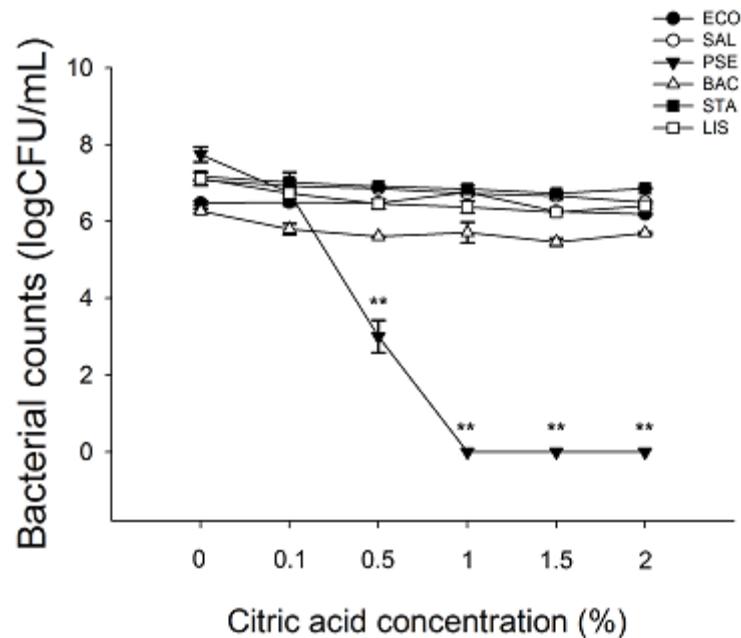
#### **Effect of Citric and Acetic Acid on Anti-bacterial Activities against Gram-negative and Gram-positive Bacteria**

Bacteria typically maintain a cytoplasmic pH close to neutrality (Baronofsky et al., 1984; Krulwich et al., 2011). Survival of bacteria is pH specific (Alegre et al., 2010;

Cotter and Hill, 2003). Based on our results, we hypothesized that bacterial growth is regulated in a pH-specific manner on ginseng. We examined the effect of citric and acetic acids on bacteria. Figure 2 depicts the changes in *P. aeruginosa* counts. This Gram-negative strain was suppressed by 50% in 0.5% citric acid, and growth was virtually absent in 1% citric acid. As shown in Figure 3, acetic acid also strongly inhibited *P. aeruginosa* growth at a 2% concentration and reduced *L. monocytogene* viability because of acidification or excess of cationic charges. The growth of the other bacteria was unaffected. These results suggest that bacterial viability is modulated according to the pH and the type of organic acid present.

**Table 2.** Changes of pH values of sodium hypochlorite and various organic acids at various concentrations.

	Concentration (%)							
	0.0	0.1	0.5	1.0	1.5	2.0	3.0	5.0
Sodium hypochlorite	5.90	8.40	10.00	10.40	10.52	10.63	10.85	11.50
Lactic acid	5.90	2.82	2.39	2.20	2.09	2.04	1.93	1.80
Citric acid	5.90	2.73	2.31	2.13	2.04	1.96	1.86	1.72
Acetic acid	5.90	3.24	2.86	2.70	2.60	2.43	2.43	2.31



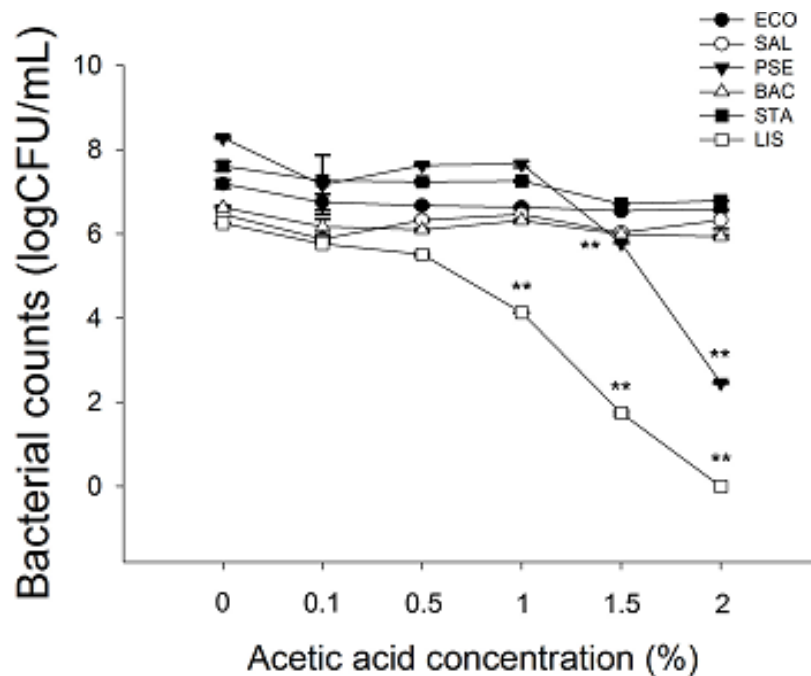
**Figure 2.** Effect of citric acid on bacterial viability on ginseng root-derived medium treated at 20°C for 10 min. Viability was determined via bacterial count assay. All values are mean  $\pm$  standard deviation of five independent experiments (\*P < 0.05; \*\* P < 0.001, respectively).

### Effect of lactic acid on anti-bacterial activity

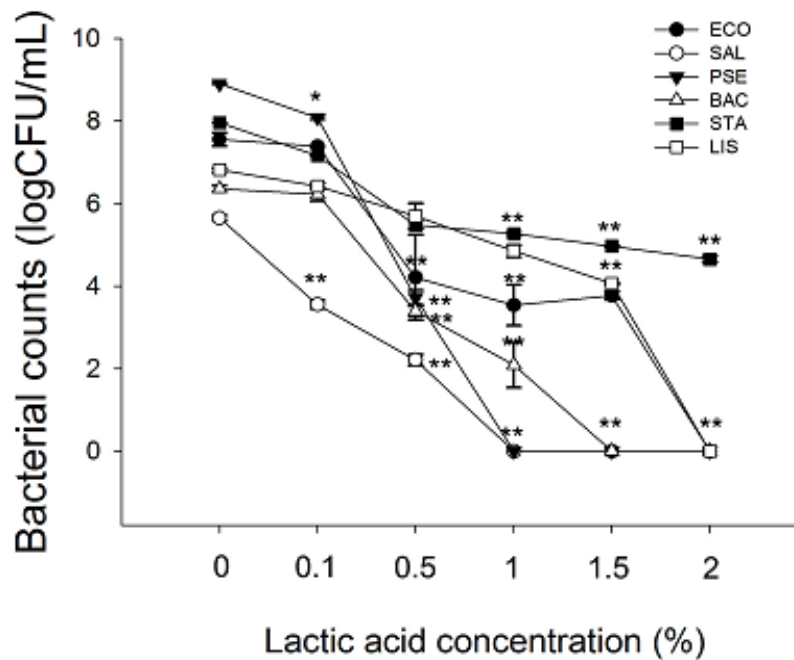
Lactic acid functions as a natural anti-microbial with a generally recognized safe status and is able to inhibit growth of many types of food bacteria including Gram-negative bacteria species (Greer and Dilts, 1995; Rizzello et al., 2013). We examined lactic acid-treated ginseng root to test anti-bacterial activity. The data in Figure 4 showed that 2% lactic acid significantly suppressed growth of all tested bacteria. Most interestingly, Gram-negative bacteria showed a more sensitive response to lactic acid treatment than Gram-positive bacteria. Lactic acid treatment produced an immediate reduction in the pH of ginseng root, which recovered to 2.04 within 10 min (Table 2). Ginseng is sliced from whole roots to increase productivity. However, this process results in a higher possibility for infection from bacteria and decreases storage time. Therefore, we investigated the effect of

lactic acid on whole ginseng root compared to the sliced ginseng. As shown in Figure 5, lactic acid inhibited bacterial colonies at a 2.5% concentration in both groups, indicating that treatment time is important to reduce bacterial colonization of ginseng roots. Interestingly, bacterial growth on sliced ginseng root was strongly suppressed at both 10 and 30 min compared to that of whole ginseng root.

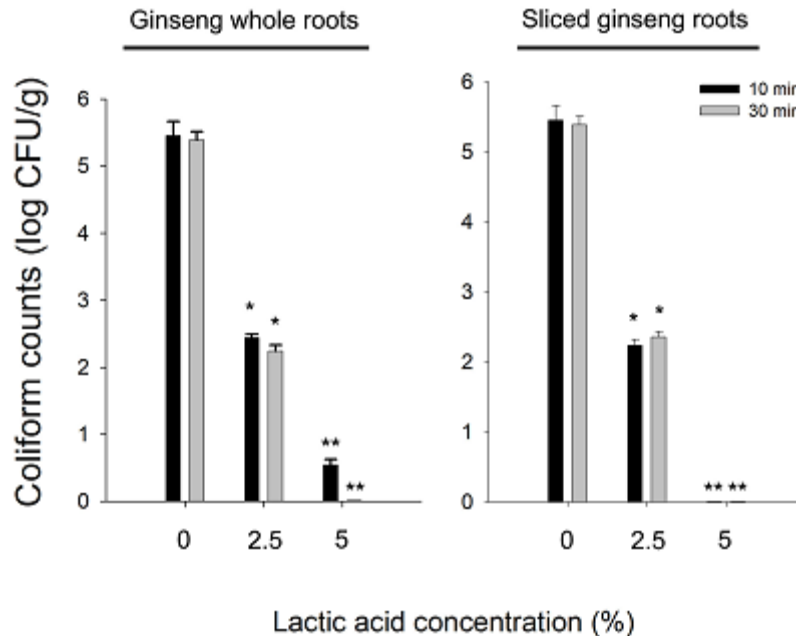
In conclusion, the different inhibitory effects may be attributed to differences in the biological properties of the pH values of the ginseng root. Sodium hypochlorite showed higher antibacterial activity against the tested bacteria (except *E. coli*). Its strong inhibitory effect appeared to be due to a higher pH. However, sodium hypochlorite was less potent against the tested Gram-negative bacteria. In addition, we showed the effect of organic acids (acetic acid, citric acid, and lactic acid) on bacteria growing on ginseng roots. These organic acids



**Figure 3.** Effect of acetic acid on bacterial viability on ginseng root-derived medium treated at 20°C for 10 min. Viability was determined via bacterial counts assay. All values are mean  $\pm$  standard deviation of five independent experiments (\*  $P < 0.05$ ; \*\*  $P < 0.001$ , respectively).



**Figure 4.** Effect of lactic acid on bacteria viability on ginseng root-derived medium treated at 20°C for 10 min. Viability was determined via bacterial counts assay. All values are mean  $\pm$  standard deviation of five independent experiments (\* $P < 0.05$ ; \*\* $P < 0.001$ , respectively).



**Figure 5.** Effect of lactic acid on bacterial growth on ginseng whole and sliced roots. Data are representative of three independent experiments (\* $P < 0.05$ ; \*\* $P < 0.001$ , respectively).

showed anti-bacterial activity against the tested Gram-negative bacteria. Lactic acid strongly inhibited bacterial growth from sliced ginseng, rather than ginseng whole root. The results indicate the potential of the combination of a sodium hypochlorite and an organic acid against both Gram-negative and Gram-positive bacteria.

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