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Effects of NaCl and Organic Acids on the Antimicrobial Activity of Chitosan

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The antibacterial activity of chitosan against *Escherichia coli* and *Staphylococcus aureus* was investigated in the presence of NaCl, acetic acid, lactic acid, and citric acid to assess its potential use as a food preservative. The inhibitory activity of chitosan decreased slightly upon adding NaCl to culture broth containing 100 ppm of chitosan (MW 3,000), while adding acetic acid, lactic acid, or citric acid enhanced the inhibitory activity of chitosan on growing cells. Our results indicate that food components, such as NaCl, acetic acid, lactic acid, and citric acid, can significantly affect the bactericidal activity of chitosan.

Keywords: Antibacterial activity, chitosan, *Escherichia coli*, food components, *Staphylococcus aureus*

Previously, many studies have reported the antimicrobial effects of organic acid. In et al. showed that the antimicrobial activities of some organic acids such as acetic acid, citric acid, and lactic acid, against *Shigella* spp. differed [5]. They showed that the citric acid weakly inhibited the growth of *Shigella flexneri*, but it strongly inhibited the growth of *S. dysenteriae*. On the other hand, acetic acid exhibited the weakest antimicrobial activity among the tested organic acids but produced the highest ratios of injured cells. In their study, lactic acid was the most effective antimicrobial agent against *Shigella* spp.. In another study, acetic acid showed the most effective acid on *Staphylococcus aureus* and *Escherichia coli* DH5a followed by lactic acid and ascorbic acid [3]. Organic acids, such as lactic acid and ascorbic acid, reduce pH and the buffering capacity of the feed while their antibacterial effect inhibits the growth of bacteria, yeasts and moulds. The study have shown that lactic, acetic and ascorbic acids lowered pH of the Brain Heart Broth liquid media. They also have reported that ascorbic acid made the strongest cytotoxic effect on mammalian cells. On the contrary, its antibacterial effect was weaker than the other acids. On the other hand, acetic and lactic acids did not lead to any colony formation on solid media for *S. aureus* and *E. coli*. These organic acids had a less cytotoxic effect on murine fibroblast NIH 3T3 cell line as compared to ascorbic acid.

Chitosan (poly β-(1-4)*N*-acetyl-d-glucosamine), a deacetylated form of chitin, is a natural antimicrobial compound that is widely used in pharmaceuticals, agriculture, and the food industry [1, 9, 14, 15, 18]. The antimicrobial activity of chitosan against food-borne pathogens has been actively studied over the past 15 years [12, 16, 17, 19], though the presence of several food components could affect chitosan activity. In this study, Chitosan inhibition of *E. coli* and *S. aureus* growth was measured in the presence of NaCl and organic acids (Fig. 1A and B). NaCl without chitosan had

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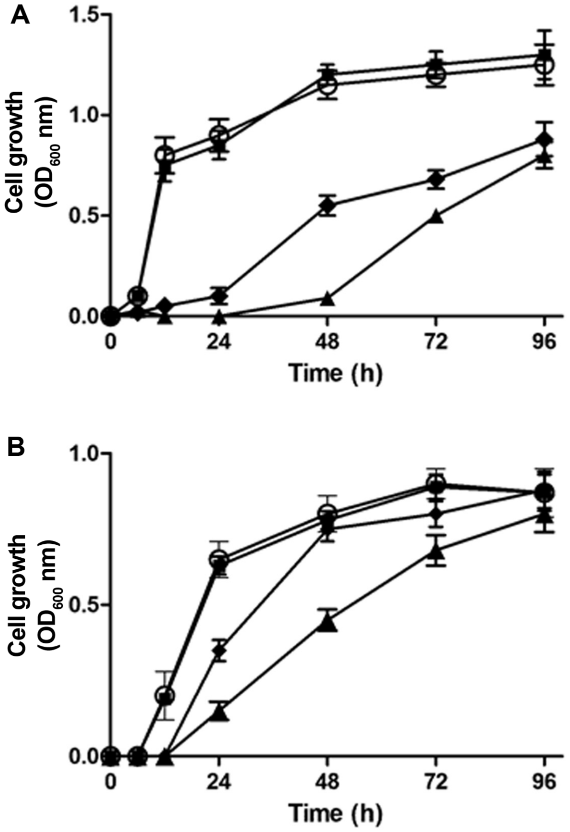

Fig. 1. Effect of NaCl on chitosan activity against pathogens. 2% NaCl was treated together with chitosan (MW 3,000; 100 ppm) to examine the activity of chitosan against growing *E. coli* (a) and *S. aureus* (b). Cells were cultured in TS broth at 37°C in the presence of (▲) chitosan only; (◆) chitosan with NaCl; (n) NaCl only. Open symbols indicate the control without chitosan.

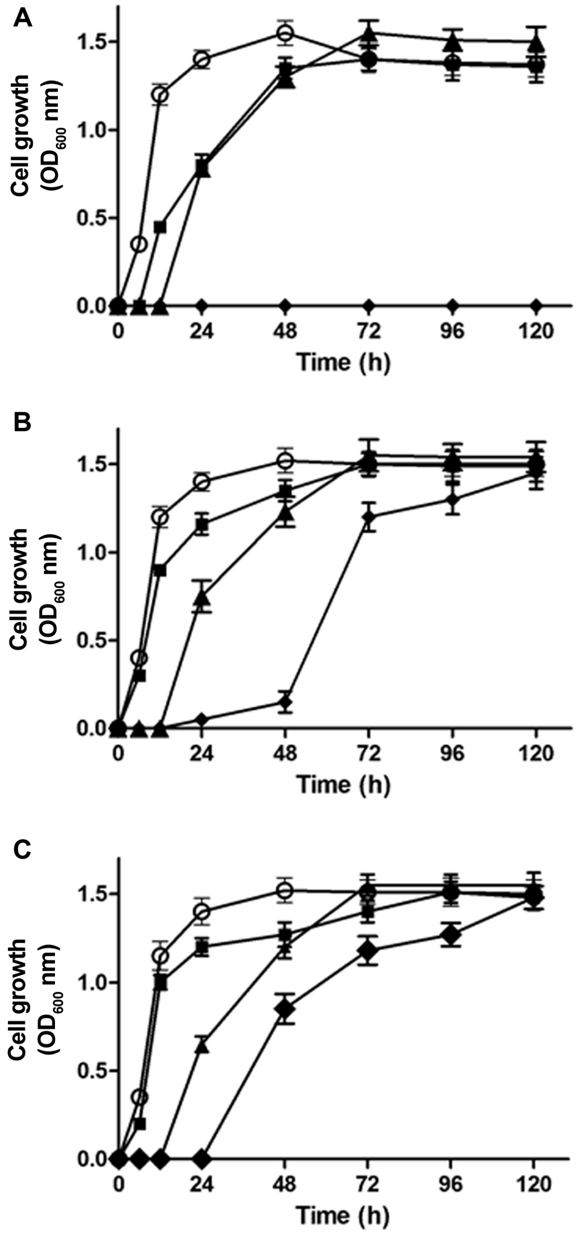

Fig. 2. Effect of organic acid on chitosan activity against pathogen. 0.1% acetic acid (a), 0.1% lactic acid (b), and 0.1% citric acid (c) was treated together with chitosan (MW 3,000; 100 ppm) to examine the activity of chitosan against growing *E. coli*. Cells were cultured in TS broth at 37°C in the presence of (▲) chitosan only; (◆) chitosan with acetic acid (a), lactic acid (b), or citric acid (c); (n) acetic acid (a), lactic acid (b), or citric acid (c). Open symbols indicate the control without chitosan.

no significant effect on both bacterial growth. When NaCl was added to Tryptic Soy (TS) broth (Sigma Aldrich, USA) containing chitosan, the inhibitory activity of chitosan on *E. coli* and *S. aureus* growth was decreased at 48 h incubation as previously reported [2, 10, 11]. Adding NaCl to the medium could decrease chitosan activity since Na^+ ions can compete with chitosan to interact with negative charges on the cell surface. Adding organic acids drastically increased the antibacterial activity of chitosan (Fig. 2). Among the three acids, acetic acid had the largest effect. Acetic acid with 100 ppm of chitosan completely inhibited *E. coli* growth for 120 h (Fig. 2A). Adding lactic acid and citric acid with chitosan inhibited *E. coli* growth for 48 h and 24 h, respectively, as compared to chitosan only (Fig. 2B and 2C). Thus, adding organic acids enhanced chitosan activity. The enhanced activity could be due to organic acids penetrating into the cell or changing the permeability of the cell membrane. Therefore, organic acids had a synergistic effect with chitosan.

The effects of NaCl and organic acids on chitosan could be explained by two possible mechanisms. First, these

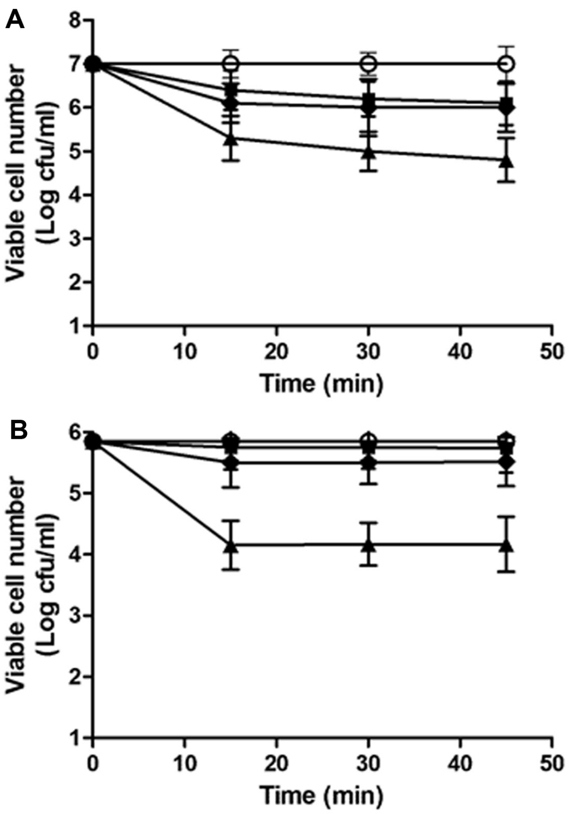

Fig. 3. Viability of pathogens treated with chitosan and NaCl. Survival of non-growing *E. coli* (a) and *S. aureus* (b) with 100 ppm chitosan after adding 2% NaCl: (▲) chitosan only, (◆) chitosan after adding NaCl; or (n) NaCl alone. Open symbols indicate the control without chitosan.

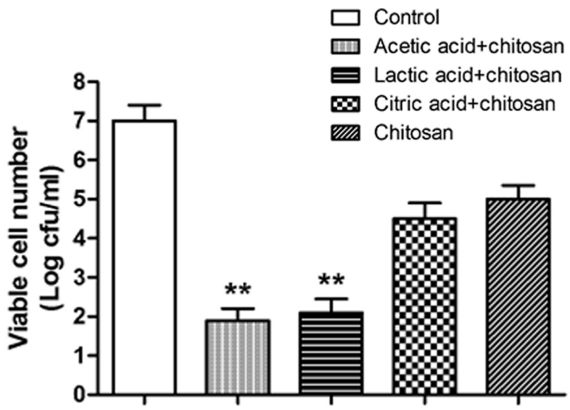

Fig. 4. Survival of non-growing *E. coli* with 100 ppm chitosan after adding 0.1% organic acids. Results were expressed as the mean ± standard deviation (SD), and statistical analyses were performed using a two-tailed unpaired Student *t* test. **, $p < 0.01$ as compared to chitosan only.

substances could act on the cell surface and alter the sensitivity of the cells to chitosan. Second, the food components could directly interact with chitosan. To determine the actual mechanism we tested the effects of NaCl and organic acids on chitosan activity in non-growing cells. The viability of non-growing cells after chitosan treatment in the presence of various food components is shown in Figs. 3 and 4. The viable cell count of *E. coli* (Fig. 3A) and *S. aureus* (Fig. 3B) was nearly ten-fold higher when NaCl was added to the cell suspension prior to treating with chitosan. Because NaCl alone inhibits cells, the actual effect is likely much higher. This result clearly demonstrates that NaCl represses chitosan activity by acting directly on the cells. Fig. 4 shows the effects of organic acids on chitosan activity. Treating with chitosan alone resulted in 5 log cfu/ml viable cells, while chitosan together with acetic acid or lactic acid decreased approximately 3 log cfu/ml of the *E. coli* cells. Treating with citric acid resulted in 4.6 log cfu/ml viable cells. Therefore, organic acids are the most compatible food components with chitosan as a bactericide against the food-borne pathogens used in this study. On the other hand, NaCl inhibits chitosan-mediated antibacterial activities. These results indicate that food components can significantly affect the bactericidal activity of chitosan. For example, adding NaCl decreased chitosan activity, while the addition of organic acids significantly enhanced chitosan activity. Chitosan itself has been developed as a new physiologically bioactive material, which has been touted as a treatment for various disorders, including asthma, atopic dermatitis, arteriosclerosis, hypertension, macular degeneration, arthritis, cancer, diabetes, and osteoporosis, among others [6-8]. Administration of NaCl with chitosan decreases the systolic blood pressure in spontaneously hypertensive rats (SHRs), suggesting that the consumption of NaCl plus chitosan-based functional dietary salt should be encouraged as part of an overall lifestyle medicine approach for the prevention of hypertension [13]. The microcapsule prepared with chitosan, tripolyphosphate, and NaCl showed a solid particle structure with a thicker wall and good wall-core interaction, while the microcapsule without NaCl in the formulation had a thin and transparent layer on the surface [4]. These data suggest that NaCl affects chitosan structure formation or activity. Our study also showed that NaCl decreases the anti-microbial activity of chitosan.

In conclusion, this study elucidated the effects of NaCl and organic acids on the inhibitory activity of chitosan. In addition, this study provides indirect evidence suggesting that these food components affect chitosan activity by interacting with the cells, rather than chitosan. These results are of practical interest for determining the optimal amount of chitosan to use as a food preservative in products containing these components.

Acknowledgments

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국문초록

키토산의 항균활성에 미치는 염화나트륨 및 유기산의 영향

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식품용 방부제로써의 가능성을 평가하기 위하여 염화나트륨, 아세트산, 젖산, 구연산이 포함된 배지를 사용하여 대장균 및 황색포도상구균에 대한 키토산의 항균성을 조사하였다. 키토산의 억제활성은 100 ppm의 키토산(분자량 3,000)이 포함된 배양배지에 염화나트륨을 첨가시킴으로써 미세하게 감소하였다. 반면, 아세트산, 젖산, 구연산 등은 세균의 성장에 대한 키토산의 억제활성을 증가시켰다. 이러한 결과는 염화나트륨, 아세트산, 젖산, 구연산과 같은 식품 첨가 성분이 키토산의 항균활성에 심각한 영향을 미칠 수 있다는 것을 보여준다.