Available online at www.tox.or.kr

MCT

The Intrinsic Antimicrobial Activity of Bamboo Salt against *Salmonella enteritidis*

Ji Hea Moon[1], **Hyun Ah Shin**[1], **Young Ah Rha**[2,*] **& Ae Son Om**[1,*]

[1]Department of Food and Nutrition, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea
[2]Department of Food Services and Management, Eulji University, 212 Yangji-dong, Sujeong-gu, Seongnam-si, Gyeonggi-do 461-713, Korea
*These authors contributed equally to this work
Correspondence and requests for materials should be addressed to Y. A. Rha (jyana@eulji.ac.kr) and A. S. Om (aesonom@hanyang.ac.kr)

Accepted 17 November 2009

Abstract

The object of this study was to examine the antimicrobial properties of commercial bamboo salts and other commercial table salts (refined salts, roasted salts, processed salts, imported salts, and sea salts) for *Salmonella enteritidis*. Values of the oxidation-reduction potential (ORP), pH were measured and the minimum inhibitory concentration (MIC) of 20 salts against *S. enteritidis* were measured. The results showed that notably, the ORP and pH values of bamboo salt groups stayed in reduced (−105.7-−52.5 mV) and alkaline states (pH 8.4-9.3), while other salt groups did in oxidized (182.6-248.0 mV) and acidic or neutral states (pH 5.49-7.77). The MIC values of 5.0% against *S. enteritidis* were obtained for bamboo salts. On the other hand, MIC values of 5.0% were not observed for other salts. These data indicated that bamboo salt has its strong bactericidal activity and should need further in-depth study.

Keywords: Antimicrobials, Bamboo salt, *S. enteritidis*, ORP, MIC

Salmonellosis is one of the leading food-borne illness in Asia, the European Union (EU) and the USA[1]. In Korea, Salmonella caused 10.1% of food-borne illnesses recorded during 2006-2008 (6.4% in 2006, 12.3 % in 2007, and 13.04% 2008)[2]. In France, salmonellosis is reported to be responsible for 87% of cases of food-borne illness[3]. In the US, 40,000 cases of salmonellosis are reported[4].

Recently, food poisoning often occurs due to increased intake of non-heat treating food like fresh-cut vegetables and fruits. Accordingly, people are overly concerned about food safety, with such concerns leading to great demand for natural antimicrobial ingredients scientifically proven by many other studies.

Salt has been used as a natural antimicrobial agent for many years. However, there has been little study into the effects of salt as an antimicrobial agent. Especially, bamboo salt has been used as a folk medicine for the purposes of prevention and the treatment of various diseases in Korea for hundreds of years[5]. Some studies proved the anticancer[6,7], antioxidant[7,8], and anti-inflammatory[9] effects of bamboo salt but there have been few studies of its antimicrobial properties[10-13]. Certain studies showed the growth of food poisoning bacteria to be significantly inhibited when bamboo salt was used in salted cabbage[14-16]. Although salt is generally thought to be harmful for human health, bamboo salt is distinct in that it actually treats human diseases[5]. Bamboo salt is specially processed according to a traditional recipe, using normal salt, bamboo, pine tree firewood, pine resin, and yellow soil, combined at very high temperature. Compared with sea salt, the contents of iron, silicon, potassium and phosphate in the purple bamboo salt were higher, whereas the sulfate content was lower[8]. In this regards, these studies provide an possibility of antimicrobial activity relevant to its high mineral contents.

Nowadays, hurdle technology is developed for effective management of food safety. It is included to combine with different antimicrobial factors and can reduce the growth of bacteria chemically or physically treated. In this view, it is important that the growth rate of bacteria is depended on intrinsic factors (pH, oxidation-reduction potential (ORP), concentration of NaCl and water activity) and extrinsic factors (temperature and composition of atmosphere)[17]. Therefore, it should be important to determine its intrinsic or extrinsic factors to effect microbiological environment for proving the antimicrobial property of specific material.

In this study, it was to evaluate the bactericidal ef-

Table 1. Oxidation-reduction potential (ORP) values of different salt groups.

Groups	No. of salt products	Salt concentration	
		1.0 mg/mL	5.0 mg/mL
Bamboo salt	4	−52.50 ± 15.19[a]	−105.75 ± 17.97[a]
Refined salt	4	223.50 ± 25.17[bc]	243.50 ± 17.02[b]
Roasted salt	3	192.67 ± 18.01[b]	182.67 ± 75.48[b]
Processed salt	3	205.00 ± 6.25[bc]	233.00 ± 12.53[b]
Imported salt	3	212.00 ± 23.58[bc]	240.33 ± 22.48[b]
Sea salt	2	231.00 ± 25.46[c]	248.00 ± 22.63[b]

[a,b,c]Means with the different letters are significantly different among different treatment at the same time ($P < 0.001$).
Each experiment was conducted in triplicate.

Table 2. pH values of different salt groups.

Groups	No. of salt products	Salt concentration	
		1.0 mg/mL	5.0 mg/mL
Bamboo salt	4	8.40 ± 0.52[d]	9.30 ± 0.27[a]
Refined salt	4	5.82 ± 0.12[ef]	6.59 ± 0.19[bc]
Roasted salt	3	7.24 ± 1.71[de]	7.77 ± 1.86[b]
Processed salt	3	5.87 ± 0.61[ef]	6.08 ± 1.00[c]
Imported salt	3	5.86 ± 0.11[ef]	6.06 ± 0.25[c]
Sea salt	2	5.49 ± 0.19[f]	5.90 ± 0.04[c]

[a,b,c]Means with the different letters are significantly different among different treatment at the same time ($P < 0.05$). [d,e,f] Means with the different letters are significantly different among different treatment at the same time ($P < 0.001$).
Each experiment was conducted in triplicate.

fect of bamboo salt and other salts by proving their intrinsic antimicrobial factors (ORP and pH) and the inhibiting effect on the growth of Salmonella.

Intrinsic Factors of Salts

The ORP and pH values for the different salt groups were analyzed in proc glm (Duncan's test for group comparisons). ORP provides an instantaneous measure between a standard redox potential and the concentration ratio of oxidizers and reducers. Simply stated, redox potential can be used to determine whether a system is in an oxidized or a reduced state[18]. The ORP values for the different salt solutions (Table 1) ranged from −105 mV to 248 mV. The values of bamboo salt groups were below −50 mV. However, the ORP of other groups were above 180 mV. Therefore, at salt concentrations of 1.0 and 5.0 mg/mL, the ORP of the bamboo salt group was significantly lower than that of the other salt groups ($P < 0.001$).

The corresponding pH variations ranged from 5.82 to 9.30 (Table 2). The pH of processed salt, imported salt or sea salt group was below 7.0. However, the pH of the bamboo salt group was up to 8.0. At concentrations of 1.0 and 5.0 mg/mL, the pH of the bamboo salt group was significantly higher than other salt groups (in case of 5.0 mg/mL, $P < 0.005$; 1.0 mg/mL, $P < 0.01$).

Table 3. Minimum inhibitory concentration (MIC) of the natural antimicrobials against *Salmonella enteritidis*.

Groups	No. of salt products	MIC (mg/mL)
Bamboo salt	4	50.0
Refined salt	4	50.0[a]
Roasted salt	3	No effect
Processed salt	3	No effect
Imported salt	3	No effect
Sea salt	2	No effect

[a]Means that only one refined salt among four refined salts was the significantly MIC against *S. entertidis* at 37°C ($P < 0.0001$).
Each experiment was conducted in triplicate.

Minimal Inhibitory Concentration (MIC) Evaluation of Salts

The MICs of commercially natural antimicrobials (19 salts; 4 bamboo salts, 4 refined salts, 2 sea salts, 3 roasted salts, 3 imported salts and 3 processed salts) and NaCl against *S. enteritidis* are presented in Table 3. Among the salts, bamboo salts and NaCl were found to be the most effective, with the lowest MIC values of 50 mg/mL against *S. entertidis*. Except for bamboo salts and NaCl, the antimicrobial order of the compounds tested was: refined salts > imported salts > roasted salts > processed salts > sea salts. The inhibition profiles (growth curves) of all the antimicrobial

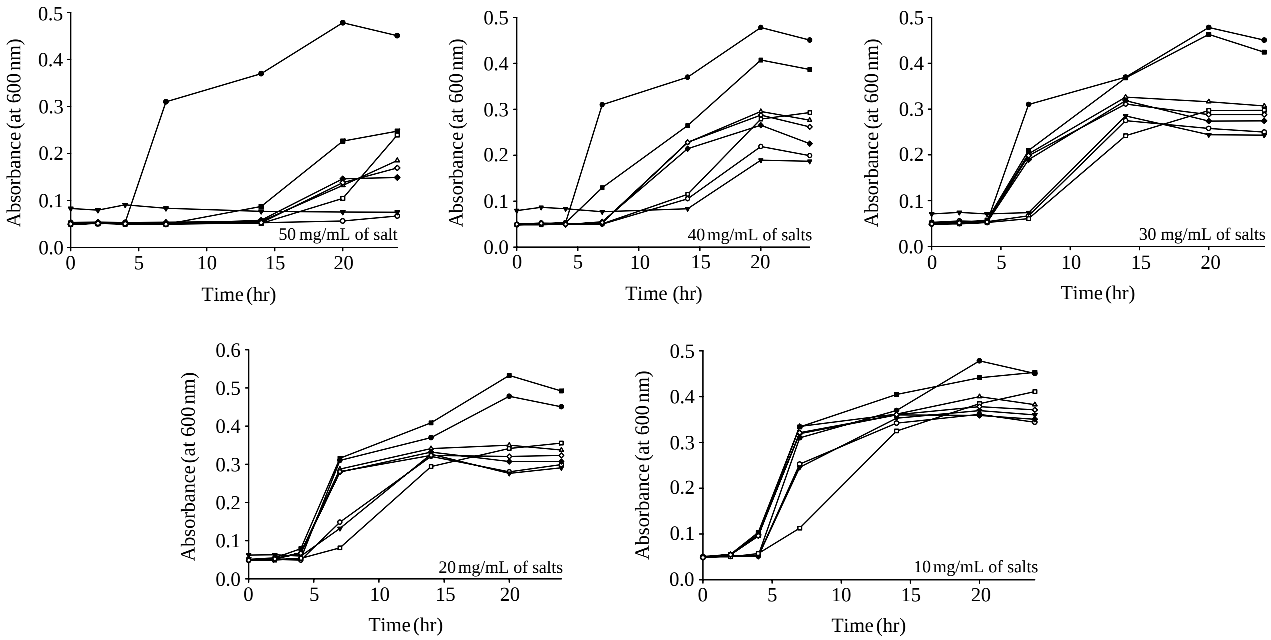

Figure 1. The growth of *Salmonella enteritidis* in the absence (●) or presence of NaCl (○), bamboo salts (▼), roasted salts (△), processed salts (■), refined salts (□), sea salts (◆) or imported salts (◇) in Muller-Hinton broth at 37°C. Each experiment was conducted in triplicate.

compounds are presented in Figure 1.

Discussion

Salt is one of the major factors influencing bacterial growth and survival, and is used as a natural preservative[19]. The main effect is caused by decreasing the water activity level (Aw) of bacteria, with a decline in Aw giving hyperosmotic shock to bacteria. As a result, the number of bacteria is reduced by decreasing the cytoplasmic volume of the cell[20]. Especially, gram-negative bacteria are affected more by osmotic stress than are gram positives, due to differences in transfer system. It can be stated that Salmonella is more affected by stress than other gram positive bacteria. However, there have been limited studies of relationships between the survival of pathogens and salt. Therefore, the objective of this study was to evaluate the antimicrobial properties of bamboo salts in comparison with common salts by measuring their intrinsic factors (ORP and pH values) and then confirming MIC concentrations against *S. enteritidis*.

ORP represents the relationship between a standard redox potential and the concentration of oxidizers and reducers[21]. Therefore, decline of ORP value should not be oxidized but reduced. However, Liao (2007)[22] suggested that ORP could damage cell membranes of gram negative bacteria. It means that high ORP values (reduced state) in solution could be main factor of cellular redox signaling related to the control of cellular life cycle. This redox reaction should effect cellular environments and then be associated with apoptosis. Also, it was reported that negative values of ORP were not suitable to the existence of pathogenic bacteria (*E. coli* O157 : H7)[23].

Table 1 shows that ORP values in bamboo salts group were only reduced. Schreyer *et al.* (2008) explained that electroreduction has also been put forward as a means of inhibiting and/or reducing oxidation phenomena in food by modifying its redox potential. Therefore, these declines in the ORP of bamboo salt groups can be explained by electroreduction generating electrons. Also, the reason for this electroreduction could be related to higher mineral contents (Ca, P, K, Mg, Fe, Mn, Si and Zn) than in other salts[24]. Gallaghter and Cutress (1977)[25] have found that Mn and Zn inhibit the growth of bacteria. Other study has reported that metallic cation has antimicrobial activities[26]. In this study, the antimicrobial activity of bamboo salt should be caused from high contents on metal ions and reduced values of ORP.

In case of pH, bamboo salts group was alkaline state and other salts were acidic. This agrees with reports that the pH of bamboo salts was higher than that of sea salts or refined salt[24,27]. In general, Salmonella and other gram negative bacteria prefer slightly acidic or neutral state[17]. Therefore, these alkaline property of bamboo salts group could be another intrinsic factor for antimicrobial activity.

Bamboo salts and refined salts were composed of NaCl ($>$90%), but the other salts were $<$90% NaCl. As NaCl was one of the environmental factors for the microbial growth, Salmonella could be affected by changes in the NaCl concentration of their surroundings[28]. At the sub-MIC levels, the lag phase of growth was extended and both the growth rate and final cell density were reduced with increasing concentrations of salts. Complete inhibition of *S. enteritidis* was only achieved with 5.0% NaCl and with bamboo salts. These results agreed with the reports that Salmonella declines at bamboo salt levels $\geq$5.0%[14].

Our observations of the bacteriostatic effect of the salts alone against *S. enteritidis* were consistent with several earlier studies. Previously, Son *et al.* (1991) reported that bamboo salts had better growth inhibition of lactic acid bacteria and oral bacteria than other salts at the same concentrations. They explained that these inhibitions of bamboo salt were caused by its content, like minerals or metal ions. Also, reduced and alkaline state of bamboo salt could co-affect to inactivate on bacteria by damaging the membranes of pathogen.

In the present study, the antimicrobial activity of bamboo salts (the concentration above 50 mg/mL) was observed against *Salmonella* and this may reflect the optimal inhibitory concentration.

In conclusion, the antimicrobial activities of bamboo salts as found in our study are clearly higher than that of other salt groups. The results found in this study suggest that bamboo salt can be used as a natural antimicrobial. Also, the antimicrobial properties of bamboo salt mean that it can be used as a functional food ingredient. However, as illustrated in the introduction, there have been very few studies done about properties of salts. Consequently, much more research must be done on the antimicrobial properties of bamboo salt.

Materials & Methods

Preparation of Salts

Nineteen kinds of salts were purchased from the local market in Seoul, Korea: four bamboo salts, four refined salts, three roasted salts, three processed salts, three imported salts and two sea salts. Each of the salts was dissolved in distilled water to reach appropriate concentrations, filtered through 0.45 μm filter, and kept at 4°C.

ORP Analysis and pH Analysis

An HI 98201 Pocket-sized ORP meter was calibrated with 200/275 mV test solution (Hanna Instruments, Singapore). Data were collected at 30s intervals for 90 minutes and 3 replicates were performed. Additional pH data was collected simultaneously. The experiment was repeated using a 50 mM Tris-HCl buffer, pH 7.8, using aforementioned methodology.

Bacterial Strains and Inoculum Preparation

The strain of *Salmonella enteritidis* (KCCM 12021) used in experimental studies was obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). Stock cultures were preserved by freeze drying. The organism was pre-cultured in Rappaport-Vassiliadis broth (RV broth, Oxoid, England) and then Xylose Lysine Deoxycholate medium (XLD medium, Oxoid, England). The inoculum was transferred to Tryptic Soy broth (TS broth, Difco, USA) at two consecutive 24 h intervals immediately before their use in the experiment. Fresh *S. enteritidis* cultures were prepared by inoculating 0.1 mL into 10 mL TS broth and incubation for 18 to 24 h at 35°C. The cultures were centrifuged at 5000 × g for 15 min at 4°C. The supernatant was discarded and the bacterial cell pellet was reconstituted in 9 mL of 0.1% sterile peptone water. Cell suspension was adjusted to an absorbance at 600 nm using a spectrophotometer (Beckman, USA) so that the *S. enteritidis* concentration was between 2 and 3 log CFU/mL.

Minimal Inhibitory Concentration (MIC) Evaluation by Spectrophotometric Analysis

The minimum inhibitory concentrations (MICs) were measured as described by Olasupo *et al.* (2003)[29]: determined using an ELX 800UV universal microplate reader (Bio-TEK, Winooski, Vermont, USA), which kinetically measures, the development of turbidity (i.e. growth) by vertical photometry. The test organisms were grown for 24 h in Muller-Hinton broth (Difco Laboratories, Detroit, MI) and incubated with continuous agitation at 37°C. The optical density of the cultures was 600 nm (O.D.).

Antimicrobial assays were performed in Falcon 96-well plates containing MH-broth with salt compounds whose final concentrations were: 0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg/mL with different salts. NaCl (Sigma Inc., USA) having a purity greater than 99.9% was used. Final volume of Cell cultures was 200 μL per well. The plates were incubated at 37°C for 24 h. The MIC of each salt was taken as the lowest concentration that completely inhibited bacterial growth after 2 h. In all cases, the assays were performed in triplicate.

Statistical Analysis

For the determination of antioxidant and antimicrobial activity, the significance of differences among salt groups was analyzed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA) by means of Analysis of Variance (ANOVA) and Duncan's multiple test. *P*-value less than 5% were considered significant.

References

1. Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, R. R. & Froning, G. Dynamic predictive model for growth of *S. enteritidis* in egg yolk. *J Food Sci* **72**:M254-M262 (2007).
2. Korea Food & Drug Administration. Department of Preventaion and management of Food-borne disease. http://www.foodnara.go.kr/portal/site/kfdaportal (2009).
3. Oscar, T. P. A quantitative risk assessment model for *Salmonella* and whole chickens. *Int J Food Microbiol* **93**:231-247 (2004).
4. Centers for Disease Control and Prevention. Department of Health and Human Services. http://www.cdc.gov/nczved/dfbmd/disease_listing/salmonellosis_gi.html (2009).
5. Shin, H. Y. *et al*. Biological activity of bamboo salt. *Food Industry & Nutrition* **9**:36-45 (2004).
6. Hwang, K. M., Oh, S. H. & Park, K. Y. Increased antimutagenic and in vitro anticancer effects by adding green tea extract and bamboo salt during Doenjang fermentation. *J Korean Soc Food Sci Nutr* **36**:1-7 (2007).
7. Jung, K. O., Lee, K. Y., Rhee, S. K. & Park, K. Y. Effects of various kinds of salt on the tumor formation, NK cell activity and lipid peroxidation in Sarcoma-180 cell transplanted mice. *Cancer Prev Resear* **7**:134-142 (2002).
8. Om, A. S. & Jeong, J. H. Bamboo salts have antioxidant activity and inhibit ROS formation in human astrocyte U373MG cells. *Cancer Prev Resear* **12**:225-230 (2007).
9. Son, W. S., Yoo, Y. C. & Kim, C. Y. The effect of NaCl and bamboo salt on the growth of various oral bacteria. *J Kor Aca Dental Health* 255-268 (1991).
10. Kim, J. B., Paik, D. I., Moon, H. S., Jin, B. H. & Park, D. Y. A study on the effects of cetylpyridinim chloride and bamboo salt containing mouth rinse on the number of Streptococcus mutants and plaque index. *J Kor Aca Dental Health* **17**:176-186 (1993).
11. Min, B. S. *et al*. The reducing effects on dental plaque formation and gingiritis of toothpastes containing bamboo salt and several herb medicines. *J Kor Dental Assessment* **33**:65-71 (1995).
12. Yang, J. S. *et al*. Pharmacological evaluation of bamboo salt. *J Appl Pharmacol* **7**:178-184 (1999).
13. Hun, K., Kim, Y. H. & Jin, D. Q. Protective effect of an aged garlic-bamboo salt mixture on the rat with the alcohol-salicylate induced gastropathy. *Yakhak Hoeji* **45**:258-268 (2001).
14. Hahn, Y. S. Effect of salt type and concentration on the growth of lactic acid bacteria isolated from *Kimchi*. *Kor J Food Sci Technol* **35**:743-747 (2003).
15. Park, S. J., Park, K. Y. & Jun, H. K. Effects of commercial salts on the growth of *Kimchi*-related microorganisms. *J Kor Soc Food Sci Nutr* **30**:806-813 (2001).
16. Hahn, Y. S., Kwon, M. K., Hyun, Y. H., Song, J. E. & Oh, J. Y. Effects of kinds and concentration of salts on the growth of yeasts isolated from *Kimchi*. *J East Asian Soc Dietary Life* **11**:393-399 (2001).
17. Kwon, H. J., Kim, J. W. & You, H. C. Food Safety First. Kyomunsa. Seoul. 20-31 (2003).
18. Schreyer, A., Britten, M., Chapuzet, J. M., Lessard, J. & Bazinet, L. Electrochemical modification of the redox potential of different milk products and its evolution during storage. *Innov Food Sci Emerg Tech* **9**:255-264 (2008).
19. Wijinker, J. J., Koop, G. & Lipman, L. J. A. Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. *Food Microbiol* **23**:657-662 (2006).
20. Mellefont, L. A., McMeeking, T. A. & Ross, T. The effect of abrupt osmotic shifts on the lag phase of food-borne bacteria. *Int J Food Microbiol* **83**:281-293 (2003).
21. Morris, G. J. The effect of redox potential. The microbiological safety and quality of food, Vol. 1. Aspen Publishers, Inc. Maryland. 235-250 (2000).
22. Liao, L. B., Chen, W. M. & Xiao, X. M. The generation and inactivation mechanism of oxidation-reduction potential of electrolyzed oxidizing water. *J Food Eng* **78**:1326-1332 (2007).
23. Brookers, P. & Darley-Usmar, V. M. Hypothesis: the mitochondrial NO signaling pathway, and the transduction of nitrosative to oxidative cell signals: and alternative function for cytochrome C oxidase. *Free Radical Biol & Med* **32**:370-374 (2002).
24. Kim, S. H. *et al*. Characterization and anti-gastric ulcer activity of bamboo salt. *J Fd Hyg Safety* **13**:252-257 (1998).
25. Gallaghter, I. H. C. & Cutress, T. W. The effect of trace elements on the growth and fermentation by oral streptococci and actinomyces. *Archs Oral Biol* **22**:555-562 (1977).
26. Eisenberg, A. D. Interactions of Sanguinarine and Zinc on oral streptococci and actinomyces species. *Caries Res* **25**:185-190 (1991).
27. Shin, H. Y. *et al*. Inhibition of mast cell-dependent immediate-type hypersensitivity reactions by purple bamboo salt. *J Ethnopharmacol* **91**:153-157 (2004).
28. Prescott, L. M., Harley, J. P. & Klein, D. A. Microbial Growth. Microbiology. Mc Graw Hill Higher Education. USA. 109-132 (2005).
29. Olasupo, N. A., Fitzgerald, D. J., Gasson, M. J. & Narbad, A. Activity of natural antimicrobial compounds against Escherichia coli and Salmonella enterica serovar Typhimuium. *Let Appl Microbiol* **37**:448-451.