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Inhibition of *Vibrio vulnificus* in Oysters using Organic Acids

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유기산을 이용한 생굴의 *Vibrio vulnificus* 억제

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Abstract

The number of *Vivrio vulnificus* strain 29307 was assessed for oysters treated with acetic, lactic, citric, and alginic acids during storage at 15℃. When oysters were dipped with 0.5% acetic, 0.5% lactic, or 0.5% citric acids for 3 min, *V. vulnificus* was not detected after 4 days of storage. *V. vulnificus* in the treatment of 3% alginic acid (AL) containing 2% acetic acid (AA) was not detected after 2 days of storage, while it was isolated in the controls for 4 days of storage. Based on these results, the combination of AL and AA was more effective for preventing the growth of *V. vulnificus* in oysters than the treatments of the acid alone.

Key words : *Vibrio vulnificus*, oyster, organic acids

INTRODUCTION

Vibrio vulnificus is a human pathogen capable of causing death in humans, which have been found in estuarine waters, sediments, and shellfish[3,4,12,18]. This pathogen in shellfish grew rapidly during storage in the summer and temperature abuse, and it was found to survive for at least 6 days in shucked oysters under refrigeration[3,4,7,17]. The organism is a gram-negative bactreia that typically results from infection of a wound, frequently of oyster and fish origin[3].

The use of food preservatives such as acetic, lactic, and citric acids as antimicrobial surface sanitizer of fresh fish and meat has been reported[1,5,6,7,10,11,13,16]. The acids are known as a Generally Recognized as Safe (GRAS) substances in the United States, which has received approval for use as a preservative of fish and meat[6]. Organic acids could be used to controlling gram-negative bacteria on refrigerated fish and meat, which could be applied to suppress the growth of this organism in fish and oyster[8,13]. Studies have shown that sanitizing fish and meat surfaces with organic acids cause sublethal injury or death to aerobic spoilage bacteria[1,8,9,11,19]. In previous study, Marshall and Kim[11] noted that catfish fillets treated with acetic and lactic acids prolonged

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microbiological shelf-life to 12 days at 4℃. Anderson and Marshall[2] noted that when a mixture of 2% acetic, 1% lactic, 0.25% citric and 0.1% ascorbic acids was applied to beef core samples of muscle inoculated with bacteria, resulting in reductions in counts of about one log unit for aerobic bacteria.

Little published information is available to prevent on the growth of *V. vulnificus* in oysters, which is the effects of acetic, lactic, citric, and alginic acids. The objective of this study was to assess the antimicrobial effect on the growth of *V. vulnificus* in oysters treated with those acids during storage at 15℃.

MATERIALS AND METHODS

1. *Vibrio vulnificus* and growth

V. vulnificus strain 29307 were obtained from National Institute of Health in Kwang Ju. The cultures were maintained in trypticase soy agar (Difco Laboratories, Detroit, MI), and propagated in Brain Heart Infusion (BHI-salt, BBL) broth with 3.0% NaCl at 37℃ for 24 hr. The suspension was diluted with 1.0%(w/v) peptone water. The concentration of cells was estimated against a standard curve by absorbance readings at 550 nm using spectrophotometer (Model, Beckman BU^R 650 spectrophotomer). The enumeration of *V. vulnificus* was confirmed by duplicate plate counts on thiosulfate-citrate-bilesalts-sucrose (TCBS, Difco) agar[7,15].

2. Preparation of oysters

Fresh oysters(*Crassostrea gigas*) were obtained from commercial source, transported to Seo Kang College on ice, and used within 2 hr of collection. For each treatment, 1 kg of oysters were washed under running cold tap water to remove surface dirt and debris. Each two hundred gram of oysters was then placed in an stainless steel grill for 5 min and submerged in 1 L tap water with 0.5% of acetic, lactic, and citric acids, respectively.

For preparation of the sodium alginate solution (3%, w/v), 3 g of sodium alginic acid(Sigma Chemical Co, USA) were dissolved in 100ml of tap water heated up to 80℃, left to stand for 30 min to allow air bubbles to escape, and then autoclaved for 10 min at 121℃. Thirty gram of the alginates were put into the plastic container in the size of 7×10cm. The container were placed into the refrigerator adjusted to 15℃ for completion of the process of gel formation ranging to 2mm in diameter for 15 min. Ninty milliliter of tap water were added to the container coated with the alginate gel and then two hundred gram of oysters were put into the container. Duplicate experimental trials consisted of the following treaments :

(1) Oysters were dipped in 0.5%(v/v) acetic acid(AA; Sae Won Chemical Co., Korea) for 3 min, 0.5%(v/v) lactic acid(LA; Moo Jang Ya Chemical Co., Japan) for 3 min, and 0.5% (w/v) citric acid(CA; Dong Yang Gloval Chemical Co., Korea) dip for 3 min. (2) Oysters were dipped in 0.5% AA for 1~5 min. (3) Oysters were placed into the containers coated with thirty gram of 3.0% alginate gel with or without 2% AA containing 90 ml of tap water. Two hundred gram of each oysters placed into the plastic container in the size of 7×10cm were covered with the Clean Wrap (Clean Wrap Co, Korea), and then stored at 15℃. The number of *V. vulnificus* was determined on samples from each treatment at 2 day intervals.

3. Microbiological analyses

Duplicate 25g samples from each oysters treated with the acidulants were inoculated with approximately 10^5~10^7 cells/ml of *V. vulnificus*. An additional 25g samples treated with tap water only were analyzed as the controls. The samples were blended for 2 min, serially diluted in 1.0% (w/v) sterile peptone water, and then streaked onto of the TCBS agar using 0.1ml of each dilution[7,15]. Inoculated plates of TCBS agar were incubated at 37℃ for 24 hr, respectively. On TCBS agar, typical colonies of *V. vulnificus* were green, and 1~2 mm in diameter. The number of *V. vulnificus* was expressed as Log_{10}CFU/g.

4. Stastistical analyses

The average number of *V. vulnificus* was calculated for each replicate determination. All data were analyzed using ANOVA and means were seperated by LSD at a probability level of 0.05[11].

RESULTS AND DISCUSSION

During this study, *V. vulnificus* strain 29307 was affected by the type and exposure time of acidulant used. The effect of different acidulant on the numbers of *V. vulnificus* containing 1.0×10^6/ml viable cells is shown in Table 1. On day 2, treatment of 0.5% AA for 3 min reduced *V. vulnificus* by 0.6 log units compared to the initial controls. Samples treated with 0.5% AA for 3 min were a significant lower levels($P<0.05$) of *V. vulnificus* compared to those of CA and LA. On day 4, samples treated with the acids proved adequate to reduce *V. vulnificus* to a non-detectable level. However, *V. vulnificus* in control oysters was lowered by 2.2 log units compared to the initial controls after 4 days at 15℃. Results showed that the highest level of antimicrobial activity was observed in AA treatments. Similarly, studies have shown that AA on an equimolar basis generally has greater antimicrobial activity than other organic acids[5,13,16].

On day 0, *V. vulnificus* containing 2.0×10^4/ml viable cells was reduced about 0.75~0.80 log units in oyster treated with 0.5% AA for 3~5 min (Table 2). On day 2, *V. vulnificus* was reduced 1.0 log units in oysters treated with 0.5% AA for 5 min. After 4 days at 15℃, *V. vulnificus* in oysters treated with 0.5% AA for 1~5 min was not detected, whereas in the control was 3.4 log units. Results indicated that the reduction rate of *V. vulnificus* depended on the exposure time of acid. Marshall and Kim[11] and Kim et al.[8] noted that aerobic spoilage bacteria in refrigerated(4℃) catfish fillets subjected to AA and/or LA were prone to sublethal injury and/or death, with inhibitory effects increasing with increasing exposure time. Furthermore, the duration of dipping was more influencial in inhibiting microbial growth

Table 1. Changes in counts of *V. vulnificus* strain 29307 in oysters treated with different acidulants during storage at 15℃

Treatment \ Storage day	Log CFU/g		
	0 day	2 day	4 day
Control	5.24[b]	7.78[a]	3.0
0.5% AA[1] /3 min	5.37[b]	4.61[b]	ND
0.5% LA[2] /3 min	5.62[a]	5.02[b]	ND
0.5% CA[3] /3 min	5.63[a]	5.05[b]	ND

AA[1] = acetic acid, LA[2] = lactic acid, CA[3] = citric acid.
[a-b] Counts within the same column with different superscripts are significantly different($P<0.05$).
[c] ND = not detected.

Table 2. Changes in counts of *V. vulnificus* strain 29307 in oysters treated with different exposure time of acetic acid during storage at 15℃

Treatment \ Storage day	Log CFU/g		
	0 day	2 day	4 day
Control	3.30[a]	7.22[a]	3.40
0.5% AA[1] /1 min	2.75[b]	4.66[b]	ND
0.5% LA[2] /3 min	2.45[b]	3.29[c]	ND
0.5% CA[3] /5 min	2.50[b]	2.40[c]	ND

AA[1] = acetic acid, LA[2] = lactic acid, CA[3] = citric acid.
[a-c] Counts within the same column with different superscripts are significantly different($P<0.05$).
[d] ND = not detected.

Table 3. Changes in counts of *V. vulnificus* strain 29307 in oysters treated with alginic acid, either alone or combined with acetic acid during storage at 15℃

Treatment \ Storage day	Log CFU/g		
	0 day	2 day	4 day
Control	6.06[a]	6.31[a]	5.12
3 AL[1]	4.84[b]	5.76[b]	ND
3% AL /2% AA[2]	4.52[b]	ND	ND

AL[1] = alginic acid, AA[2] = acetic acid.
[a-b] Counts within the same column with different superscripts are significantly different($P<0.05$).
[c] ND = not detected.

than was amount of acid.

V. vulnificus in oysters treated with 3.0% AL and 2.0% AA were not detected after storage of 2 days at 15℃(Table 3). On day 0, samples treated with either AL or AL combined with AA were lower levels ($P < 0.05$) of *V. vulnificus* by approximately about 1.0 log unit compared to the controls. On day 2, *V. vulnificus* in samples treated with AL only was detectable, whereas after 4 days, in treatments of AL only was not detectable. Results showed that antimicrobial activity of the acidulants was enhanced when it was combined with AA and AL. The combined treatments could increase antimicrobial activity by an additional 2 days compared to the treatments of AL. Hence, inhibitory results in our study were likely due to pH and to the specific action of AA and AL[11]. Similarly, Marshall and Kim[11] reported that antimicrobial activity of organic acids can be increased when combined with other preservatives. They observed that refrigerated(4℃) catfish fillets treated with 2% AA and 2% LA prolonged microbiological shelf-life to 4 additional days compared to those of 4% LA.

CONCLUSION

Results of this investigate demonstrated that treatments of AA were more effective than those of either LA or CA in suppressing *V. vulnificus* on oysters during storage at 15℃. Treatments of AL combined with AA were more effective than those of AL in suppressing *V. vulnificus*. Results show that the acids should be considered as a potential antimicrobial preservative for preventing on the growth of *V. vulnificus* in oysters.

요 약

유기산 처리법을 이용하여 15℃ 저장 동안 생굴의 *V. vulnificus* strain 29307의 세균수 변화에 대한 영향을 조사하였다. 각각 3분 동안 0.5% 초산, 0.5% 유산, 또는 0.5% 구연산으로 침지한 생굴은 저장 4일 후부터 *V. vulnificus*가 검출되지 않았다. 2% 초산 함유한 3% 알지닉산 처리구는 저장 2일 후부터 *V. vulnificus*가 검출되지 않았으며, 수도물로 처리한 대조구는 저장 4일 동안 *V. vulnificus*가 분리되었다. 본 연구의 결과를 토대로 알지닉산과 초산의 조합은 각 유기산 처리구보다 *V. vulnificus*에 대한 항균력을 증진하였다.

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REFERENCES

1. Acuff, G. R., Vanderzant, C., Savell, J. W., Jones, D. K., Griffin, D. B. and Ehlers, J. G. : Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. *Meat Sci.*, **19**, 217 (1987).
2. Anderson, M. E. and Marshall, R. T. : Reducing microbial populations on beef tissues: Concentration and temperature of an acid mixture. *J. Food Sci.*, **55**, 903 (1990).
3. Blake, P. A., Merson, M. H., Weaver, R. E., Hollis, D. G. and Heublein, P. C. : Disease caused by a marine *Vibrio*. *N. Engl. J. Med.* **300**, 1 (1979).
4. Boutin, B. K., Reyes, A. L., Peeler, J. T. and Twedt, R. M. : A Research Note, Effect of temperature and suspending vehicle on 10 survival of *Vivrio parahaemolyticus* and *Vibrio vulnificus*. *J. Food Prot.*, **48**, 875 (1985).
5. Conner, D. E., Scott, V. N. and Bernard, D. T. : Growth, inhibition, and survival of *Listeria monocytogenes* as affected by acidic conditions. *J. Food Prot.*, **53**, 652 (1990).
6. Federal Register, Vol. 47, No. 123, Rules and Regulations, Part 184, USA (1982).
7. Kaysner, C. A., M. L. Tamplin, M. M. Wekell, R. F. Stott, and K. G. Colburn : Survival of *Vivrio vulnificus* in shellstock and shucked oysters (*Crassostrea gigas* and *Crassostrea virginica*) and effect of isolation medium on recovery. *Appl. Environ. Microbial.* **55** : 3072~3079 (1989).
8. Kim, C. R., Hearnsberger, J. O. and Eun, J. B. : Gram-negative bacteria in refrigerated catfish fillets treated with lactic culture and lactic acid. *J. Food Prot.*, **58**, 639 (1995).
9. Kim, C. R., Lee, J. I., Kim, K. H., Kang, C. K., Rhie, S. C., Moon, S. J. and Lee, Y. K. : Microbiological and sensory evaluations of refrigerated pork loins treated with citric acid. *Kor. Vet. Publ. Hlth.*, **20**, 329 (1996).
10. Marel, G. M. Van Der, Logtestijn, J. G. Van and Mossel, D. A. A. : Bacteriological quality of broiler carcasses as affected by inplant lactic acid decontamination. *Intl. J. Food Microbe.* **6**, 31 (1988).
11. Marshall, D. L. and Kim, C. R. : Microbiological

and sensory analyses of refrigerated catfish fillets treated with acetic and lactic acids. *J. Food Qual.*, **19**, 317 (1996).
12. Oliver, J. D., Warner, R. A. and Cleland D. R. : Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. *Appl. and Environ. Microbiol.*, **45**, 985 (1983).
13. Ray, B. and Sandine, W. E. : Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives. *In Food Biopreservatives of Microbial Origin.* Ray, B., and M. Daeschel. *CRC Press, Inc.*, Boca Raton, Florida (1991).
14. SAS : SAS User's Guide : Stastics. SAS Institute Inc., Cary, N. C. (1993).
15. Sloan, E. M., Hagen, C. J., Lancette, G. A., Peeler, J. T. and Sofos, J. N. : Comparison of five selective enrichment broths and two selective agars recovery of *Vibrio vulnificus* from oysters. *J. Food Prot.*, **55**, 556 (1992).
16. Sorrell, K. M., Enigel, D. C. and Hatfield, J. R. : Effect of pH, acidulant, time, and temperature on the growth and survival *Listeria monocytogenes. J. Food Prot.* **52**, 571 (1989).
17. Sun, Y. and Oliver, J. D. : A Rsearch Note, Value of Cellobiose-Polymyxin B-Colistin agar for isolation of *Vibrio vulnificus* from oysters. *J. Food Prot.* **58**, 439 (1995).
18. Wong, H. C., Shieh, W. R. and Lee, Y. S. : Toxigenic characterization of *Vibrios* isolated from foods available in Taiwan. *J. Food Prot.*, **56**, 980 (1993).
19. Woolthuis, C. H. J. and Smulders, F. J. M. : Microbial decontamination of calf carcasses by lactic acid sprays. *J. Food Prot.*, **48**, 832 (1985).

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