KOREAN J. FOOD & NUTR.
Vol. 11, No. 6, 662~666(1998)

Influence of Acetic Acid, Lauric Acid and Monolaurine Treatments on Survival of *Vibrio cholerae* in Refrigerated Flatfish

Jae-Il Lee, Byung-Chul Chung and Chang-Ryoul Kim*

College of Veterinary Medicine, Chonnam National University, Kwangju 500-757, Korea

**Dept. of Food Science and Nutrition, Seo Kang College, Kwangju 500-742, Korea*

냉장광어에서 *Vibrio cholerae*에 대한 아세트산, Lauric acid 및 Monolaurine의 영향

이재일 · 정병철 · 김창렬*

전남대학교 수의과대학, *서강정보대학 식품영양과

Abstract

The effects of 0.5~1.0% acetic acid, 0.5% lauric acid, or 0.5% monolaurine against *Vibrio cholerae* non 01 in flatfish strips stored at 15℃ were assessed. Control strips were dipped in distilled water only for 3 min. All treatments significantly ($P<0.05$) reduced the levels of *V. cholerae* at initial day. The counts of *V. cholerae* in flatfish treated with either lauric acid or monolaurine were a significantly different ($P<0.05$) from those of acetic acid treatment after 2 days of storage. The counts of *V. cholerae* in treatments of 0.5% lauric acid after dipping in 1.0% acetic acid for 3 min were lower than those of treatments with 0.5% lauric acid for 3 min after dipping in 0.5% acetic acid for 3 min. Treatments with 0.5% monolaurine for 3 mun were not effective in lowering ($P<0.05$) the counts of *V. cholerae* after 3 days compared to the control.

Key words : acetic acid, lauric acid, monolaurine, *Vibrio cholerae*, flatfish.

INTRODUCTION

Vibrio cholerae survives in seawater and freshwater, which has been found in fish and shellfish[1,2]. It is transmitted by contaminated food and water, and through infected persons. Since this organisms is a pathogen and can grow at refrigeration temperatures, fish and shellfish from refrigerated storage cannot be a safeguard against its growth[1~5].

Decontamination of *V. cholerae* is an important critical control point which can increase the safety and value of live fish and its products. Many researchers[6~12] have reported that organic acids had an antimicrobial activity for preventing the growth of undesirable microorganisms in fish and meat. Kim *et al.*[6] observed that red seabream treated with a combination of 3% alginic acid and 0.5% acetic acid caused sublethal injury or death to *Vibrio vulnificus*. Kim *et al.*[8] noted that catfish fillets treated with a combination of lactic acid and lactic culture extended microbiological shelf-life for an additional 3 days of storage at 4 and 10℃. However, chemical preservatives in foods have been seriously considered for removal from market. Fatty acids as antimicrobial preseratives have been studied to public health, because it is naturally occurring nontoxic substances in foods[13].

Corresponding author : Chang R. Kim

Few studies have been reported on the antimicrobial effect of fatty acids against pathogenic microorganisms. Ababouch *et al.*[14] noted that linolenic acid was the most inhibitory unsaturated fatty acid and lauric acid was the most inhibitory saturated fatty acid against *Clostridium sprogenes* and *Bacillus cereus*. Oh and Marshall[19] noted that antilisterial effects of glycerol monolaurate (monolaurine) have been related to temperature and pH.

The present investigation was to study the antimicrobial effect of acetic acid, lauric acid, and monolaurine on the growth of *V. cholerae* on flatfish during storage at 15℃.

MATERIALS AND METHODS

1. Bacterial maintenance and culture

Vibrio cholerae non 01 was maintained in trypticase soy agar (Difco Laboratories, Detroit, MI, USA), and cultured in Brain Heart Infusion (BHI-salt, BBL) broth with 3.0% NaCl at 37℃ for 12hr. One ml of the suspension was withdrawn and added to a test tube containing 9ml of sterile 1.0% (w/v) peptone water for sequential ten-fold dilutions, which was diluted to 10^6. The cell concentration was estimated using a standard curve by readings absorbance at 600nm using spectrophotometer (Model, Beckman BU^R 650 spectrophotomer). The enumeration of *V. cholerae* was confirmed by duplicate plate counts on thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Difco Laboratories, Detroit, MI, USA).

2. Preparation of flatfish

Fresh flatfish strips were purchased from commercial source less than 1hr postmortem, transported to laboratory on ice, and used within 2hr. Three treatment solutions were prepared by mixing 100ml distilled water with appropriate amounts of acetic acid (Sae Won Chemical Co., Korea), lauric acid (Sigma Chemical Co., USA), and monolaurine (Sigma Chemical Co., USA), respectively. Each five hundred gram of strips (average weight 10g per strip) was dipped in 500ml sanitizer for 1 or 3min. Strips were allocated to the following experimental trials : (1) 0.25~1.0% (v/v) acetic acid dipping for 1 or 3min, (2) 0~0.5% (w/v) lauric acid dipping for 3min after 0.5~1.0% acetic acid dip for 3 min, (3) 0~0.5% (w/v) monolaurine (containing 3% ethanol) dipping for 3min after 0.5~1.0% acetic acid dipping for 1 or 3min.

3. Inoculation of *V. cholerae*

Fifity gram samples from each flatfish were in duplicate inoculated with 1ml of *V. cholerae* which was diluted to approximately 4 to 5 $\log_{10}$ CFU/ml. Strips were placed in Whirl-Pak sample bags (Fisher Scientific Chemical Co., Norcross, GA, USA) and stored at 15℃.

4. Microbiological analysis

Individual strips were aseptically transferred to Whirl-Pak sample bags and weighed. Each samples was blended for 2min using a Stomacher LabBlender 400 (Tekmar, Cincinnati, OH, USA), serially diluted in 1.0% (w/v) sterile peptone water, and then streaked onto the TCBS agar using 0.1ml of each dilution. Inoculated plates of TCBS agar were inoculated at 37℃ for 24hr. The number of *V. cholerae* was expressed as a mean Log_{10} CFU/g for the duplicate treatments.

5. Stastistical analysis

All data were analyzed using ANOVA and means were seperated by LSD at a probability level of 0.05[15].

RESULTS AND DISCUSSION

During storage at 15℃, the bactericidal efficiency of acetic acid, lauric acid, and monolaurine was tested by inoculating 10^4~10^5 *Vibrio cholerae* non 01/ml on flatfish.

The antimicrobial effect of acetic acid on the growth of *V. cholerae* containing 7.3×10^5

CFU /ml viable cells in flatfish strips is shown in Table 1. Treatment with 0.5~1.0% acetic acid for 3 min significantly ($P<0.05$) reduced the counts of *V. cholerae* during storage at 15 ℃. After 3 days of storage, the counts of *V. cholerae* in the strips treated with 0.5~1.0% acetic acid for 3 min were lower by 0.68~1.13 log units than those of control. However, the counts of *V. cholerae* treated with 0.5~1.0% acetic acid for 1min were not significantly different from those of controls after 3 days of storage. Kim *et al.*[6] and Marshall and Kim[11] reported that most of aerobic spoilage microorganisms were sensitive to increasing levels of acetic acid and exposure times. Similarly, in a previous work in our laboratories[6], we observed that the counts of *Vibrio vulnificus* and aerobic spoilage bacteria on red seabream treated with alginic acid in combination with increasing levels of acetic acid were significantly influenced by their combined interaction. Ingham[7] reported that catfish fillets treated with 1.77 and 2.55% lactic acid dips significantly reduced aerobic bacterial counts on fish compared to untreated samples.

Treatments with 0.5% lauric acid after dipping in 0.5~1.0% acetic acid for 3 min were effective in lowering ($P<0.05$) the level of *V. cholerae* during storage at 15℃ (Table 2). All treatments with lauric acid after dipping in acetic acid significantly ($P<0.05$) inhibited the growth of *V. cholerae* for 3 days. Treatments with lauric acid after dipping in 1.0% acetic acid had a lower counts of *V. cholerae* than those after dipping 0.5% acetic acid. The results indicated that the antimicrobial activity of lauric acid depended on the concentration of acetic acid. Similarly, Marshall and Kim[11] reported that antimicrobial activity of organic acids on refrigerated catfish fillets was dependent on the type and concentration of acidulant used.

Treatments with 0.5% monolaurine dissolved in 3% ethanol solution for 3 min after dipping in 1.0% acetic acid for 3 min had a lower levels of *V. cholerae* than those of control after 1 day of storage at 15℃ (Table 3). However,

Table 1. Changes in counts of *V. cholerae* in flatfish strips dipped in 0.5~1.0% acetic acid solution for 1 or 3 min during storage at 15℃

Treatments	Log CFU /g		
	Storage time (days)		
	0	1	3
Control	5.86[a]	5.36[a]	2.46[a]
0.5% AA[1] /1min	4.68[b]	4.62[b]	2.46[a]
1.0% AA /1min	4.79[b]	4.74[b]	2.04[ab]
0.5% AA /3min	4.86[b]	4.90[b]	1.30[b]
1.0% AA /3min	4.67[b]	4.96[b]	1.78[b]

AA[1] = acetic acid. [a-b] Counts within the same column with different superscripts are significantly different ($P<0.05$).

Table 2. Changes in counts of *V. cholerae* in flatfish strips treated with 0.5% lauric acid dipping in combination with 0.5~1.0% acetic acid dipping for 3 min during storage at 15℃

Treatments	Log CFU /g		
	Storage time (days)		
	0	1	3
Control	5.86[a]	5.36[a]	2.46[a]
0.5% LA[1] /3min, 0.5% AA[2] /3min	3.36[b]	3.18[b]	0.70[b]
0.5% LA /3min, 1.0% AA /3min	2.15[c]	2.30[c]	ND

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

Table 3. Changes in counts of *V. cholerae* in flatfish strips treated with 0.5% monolaurine dipping in combination with 0.5~1.0% acetic acid dipping for 3 min during storage at 15℃

Treatments	Log CFU /g		
	Storage time (days)		
	0	1	3
Control	5.86[a]	5.36[a]	2.46[a]
0.5% ML[1] /3min, 0.5% AA[2] /3min	4.36[b]	4.62[b]	5.32[b]
0.5% ML /3min, 1.0% AA /3min	4.20[b]	4.83[b]	3.76[c]

ML[1]=monolaurine, AA[2]=acetic acid. [a-b]Counts within the same column with different superscripts are significantly different ($P<0.05$).

Table 4. Changes in counts of *V. cholerae* in flatfish strips treated with 0.5% lauric acid, 0.5% monolaurine, and 0.5% acetic acid after dipping for 3 min during storage at 15℃

Treatments	Log CFU /g		
	Storage time (days)		
	0	1	2
Control	4.84[a]	3.90[a]	3.30[a]
0.5% AA[1] /3min	3.40[b]	3.63[a]	3.15[a]
0.5% LA[2] /3min	2.23[c]	2.41[b]	1.78[b]
0.5% ML[3] /3min	2.16[c]	2.38[b]	1.60[b]

AA[1]=acetic acid, LA[2]=lauric acid, ML[3]=monolaurine. [a-b] Counts within the same column with different superscripts are significantly different ($P<0.05$).

the antimicrobial effect of monolaurine on the counts of *V. cholerae* did not last after 3 days. Oh and Marshall[13] reported that the effect of monolaurine on the growth and survival of *Listeria monocytogenes* in tryptic soy broth with yeast extract increased as temperature increased at constant pH. At constant temperature the bactericidal and bacteriostatic effects of monolaurine increased as the pH of the medium decreased.

The antimicrobial effect of lauric acid, monolaurine, and acetic acid on the number of *V. cholerae* containing 6.9×10^4 CFU /ml viable cells in flatfish strips is shown in Table 4. All the treatments initially were lower in the counts of *V. cholerae* than control. Treatment of either 0.5% lauric acid or 0.5% monolaurine for 3 min significantly reduced the counts of *V. cholerae* during storage at 15℃. The results indicated that the antimicrobial activity of lauric acid and monolaurine was greatly higher than that of acetic acid.

Chaibi *et al.*[16] observed that monolaurine, monomyristin, monolinolein, and monolinolenin inhibited cell growth from *Bacillus cereus* T, *Clostridium botulinum* 62A, and *C. sporogenes* PA 3679 spores and vegetative cells. Kim *et al.*[6] found that *V. vulnificus* in treatment of 3% alginic acid containing 1.5~2.0% acetic acid after 0.5% acetic acid dip for 5 min was not detected after 2 days of storage, while treatment dipped in 0.5% acetic acid only for 5 min reduced *V. vulnificus* by 1.0 log units compared to the initial controls. They reported that antimicrobial activity of organic acids on refrigerated catfish was dependent on the type and concentration of acidulant used. Furthermore, Ababouch *et al.*[14] found that lauric acid dissolved in 95% ethanol solution to a final concentration of 100mg /ml was the most inhibitory of the saturated fatty acids on spores of *C. botulinum* 62A, *C. sporogenes* PA 3679, and *Bacillus cereus* F4165 /75. They reported that fatty acids may inhibit spore germination by blocking one or more post-triggering steps, i. e., connecting reactions, in the germination process. They reported that fatty acids inhibited spore germination, possibly by binding to spore envelopes and thus inhibiting the binding of germinants to germination sites.

Marshall and Kim[11] and Anderson and Marshall[9] reported that a combination of acetic acid with lactic acid was effective for increasing shelf-life of fish and meat. Oh and Marshall reported[13] that the most rapid inactivation of the pathogen by monolaurine occurred with the highest temperature and lowest pH combination. They found that bacteriostatic effects of sublethal monolaurine concentrations increased as temperature and pH decreased. Furthermore, Oh and Marshall[17] observed that synergistic effects against growth of *Listeria monocytogenes* when monolaurine was combined with organic acids such as acetic, benzois, and lactic acids.

요 약

아세트산, lauric acid, monolaurine 그리고 이의 조합에 의한 침지법을 이용하여 15℃ 저장 동안 광어의 *V. cholerae*의 세균수 변화에 대한 영향을 조사하였다. 대조구는 증류수에 3분 침지후 실험에 사용하였다. 모든 처리구에서 처리직후 *V. cholerae* 의 세균수는 유의적 ($P<0.05$) 으로 감소하였다. 저장 2일 이후 lauric acid 및 monolaurine 의 처리구는 *V. cholerae*의 수를 감소하는데 있어서 초산의 처리구와

유의적 차이를 나타내었다. 1.0%의 초산에 3분 침지 후 0.5% lauric acid 로 3분 침지한 처리구는 0.5%의 초산에 3분 침지후 0.5% aluric acid 로 3분 침지한 처리구 보다 *V. cholerae*의 세균수를 유의적 ($P<0.05$) 으로 감소하였다. 0.5%~1.0% 의 초산침지후 0.5% monolaurine 으로 3분 침지한 처리구는 저장 3일 이후 *V. cholerae*에 대한 세균수 감소효과가 나타나지 않았다.

ACKNOWLEDGEMENTS

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1997.

REFERENCES

1. Klontz, K. C., Williams, L., Baldy, L. M. and Campos, M. : Raw oyster-associated *Vibrio* infections : Linking epidemiologic data with laboratory testing of oysters obtained from a retail outlet. *J. Food Prot.*, **56**, 977~979 (1993).
2. Kaysner, C. A., Abeyta, C., Wekell, M. M., Depaola, A., Stott, R. F. and Leitch, J. M. : Incidence of *Vibrio cholerae* from estuaries of the United States west coast., *App. Environ. Microbial.*, **53**, 1344~1348 (1987).
3. Sang, F. C., Hugh-Jones, M. E. and Hagstad, H. V. : Viability of *Vibrio cholerae* 01 on frog legs under frozen and refrigerated conditions and low dose radiation treatment. *J. Food Prot.*, **50**, 662~664 (1987).
4. 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**, 1~5 (1979).
5. Massad, G. and Oliver, J. D. : New selective and differential medium for *Vibrio cholerae and Vibrio vulnificus*. *Appl. Environ. Microbiol.* **53**, 2262~2264 (1987)
6. Kim, C. R., Lee, J. I., Shin, E. H., and Kim, K. H.: Antibacterial activity of alginic and acetic acids on refrigerated red seabream. *Kor. J. Vet. Publ. Health.* 21, 363~368 (1997).
7. Ingham, S. C. : Lactic acid dipping for inhibiting microbial spoilage of refrigerated catfish fillet pieces. *J. Food Qual.*, 12, 433~443 (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**, 639~643 (1995).
9. Anderson, M. E. and Marshall, R. T. : Reducing microbial populations on beef tissues: Concentration and temperature of an acid mixture. *J. Food Sci.* **55**, 903~905 (1990).
10. Kim, C. R., Kim, J. S., Koh, D. H., Rhie, S. C. and Eun, J. B. : Microbial decontamination of refrigerated red seabream by acetic, lactic, and citric acids. *Kor. J. Food & Nutr.*, **10**, 263~267 (1997).
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: 317~329 (1996).
12. 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, p. 103~133 (1991).
13. Oh, D. H. and Marshall, D. L. : Influence of temperature, pH, and glycerol monolaurate on growth and survival of *Listeria monocytogenes*. *J. Food Prot.* **56**, 744~749 (1993).
14. Ababouch, L, Chaibi, A., and Busta, F. F.: Inhibition of bacterial spores growth by fatty acids and their sodium salts. *J. Food Prot.* **55**, 980~984 (1992).
15. SAS User's Guide : Stastics. Version 10 edition, SAS Institute Inc., Cary, N. C., (1991).
16. Chaibi, A. Ababouch, L. H., and Busta, F. F.: Inhibition of bacterial spores and vegetative cells by glycerides. *J. Food Prot.* **59**, 716~722 (1996).
17. Oh, D. H. and Marshall, D. L. : Enhanced antimicrobial activity of monolaurine combined with organic acids against *Listeria monocytogenes*. Abstracts of 53rd of the Annual Meeting Institute of Food Technologists. June 20-24, New Orleans, LA (1992).

(Received November 23, 1998)