

## Reducing Microbial Populations on Refrigerated Pork Hams Treated with Acetic Acid

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### 초산으로 처리한 냉장 돼지고기햄의 미생물 증식억제

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#### Abstract

The influence of acetic acid (AA) dips on aerobic plate counts (APC), gram-negative bacterial counts (GNC), pH, and sensory tests of pork hams was studied during storage at 4°C. Pork hams were treated by dipping in 0.5~3.0% (v/v) AA for 0~3 min. Treatments of 1.0~3.0% AA for 3 min completely ( $P<0.05$ ) inhibited the GNC for 12 days of storage. Treatments of 3.0% AA for 3min completely ( $P<0.05$ ) inhibited the APC for 12 days of storage. Treatments with 1.0~3.0% AA for 0~3 min prolonged the microbiological shelf-life to 12 days of storage. Sensory evaluations of pork hams treated with acetic acid were liked less than the fresh controls due to acidic odor and whiteness.

Key words : acetic acid, pork ham, aerobic bacteria, gram-negative bacteria

#### Introduction

Refrigerated meat can be unsafe for consumption because of the growth or presence of meat spoilage and pathogenic bacteria<sup>1,9,13</sup>. Aerobic meat spoilage bacteria in refrigerated food can reduce keeping quality and microbiological shelf-life<sup>13,19</sup>. Organic acids such as acetic, lactic, and citric acids, either individually or combined, could be used to controlling undesirable microorganisms on refrigerated meat and fish<sup>9,10,13,16,18,19</sup>. Acetic acid has antimicrobial properties due to the undissociated acid molecules as well as the dissociated molecules<sup>14,15</sup>. Gram-negative bacteria was the majority of psychrophilic meat spoilage bacteria<sup>3</sup>, which was normally more sensitive than gram-positive bacteria<sup>14,15</sup>. Previous work in our laboratories showed that catfish fillets treated with 3.0% acetic acid for 30~60 sec were

more effective to suppress the growth of aerobic microorganisms than those of 3.0% lactic acid<sup>11</sup>. Furthermore, the duration of dipping was more influential in inhibiting microbial growth than was the amount of acid. Acetic acid on an equimolar basis has generally greater antimicrobial activity than other organic acids<sup>5,15</sup>. Several researchers<sup>1,5,9</sup> reported that sanitizing meat and fish surfaces with organic acids cause sublethal injury or death to undesirable microorganisms. The degree of injury varies with type and concentration of acid, microbial species, product, and storage condition.

Although most research on effects of organic acids has been focused on surface treatments of fresh meat<sup>7,16</sup>, there are limited published reports on the use of AA as meat preservatives for refrigerated pork hams. The objective of the present study was to evaluate the microbiological shelf-li-

fe, odor, appearance, and pH of pork hams treated with acetic acid for storage at 4°C.

## Materials and Methods

### 1. Sample preparation

Fresh pork hams were purchased from a commercial source less than 8h postmortem, transported on ice to laboratory, and used within 3h. The pork hams were cut into 3.5 Cm × 3.5 Cm squares at meat laboratory in the Chonnam National University. For each treatment, 1 Kg of pork hams (average weight 25g per pork ham) were submerged in 1 L tap water with appropriate amounts (v/v) of acetic acid(AA) (Moo Jang Ya Chemical Co, Japan). Duplicate experimental trials consisted of the following chemical treatments : (1) 0.5~3.0% AA dipping for 30 sec, (2) 0.5~3.0% AA dipping for 1 min, (3) 0.5~3.0% AA dipping for 2 min, (4) 0.5~3.0% AA dipping for 3 min. Treated pork hams drained on a sanitized stainless-steel grill for 2 min at room temperature. Control pork hams not treated with AA were dipped in 1 L tap water for required times and drained for 2 min to compensate for possible physical removal of bacteria and for moisture uptake. After dipping, pork hams were placed individually into plastic bags (Clean Zipper bags, Clean Wrap Co, Busan), stored at 4°C, and removed for analysis at 3 day intervals thereafter.

### 2. Microbiological analyses

Each pork hams was weighed and 0.85% (w/v) NaCl was added to make a 1 : 1 dilution (w/v). Samples were shaken for 50 times using standard rinse method<sup>2)</sup>. Aerobic plate counts (APC) and gram-negative bacterial counts (GNC) were determined by spread platings on standard plate count agar (Difco, Detroit, MI) and MacConkey agar (Difco, Detroit, MI), respectively, which were incubated at 30°C for 48 h. The number of bacteria was expressed as Log<sub>10</sub> CFU/g. Mean values were reported of duplicate platings of each sample. Growth rate of bacteria, expressed as generation times, was de-

termined using a procedure of Marshall and Schmidt<sup>12)</sup>. Two points on the logarithmic growth phase of each curve were used in the calculation.

Generation time (GT) =  $(0.301(T_2 - T_1)) / (\text{Log } P_2 - \text{Log } P_1)$ , where :  $T_1$  = time of  $P_1$ ,  $T_2$  = time of  $P_2$ ,  $P_1$  = CFU/g at  $T_1$  and  $P_2$  = CFU/g at  $T_2$ .

### 3. pH values

pH of pork hams was measured with standardized pH meter (Beckman ZeromaticR IV, Model 34, Irvine, CA) using a flat type surface electrode. Mean pH values were reported as the average of quadruplicate readings for each duplicate pork ham.

### 4. Sensory evaluation

Sensory evaluation of pork hams was performed by a ten member trained panel. Odor and appearance of uncooked pork hams were evaluated during storage. Treated pork hams were judged against fresh control pork hams (fresh daily), which were assigned a score of 5. Samples liked less than the control were scored 1 to 4, where 1 = disliked most. Samples liked more than the control were scored 6 to 9, where 9 = liked most. Untreated control pork hams also were stored at 4°C for comparison against fresh control and treated pork hams. For each treatment, duplicate pork hams per sampling day were analyzed.

### 5. Statistical analyses

Two replications of each trial were performed. APC, GNC, pH, and sensory data were analyzed using ANOVA and means were separated by LSD at a probability level of 0.05<sup>17)</sup>.

## Results and Discussion

### 1. Microbiological changes

Increasing acid contrations from 1.0% AA to 3.0% AA at dipping times of 30~60 sec were more influential in inhibiting microbial growth than was the controls (Fig. 1 and 2). Lag phase in-

creases of aerobic spoilage bacteria at treatments of 2.0~3.0% AA were observed for 9 days of storage. After 12 days at 4°C, APC in treatments with dipping in 3.0% AA for either 30 or 60 sec was higher than initial control samples by 1.3 and 1.5 log cycles, respectively. The meat industry generally considers that spoilage occurs when the total aerobic plate count (APC) reaches 106 to 107 CFU/g. Microbiological shelf-life of pork hams treated with 1.0~3.0% AA for 30~60 sec could be extended by 12 days of storage.

The duration of dipping was influential as much as amount of AA (Fig. 3 and 4). Results indicate that treatment of 0.5~3.0% AA for 2~3 min reduced ( $P<0.05$ ) initial microbial numbers immediately following dipping in acids by 0.7~1.

7 log cycles. When pork hams were treated with 3.0% AA for 3 min, APC was completely ( $P<0.05$ ) inhibited for 12 days of storage. Treatments dipped with 2.0~3.0% AA for 2~3 min effectively ( $P<0.05$ ) reduced APC by about 2 log cycles compared to the controls after 12 days of storage. Results show that treatment of 3.0% AA caused the greatest reduction in APC, there was no significant ( $P>0.05$ ) difference between 2.0% AA and 3.0% AA treatments after dipping for either 1 or 2 min. Kim et al.<sup>9)</sup> noted that when citric acid was used to pork loins, treatments of 2.0% citric acid dipped for 5 min reduced the APC by 0.8 log cycles more than did the controls after 12 days of storage. Ray<sup>14)</sup> noted that antimicrobial effects of organic acid related to the type and

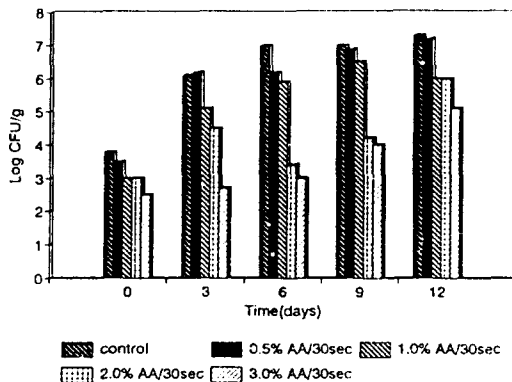


Fig. 1. APC of refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 30 sec.

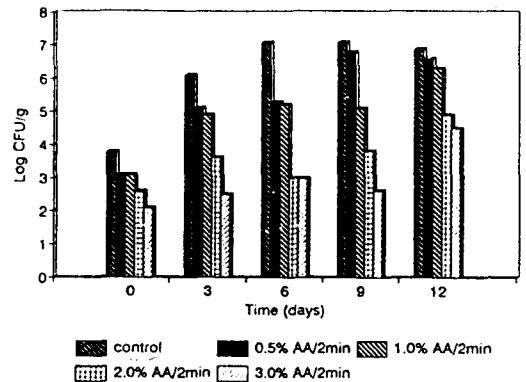


Fig. 3. APC of refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 2 min.

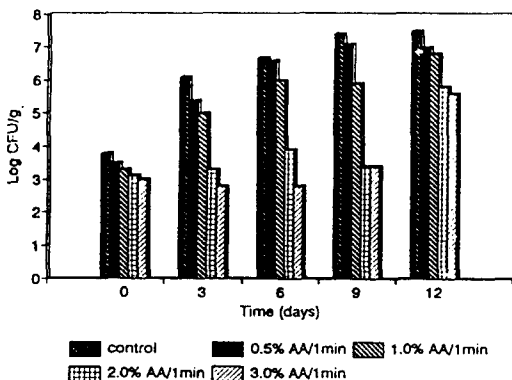


Fig. 2. APC of refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 1 min.

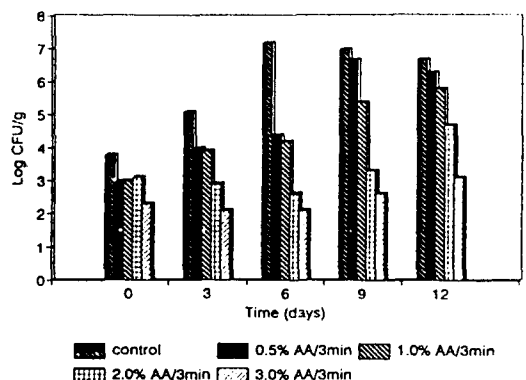


Fig. 4. APC of refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 3 min.

concentration of acids, and the product and storage condition. He reported that organic acids would sublethally injure bacteria on meat surface. The data in Fig. 5 show that treatment after dipping in 0.5~3.0% AA for 3 min effectively (0.05) reduced GNC for 12 days of storage at 4°C. All treatments completely inhibited ( $P<0.05$ ) the growth of gram-negative bacteria for storage at 4°C, while GNC in untreated pork hams raise about 1.3 log cycles in the same period. Results indicate that gram-negative bacteria are sensitive to AA, when exposure times in AA increased by 3 min, even in the low concentration of AA by 0.5%. Increasing dipping time in 0.5~3.0% AA solutions for 1 min significantly increased ( $P<0.05$ ) generation times (GT) (Fig. 6). GT of control and 0.5~3.0% AA treatments for 1 min were 14.9 h, 18.1 h, 24.7 h, 32.0 h, and 33.2 h, respectively. Results indicate that most aerobic spoilage microorganisms subjected to AA and were prone to death and sublethal injury, with increasing exposure times and acid concentrations<sup>9,10,11,13,19</sup>. Another notable observation relates to the 9 day lag phase extension of microbial growth on all treatments after dipping in 0.5~3.0% AA for 2~3 min.

## 2. Changes in pH

pH of pork hams after dipping AA declined ( $P<0.05$ ) immediately by 0.5 to 0.7 log cycles compared with the controls (results not shown). Although the pH increased after 3 days, this is marginal for inhibiting growth of psychrotropic pseudomonads<sup>1</sup>. Other researchers<sup>6,19,20</sup> noted that antimicrobial effect of weak acids was due to the presence of undissociated molecules as well as dissociated molecules.

## 3. Sensory Evaluations

Sensory data of pork hams dipped in AA for 60 sec are shown in Table 1 and 2. Sensory scores indicate that treated pork hams were in the "liked less" to "typical" category in appearance and odor compared with fresh control pork hams. During storage at 4°C, typical comments were that

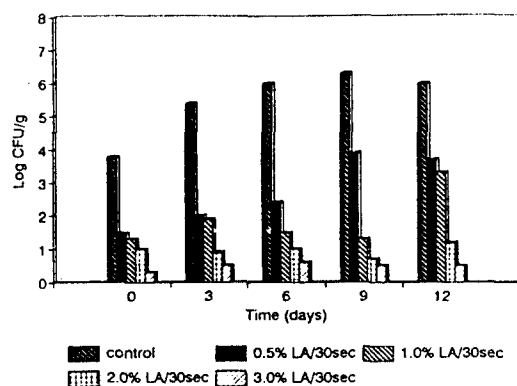


Fig. 5. GNC of refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 3 min.

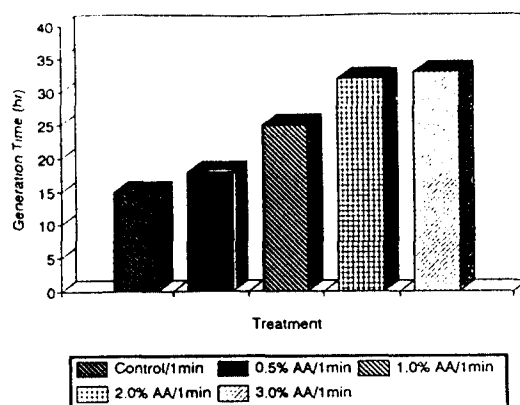


Fig. 6. Generation times (GT) of aerobic spoilage bacteria on refrigerated (4°C) pork hams treated with 0.5~3.0% acetic acid (AA) for 1 min.

treated pork hams were acidic odors or vinegar-like odor. Typical appearance comments were gray or white appearance. It was found that when AA was used at a concentrations greater than 2.0% or for longer exposure times than 1 min, there was some evidence of the muscle by the acid. Similarly, Reynolds and Carpenter<sup>16</sup> and Mendonca et al.<sup>13</sup> have reported that discoloration of meat surfaces after dipping or spraying with 1.0~3.0% acetic or lactic acids. Cacciarelli et al.<sup>4</sup> reported that if the discoloration could be prevented, acetic acid would be useful in extending the shelf-life of pork loins. However, when the surface of pork hams is coated with batter

**Table 1. Mean odor scores of pork hams treated with acetic acid(AA) during storage at 4℃**

Treatment	Storage time(days)				
	0	3	6	9	12
Fresh control	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>
0.5% AA	3.84 <sup>c</sup>	4.84 <sup>a</sup>	3.67 <sup>b</sup>	3.67 <sup>b</sup>	3.17 <sup>b</sup>
1% AA	4.33 <sup>b</sup>	4.0 <sup>b</sup>	3.67 <sup>b</sup>	3.84 <sup>b</sup>	3.0 <sup>b</sup>
2% AA	3.84 <sup>c</sup>	4.0 <sup>b</sup>	3.67 <sup>b</sup>	3.5 <sup>b</sup>	2.83 <sup>b</sup>

a,b,c : Means in the same column with different superscripts are significantly different ( $P < 0.05$ )

**Table 2. Mean appearance scores of pork hams treated with acetic acid(AA) during storage at 4℃**

Treatment	Storage time(days)				
	0	3	6	9	12
Fresh control	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>
0.5% AA	3.34 <sup>b</sup>	3.5 <sup>c</sup>	4.0 <sup>b</sup>	3.83 <sup>ab</sup>	2.5 <sup>b</sup>
1% AA	4.0 <sup>b</sup>	4.0 <sup>b</sup>	3.5 <sup>bc</sup>	4.0 <sup>ab</sup>	3.0 <sup>b</sup>
2% AA	3.84 <sup>b</sup>	4.0 <sup>b</sup>	3.0 <sup>c</sup>	3.33 <sup>b</sup>	2.67 <sup>b</sup>

a,b,c : Means in the same column with different superscripts are significantly different ( $P < 0.05$ )

and breading prior to cooking, the discoloration is believed to have little detrimental effect on consumer appeal<sup>8)</sup>.

## 요 약

초산침지법을 이용하여 4℃ 냉장시의 돼지고기 햄의 APC, GNC, pH, 및 관능평가에 대한 영향을 조사하였다. 3분동안 1.0~3.0% (v/v) 초산을 처리한 구는 12일 저장동안 GNC를 완전히 억제하였다. 3분동안 3.0% 초산을 처리한 구는 12일 저장동안 APC를 완전히 억제하였다. 0~3분동안 1.0~3.0% 초산을 처리한 구는 12일까지 미생물학적 저장안정성을 확장하였다. 초산으로 처리한 돼지고기햄의 관능평가는 유기산 냄새 및 표백으로 인하여 신선한 돼지고기 햄보다 낮은 등급의 기호성을 보였다.

## References

- 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).
- AOAC. : Official Methods of Analysis, 14th ed., Association of Official Analytical Chemists, Washington, DC(1984).
- Brown, A. D. and Weidemann, J. F. : The taxonomy of psychrophilic meat spoilage bacteria. A reassessment. *J. Appl. Bacteriol.* **21**, 11(1958).
- Cacciarelli, M. A., Springer, W. C., Anderson, M. E. and Naumann, H. D. : Effects of washing and sanitizing on the bacterial flora on vacuum-packaged pork loins. *J. Food Prot.*, **46**, 231(1989).
- 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).
- El-Shenawy, M. A. and Marth, E. H. : Organic acids enhance the antilisterial activity of potassium sorbate. *J. Food Prot.*, **54**, 593(1991).
- Hamby, P. L., Savell, J. W., Acuff, G. R., Vanderzant, C. and Cross, H. R. : Spray-chilling and carcass decontamination systems using lactic and acetic acids. *Meat Sci.*, **21**, 1(1987).
- Ingham, S. C. : Lactic acid dipping for inhibiting microbial spoilage of refrigerated catfish fillet pieces. *J. Food Qual.*, **12**, 433(1989).
- Kim, C. R., Lee, J. I., Kim, K. I., 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. J. Vet. Publ. Hlth.*, **20**, 329(1996).
- Kim, C. R., Hearnberger, 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).
- 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).
- Marshall, D. L. and Schmidt, R. H. : Growth of *Listeria monocytogenes* at 10℃ in milk preincubated with selected pseudomonads. *J. Food Prot.*, **51**, 277(1988).
- Mendonca, A. F., Molins, R. A., Kraft, A. A. and Walker, H. W. : Microbiological, chemical, and physical changes on fresh vacuum packaged pork treated with organic acids and salts. *J. Food Sci.*, **54**, 18(1989).
- Ray, B. : Impact of bacterial injury and repair in food microbiology : its past, present, and future. *J. Food Prot.*, **49**, 651(1986).
- Ray, B. R. and Sandine, W. E. : Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives. p. 103~106, In B. ray and M. Daeschel (ed.), Food Biopreservatives of Microbial Origin, CRC Press, Inc., Boca Raton, FL(1992).
- Reynolds, A. E. and Carpenter, J. A. : Bactericidal properties of acetic and propionic acids on pork carcasses. *J. Animal Sci.*, **38**, 515(1974).
- SAS. : SAS User's Guide : Stastics, SAS Institute Inc., Cary, NC(1993).
- Visser, I. J. R., Koolmees, P. A. and Bijker, P. G. H. : Microbiological conditions and keeping quality of veal tongues as affected by lactic acid decontamination and vacuum packaging. *J. Food Prot.*, **51**, 208(1988).

19. Woolthuis, C. H. J. and Smulders, F. J. M. : Microbiological decontamination of calf carcasses by lactic acid sprays. *J. Food Prot.*, **48**, 832(1985).
  20. Zamora, M. C. and Marth, N. E. : Antimicrobial activity of undissociated sorbic acid in vacuum packaged beef. *J. Food Sci.*, **52**, 1449(1987).
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(1996년 11월 29일 접수)