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Microbiological Evaluations of Refrigerated Chicken Wings Treated with Acetic Acid

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초산으로 처리한 냉장 닭고기 날개의 미생물 평가

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ABSRACT — The effects of acetic acid (AA) on aerobic plate counts (APC), gram-negative bacterial counts (GNC), and generation time (GT) in chicken wings stored at 4°C were assessed. Chicken wings were treated with 0.5~1.5% (v/v) AA at exposure times of 5 min. Treatments of AA for 5 min significantly ($P<0.05$) reduced aerobic plate counts (APC) and gram-negative bacterial counts (GNC) on the surface of chicken wings for 8 days, respectively. After 4 days of storage, treatments of 1.0% AA and 1.5% AA for 5 min completely ($P<0.05$) inhibited APC and GNC compared to initial controls. Based on these results, treatments of 1.0% AA and 1.5 % AA for 5 min prolonged the microbiological shelf-life for 8 days compared to those of 0.5% AA and the controls. All treatments of AA increased the lag phase and GT of aerobic microorganisms.

Key words □ Acetic acid, chicken wing, aerobic plate count, gram-negative bacterial count

Microbiological safety of poultry products is mainly due to the growth of aerobic spoilage bacteria and foodborne pathogens during storage and handling.[1,3,4,8,9,11,15] Extended storage under refrigerated temperature without any suitable preservatives in refrigerated foods can allow aerobes to grow and among those organisms gram-negative bacteria are the most indicative.[3,5,6,10]

Recently various organic acids have been utilized in a manner to inhibit growth of those aerobes on the surfaces of fresh meat and meat products at the retail level.[3,4,10,11,15,16] Several researchers[2,4,6,7,11,12] suggeated that decontamination of refrigerated meat and fish with undesirable microorganisms was highly dependent on the concentration, type, and exposure times of acidulant used.

Hardin *et al.*[5] noted that beef carcasses surfaces treated with 2% lactic or acetic acid spray significantly reduced fecal microorganisms. Previous work[7] in our laboratories found that pork loins treated with 1~2% citric acid significantly reduced aerobic spoilage bacteria during storage at 4°C. Dickens *et al.*[3] found that gram-negative bacteria in poultry carcasses dipped in 0.6% acetic acid have a higher susceptibility or death rate than gram-positive bacteria due to the decrease in pH. Kim *et al.*[6] noted that catfish fillets treated with either 2% lactic acid or 3% lactic acid for 1 or 5 min significantly reduced gram-negative bacterial counts for 9 days at 4°C.

Studies[2,14,15] have shown that acetic acid on an equimolar basis has generally greater antimicrobial activity than other organic acids.

Although organic acids have an antimicrobial effectiveness on fresh meat and its product,[3,4,10,17,18] there are limited studies on the effects of acetic acid in refrigerated chicken wings. Our objective was to determine the bactericidal effectiveness of acetic acid in chicken wings for

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storage at 4°C.

MATERIALS AND METHODS

Chicken preparation and treatments

Fresh chicken wings were obtained from commercial source, transported to the laboratory on ice and used within 2 hr. For each treatment, six hundred gram of chicken wings (average weight 25 g per wing) were washed under running cold tap water to remove surface dirt and debris. Each chicken wings was submerged in 2L of 0.5~1.5% (v/v) AA (food grade acetic acid, Sae Won Chemical Co., Korea) for 5 min, and drained on a sanitized stainelesssteel grill for 2 min. Controls were dipped in 2L tap water only for 5 min to compensate for possible physical removal of bacteria and for moisture uptake. Treated chicken wings were packed in groups of 4 in large Whirl-Pak bags (Fisher Chemical Co., USA), respectively. The bags were closed and stored at 4°C. Samples were removed from storage at appropriate times for analyses.

Microbiological analysis

The Whirl-Pak bags were removed one by one from refrigerator. Each chicken wing was weighed, 0.1% (w/v) sterile peptone water was added to make a 1:1 dilution (w/v), which was shaken 60 times.[9] The liquid from each sample was diluted and plated in volumes of 0.1 m*l* on plate count agar (Difco Laboratories, Detroit, MI) for aerobic plate count (APC) and MacConkey agar (Difco) for gram-negative bacterial count (GNC), respectively. The plates were incubate for 48 hr at 30°C before colonies were counted. The number of bacteria was expressed as mean Log_{10} CFU/g for the duplicate treatments.

Calculation of growth curves

The growth rates of aerobes were determined using the following to calculate generation times.[13] Two points on the logarithmic growth phase of each curve were used in the calculation.

Generation time (GT)=$(0.301(T_2-T_1))/(\log P_2-P_1)$

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

Statistical analyses

APC, GNC, and GT were analyzed using ANOVA, and means were seperated by the least significant difference test at $P<0.05$.[16]

RESULTS AND DISCUSSION

APC and GNC of chicken wings treated with 0.5~1.5% AA were significantly lower ($P<0.05$) than water-only controls during storage at 4°C (Table 1). Treatments of 1.5% AA for 5 min were effective in lowering ($P<0.05$) the initial level of APC and GNC by 1.3 and 1.5 log units compared to controls, respectively (Table 1 and 2). Chicken wings treated with 1.0~1.5% AA for 5 min completely ($P<0.05$) inhibited growth of aerobes for 4 days of storage, while in the controls were rapidly spoiled as evidenced by microorganismal growth (Table 1). After 8 days of storage, treatements of either 1.0% AA or 1.5% AA for 5 min allowed growth of aerobes by 0.3 and 0.1 log units, respectively. Results indicate that increasing concentration of AA by 1.5% was strongly inhibitory against the growth of aerobes in chicken wings. Aerobic microorganisms subjected to AA were prone to sublethal injury and/or death while being retained in AA.[3,4,9] Visser *et al.*[17] noted that veal calf tongues treated with 2% L-lactic acid before vacuum-packaged had an inhibitory effect on the growth of mesophlic aerobic bacteria for 14 days at 2 or 4°C. Woolthuis and Smulders[18] reported that an extended lag phase resulting from acid injury in calf carcasses treated with lactic acid may result in a longer storage life.

Spoilage of chicken meat generally occurs when APC reach 10^7 CFU/g or greater. Thus, microbiological shelf-life of chicken wings treated with either 1.0 or 1.5% AA for 5

Table 1. Changes of APC in refrigerated (4°C) chicken wings treated with different concentrations of acetic acid (AA) for 5 min

Treatment \ Storage day	Log CFU/g		
	0 day	4 day	8 day
Control	5.94[a]	6.95[a]	8.50[a]
0.5% AA[1]/5 min	5.14[b]	6.07[b]	7.31[b]
1.0% AA/5 min	4.85[bc]	5.28[c]	6.25[c]
1.5% AA/5 min	4.62[c]	4.78[d]	6.00[c]

AA[1]=acetic acid.

[a-d]Counts within the same column with different superscripts are significantly different ($P<0.05$).

Table 2. Changes of GNC in refrigerated (4°C) chicken wings treated with different concentrations of acetic acid (AA) for 5 min

Treatment \ Storage day	Log CFU/g		
	0 day	4 day	8 day
Control	5.63[a]	6.54[a]	8.19[a]
0.5% AA[1]/5 min	4.61[b]	5.28[b]	7.40[a]
1.0% AA/5 min	4.29[bc]	4.80[b]	6.00[b]
1.5% AA/5 min	4.15[c]	4.00[c]	5.80[b]

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

min would be 8 days, respectively, than untreated chicken wings.

Treatments of either 1.0% or 1.5% AA for 5 min increased GNC by 0.37 and 0.17 log units during storage of 8 days, respectively, compared to initial controls (Table 2). Chicken wings treated with 0.5~1.5% AA for 5 or 10 min completely inhibited growth of GNC during storage of 4 days, compared to initial controls. Treatments of both 1.0% and 1.5% AA significantly (P<0.05) reduced GNC during storage of 8 days compared to those of 0.5% AA and controls. Results indicate that microbial spoilage of chicken wings is mainly caused by gram-negative bacteria. Similarly, Ray and Sandine[15] noted that gram-negative bacteria were normally more sensitive to lower pH than gram-positive bacteria. During 8 days of storage treatments of AA had a significant (P<0.05) effect on preventing the growth of aerobes, gram-negative bacteria were most indicative. Dickens *et al.*[3] reported that gram-negative bacteria have a higher susceptibility or death rate due to the sharp decrease in pH caused by the introduction of the acetic acid in processed poultry carcasses. Similarly, Lee *et al.*[10] noted that pork hams dipped in 0.5~3% AA for 3 min effectively reduced GNC for 12 days at 4°C. They reported 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%.

Chicken wings treated with AA for 5 min had GT higher (P<0.05) than controls during storage at 4°C (Fig. 1). Treatments of 1.5% AA increased GT by approximately 19.3 hr and 15.3 hr compared to the controls and treatment of 0.5% AA, respectively. There was no significant (P>0.05) difference between 1.0% AA and 1.5% AA.

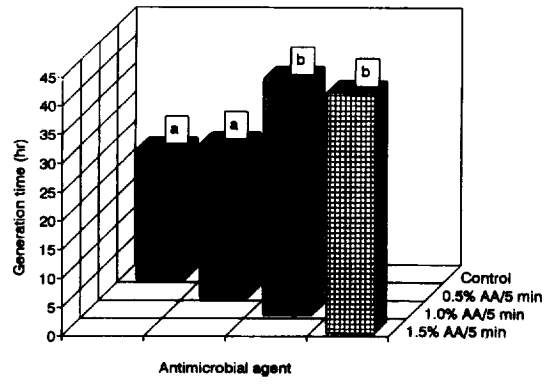

Fig. 1. Generation time (GT) of aerobic spoilage bacteia in refrigerated (4°C) chicken wings treated with different concentrations of acetic acid (AA) for 5 min.
[a,b] Bars with different superscripts are significantly different (P<0.05).

Results suggested that the effectiveness of AA as an antimicrobial surfece treatment attributed to increasing levels of AA by 1.5% when chicken wings were dipped in AA. Previous work in our laboratories[10] found that GT of aerobic microorganisms in pork hams treated with 0.5% acetic acid for 1 min was not significant difference (P>0.05) compared to control. They noted that GT in pork hams treated with 1.0~3.0% acetic acid for 5 min was higher than control and 0.5% acetic acid treatment. Similarly, Marshall and Kim[12] noted that catfish fillets dipped in either 3% acetic acid or 2% acetic acid and 2% lactic acid for 30~60 sec increased GT and extended shelf-life to 16 days during storage at 4°C. Other researchers[12,15,18] suggested that organic acids had antimicrobial properties due to the presence of undissociated molecules as well as dissociated molecules.

CONCLUSIONS

Microbiological spoilage of refrigerated chicken wings was mainly dependent on the growth of gram-negative bacteria. Antimicrobial activity of AA on aerobic microorganisms enhanced by increasing concentrations of the acid during storage at 4°C. Treatments of 1.0~1.5% AA for 5 min effectively inhibited APC and GNC in chicken wings during storage at 4°C and should be considered as a potential method for shelf-life extension.

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국문요약

초산 침지법을 이용하여 4℃ 냉장동안 닭고기 날개의 세균오염을 줄이고자 호기성 총균수(APC), 그람음성균수(GNC) 및 세대시간(GT)에 대한 영향을 조사 하였다. 저장 4일후 5분 동안 1.0~1.5%의 초산 처리구는 APC 및 GNC의 증식을 완전히 억제 하였다. 저장 8일후 5분 동안 1.0~1.5% 초산 처리구는 APC의 증식를 0.1~0.3 log unit 그리고 GNC의 증식를 0.17~0.37 log unit까지 허용한 반면, 대조구와 0.5% 초산 처리구는 대수적 증식을 보였다. 1.0~1.5%의 초산 처리구는 대조구와 0.5% 초산 처리구보다 유의적($P<0.05$)으로 GT를 증가하였다.

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