PE8.32 Application of Organic Acids and Supercritical Carbon Dioxide Treatments to Decontaminate Microorganisms in Fresh Pork 357.00

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Abstract

This study was conducted to examine the inhibitory effects of organic acids and/or supercritical carbon dioxide (SC-CO_2) treatment on generic *Escherichia coli* and three foodborne pathogens, including *Listeria monocytogenes*, *Salmonella typhimurium, and E. coli* O157:H7. The different treatment conditions were as follows: (1) treated with 3% acetic or 3% lactic acid only, (2) treated with SC-CO_2 at 14 MPa and 35 °C for 30 min only and (3) treated with 3% acetic or 3% lactic acid followed by treatment with SC-CO_2. For any given treatment conditions, the level of generic *E. coli* and three foodborne pathogens were significantly reduced by organic acids and/or SC-CO_2 treatment. Within the same organic acid concentration (3%), the acetic and lactic acid treatments had similar reductions. Moreover, although no significant differences were observed within the combined treatments, the combined treatments were more greatly reduced than the SC-CO_2 treatment only. Moreover, in case of the combined treatment, levels of generic *E. coli* and three foodborne pathogens showed maximum reductions, and were reduced by 2.78 to 3.30 log CFU/cm^2 (generic *E. coli* = 3.25 log CFU/cm^2; *L. monocytogenes* = 2.78 log CFU/cm^2; *S. typhimurium* = 3.15 log CFU/cm^2; *and E. coli* O157:H7 = 3.30 log CFU/cm^2).

***Index Terms*— Organic acids, supercritical carbon dioxide treatment, microorganisms, pork**

I. INTRODUCTION

Generally, fresh meat is prone to microbial spoilage, because meat provides suitable conditions for microbial proliferation. Therefore, sterilization methods for the inhibition of pathogenic bacteria of fresh meat have attracted a great deal of attention. Weak food-grade organic acids, such as acetic and lactic acids, are the most common and classical agents for the inhibition of microorganisms in meat industry [2]. According to report of Surve et al. [7] the treatment of buffalo steaks with 3% acetic and lactic acid solutions achieved the microbial reduction without adverse effect on surface color. However, Acuff et al. [1] applied organic acids to beef and found no difference aerobic plate counts between the control and treatment samples. On the other hand, supercritical carbon dioxide (SC-CO_2) is an effective treatment for destroying pathogens in solid and liquid foods, and is relatively inert, inexpensive, non-toxic, non flammable, and leaves no residue [5]. According to Choi et al. [3], SC-CO_2 has been applied to marinated pork at 14 MPa, the reduction level of *Listeria monocytogenes* was 2.49 log CFU/cm^2. However, the combined effects of organic acids and SC-CO_2 on the inactivation of microorganisms in fresh pork have not been extensively studied to date. Therefore, the overall objective of this study was to develop the application of combined treatment of organic acids and SC-CO_2 for improving the food safety in fresh pork, especially the inhibition of generic *Escherichia coli*, *L. monocytogenes*, *Salmonella typhimurium, and E. coli* O157:H7.

II. MATERIALS AND METHODS

A. Bacterial strains

Four bacterial strains each, including generic *E. coli* (ATCC 25922, BE4a, and K-12 2B), *L. monocytogenes* (ATCC 191413, ATCC 19114, and ATCC 19115), *S. typhimurium* (ATCC 19585, ATCC 43174, and DT104 Killercow), and *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), were obtained from the Food Microbiology Culture Collection at Korea University (Seoul, Korea). All cultures were suspended in fresh tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) containing 10% glycerol and were stored at –80 °C until subsequent analysis.

B.

C. Culture and cell suspension

Each strain of microorganisms was cultured in TSB at 37 °C for overnight. The cultures from each group were then combined in plastic centrifuge tubes (Corning Inc., NY), after which the cells were harvested by centrifugation (Centri-CL2; IEC, Needham Heights, MA) at 2,600 × g for 30 min. The supernatant was discarded and the pellet was washed twice with 0.2% sterile peptone water. The final pellet was then re-suspended in 0.2% sterile peptone to give a final concentration that ranged from 10^9 CFU/ml of

sample. Each culture cocktail of microorganisms was used for further experiments.

D. Fresh pork treatment

Twenty-four boneless pork loins were obtained from a local abattoir at 24 h post-slaughter. Samples of muscle were cut into 20 mm of intact shaped thickness (each weighing 70 ± 2 g), and were randomly selected to minimize bias.

In each experiment, the meat samples inoculated with generic *E. coli* or the three pathogens cocktail to yield approximately 10^7 CFU/cm^2. After spot inoculation, each microorganism cocktail was spread using a sterilized spatula and each sample was put into culture flask and incubated at 4 °C overnight.

The samples were then subjected to organic acid and/or SC-CO_2 treatments. For the organic acid treatments, the pork chunks soaked with 3% acetic or 3% lactic acid solutions (w/w) in polyethylene bags at 4 °C for 1 min. The SC-CO_2 treatments were performed with a SC-CO_2 system (Supercritical system, Tharex Co., Korea), and were performed at 14 MPa and 35 °C for 30 min. The various treatment conditions were as follows: treated with 3% organic acid only, treated with SC-CO_2 only, and treated with 3% acetic or 3% lactic acid followed by treatment with SC-CO_2. The whole experiment was replicated three times.

E. Microbiological analysis

The treated samples were placed in stomacher bags containing 180 ml of buffered peptone water, and homogenized for 2 min (Seware, Stomacher 400, U.K.). After homogenization, the samples underwent serial 10-fold dilutions with 9 ml of sterile buffered peptone water. Eosin-Methylene Blue agar (Difco), Oxford agar base (Difco), Xylose lysine desoxycholate agar, and Sorbitol MacConkey agar (Difco) were used as selective media for the enumeration of generic *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7, respectively, and were incubated at 37 °C for 24 h. These microbial analyses were performed before and after treatment for each treatment condition.

F. Statistical analysis

The average of the duplicate plate counts for three replications was converted to units of log CFU/cm^2. All experiments were conducted in triplicate with duplicate samples analyzed at each treatment. All data were analyzed by analysis of variance using the ANOVA contained in the SAS statistical package [6], and significance is reported at $P < 0.05$ level.

III. RESULTS AND DISCUSSION

Table 1 shows the inhibitory effects of the organic acids and/or SC-CO_2 treatment at various conditions on generic *E. coli*, *L. monocytogenes*, *S. typhimurium*, *and E. coli* O157:H7. For any given treatment conditions, the level of microorganisms were significantly reduced by the organic acids and/or SC-CO_2 treatment. However, no significant differences were observed between the samples treated with 3% acetic and 3% lactic acids. When SC-CO_2 was applied at 14 MPa and 35 °C for 30 min, a significantly lower number of generic *E. coli* (6.42 vs. 8.63 log CFU/cm^2), *L. monocytogenes* (5.93 vs. 7.76 log CFU/cm^2), *S. typhimurium* (4.91 vs. 6.87 log CFU/cm^2)*, and E. coli* O157:H7 (5.67 vs. 7.20 log CFU/cm^2) was observed compared to the control. Although no significant differences were observed between the samples combined treated with acetic acid and SC-CO_2 and the samples combined treated with lactic acid and SC-CO_2, the combined treatments were more greatly reduced than the organic acids only or SC-CO_2 treatment only. In general, the antimicrobial activity of organic acids increases as the pH decreases, however Van Netten et al. [8] reported that the application of 5% organic acid resulted in unacceptable sensory quality, especially meat color. Moreover, the treatment pressure and temperature of SC-CO_2 can affect molecular interaction and protein conformation, leading to protein denaturation [4]. For this reason, in this study, the treatment conditions were minimized. In case of the combined treatments, levels of generic *E. coli* and three foodborne pathogens showed maximum reductions, and were reduced by 2.78 to 3.30 log CFU/cm^2.

IV. CONCLUSION

Base on these results, the combined treatments of the organic acids and SC-CO_2 were more effective at the reduction of pathogens than the treatment of organic acid only or SC-CO_2 only, and these treatments are an effective method for improving the microbial safety of fresh meat and meat products.

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Table 1

Inhibitory effects of organic acids and/or supercritical carbon dioxide (SC-CO_2) treatment at 14 MPa and 35 °C for 30 min on microorganisms in fresh pork

	Control	Organic acid treatment		$SC\text{-}CO_2$ Treatment	Combined treatment		Level of significance
		3% AA	3% LA		3% AA + $SC\text{-}CO_2$	3% LA + $SC\text{-}CO_2$	
Generic *Escherichia coli*	8.63^{a} ±0.24	7.62^{b} ±0.38	7.48^{b} ±0.31	6.42^{c} ±0.33	5.38^{d} ±0.24	5.46^{d} ±0.29	***
Listeria monocytogenes	7.76^{a} ±0.10	6.87^{b} ±0.17	6.57^{b} ±0.09	5.93^{c} ±0.24	4.93^{d} ±0.24	4.98^{d} ±0.22	***
Salmonella typhimurium	6.87^{a} ±0.27	5.86^{b} ±0.35	5.76^{b} ±0.53	4.91^{c} ±0.44	3.84^{d} ±0.41	3.72^{d} ±0.32	***
***E. coli* O157:H7**	7.20^{a} ±0.17	6.60^{b} ±0.23	6.28^{b} ±0.29	5.67^{c} ±0.14	4.03^{d} ±0.16	3.90^{d} ±0.32	***

Values are means±SD.

Level of significance: *** $P < 0.001$.

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

Abbreviations: AA, acetic acid; LA, lactic acid.