

学 位 論 文 要 旨

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[論文題名]			
<p>Evaluation of chemical treatment combined with vacuum and ultrasonication with a water resonance system for reducing <i>Campylobacter</i> on naturally contaminated chicken carcasses 処理工程で鶏と体に付着したカンピロバクターを減少させるための吸引と共振超音波を組み合わせた薬剤処理の評価</p> <p>Food Control, Vol. 112, 2020, in press, DOI: 10.1016/j.foodcont.2020.107087 (参考論文)</p> <p>Current status of <i>Campylobacter</i> food poisoning in Japan Food safety, 7(3):61-73,2019, DOI: 10.14252/foodsafetyfscj.D-19-00001</p>			
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<p>Introduction</p> <p><i>Campylobacter jejuni</i> and <i>C. coli</i> are the important zoonotic pathogen that is often recognized as the leading cause of bacterial foodborne illness worldwide. Epidemiological investigations of <i>Campylobacter</i> food poisoning in Japan have shown that the improper handling or consumption undercooked chicken meat and its products are the most important sources of infection, as is the other developed countries. Over the last two decades, the consumption of fresh raw chicken meat and liver has been increasing and it is likely to account for much of the increased incidence of human campylobacteriosis in Japan. <i>Campylobacters</i> colonize the intestinal tract of healthy chickens and frequently contaminate carcasses and raw meat products during processing. To reduce the numbers of bacteria, sodium hypochlorite (NaOCl) is widely used at the chilling step in most processing plants. However, the bactericidal effect of NaOCl rapidly decrease due to the presence of organic matters. Cetylpyridinium chloride (CPC) is a cationic surface active agent which has a strong bactericidal effect and stable under organic matters conditions. Previous studies has demonstrated that CPC is highly effective for reducing the number of various pathogenic bacteria attached to chicken skin. However, microorganisms could be entrapped in skin crevice, folds, and feather follicles. As the conventional method such as immersion with a disinfectant is only partially effective for reduction of contaminating bacteria, alternative interventions are need to be developed.</p> <p>Recently, physical treatments such as ultrasonication have been applied as an alternative approach to inactivate microorganisms on raw poultry products. Ultrasonication with a water resonance system (WRS) has been used as an industrial cleaning technology for detachment of complex surface material. Although a general ultrasonicator produces waves going in one direction, the aim of the WRS system is to control the properties of dissolved oxygen in liquid medium, thus improving the intensity and uniformity of ultrasound waves which are able to propagate in every</p>			

direction. Therefore, we considered applying this technology combined with chemical treatment to enhance the effectiveness of bacterial removal from chicken carcasses. Furthermore, we introduced the vacuum step in the processing of carcasses prior to ultrasonication treatment for improving the effectiveness of immersion in sanitizers, even for feather follicles.

The objective of this study was done to evaluate the effectiveness of vacuum and ultrasonication with a WRS in combination with CPC or NaOCl for removal of *Campylobacter* on naturally contaminated chicken carcasses.

Material and methods

Free-range, often eaten undercooked or raw in Kyushu, and broiler chicken carcasses, which is consumed extensively worldwide, were obtained from two different commercial processing plants after evisceration. Each carcass was immersed in a 0.1% CPC or 0.01% NaOCl and exposed to a vacuum of -0.02 MPa for 10 min, and then returned to the atmosphere. This process was repeated three times. The carcasses were then subjected to ultrasonication with a WRS at 130 kHz for 15 min. The chicken carcasses were rotated at 30 rpm using a rotation machine during ultrasonication with the WRS to avoid a high temperature developing on the meat surfaces due to cavitation power. Tap water was also used instead of the chemical, and other carcasses were immersed in each chemical alone. The *Campylobacter* count on breast and back skin was determined by the most-probable-number (MPN) method. Moreover, breast skin from free-range and broiler chicken carcasses was examined by scanning electron microscopy (SEM)

Results

Immersion in CPC was very effective on free-range chicken breast skin, achieving a 0.87 log₁₀ MPN/10 g reduction, which was significantly ($p<0.05$) more effective than tap water. For broiler chicken carcasses, however, neither CPC nor NaOCl treatment resulted in a significant reduction of *Campylobacter*, and immersion in tap water had no effect. Combination treatments significantly ($p<0.01$) reduced the number of *Campylobacter* on back and breast skin in overall. For free-range chicken carcasses, combination of CPC with physical treatments achieved a reduction of 1.36-1.62 log₁₀ MPN/10 g, whereas NaOCl and physical treatments achieved a reduction of 1.0-1.26 log₁₀ MPN/10 g. For broiler chicken carcasses, CPC combined with physical treatments achieved a reduction of 0.94-1.16 log₁₀ MPN/10 g, whereas NaOCl with physical treatments achieved a 0.74-1.05 log₁₀ MPN/10 g reduction in *Campylobacter* levels. However, tap water combined with physical treatments achieved no significant reduction on either free-range or broiler chicken carcasses. SEM confirmed that ultrasonication with a WRS treatment successfully removed debris from breast skin in comparison to untreated skin.

Discussion

Immersion in 0.1% CPC or 0.01% NaOCl alone achieved a significant reduction of *Campylobacter* levels on free-range chicken, but not broiler chicken carcasses. The possible reason might be differences in the properties of skin between free-range and broiler chickens. Compared to free-range chickens, which are raised in the free environment, broiler chickens kept in houses with

limited access to an open field show higher fat accumulation in both the skin and body due to their lower degree of physical movement. Accordingly, the higher level of fat deposited in broiler skin and the higher amount of organic matter released from broiler carcasses might reduce the effectiveness of sanitizers on the skin. Overall, treatment with 0.1% CPC was more effective for reducing *Campylobacter* populations than 0.01% NaOCl, which was as ineffective as tap water. The bactericidal effect of NaOCl is well known to be reduced in the presence of organic matter. CPC, on the other hand, has potential applications for poultry processing due to strong germicidal activity, lack of any influence of organic matter, and low toxicity when used at the recommended doses.

A combination of 0.1% CPC or 0.01% NaOCl, vacuum, and ultrasonication with a WRS achieved a significant reduction of the *Campylobacter* count. In this study, we introduced a vacuum process to improve the effectiveness of immersion in sanitizers. Deaeration of chicken carcasses immersed in a sanitizer was observed during application of negative pressure, and restoration of ambient pressure allowed penetration of the sanitizer into crevices, folds, and feather follicles. Ultrasound decontamination technology is based on physical detachment of bacterial cells and the cleaning efficiency of ultrasound is related to the dissolved gas content in the system. The concentration of dissolved oxygen in general tap water is about 8 mg/L and many bubble nuclei usually dissipate the radiation power. On the other hand, the cavitation effect in terms of both intensity and distribution would be higher if the oxygen content of the water could be controlled to within an optimal range of around 3.17-5.02 mg/L. In our method, the concentration of dissolved oxygen in the sanitizer solutions was regulated to within 2.5-3.5 mg/L by the WRS, allowing cavitation bubbles to strongly clean the skin surface and uniform ultrasonic irradiation of the carcass. SEM observation confirmed that debris was removed from the surface of breast skin after treatment with either CPC or NaOCl, relative to untreated skin samples, showing that ultrasonication with a WRS was able to detach bacteria effectively.

The results of the present study suggest that the use of sanitizers employing a combination of vacuum and ultrasonication with a WRS is highly effective for reducing the count of campylobacters on chicken carcasses.