Food Control 67 (2016) 1–8

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effects of combined organic acid treatments during the cutting process on the natural microflora and quality of chicken drumsticks

Yuanting Zhu [a], Xiaolong Xia [a], Aiping Liu [a], Likou Zou [b], Kang Zhou [a], Xinfeng Han [a], Guoquan Han [a], Shuliang Liu [a, *]

[a] College of Food Science, Sichuan Agricultural University, Ya'an, Sichuan 625014, PR China
[b] The Laboratory of Microbiology, Dujiangyan Campus, Sichuan Agricultural University, Dujiangyan, Sichuan 611830, PR China

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form
18 February 2016
Accepted 19 February 2016
Available online 23 February 2016

Keywords:
Chicken drumsticks
Organic acid
Natural microflora
Decontamination
PCR-DGGE
Sensory evaluation

ABSTRACT

Reducing the microbial load on broiler chicken carcasses at each stage of poultry meat processing, is highly important for hygienic meat production. This study was conducted to assess the efficacy of various combined organic acid treatments during the cutting process on the microbial decontamination of chicken drumsticks. Changes in naturally occurring microflora before and after treatment were analyzed through microbiological counting and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE). Results revealed that the most effective treatments obtained through the combination of orthogonal design and sensory evaluation were as follows: 0.5% lactic acid (w/v), 1% citric acid (w/v), and spray-washing for 30 s. Microbiological counting results and PCR-DGGE analysis indicated that the microbial load on the chicken drumsticks decreased significantly after the treatment was administered. The treatment did not affect the physicochemical properties and sensory attributes of the quick-frozen chicken drumsticks during storage. Therefore, the proposed technique could be used to improve the microbial quality of chicken drumsticks. The technique could also be employed in poultry meat production chains.

1. Introduction

A significant increase in global poultry meat consumption has been observed. This increase is likely to continue into the future (Henchion, McCarthy, Resconi, & Troy, 2014). During the conversion of chicken into meat for consumption, broiler carcasses are at a high risk of contamination from fecal soiling on feathers and skin during slaughtering, intestinal content leakage during evisceration, and processing equipment and general processing environments (Owens, Alvarado, & Sams, 2010). Therefore, poultry meat can become contaminated with various food-borne pathogens, including *Salmonella*, *Campylobacter* spp., *Listeria monocytogenes*, *Clostridium perfringens*, and *Staphylococcus aureus* (Bohaychuk et al., 2006; Ternström & Molin, 1987). Various interventions have been implemented to reduce the number of food-borne pathogens on surfaces of broiler carcasses (Bolton, Meredith, Walsh, & McDowell, 2014; Koolman, Whyte, Meade, Lyng, & Bolton, 2014a, 2014b; Musavian, Krebs, Nonboe, Corry, & Purnell, 2014). Furthermore, manufacturers should reduce or eliminate carcass contamination of psychrotrophic spoilage bacteria to ensure adequate shelf life of raw chilled products (Owens et al., 2010).

Hazard analysis and critical control point (HACCP) systems have been widely used in poultry meat industries to minimize product contamination (Tompkin, 1994). HACCP includes critical control points (CCP) at which an intervention may be implemented to prevent, reduce, or eliminate microbial contamination. Decontamination is a bactericidal treatment applied to reduce pathogenic and spoilage organisms (Bolder, 1997). Chemical treatments can be used to eliminate the presence of pathogens and spoilage microorganisms and thus may provide a basis of an effective CCP intervention (Acuff, 2005). The United States Food and Drug Administration has approved the use of a number of chemicals as decontaminants of poultry meat. Several organic acids, such as lactic acid and acetic acid, have been generally recognized as safe substances for use in poultry processing plants. These acids could inhibit subsequent microbial growth and thus extend shelf life.

The efficiency of organic acid solutions in reducing artificially

* Corresponding author.
E-mail address: lsliang999@163.com (S. Liu).

http://dx.doi.org/10.1016/j.foodcont.2016.02.031
0956-7135/

inoculated pathogens has been extensively investigated (Koolman et al., 2014a, 2014b). Few have been evaluated against naturally occurring microflora on carcasses after treatment. Muyzer, De Waal, & Uitterlinden (1993) proposed denaturing gradient gel electrophoresis (DGGE) based on the separation of PCR amplicons with the same size but with different 16S rDNA sequences; this technique can be used to overcome the limitations of culture-dependent techniques, to reveal microbial communities, and to analyze microbial diversity. As a well-established tool, this technique has also been applied to numerous fields. For instance, Li, Zhou, Xu, Li, and Zhu (2006) employed PCR-DGGE to investigate the bacterial diversity and the main flora in chilled pork.

Cutting is the last step before broiler chicken products are quickly frozen. Therefore, the microbial decontamination efficacy in this process has an immediate influence on the quality of chicken products. This study aimed to evaluate the efficacy of microbial decontamination treatment during the cutting process in the slaughtering and processing chains of poultry meat. Combined organic acid treatment conditions for chicken drumsticks were optimized. Changes in naturally occurring bacterial community on chicken drumsticks before and after treatment were analyzed through PCR-DGGE. The effects of the optimum treatment on the physiochemical properties and sensory attributes of chicken drumsticks were also investigated. This study may provide a basis of chicken slaughtering and processing methods.

2. Materials and methods

2.1. Sampling

2.1.1. Sampling of chicken drumstick surface for microbial analyses

Chicken drumstick samples were obtained in three stages, namely, earlier, middle, and later stages, of cutting process in the broiler cutting workshop at a local chicken slaughtering plant where about 30,000,000 broiler chickens are processed each year. The chicken drumstick surfaces were sampled in accordance with previously described methods (Gill, Badoni, Moza, Barbut, & Griffiths, 2005). Briefly, the surface covering 25 cm^2 of each chicken drumstick sample was swabbed by using sterilized cotton swabs moistened with 0.1% peptone water. The swabs obtained after sampling were placed in stomacher bags and stored in an ice bath during transportation to our laboratory, and were determined within 3 h. The samples were collected for control and treated groups.

2.1.2. Sampling of quick-frozen chicken drumsticks for pathogen detection

For each sample from control and treated groups, 25 g of thawed chicken drumsticks was incised, collected in a sterile plastic stomacher bag containing 225 mL of sterile saline water, and pummeled for 2 min. A 1-mL aliquot of each homogenate from control and treated drumsticks was subjected to enrichment for detection of *S. aureus* and *Salmonella*.

2.1.3. Sampling of quick-frozen chicken drumsticks for physicochemical and sensory indexes evaluation during storage

The quick-frozen chicken drumstick samples, vacuum-packaged and stored at −18 °C in a freezer, were collected on days 5, 10, 15, 20, 30, 40, 50, and 60, and then subjected to physicochemical index analysis and sensory evaluation. The samples were collected for both control and treated groups.

2.2. Optimization of combined organic acid-based spray washing during cutting to reduce microbial load on chicken drumsticks

The optimal conditions for combined organic acid-based spray washing were investigated by using an orthogonal experimental design [$L_{16}(4^5)$], and the effects of lactic - citric acid concentrations [food-grade, Jindan Company (Henan, China)], and spray-washing time were analyzed. Table 1 lists the factors and levels of the tests. After spray washing was administered, total viable counts (TVC) of chicken drumsticks in treated groups and control groups were performed. The control group received no treatment. In addition, the sensory attributes of chicken drumsticks in the treated groups were evaluated. The most promising treatments were obtained on the basis of both decontamination effects and sensory evaluation.

2.3. Microbiological analysis

The cotton swabs in stomacher bags as described in Section 2.1.1 were immersed for 30 min with sterile saline solution. Serial ten-fold dilutions were then prepared and plated onto the appropriate media. All media used in the microbiological experiments were purchased from Hangzhou Microbial Reagent Co., Ltd. (Hangzhou, China).

For enumeration, the results were reported as the logarithm of colony forming units (cfu) per cm^2 (lg cfu/cm^2). TVC were performed using the pour-plate method with a plate count agar (PCA) referring to the National Standard of China (GB 4789.2-2010). Coliform bacteria were enumerated via a violet red bile agar (VRBA) method, and a minimum of 10 representative colonies from VRBA plates were inoculated into brilliant green lactose bile (BGLB) and incubated to confirm the coliforms (GB 4789.3-2010). Intestinal enterococci were cultivated at 35 ± 2 °C for 24 h (SN/T, 1933.1-2007). Enumeration of *Pseudomonas* spp. was performed on cetrimide fucidin cephaloridine (CFC) agar plate and incubated at 25 °C for 48 h in accordance with the ISO 13720: Meat and meat product enumeration of *Pseudomonas* spp (Talon et al., 2007).

The prevalence of *Salmonella* was performed according to the National Standard of China (GB 4789.4-2010). 1 mL of homogenate in Section 2.1.2 was inoculated into BPW solution (buffered peptone water, 2%), and cultured at 37 °C for 24 h and the presence of *Salmonella* was confirmed through selective enrichment and further PCR assays of presumptive colonies based on specific virulence genes (*inv* A and *hut* gene) of *Salmonella*. Occurrence of *S. aureus* was detected by surface plating on Baird−Parke Agar plate and incubated at 37 °C for 45−48 h in accordance with the methods described in Chinese standards (GB 4789.10-2010). The prevalence of *Salmonella* and *S. aureus* in quick-frozen drumsticks after the treatment were calculated by dividing the total number of samples by the number of positive samples.

2.4. PCR-DGGE analysis

2.4.1. DNA extraction

The cotton swabs after sampling as described in Section 2.1.1

Table 1
Factors and levels of orthogonal test [$L_{16}(4^5)$].

Factors	Levels			
	1	2	3	4
A Lactic acid concentration (w/v, %)	0.5	1.0	1.5	2.0
B Citric acid concentration (w/v, %)	0.5	1.0	1.5	2.0
C Spray washing time (s)	15	30	45	60