LWT - Food Science and Technology 87 (2018) 464–469

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Enhancing the bactericidal efficacy of lactic acid against Salmonella *typhimurium* attached to chicken skin by sodium dodecyl sulphate addition

Hussein M.H. Mohamed, Heba H.S. Abdel-Naeem*

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt

ARTICLE INFO

Article history:
Received 16 April 2017
Received in revised form
13 September 2017
Accepted 15 September 2017
Available online 18 September 2017

Keywords:
SDS
Lactic acid
Salmonella *typhimurium*
Chicken skin
SEM

ABSTRACT

The main objective of the current study was to investigate the mechanism by which sodium dodecyl sulphate (SDS) can enhance the bactericidal efficiency of organic acids through revealing the topographical changes of the skin follicles after treatment with SDS and organic acids. Cell suspension of S. *typhimurium* was prepared, attached into the chicken skin and treated by SDS (10 g/kg), lactic acid (LA; 10 and 20 g/kg) and combinations between SDS (10 g/kg) and LA (20 g/kg) for 3 min. Both control and treated chicken skin were exposed to bacterial counting and ultrastructural examination using SEM. Treatment of S. *typhimurium* attached to skin attachment model (SAM) with SDS 10 g/kg, LA 10 g/kg and LA 20 g/kg for 3 min resulted in 0.3 log, 1 log and 3.3 log cfu/cm^2 reduction in the count of microorganism, respectively. A synergistic inactivation of microorganism has been obtained by combining SDS 10 g/kg with LA 20 g/kg. The topographical structure of SAM treated with SDS and with combination between LA and SDS showed open feather follicles. Therefore, SDS enhanced the bactericidal effect of organic acids by opening the follicles and allowing their direct contact with the embedded microorganism with subsequent higher reduction rates of the bacterial populations.

1. Introduction

Chicken carcasses can be contaminated through many sources from which chicken skin was considered to be the highest source that is difficult to be controlled due to its presence in the outer layer which makes it easily to come in contact with different contamination sources. Chicken skin contains microfolds, feather follicles and microcracks that facilitate bacterial attachment and its colonization (Lecompte, Collignan, Sarter, Cardinale, & Kondjoyan, 2009). Salmonella is one of the most important microorganisms that can persist on chicken skin during poultry processing because of their ability to attach to skin and be captured in deeper skin layers, crevices, or feather follicles (Lillard, 1986). These sites may provide a suitable microenvironment for bacteria to lodge (Chantarapanont, Berrang, & Frank, 2003) and create a physical defense against antimicrobial agents (Chantarapanont, Berrang, & Frank, 2004; Yang, Li, & Johnson, 2001). The attached bacteria can lodge deep in these sites and become difficult to be removed (Notermans & Kampelmacher, 1974). This fact recommends a need for potential carcass treatments that will reduce or eliminate Salmonellae which attached to chicken skin.

The levels of Salmonellae attached to poultry carcasses can be reduced by using different types of decontamination methods, however, an effective, gentle and inexpensive method that will not adversely affect the chicken quality should be used (Huffman, 2002). One of these methods is application of organic acids which, characterized by their antimicrobial efficiency and application simplicity (Cosansu & Ayhan, 2010; Sumarmono & Rahardjo, 2008). Moreover, organic acids are generally recognized as safe substances (GRAS) and approved as food preservatives by European committee, FAO/WHO and FDA (Surekha & Reddy, 2000). The effective application of these acids against Salmonellae attached to chicken skin requires high concentrations which, adversely affect the carcass appearance and result in bleaching of the skin. Lower concentrations of these acids are ineffective due to the topographical structure of chicken skin and its high lipid content which

* Corresponding author. Current address: Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Beside Students Hospitals, Giza Square, Giza 12211, Egypt.
E-mail addresses: h.hussein@cu.edu.eg, dr_hoba.h106@yahoo.com (H.H.S. Abdel-Naeem).

http://dx.doi.org/10.1016/j.lwt.2017.09.022
0023-6438/

provide protective elements for microorganisms (Tamblyn & Conner, 1997). Therefore, accelerating organic acid delivery to the attached bacterial cell is important for improving their bactericidal efficacy with lower concentrations. This may be achieved by using surfactants which have the ability to change cell membranes permeability of the skin. Consequently, they can enhance the skin penetration power of other compounds used in the formulation (Lopez, Llinares, Cortell, & Herraez, 2000).

Sodium dodecyl sulfate (SDS) is one of the transdermal surfactant, which is generally recognized as safe (GRAS) (FDA, 2007) and could be applied on wide variety of foods as additive. It has the ability to denature proteins and damage cell membranes, and its effectiveness increases with lowering the pH (Zhao, Zhao, & Doyle, 2009). It has been proposed that SDS increases the surface tension of the skin with subsequent opening of the skin follicle and allowing the contact of the acid with the microorganism. Combining SDS with lower concentrations of organic acids has been applied previously with synergistic inactivation of Salmonella attached to chicken skin has been observed (Zaki, Mohamed, & El-Sherif, 2015; Zhao et al., 2009). In a previous study conducted by. Zaki et al. (2015), Salmonella *enteric*a Kentucky attached to SAM has been treated by dipping in organic acids, SDS and their combinations and the results revealed that the highest bactericidal efficacy was obtained when lactic acid was combined with SDS. The most effective inactivation of the organism was achieved when the concentrations of organic acids and SDS were in the ratio of 2:1. Moreover, the sensory characteristics of chicken drumsticks treated with organic acids and SDS were satisfactory. To the best of our knowledge, there is no any previous study revealed the chicken skin follicles inoculated with Salmonella after treatment with organic acid or SDS. Therefore, the main goal of the current study was to reveal the topographical structure of skin follicle inoculated with Salmonella *typhimurium* after treatment with SDS, lactic acid and their combination. To achieve this goal an advance technology using scanning electron microscope has been used to reveal the inoculated and treated follicles.

2. Materials and methods

2.1. Preparation of S. typhimurium cell suspension

S. *typhimurium* strain was obtained from the culture collection of the department of microbiology at the faculty of veterinary Medicine, Cairo University. Cultures were kept at −18 °C in brain heart infusion (BHI, LAB M, 49) containing 100 g/kg glycerol (Sigma Aldrich, G5516) until use. Stock culture of the microorganism was propagated in tryptic soy broth (Oxoid, CM 129) twice at 37 °C for 24 h before use. The culture was harvested by centrifugation at 7600 × g and 4 °C for 15 min and then washed several times with sterile peptone water (1 g/kg). Finally, the resulting cell pellet was re-suspended in the peptone water.

2.2. Treatment solutions

Sodium Dodecyl Sulfate (SDS) and Lactic acid (LA) were obtained from Sigma Aldrich (St.Louis, MO) and used in this experiment. Preliminary experiments have been conducted to study the effect of different concentrations of SDS and lactic acid on the sensory characteristics (color, odor and taste) of drumsticks and the chosen concentrations in this study have been proved to give satisfactory sensory attributes and do not attack chicken skin.

2.3. Sensory evaluation of drumsticks treated with SDS and lactic acid

Fresh drumsticks were obtained from local retail market and divided in 5 groups where each group was treated with one of the following solutions: distilled water (washing control), SDS (10 g/kg), LA (10 g/kg), LA (20 g/kg) and combination of SDS (10 g/kg) and LA (20 g/kg) for 3 min. Treated drumsticks were sensory evaluated immediately after treatment. Sensory evaluation was conducted on raw treated drumstick or drumsticks cooked in a forced draught oven at 220° C for 30 min according to the scheme of Baston and Barna (2010). Nine experienced panelists from both sexes in the age range of 25–40 years old from the staff members of the Department of Food Hygiene and Control at Faculty of Veterinary Medicine, Cairo University, Egypt were selected on the basis of previous experience in consuming dressed chicken. The panelists evaluated treated raw drumsticks in a randomized order and asked to assign a numerical value between 1 and 7 for the color and odor where 1 is very poor (I dislike it very much) and 7 is excellent (I like it very much). After cooking, the panelists were asked to assign the same numerical values for color where 1 (very poor) and 7 (excellent) as well as Flavor where 1 (imperceptible) and 7 (extremely intense). Tap water was provided between samples to cleanse the palate.

2.4. Preparation of skin attachment model (SAM)

Fresh chicken breast skin was obtained from a local dressed chicken plant. The skin was washed several times with sterile distilled water and then cut into 2 × 5 cm pieces then the skin was rewashed again with sterile distilled water as a tool for decreasing the chance of contamination. Washed skin was exposed to UV light under U.V. cabinet (Cole-Parmer 9818 Series-Darkroom) at 356 nm for 2.5 h to remove the attached micro-flora. Samples were taken from SAM and examined for presence of Salmonella spp. in addition to perform enumeration of aerobic mesophilic bacteria. Prepared SAM was free from Salmonella spp. and the aerobic mesophilic bacterial count was under the detectable limit of spread plating (2 log cfu/cm^2).

2.5. Inoculation of SAM with S. typhimurium

The culture of S. *typhimurium* were inoculated into 10 cm^2 SAM by immersion in the previously prepared cell suspension containing 9.35 log cfu/mL for 20 min. The SAM were removed from the cell suspension and left for 20 min under aseptic condition at room temperature to allow the attachment of S. *typhimurium* into skin. The final inoculation level of S. *typhimurium* on SAM was 8.41 log cfu/cm^2.

2.6. Washing tests of SAM

Four treatment solutions were prepared from SDS and lactic acid. The first one was containing SDS at concentration of 10 g/kg, the second and third ones were containing lactic acid at concentrations of 10 g/kg and 20 g/kg, respectively and the fourth one was containing combinations of SDS 10 g/kg and lactic acid 20 g/kg. Each inoculated SAM was washed with 100 mL of one of the washing solutions for 3 min in a sterile stomacher bag (12 × 20 cm). An inoculated SAM was placed in bags containing 100 mL sterile distilled water and used as control; moreover, a negative control of prepared SAM was tested without washing. The treated SAM was homogenized in a stomacher bag containing 90 mL sterile peptone water (1 g/kg) using stomacher (Lab blender 400, Seward lab. Model No. AB 6021) at high speed for 2 min. Homogenates were