J. Fd Hyg. Safety 11(1), 7-10(1996)

Grapefruit Seed Extract (DF-100) Treatment of Poultry to Reduce Attached *Salmonella*

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ABSTRACT—Chicken skins or carcasses inoculated with *Salmonella typhimurium* were exposed to 0.1 or 0.5% grapefruit seed extracts (DF-100) for 1 or 3 min to evaluate antibacterial activity of DF-100 and its possible application in proultry processing. The numbers of live salmonellae on chicken skins were reduced by 0.8-1.2 logs/cm^2 with 0.1% and by 1.6-1.7 logs/cm^2 with 0.5% DF-100. Dipping chicken carcasses into 0.5% DF-100 for 3 min reduced salmonellae by 4.3 logs/carcass. Scanning electron microscopy showed that DF-100 killed the cells attached but did not detach cells from the skin. No odor or changes in the color of chicken skin were detected after DF-100 treatment.

Key words □ Grapefruit seed extract, *Salmonella*, Poultry.

INTRODUTION

Grapefruit seed extract has been reported to have antimicrobial activities against a wide variety of fungi and bacteria.[1,2] Although the exact mechanisms of antimicrobial activity have not been elucidated, grapefruit seed extract has been evaluated for storage of various fruits and vegetables as a natural antimicrobial agent.[1] In U.S., foodborne illness caused by salmonellae infection is estimated 1~5 millions annually, and an important portion of these salmonelloses was traced to consumption of contaminated poultry.[6] Thus, numerous studies have been conducted to reduce the contamination level of salmonellae on poultry; however, there are still demands for better and safer methodologies directly applicable to poultry processing.[3]

The purpose of this study was to measure the efficacy of grapefruit seed extract (DF-100) for the reduction of *Salmonella* on chicken skins or on whole chicken carcasses for future application in poultry processing.

MATERIALS AND METHODS

Grapefruit seed extract

Grapefruit seed extract (DF-100, pH 2.75) produced by Chemie Research & Manufacturing Co., Inc. (Casselberry, FL) was diluted with deionized water to 0.1 or 0.5% (v/v), and used for treating chicken skins or chicken carcasses.

DF-100 is a very viscous, lemon-yellow colored liquid having specific gravity 1.18, pH 2.75, and flash point 144.4°C. DF-100 is composed of 16.5% ascorbic acid, 2.0% pro'ein, 1.0% fat, and 39.6% nitrogen free extract dissolved in glycerine (30%) and water (10%). DF-100 also contained 0.5% mineral ash and 0.4% fibers (personal communication).

Determination of bactericidal activity of DF-100

Salmonella typhimurium (ATCC) and *Escherichia coli* 0157:H7 (USDA, FSIS 45956) cultured in brain heart infusion broth (Difco, Detroit, MI) for 20 h at 37°C were washed and suspended in various concentrations (0.5, 0.1, 0.005, 0.01, 0.001%) of DF-100 solutions for 1 min. Serial dilutions were made immediately and plated on tryptic soy agar (TSA, Difco). Cells were counted after 24 h incubation at 37°C. This trial was repeated three times.

Exposure of chicken skin surface to DF-100

Breast skins were cut from pre-chill chicken carcasses,

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placed on skin holder, and the skin surfaces (10 cm^2/piece) were inoculated with S. *typhimurium* (10^8 CFU/m*l*) for 30 min. After rinsing with deionized water to remove unattached cells, each skin surface was exposed to 5 m*l* of 0.1% or 0.5% DF-100 solution for 1 or 3 min. Four skin pieces were used for each treatment. After rinsing with water, each skin piece was stomached with buffered peptone water (BPW, Difco), and serial dilutions were plated on TSA and xylose lysine desoxycholate agar (XLD, Difco). This trial was repeated.

Scanning electron microscopy

After exposing skin pieces to DF-100 solutions as above, skin samples were fixed by dipping into Karnovsky's fixative. They were dehydrated with graded ethanols (30, 50, 70, 80, 95%, and 3 changes of 100%) for 10 min each, washed with hexamethyldisilazane (Electron Microscopy Sciences, Fort Washington, PA) for 5 min 3 times, and sputter-coated with gold. Skin surfaces were examined by using ISI-60 scanning electron microscope (International Scientific Instruments, Japan) at 30 kV.

Dipping chicken carcasses into 0.5% DF-100 solutions

Twelve pre-chill chicken carcasses were inoculated with *S. typhimurium* (10^5 CFU/m*l*) by spraying on breast and back areas, incubated for 30 min at room temperature, and rinsed with water to remove unattached cells. Six carcasses were dipped in 16 L of 0.5% DF-100 solution (15~16°C) for 3 min. Control carcasses were dipped in plain tap water for 3 min. Then, each carcass was rinsed again to remove residual DF-100 on the carcass, bagged individually, mechanically shaken with 100 m*l* BPW. Salmonellae was cultured using conventional method, and the number of salmonellae on each carcass was enumerated by using most probable number technique[4, 5]. This trial was repeated.

RESULTS AND DISCUSSION

Before applying DF-100 on poultry, pure cultures of S. *typhimurium* and *E. coli* 0157:H7 were exposed to various concentrations of DF-100 to measure the reduction levels obtained in a short period of time (Table 1). Up to 7 log (99.99999%) reduction was achieved at 0.5% on *S. typhimurium*. Even though 0.01-0.001% DF-100 reduced salmonellae by 50~60% in 1 min, DF-100 at 0.1% and 0.5% were chosen to apply on poultry skins and carcasses, because cells attached to poultry skins are much more resistant to antimicrobial agents than suspended cells.

Table 1. Reduction of *Salmonella typhimurium* in DF-100 solution for 1 min.

DF-100 Conc.(%)	pH	*Salmonella* Reduction (log CFU/ml/min)
0.50	4.24	7.0
0.10	4.98	4.9
0.05	5.21	2.9
0.01	5.70	0.3
0.001	6.21	0.4

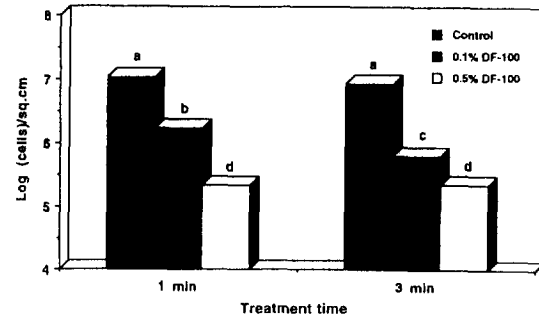

Fig. 1. Reduction of *salmonellae* attached to chicken skin by DF-100 treatment. Bars with different letters are significantly different (P<0.05).

Compared to controls, chicken skins treated with DF-100 had 0.8-1.2 log lower number of salmonellae at 0.1% and 1.6~1.7 log lower at 0.5%, respectively (Fig. 1). At 0.1% DF-100, the number of salmonellae decreased as the exposure time increased; however, the effect of exposure time was not clear ($p>0.05$) at 0.5% DF-100 since the reduction levels at 3 min were not higher than reduction levels at 1 min.

After DF-100 treatment, skin surfaces were examined to monitor any changes caused by DF-100. No difference was noticed between control and DF-100 treated skins. When the cells were directly counted from the skin surfaces using SEM, there were no significant differences ($p>0.05$) in cell counts and their morphologies among control and treatment groups (Fig. 2). This observation suggests that DF-100

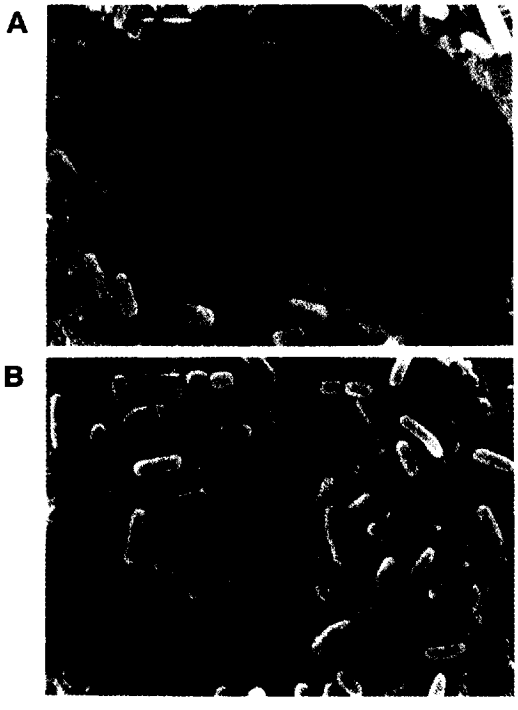

Fig. 2. Scanning electron micrographs of chicken skin inoculated with *S. typhimurium* after DF-100 treatment. A: Control, B: Skin treated with 0.5% DF-100.

causes metabolic damage to kill the cells and does not detach dead cells from skin surface.

By dipping chicken carcasses into 0.5% DF-100 solution for 3 min, more than 4.5 log reduction (>99.997%) of salmonellae was achieved per carcass (Fig. 3). It is an encouraging result since most chemicals or antimicrobial agents approved for poultry processing give about 2 log reductions at most. In addition, no odor from the carcass or visible changes in skin color were noticed after DF-100 treatment. The reason why the trials with skin pieces gave much lower reduction (<2 logs) than the trials with chicken carcasses (<4 logs) is perhaps the amount of active bactericidal ingredients per unit area of skin was much smaller in skin piece trials.

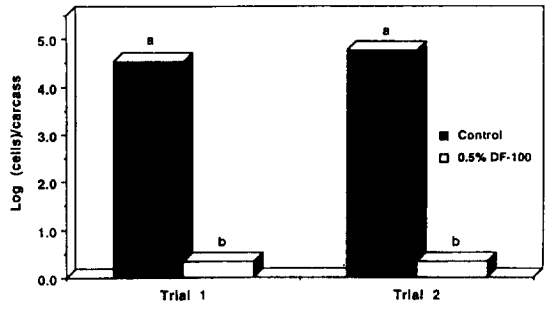

Fig. 3. Reduction of salmonellae on chicken carcasses by dipping into 0.5% DF-100 solution for 3 min. Each value is the average of 6 measurements, and the bars with different letters are significantly different (P<0.05).

The data for DF-100 treatment on chicken carcasses are promising and future studies need to be done to determine applications of DF-100 in poultry processing to reduce salmonellae level.

국문요약

Grapefruit 종자 추출물인 DF-100을 가금류 가공에 이용하여 가공된 닭의 세균오염을 줄이고자 이의 salmonellae에 대한 한균성을 측정 하였다. *Salmonella typhimurium*을 접종한 닭표피에 0.1% 또는 0.5%의 DF-100을 1분 또는 3분간 처리했을 때 0.1%에서는 salmonellae의 수가 0.8~1.2 logs/cm^2, 0.5%에서는 1.6~1.7 logs/cm^{2}만큼 줄었다. 닭 전체를 0.5% DF-100에 3분간 침지했을 때, salmonellae의 수는 4.3 logs/마리 만큼 감소하였다. Scanning electron microscopy로 DF-100를 처리한 닭표피를 관찰하였을 때, 죽은 salmonellae들이 대조수와 비교하여 큰 차이가 없이 그대로 붙어 있었다. DF-100는 닭 또는 닭표피에 어떤 냄새나 색깔변화를 일으키지 않았다.

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