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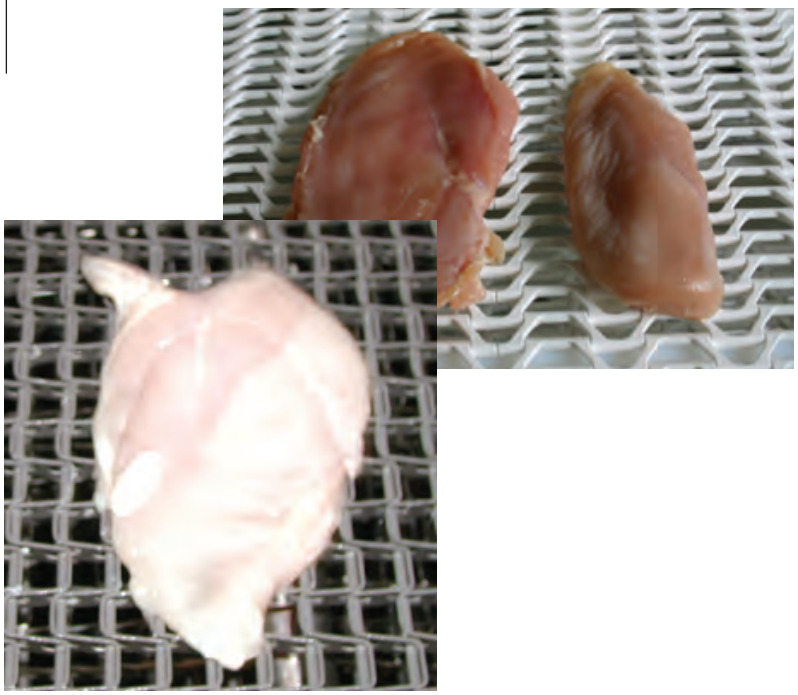

Efficacy of Quaternary Ammonium Compounds on Different Conveyor Chips Contaminated with Poultry Rinsate

ABSTRACT

The use of cleaning agents and sanitizers in routine sanitation programs to ensure food safety is necessary in the poultry industry to prevent contamination of food products with pathogens and to reduce quality problems such as spoilage. This study evaluated the efficacy of quaternary ammonium compounds (QAC) on different types of conveyor belt materials contaminated with poultry rinsate. Six different types and designs of processing conveyor belts were used in the experiment: (1) canvas (polyurethane with mono-polyester fabric <1% mesh), (2) acetal 3.2% mesh, (3) polypropylene 48% mesh, (4) polypropylene meshtop 24% mesh, (5) stainless steel-single loop 80% mesh, and (6) stainless steel-balance weave 70% mesh. Commercial conveyor belts that had never been used were cut into chips so as to include interlocking joints, after which the chips were cleaned and sanitized before being exposed to contaminated poultry rinsate for 1 h. The contaminated chips were sprayed with 200 ppm QAC and allowed to stand for 10 min prior to bacterial enumerations (aerobic mesophiles and coliforms). In general, the QAC was more effective against mesophilic bacteria on stainless steel chips (3 log CFU/cm^2 reduction) than on canvas, acetal, polypropylene, and polypropylene materials (1.5 to 2.3 log CFU/cm^2 reduction). Similarly, the sanitizer reduced the coliform counts to undetected levels (>2 log CFU/cm^2) in all the conveyor chips except those of the canvas material. The findings indicate that QAC efficacy against mesophilic and coliform bacteria is influenced by the design and type of materials, with the highest efficacy observed on stainless steel surfaces.

INTRODUCTION

Sanitation is one of the most effective preventive measures in poultry processing for ensuring product shelf life and safety. Inadequate sanitation may lead to contamination

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by spoilage or pathogenic microorganisms due to the demonstrated ability of bacteria to attach to poultry processing equipment made from stainless steel, rubber and polymers *(3, 11, 14, 19, 21, 25)*. In poultry processing, most conveyor belts are difficult and time consuming to dismantle for cleaning, and the complex structure of processing machines makes cleaning difficult. Similarly, routine sanitation procedures may be unsuccessful in eradicating pathogenic bacteria that can remain viable even on dry surface environments, necessitating an effective sanitation method *(13, 14, 29)*.

The use of sanitizing agents reduces the incidence of spoilage and pathogenic bacteria on product contact surfaces. Quaternary ammonium compounds (QAC), which are USDA approved and EPA approved sanitizers for food processing equipment and utensils, are highly used in the poultry industry because of their long history of successful use and effectiveness when used in accordance with their labels as directed in FIRFRA (Federal Insecticide, Fungicide, and Rodenticide Act) *(27)*. Quaternary ammonium compounds are cationic surfactants (or detergents) and antimicrobials used for numerous industrial purposes, including cleaning and disinfecting. Many studies have shown their effectiveness against *Salmonella* and *Listeria*, but efficacy can be affected by various factors such as time, temperature, concentration, and presence of organic matter *(5, 17, 20, 24, 26, 27)*. The antimicrobial activity of QAC primarily involves interactions with cell membranes, disruption of membrane integrity, leakage of cellular contents and eventually cell death *(6)*.

An equally important but often overlooked aspect of sanitary equipment manufacturing and application is the design and type of conveyor belts. Even though the American Meat Institute has published the Ten Principles of Sanitary Equipment Design *(1)*, which focus attention to this issue for processors, many conveyor belts and pieces of equipment are still difficult to clean and sanitize. One of the most important problems with improper sanitation is the development of biofilms on equipment surfaces and in niches. Biofilms, resulting from a self-protecting growth pattern of bacteria, may contain spoilage and pathogenic organisms that can cause post-processing contamination and pose a risk to food safety because the cells in a biofilm are unusually resistant to cleaning or disinfection *(23)*. Studies have demonstrated the attachment ability of *Listeria* and *Salmonella* to conveyor belts and formation of biofilms over time *(19, 30)*. Few studies, however, have been done to evaluate the efficacy of QAC in relation to the design and type of surface materials. In this study, six different types and designs of conveyor belts contaminated with poultry rinsate were treated with QAC and the microbial reduction was determined.

MATERIALS AND METHODS

Preparation of test surfaces

The test surfaces used in this study were conveyor belts of different designs and made from different materials. Portions of new conveyor belts were cut into pieces of varying dimensions, including the interlocking part, to ensure uniformity of area across the different conveyor belts *(Table 1)*. The dimensions were later standardized to per-square-cm surface area. The test surfaces, referred to as

TABLE 1. Materials, design and specifications of conveyor belt/chips used in the study[1]

Materials/Design	Design-% Open Mesh	Dimension (cm)
Canvas[2]	0	5.0 x 4.0
Acetal	3.2	5.0 x 5.5
Polypropylene-meshtop	24	4.5 x 5.0
Polypropylene	48	6.5 x 5.0
Stainless steel-Single loop	80	8.7 x 2.7
Stainless steel-Balance weave	70	6.0 x 6.0

[1]All belts were manufactured by Wire Belt Company of America, Londonderry, NH; belts were cut with a height dimension of 1 cm.

[2]Polyurethane with mono polyester fabric.

chips, were washed in 1% Micro cleaning solution (International Products Corp., Burlington, NJ), rinsed with distilled water, and sonicated for 30 min as described by Arnold and Silvers *(4)* prior to use.

Poultry rinse and bacterial inoculation

Raw chicken fillets (skin-off) were purchased from a local grocery store and refrigerated prior to preparation. To prepare the poultry rinse, 2 kg of fillets were placed in a stomacher bag to which was added 400 mL of phosphate-buffered saline (PBS, 0.01M, pH 7.2) with Tween 20 (pH 7.4, 0.01M, Sigma Aldrich), and rinsed by shaking ten times. All poultry rinsates were combined and served as the inoculum. The experiment was performed in 3 trials, with 2 replications per trial. Two chips per treatment were immersed in 100 mL poultry rinsate and incubated at 10°C for 1 h to allow bacterial adhesion. Fresh poultry rinsate was used in each treatment. After 1 h exposure to rinstate, the chips were removed. One contaminated chip was analyzed for aerobic mesophile and coliform counts, and the other chip was treated with sanitizer prior to bacterial enumeration.

Application of sanitizer and bacterial enumeration

Inoculated chips from the exposure step were exposed to sanitizer by spraying them with 200 ppm of Ala-Quat™ (Birko, Henderson, CO), using a common pressurized pesticide hand sprayer (0.5 ml of sanitizer/pump). The chips were then allowed to air dry for 10 min per manufacturer's instructions to allow time for the sanitizer to become effective. Each chip was placed individually into a petri dish containing 5.0 mL of PBS (0.01 M, pH 7.2) with Tween 20 (pH 7.4, 0.01M) and manually shaken in clockwise and counter-clockwise directions, ten times each, to remove unattached bacteria. Aerobic and coliform bacteria were dislodged by putting the test chip into a sterile beaker containing 5.0 g of glass beads and 25 mL of PBS with Tween 80 (0.1%), covered with parafilm and vortexed (Fisher Scientific, Touch mixer, Model 231) for 30 s as described by Anwar et al. *(2)* and Denes et al. *(8)*. Bacteria was enumerated with an AUTOPLATER (Spiral Biotech 4000, Advanced Instruments Inc., MA). Tryptic Soy Agar (TSA) and Violet Red Bile Agar (VRBA) plates were used to enumerate the total aerobic bacteria and coliforms, respectively. Colonies were counted using Q-count™ (Spiral Biotech, Advanced Instruments Inc., MA) software, and results were recorded.

Statistical analysis

The experiments were replicated in 3 trials (n = 3) and the means calculated. The thickness and mesh size of the test materials varied, which affected the total surface area of the test surfaces. This variation was taken into account, and the results reported are normalized to per-square-cm surface area *(Table 1)*. Data were analyzed by a one-way analysis of variance (ANOVA) for treatments that showed an interaction between chip and replication time. Means were separated using Duncan's multiple range tests of the Statistical Analysis System software (SAS Institute Inc., Cary, NC). A significance level of $P < 0.05$ was employed.

RESULTS AND DISCUSSION

Poultry processing conveyor belts are often exposed to mixed populations of bacteria and are potential surfaces for adherence of spoilage and pathogenic microorganisms. These bacteria can attach and behave differently. A mixed microbial population of bacteria, including pathogens, were reported to adhere and form biofilms on contact with food *(3, 4, 7, 21)*, making the cleaning and sanitation regimen a challenge to food processors. In this study, commercially available QAC was used to sanitize contaminated conveyor chips. Results indicated that all the contaminated test chips had the same initial bacterial population (mesophiles), ranging from 3.27 to 3.55 logs CFU/cm^2, following the 1 h dip in poultry rinsate *(Table 2)*. After 10 min exposure to QAC, there was a 1.5 to 2.3 log reduction in the bacterial population for canvas, acetal, polypropylene-meshtop and polypropylene test chips. A greater ($P < 0.05$) reduction, 3 logs or to an undetected level of mesophilic bacteria, was observed on the stainless steel chips, indicating greater efficacy of QAC. Both of the stainless steel chips used in this study are smoother than the polymeric materials (canvas, acetal and polypropylene). In a similar study, Yang et al. *(31)* found that sanitizer efficacies were greater on smoother surfaces of high-density polyethylene coupons. Likewise, Frank and Chmielewski *(8)* reported that polished stainless steels were more readily sanitized by QAC and chlorine than polycarbonate and mineral resin were. All findings from these studies lead to the conclusion that chemical sanitizers work better on smoother surfaces than on rough or abraded materials.

Quaternary ammonium compounds are effective sanitizers that act primarily by disrupting the permeability of the cytoplasmic membrane, leading to cell death *(16, 27)*. However, the sanitizer must reach the bacteria before it can be efficacious. Previous research has indicated that different conveyor belt materials may allow bacteria *(19, 30)* to harbor in crevices or pores so that they are not affected by the sanitizer. Results of our study indicate that QAC efficacy is somewhat affected by other factors, such as the type and makeup of the conveyor belt material. Both stainless steel materials *(Table 2)* had undetectable levels of mesophilic bacteria after the application of sanitizer. Similarly, lower ($P < 0.05$) bacterial counts were recorded for polypropylene (1.24 log CFU/cm^2) and canvas (1.32 log CFU/cm^2) than with the acetal (1.67 log CFU/cm^2) and polypropylene-meshtop (1.81 log CFU/cm^2) conveyor chips. All contaminated chips in this study had the same initial bacterial count after 1 h exposure to poultry and rinse; the extent of bacterial attachment may vary in relation to the nature of

TABLE 2. Aerobic mesophiles and coliform counts (log CFU/cm²)[1] of contaminated conveyor chips before and after treatment with QAC for 10 min

	Aerobic mesophiles(log CFU/cm²)		Coliform (log CFU/cm²)	
Belt type	Contaminated chip	Contaminated chip + sanitizer	Contaminated chip	Contaminated chip + sanitizer
Canvas	3.36[a] ± 0.20	1.32[b] ± 0.14	2.58[ab] ± 0.39	2.19[a] ± 0.26
Acetal	3.32[a] ± 0.23	1.67[a] ± 0.12	2.32[b] ± 0.28	nd[3]
Polypropylene-Meshtop	3.27[a] ± 0.56	1.81[a] ± 0.10	2.43[b] ± 0.17	nd
Polypropylene	3.55[a] ± 0.25	1.24[b] ± 0.12	2.37[b] ± 0.48	nd
Stainless steel-Single loop	3.27[a] ± 0.21	nd	2.58[ab] ± 0.50	nd
Stainless steel-Balance weave	3.28[a] ± 0.14	nd	3.21[a] ± 0.37	nd

[a-b]Means within a column with different superscripts differ ($P < 0.05$).

[1]Results are presented as the means of triplicate measurements (n = 3), followed by Standard Deviation.

[2]Not detected (limit of detection is >1 CFU/cm²).

the test surface materials. Many studies have indicated that fewer bacterial cells attached to stainless steel surfaces than to polymeric materials and, that stainless steel surfaces have weaker adhesion properties for bacteria *(4, 24, 30)*. The low adherence ability of the bacterial cells and the weaker adhesion property of the stainless steel possibly influenced the efficacy of the QAC.

The QAC used in this study was an acidified quaternary ammonium sanitizer whose individual components or composite action may be influenced by the nature of the test materials and the degree of bacterial attachment. The variation in the efficacy of QAC against mesophilic bacteria attached to the various test chips can be the attributed to the adhesion property of the bacterial population and test surface, as mentioned previously. Bacterial adhesions were reported to be affected by hydrophobicity and surface charge of the bacteria, surface conditioning and roughness, and organic load *(4, 7, 12, 18, 24)*. A previous study by Veluz et al. *(30)* confirmed that *Listeria* and *Salmonella* can attach and behave differently on different conveyor belts. A study by Taormina and Dorsa *(27)*, using knives as test surfaces, revealed that the use of sanitizers such as QAC, coupled with scrubbing, can significantly reduce numbers of pathogenic bacteria. An effective cleaning and washing, accompanied by scrubbing and sanitizers, will provide the most benefit in eliminating attached bacteria.

In the case of coliform bacteria, the initial count (3.21 log CFU/cm²) of coliforms attached to stainless steel-balance weave material was slightly higher ($P < 0.05$) than counts with the acetal (1.67 log CFU/cm²), polypropylene-meshtop (2.43 log CFU/cm²), and polypropylene (3.55 log CFU/cm²) test chips. However, most of the coliform bacteria attached to all chips were reduced to non-detectable levels by the sanitizer application, except for the canvas conveyor chip. The coliform count (2.19 log CFU/cm²) on the canvas chip was almost unchanged even after the application of the sanitizer, indicating inability of the QAC to kill the coliforms within the 10 min exposure time.

The smoothness or roughness of food contact surfaces has been reported to influence the sanitizer efficacy *(9, 29, 31*; however, some conflicting results have also been reported *(9, 10, 18, 22)*. In any case, bacterial adhesion is affected by surface finish *(4, 7, 14, 15)*. From our study, it is more likely that mesophilic bacteria and coliforms behave differently, and the composite effects of the nature of the test surface (degree of roughness or smoothness), the time of bacterial exposure, and the degree of bacterial attachment contribute to overall QAC efficacy, which is supported by a previous study *(30)*.

Many factors are involved in sanitation, and there is no single formula for effective use of sanitizer. The results of this study indicate only that conveyor chips made from stainless steel are better materials when QAC is used. Those of stainless steel belt types can possibly prevent contamination with spoilage bacteria and pathogens of concern in poultry industry. Although in this study the application of QAC sanitizer was found to be more effective in reducing aerobic mesophiles and coliforms on stainless steel than on other materials, further investigation is necessary to determine the interaction of influencing factors relevant to the nature and design of the conveyor chips.

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