

Recovery of microorganisms on fabric materials after low water temperature washing with non-oxidizing acidic bleaching formulation by culture method

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ABSTRACT

The recovery of microorganisms to different fabrics was evaluated after a washing process combined with a food-grade non-oxidizing acidic formulation and low washing water temperature. Cotton, polyester and a polyester/cotton blend fabric samples were inoculated with *Escherichia coli*, *Listeria innocua* and *Saccharomyces cerevisiae*, then dried for 1 day. They were separately placed in a simulated fabric washer and decontaminated for 1 and 10 min with the acidic formulation at 23 °C water washing temperature. The combination of direct detecting and dilution methods was used to detect survivors on fabrics. The use of $\geq 0.1\%$ acidic formulation in the washing process significantly increased the efficacy of the washing for all fabric samples. Microorganisms on the cotton and mixed fabric appeared to bind more strongly and were more resistant to the washing process. No viability was observed on the fabric swatches at 1 cfu/sample detection limit when the washing process was combined with 0.5% acidic formulation in the 10 min washing cycle. These findings can be used to increase the efficiency of sanitizing fabrics in an environmentally friendly way, for remove harmful microorganisms from them and reduce cross-contamination.

Key words | decontamination, fabric washing, food-grade acidic formulation, sanitation

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INTRODUCTION

To prevent cross-contamination and provide personnel protection from infectious microorganisms, various forms of fabric materials are used to make coats and aprons for health-care workers and laboratory personnel in the health-care industries (Swell 1995). Findings in previous studies indicated that both Gram-positive and Gram-negative pathogenic bacteria can survive for times ranging from hours to months after drying on fabric which has been commonly used in food service and health-care work places (Wilkoff *et al.* 1969; Neely 2000; Neely & Maley 2000). The literature reports that the nature of the fabric, such as origin of material, pore size, and surface tension, is strongly related to the ability of bacteria to attach to it (Leonas & Jinkins 1997).

To achieve desired minimal microbial counts on laundered fabrics, the use of appropriate qualities and

quantities of bleaching agents are needed for the laundering process in health-care and food service facilities. Bleaching agents most widely used in laundering operations include hydrogen peroxide, hydroquinone and chlorinated compounds (Belkin 1998; de Vos *et al.* 2001). When these bleaching agents are added to detergents and used at the correct temperature and exposure time on fabrics that are heavily contaminated, they are able to reduce bacterial numbers to acceptable margins of safety (CDC 1985). However, the safety associated with the use of these bleaching agents can be questioned when high residual quantities with toxic potential to humans are left on the washed fabric. Furthermore, by-products are possibly formed when they react with naturally occurring organic matters (Pontius 1996; Betts 2005). In our study, we enhanced the sanitizing efficacy of the laundry washing process by adding a food-grade

acidic chemical formulation to the washing process in order to produce microbial inactivating capabilities, but with a safe and lower residual potential (Lopes 2004).

Presently, the known methods used to monitor microbial numbers on fabric include impression sampling using RODAC plates, rinse-extraction and maceration. However, these methods are time-consuming and cumbersome and/or have low efficiencies of microbial enumeration. A procedure has been developed to directly enumerate microorganisms on fabric material by overlaying the material with a nutrient agar containing 2,3,5-triphenyltetrazolium chloride (TTC) as a vital dye indicator (Lee *et al.* 2007a, b). This method appears to be better than the widely used maceration method for the determination of bacterial density on laundered fabric materials. This is because this new method is better able to detect smaller numbers of microorganisms when compared with the traditional techniques. It is known that 10–100 pathogenic bacterial cells might be sufficient to cause disease in healthy adults (DuPont *et al.* 1989). Therefore, this detection method is advantageous to laboratory coat wearers who rely on mechanical agitation as a washing method.

The objectives of this study were: (i) to examine the attachment and reduction of microorganisms on various un-cleaned/cleaned fabrics; and (ii) to determine the efficacy of a food-grade acidic formulation for the reduction of inoculated microorganisms on various fabric materials during washing processes. For this study, *Escherichia coli* K-12 and *L. innocua* Seeliger were used as surrogates for foodborne pathogenic strains of *E. coli* 0157:H7 (Gram-negative bacteria) and *L. monocytogenes* (Gram-positive bacteria), respectively (Moce-Livina *et al.* 2003). A strain of *Saccharomyces cerevisiae* (yeast) was also selected to investigate the broad-spectrum efficacy of the acidic formulation.

METHODOLOGY

Bleaching solutions

A food-grade acidic formulation containing 66% citric acid and 3.6% sodium dodecylbenzene sulfonate (SDBS) was obtained from Microcide, Inc. (Detroit, MI, USA) and

was used as chemical washing agent. Citric acid is a non-oxidizing bleaching compound that can be used to remove certain types of stains on materials (Keshav *et al.* 2012). The stabilizers and other excipients in this formulation have a high safety and biodegradability profile. This acidic bleaching formulation was supplied as a powdered concentrate which needed reconstitution before use. Three concentrations (0.1, 0.5, and 1.0% with pHs of 3.0, 2.6, and 2.5, respectively) of this formulation were prepared by adding the powdered formulation to sterile deionized water. A commercial chlorine bleach, containing 6% sodium hypochlorite, was used as positive control washing agent. The sodium hypochlorite used in this study had 100 ± 10 ppm chlorine and this concentration was determined using a HI 95771 Chlorine Ultra High Range Meter (Hanna Instruments, Ann Arbor, MI).

Preparation of microbial cell suspensions

E. coli K-12 (ATCC 29181), *L. innocua* (ATCC 33090) and *S. cerevisiae* (OSU 211) were stored at -86°C in trypticase soy broth (Difco, Detroit, MI) supplemented with 0.3% (w/w) yeast extract (TSBYE) for *E. coli* and *L. innocua*, and Sabouraud dextrose broth (Difco, SDB) for *S. cerevisiae* with 40% glycerol. A loopful of each frozen culture was revived in 10 mL of TSA for bacteria and SDB for *S. cerevisiae*, and incubated for 24 h at 37°C for bacteria and for 36 h at 30°C for *S. cerevisiae*. Each of these cultures was then inoculated on a tryptic soy agar (TSA) slant for bacteria and Sabouraud dextrose agar (SDA) slant for *S. cerevisiae*. The bacteria were incubated at 37°C for 24 h. *S. cerevisiae* was incubated at 30°C for 36 h. The cells grown on the slant were stored at 3°C and used as a stock culture. For each experiment, a loopful of stock culture was transferred to 20 mL TSBYE or SDB and incubated for 24 h at 37°C for bacteria and for 36 h at 30°C for *S. cerevisiae*. The cells were then harvested by centrifugation (Sorvall[®] RC5C Plus, Newtown, CT, USA) at $10,000\text{ g}$ for 10 min at 4°C . The supernatant was decanted, and the pellets were re-suspended in 20 mL of 0.1 M phosphate buffer containing organic matter (0.1% tryptone, w/v), pH 7.2 to obtain cell populations of >8.0 log cfu/mL for bacteria and >7.0 log cfu/mL for *S. cerevisiae*.

Microbial inoculation of fabric materials

Three types of fabric materials, 100% cotton (C, 0.15 × 0.14 mm pore size, 0.7 mm thickness), 65% polyester +35% cotton (CP, 0.14 × 0.12 mm pore size, 0.6 mm thickness), and 100% polyester (P, 0.12 × 0.11 mm pore size, 0.4 mm thickness), were purchased from a local fabric store and used in this study. Fabric swatches were prepared by cutting them into 4 × 4 cm squares. The average weights of the swatches were approximately 0.15 g for C and CP samples, and 0.09 g for the P sample. These swatches were placed into a plastic box and sterilized prior to use by autoclaving for 15 min, then air dried. The swatches were inoculated (300 µL) with microbial cell suspensions. Each inoculum was spread evenly using a sterile pipette tip, then allowed to dry for 24 h under ambient conditions. A dilution method was used to enumerate the organisms on the swatches before and after drying. In this method, each swatch sample was transferred to 30 mL of the phosphate buffer solution containing neutralizers (0.07% lecithin, 0.5% Tween 80 and 0.1% sodium thiosulfate) in a 50 mL sterile conical tube. After vortexing vigorously to resuspend the contents in the tube, the cells were serially diluted and pour plated using an appropriate agar containing the neutralizers and TTC (Becton, Dickinson and Co., Sparks, MD, USA) as a dyeing agent. In order to enumerate the microorganisms that remained attached after vortexing, fabric swatches were placed in separate Petri dishes and directly overlaid with the appropriate agar containing the neutralizer and dyeing agent. After 36 h incubation at optimum growth temperature, culturable cells in all samples were enumerated. The number of colony-forming units on the swatch samples (cfu/sample) after the drying process was used as the initial microbial contents for the wet laundering study.

Wet fabric laundering and microbial enumeration

Two swatches from each fabric type were placed in a simulated top load single-drum mechanical fabric washing device and washed by mechanical forces created from the agitator (100 rpm) and turbulence of 5 mm glass beads in a jar containing 200 mL of washing solutions (23 °C). The swatches were washed for 1 and 10 min with 0.0, 0.1, 0.5, and 1.0%

of the acidic formulation or the chlorine bleach solution with a final concentration of 100 ppm chlorine, and then rinsed for 30 s with sterile deionized water (200 mL, 23 °C) in a second jar. After draining to remove excess washing solutions, surviving organisms on the fabric surfaces and in the washing/rinsing water were enumerated. The processed fabric swatches were placed in separate Petri dishes and directly overlaid with appropriate agar containing the neutralizers and TTC. A second set of the fabric swatches were assayed after dilution. This was done because of the difficulty in counting high microbial numbers in some samples. In the dilution method, the processed samples were transferred to sterile tubes containing 30 mL of the neutralizing buffer solution. After vigorous vortexing to resuspend the contents of the tube, the cells were serially diluted and pour plated using appropriate agar containing the neutralizers and dyeing agent.

A portion (100 or 1,000 µL) of the washing/rinsing water was serially diluted and also plated onto appropriate agar containing the neutralizers and TTC. Survivors in all samples were enumerated after incubation at 37 °C (bacteria) and 30 °C (*S. cerevisiae*) for 36 h.

Statistical analysis

The significances between the mean values of the microbial viability were analyzed by multifactor analysis of variance. The data analyses were performed by the General Linear Model function and Tukey's multiple comparison testing with SAS, version 9.2, statistical program (SAS Institute, Cary, NC) to determine the level of significance between the effects of the types of bleaching solutions, types of materials and washing time on the removal of the tested microorganisms. The level of significance was set at $P < 0.05$. All laundering processes were repeated at least three times in this study.

RESULTS

Drying effect before wet laundering process

Table 1 shows the microbial populations on the three different fabric swatches before the washing process. After drying

Table 1 | Viable counts (log₁₀ cfu/sample) of microbial strains on fabric samples after air drying at 23 °C for 24 h

Drying	<i>E. coli</i>			<i>L. innocua</i>			<i>S. cerevisiae</i>		
	C	CP	P	C	CP	P	C	CP	P
Before	7.9 ± 0.3	7.9 ± 0.3	7.9 ± 0.3	8.2 ± 0.2	8.2 ± 0.2	8.2 ± 0.2	7.2 ± 0.2	7.2 ± 0.2	7.2 ± 0.2
After	5.2 ± 0.3	5.3 ± 0.2	4.8 ± 0.2	5.4 ± 0.1	5.1 ± 0.2	4.5 ± 0.4	3.0 ± 0.2	3.1 ± 0.1	2.8 ± 0.5

on the fabric swatches for 24 h, the reduction in culturable cell numbers of the inoculated cultures ranged between approximately 2.5 and 3.5 log units for the *E. coli* and *L. innocua*, and >4 log units for *S. cerevisiae*. When the fabric types were compared, lower culturable cell reductions were found on the 100% cotton (C) and 35% cotton + 65% polyester (CP) for *E. coli* (2.5 log cfu/sample) and *L. innocua* (3.0 log cfu/sample) while 100% polyester (P) showed cell reductions of >3.0 log cfu/sample for *E. coli* and >3.5 log cfu/sample for *L. innocua*. There was no significant ($P > 0.05$) difference in cell reduction among the three types of fabrics for *S. cerevisiae*.

Effect of wet laundering process

The effectiveness of washing times for reduction of the culturable microorganisms attached to the fabric swatches after the laundering process at 23 °C water temperature is shown in

Table 2. The wet laundering process without bleaching solutions caused ~1.5 log cfu reduction in bacteria after 1 min washing time and ≤1 log cfu reduction in the *S. cerevisiae* samples. After the 10 min washing time in water for the fabric swatches, there were additional (~0.5 log cfu) reductions in the organisms on samples C and CP, while >1.0 log cfu reductions were found for the organisms on sample P.

In comparison with the result for the wet laundering process without the acidic formulation for the 1 min washing, the reduction in culturable cell numbers on the C swatches after the addition of 0.1% acidic formulation was ~1.0 log cfu greater for the bacterial strains and <0.5 log cfu for *S. cerevisiae*. Similar reductions were obtained for the CP swatches for *E. coli*, while the reduction was 2.0 log cfu greater for *L. innocua* and 1.2 log cfu for the *S. cerevisiae* organism. Among the fabrics, the greatest reductions were achieved on the P swatches – *E. coli* (2.5 log cfu), followed by *L. innocua* (3.0 log cfu), and *S. cerevisiae* (1.3 log

Table 2 | Viable counts (log₁₀ cfu/sample) of microbial strains in the fabric swatches after wet laundering process under various conditions

Organisms	Wipes	Unwashed ^a	Types of washing solution									
			Water only		0.1% AF ^b		0.5% AF		1.0% AF		Chlorine bleach ^c	
			1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min
<i>E. coli</i>	C	5.2 ± 0.3	3.9 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	0.4 ± 0.1	1.4 ± 0.2	^d ND	ND	ND	2.7 ± 0.3	ND
	CP	5.3 ± 0.2	3.9 ± 0.1	3.4 ± 0.0	3.0 ± 0.1	0.4 ± 0.3	1.8 ± 0.1	ND	ND	ND	1.1 ± 0.2	ND
	P	4.8 ± 0.2	2.6 ± 0.1	1.0 ± 0.2	0.2 ± 0.2	ND	ND	ND	ND	ND	ND	ND
<i>L. innocua</i>	C	5.4 ± 0.1	4.0 ± 0.1	3.5 ± 0.1	3.2 ± 0.2	0.2 ± 0.1	1.0 ± 0.3	ND	ND	ND	2.8 ± 0.2	ND
	CP	5.1 ± 0.2	3.9 ± 0.2	3.5 ± 0.1	1.7 ± 0.1	ND	ND	ND	ND	ND	1.9 ± 0.3	ND
	P	4.5 ± 0.4	3.1 ± 0.3	1.6 ± 0.4	0.2 ± 0.1	ND	ND	ND	ND	ND	ND	ND
<i>S. cerevisiae</i>	C	3.0 ± 0.2	2.8 ± 0.3	2.2 ± 0.2	2.4 ± 0.1	2.1 ± 0.1	0.3 ± 0.3	ND	ND	ND	ND	ND
	CP	3.1 ± 0.1	2.7 ± 0.5	2.4 ± 0.1	1.5 ± 0.5	0.2 ± 0.2	ND	ND	ND	ND	ND	ND
	P	2.8 ± 0.5	1.7 ± 0.1	0.9 ± 0.3	0.4 ± 0.4	ND	ND	ND	ND	ND	ND	ND

^aMicrobial numbers in fabric swatches before wet laundering process.

^bAcidic formulation.

^cBleach solution containing 100 ppm chlorine.

^dNo colony was detected on plates from sample replicates (detection limit was 1 cfu/swatch sample).

cfu). The mean reductions of the microorganisms after chlorine-bleach washing were slightly higher than those obtained by the acidic formulation. The direct detecting method allowed us to detect 1 cfu/fabric swatch for both bacteria and *S. cerevisiae* organisms (Figure 1). However, the presence of 0.01% TTC only assisted in the detection and counting of the bacterial cells.

The application of a longer washing time (10 min) significantly reduced ($P < 0.05$) the survival numbers of most microorganisms on the fabric swatches washed in the 0.1% acidic formulation. Except for the *S. cerevisiae* on the C swatches, in which ~ 2.0 log cfu were observed, less than 1.0 log cfu survivors were detected on all swatch samples. The samples washed with chlorine bleach had no culturable microorganisms. The reduction in culturable cell numbers on the C swatches after the addition of the 0.5% acidic formulation for 1 min washing time was >2.5 log cfu greater for all organisms. Except for *E. coli* on the CP swatches, no culturable cells were detected on the CP and P swatches. At the same time, the samples treated with the 1.0% acidic formulation had no culturable microorganisms (Table 2).

Tables 3 and 4 show the number of microorganisms that survived in the washing solution and rinsing water after the wet laundering process at various conditions. There was no significant ($P > 0.05$) difference in the microbial numbers in the washing solution (Table 3) and the numbers on the unwashed swatches (unwashed column in Table 2) for all samples when no bleaching solution was used. No colonies were detected on the agar plates associated with the washing solution and rinsing water after wet laundering with the

acidic formulation or chlorine bleach, indicating less than 20 cells remain culturable after the washing process (Table 3).

In comparing the culturable cell counts of the washing solutions, less than 1 log cfu reduction occurred in the counts in any rinsing water after the swatches were wet laundered without washing agents (Tables 3 and 4). The application of the longer washing time (10 min) reduced the surviving number of bacteria in the rinse water, while that of *S. cerevisiae* remained the same (Table 4). Only the bacteria on C and CP swatches showed detectable colonies on the agar plates after wet laundering with the 0.1% acidic formulation.

DISCUSSION

The methodology used in this study was modified from the standard test method used to evaluate laundry sanitizers and disinfectants (ASTM International 2009). This test method recommends the addition of organic matter (soil) to the experimental environment as a challenging hurdle to the sanitizer or disinfectant. NFPA (2007) reported that the higher the concentration of the organic soil, the lower will be the potency of the sanitizer. On the other hand, the presence of organic matter on the surface of a substrate will act to reduce the ability of microorganisms to attach to the underlying material (Fletcher 1976; Parker et al. 2001). In our study the organic matter used was 0.1% tryptone. This was added to the microbial cell suspensions used to contaminate the fabric samples. The contaminated samples were air-dried for 1 day to simulate

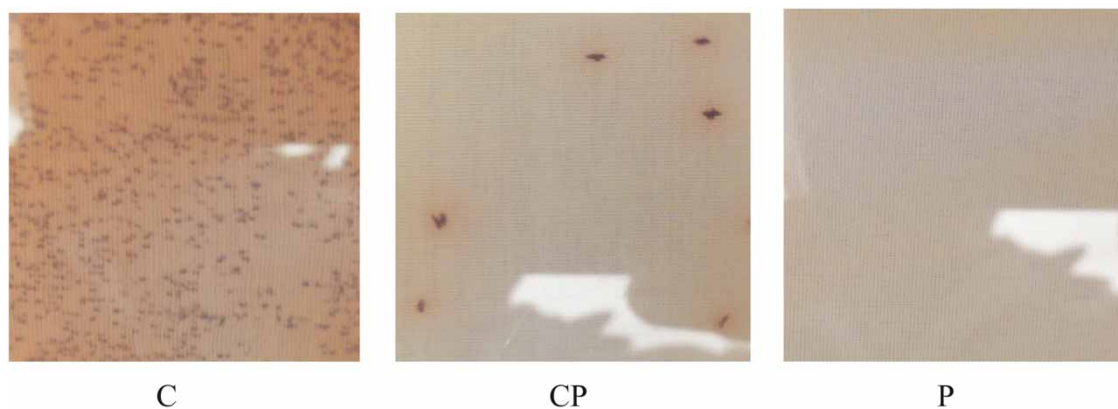


Figure 1 | The colony formation of *L. innocua* on different types of fabric swatches [cotton (C), 65% polyester/35% cotton (CP), polyester (P)] in TSA after washing with the commercial chlorine bleach for 1 min at 23 °C.

Table 3 | Viable counts (log₁₀ cfu/mL) of microbial strains in the washing solutions after wet laundering process under various conditions

Organisms	Wipes	Unwashed ^a	Types of washing solution									
			Water only		0.1% AF ^b		0.5% AF		1.0% AF		Chlorine bleach ^c	
			1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min
<i>E. coli</i>	C	^d ND	5.4 ± 0.1	5.2 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND
	CP	ND	5.5 ± 0.1	5.4 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND
	P	ND	4.8 ± 0.1	4.4 ± 0.5	ND	ND	ND	ND	ND	ND	ND	ND
<i>L. innocua</i>	C	ND	5.7 ± 0.1	5.7 ± 0.1	ND	ND	ND	ND	ND	ND	ND	ND
	CP	ND	5.5 ± 0.1	5.2 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND
	P	ND	5.3 ± 0.1	4.7 ± 0.5	ND	ND	ND	ND	ND	ND	ND	ND
<i>S. cerevisiae</i>	C	ND	2.6 ± 0.3	2.6 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND
	CP	ND	2.9 ± 0.3	3.2 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND
	P	ND	3.6 ± 0.2	3.5 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND

^aMicrobial numbers in washing solution before wet laundering process.^bAcidic formulation.^cBleach solution containing 100 ppm chlorine.^dNo colony was detected on plates from sample replicates (detection limit was 1 cfu/10 ml washing solution).**Table 4** | Viable counts (log₁₀ cfu/mL) of microbial strains in the rinsing water after wet laundering process under various conditions

Organisms	Wipes	Unwashed ^a	Types of washing solution									
			Water only		0.1% AF ^b		0.5% AF		1.0% AF		Chlorine bleach ^c	
			1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min	1 min	10 min
<i>E. coli</i>	C	ND ^d	5.0 ± 0.1	3.7 ± 0.1	3.0 ± 0.3	ND	ND	ND	ND	ND	ND	ND
	CP	ND	5.2 ± 0.1	4.3 ± 0.1	3.5 ± 0.1	ND	ND	ND	ND	ND	ND	ND
	P	ND	4.0 ± 0.0	3.2 ± 0.1	ND	ND	ND	ND	ND	ND	ND	ND
<i>L. innocua</i>	C	ND	5.4 ± 0.0	4.5 ± 0.1	4.0 ± 0.3	ND	ND	ND	ND	ND	ND	ND
	CP	ND	5.4 ± 0.1	4.1 ± 0.2	2.8 ± 0.5	ND	ND	ND	ND	ND	ND	ND
	P	ND	4.3 ± 0.1	2.7 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND
<i>S. cerevisiae</i>	C	ND	2.1 ± 0.1	2.2 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND
	CP	ND	2.3 ± 0.3	2.3 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND
	P	ND	2.1 ± 0.1	2.3 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND

^aMicrobial numbers in rinsing water before wet laundering process.^bAcidic formulation.^cBleach solution containing 100 ppm chlorine.^dNo colony was detected on plates from sample replicates (detection limit was 1 cfu/10 mL rinsing water).

the storage of contaminated laboratory coats in normal working environments.

Prior to the wet laundering process, a greater reduction in cell counts for *S. cerevisiae* on the fabric swatches after air drying (Table 1), indicated that the attachment ability and desiccation stability of the bacteria on the fabrics were greater than that of *S. cerevisiae* in this study. A similar

result was observed in a previous study for the desiccation stability of *S. cerevisiae*, showing that it was lower than that of bacteria inoculated on to plastic and metal surfaces (Lee et al. 2007a). A greater survival of all microorganisms on the C and CP swatches after the drying process suggested that the attachment of bacteria by the fabrics increased with increasing cotton contents and thickness, thus more cells

had a chance to survive the drying time. A previous study also demonstrated that *Staphylococcus aureus* showed the strongest resistance against desiccation when attached to a cotton fabric (Hsieh & Merry 2008).

In our study, the wet laundering process (washing and rinsing) at low washing temperature (23 °C), without the use of detergent (water only) was performed to properly evaluate the effectiveness of bleaching solutions on the laundering process. This approach was also taken in a hospital laundry study carried out by Blaser *et al.* (1984). The study observed that there was no noticeable difference between low washing temperature (22 °C) and high washing temperature (71 °C) for the elimination of both Gram-negative and Gram-positive bacteria, whether a detergent was used or not in the laundering process. On the other hand, the use of bleaching agents in the laundry process increases the reduction of bacteria on the fabric materials. A recent study by Ohsaki *et al.* (2007) showed that bleach containing sodium hypochlorite or hydrogen peroxide as the bacteriocidal agent was a major contributor to bacterial reduction during a laundering process. In our study, the acidic formulation used was a non-oxidizing food-grade chemical formulation. The advantage of the acidic formulation when compared with sodium hypochlorite or hydrogen peroxide is that it does not have high toxic residual potential and therefore will not irritate the skin, nor is it corrosive to the equipment, since the formulation contains a surfactant (SDBS) and a non-oxidizing substance (citric acid) as active agents. It is known that citric acid has antimicrobial activity due to its solubility and pH-lowering ability (DiPersio *et al.* 2004). In addition, citric acid does not have a negative impact on the environment since it rapidly degrades to carbon dioxide and water (Huang *et al.* 1998). At the same time, previous reports have shown that the acidic formulation has potent sanitizing properties that are comparable to those of sodium hypochlorite and hydrogen peroxide (Lee *et al.* 2007a; Handojo *et al.* 2009).

Less than a 2 log cfu decrease in microbial count on the fabrics after a wet laundering process without a bleaching solution suggested that the reduction could only have been attributed to a washing away of the un-attached or loosely attached microbial cells. The use of the >0.1% acidic formulation in the washing process significantly ($P < 0.05$) increased the efficacy of the wet laundering process for all samples (Table 2). In addition, except for the rinsing water from the 0.1% acidic formulation treatment, no culturable

cells were detected in either washing solution or rinsing water after the wet laundering process with the acidic formulation (Tables 3 and 4). These results suggested that most microbial cells on the fabric swatches were inactivated by the activity of the bleaching agent in the acidic formulation without dislodging the microbial cells during the laundering process.

In general, microorganisms are known to survive longer on more hydrophobic fabrics (such as polyester) than on cotton. This is because hydrophobic interactions have been known as major factors involved in causing microorganisms to adhere to an underlying surface (Koziazar & Yamazaki 1998; Neely & Maley 2000). However, the results of our study indicated that slightly higher numbers of the culturable microbial cells were attached to the cotton after the drying step, and this could have been one of the main reasons why these cells showed higher resistance to the mechanical force during the wet laundering process. *S. cerevisiae* showed a greater tolerance to wet laundering than the bacteria strains for all conditions (Table 2). Table 2 indicates that *S. cerevisiae* appeared to bind more strongly to the fabrics when compared with the *E. coli* and *L. innocua*, although its desiccation stability was lower than that of the bacterial strains.

The maceration method has been commonly used to count microbial numbers on fabric materials. However, the method does not recover all the microorganisms in the solution because only a part of the maceration liquid is used for plating. In addition to this, there is no certainty that all the bacteria attached to the fabrics are released by the mechanical force of the blender. In our study, a different approach was taken and the attached microorganisms on the test fabrics were clearly enumerated before and after the wet laundering process, using the combination of the direct detecting and dilution methods.

CONCLUSION

Our study demonstrated that decontamination efficacy against the test bacteria and yeast can be achieved after the wet laundering process when it is combined with a moderate concentration of the food-grade acidic formulation in washing solution. This result could be used to help improve the fabric washing for the food preparation and health-care

industries and this would help to improve the use of micro-biologically safe fabric coats and aprons. Future studies will focus on challenging the acidic formulation with different sanitizing hurdles in order to demonstrate its efficacy under various real life conditions.

ACKNOWLEDGEMENT

The authors wish to thank the Center for Innovative Food Technology for its financial support for this project.

REFERENCES

- American Society for Testing and Materials International (ASTM International) 2009 Standard test method for evaluation of laundry sanitizers and disinfectants. E2274-09.
- Belkin, N. 1998 Aseptics and aesthetics of chlorine bleach: can its use in laundering be safely abandoned? *Am. J. Infect. Contr.* **26**, 149–151.
- Betts, K. 2005 When chlorine + antimicrobials = unintended consequences. *Environ. Sci. Technol.* Available from: <http://pubs.acs.org/subscribe/journals/esthag-w/2005/apr/science/kbchlorine.html>.
- Blaser, M. J., Smith, P. F., Cody, H. J., Wang, W. L. & LaForce, F. M. 1984 Killing of fabric-associated bacteria hospital by low-temperature washing. *J. Infect. Dis.* **149**, 48–57.
- Centers for Disease Control and Prevention (CDC) 1985 *Guidelines for handwashing and hospital environmental control*. Section 6, p. 18, Publication 99-1117. Department of Health and Human Services, Atlanta (GA).
- De Vos, D. E., Sels, B. F. & Jacobs, P. A. 2001 Immobilization of homogeneous oxidation catalysts. *Adv. Catal.* **46**, 1–87.
- DiPersio, P. A., Patricia, A., Kendall, P. A. & Sofos, J. N. 2004 Inactivation of *Listeria monocytogenes* during drying and storage of peach slices treated with acidic or sodium metabisulfite solutions. *Food Microbiol.* **21**, 641–648.
- DuPont, H. L., Levine, M. M., Hornick, R. B. & Formal, S. B. 1989 Inoculum size in shigellosis and implications for expected mode of transmission. *J. Infect. Dis.* **159**, 1126–1128.
- Fletcher, M. 1976 The effect of proteins on bacterial attachment to polystyrene. *J. Gen. Microbiol.* **94**, 400–404.
- Handojo, A., Lee, J., Hipp, J. & Pascall, M. A. 2009 The efficacy of electrolyzed water and an acidic formulation compared with regularly used chemical sanitizers for tableware sanitization during mechanical and manual ware washing protocols. *J. Food Prot.* **72**, 1315–1320.
- Hsieh, Y. L. & Merry, J. 2008 The adherence of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* on cotton, polyester and their blends. *J. Appl. Microbiol.* **60**, 535–544.
- Huang, F. C., Brady, P. V., Lindgren, E. R. & Guerra, P. 1998 Biodegradation of uranium-citrate complexes: Implications for extraction of uranium from soils. *Environ. Sci. Technol.* **32**, 379–382.
- Keshav, A., Norge, P. & Wasewar, K. L. 2012 Reactive extraction of citric acid using tri-*n*-octylamine in nontoxic natural diluents: part 1 – equilibrium studies from aqueous solutions. *Appl. Biochem. Biotechnol.* **167**, 197–213.
- Koziarz, J. & Yamazaki, H. 1998 Immobilization of bacteria on to polyester cloth. *Biotechnol. Tech.* **12**, 407–410.
- Lee, J., Gupta, M. J., Lopes, J. & Pascall, M. A. 2007a Efficacy of two acidic sanitizers for microbial reduction on metal cans and low-density polyethylene film surfaces. *J. Food Sci.* **72**, M335–M339.
- Lee, J., Lopes, J. & Pascall, M. A. 2007b Development of a sanitizing fabric wipe for use on food contact surfaces. *J. Food Sci.* **72**, M375–M381.
- Leonas, K. K. & Jenkins, R. S. 1997 The relationship of selected fabric characteristics and the barrier effectiveness of surgical gown fabrics. *Am. J. Infect. Contr.* **25**, 16–25.
- Lopes, J. 2004 Are oxidizing sanitizers safe for use on fruits and vegetables? Available from: www.fda.gov/ohrms/dockets/dailys/04/july04/072904/04N-0258-emc00009-01.pdf. Last accessed: October 2013.
- Moce-Livina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F. & Jofre, J. 2003 Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. *Appl. Environ. Microbiol.* **69**, 1452–1456.
- National Food Processors Association (NFPA) 2007 Food Plant Sanitation. In: *Canned Foods, 5th edn: Principles of Thermal Process Control, Acidification and Container Closure Evaluation* (L. M. Weddig, ed.). GMA Science & Education, Washington, DC, USA, pp. 47–58.
- Neely, A. N. 2000 A survey of Gram-negative bacteria survival on hospital fabrics and plastics. *J. Burn Care Rehabil.* **21**, 523–527.
- Neely, A. & Maley, M. 2000 Survival of enterococci and staphylococci on hospital fabrics and plastics. *J. Clin. Microbiol.* **38**, 724–726.
- Ohsaki, Y., Kuroki, M., Tachibana, M., Koyano, S., Yoshida, I. & Ito, Y. 2007 Use of sodium hypochlorite (NaOCl) in laundering stops cross-contamination of *Bacillus cereus*. *J. Infect.* **55**, 283–284.
- Parker, S. G., Flint, S. H., Palmer, J. S. & Brooks, J. D. 2001 Factors influencing attachment of thermophilic bacilli to stainless steel. *J. Appl. Microbiol.* **90**, 901–908.
- Pontius, F. W. 1996 Inside the information collection rule. *J. Am. Water Works Assoc.* **88**, 16–46.
- Swell, D. L. 1995 Laboratory-associated infections and biosafety. *Clin. Microbiol. Rev.* **8**, 389–405.
- Wilkoff, L. J., Westbrook, L. & Dixon, G. J. 1969 Factors affecting the persistence of *Staphylococcus aureus* on fabrics. *Appl. Microbiol.* **17**, 268–274.

First received 9 October 2013; accepted in revised form 5 December 2013. Available online 24 January 2014