PAPER

EFFECTIVENESS OF SANITIZING AGENTS IN INACTIVATING *ESCHERICHIA COLI* (ATCC 25922) IN FOOD CUTTING BOARD SURFACES. REMOVAL *E. COLI* USING DIFFERENT SANITIZERS

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ABSTRACT

The objective of this study was to investigate *Escherichia coli* adhesion on new and used polyethylene cutting board surface and evaluate it's removal using different sanitizer (peracetic acid, chlorhexidine, sodium hypochlorite and organic acids). Results indicated that the number of adherent cells increased with time in both surfaces evaluated. Evaluating the sanitizer action, 0.5% peracetic acid was more effective in removal *E. coli* than chlorhexidine and organic acids at same concentration in both surfaces. Peracetic acid and sodium hypochlorite also showed effectiveness at concentrations of 0.2% and 0.5% on new surfaces, respectively. 0.8% of chlorhexidine and 2.0% of organic acids showed similar effectiveness in the removal *E. coli* on new and used surfaces, respectively. These results suggest that peracetic acid is considerable promise sanitizer for application in surfaces of the food processing industry.

Keywords: removal, peracetic acid, cutting boards, *Escherichia coli*

INTRODUCTION

E. coli is a gram-negative bacteria that present surface layer organizations of the type fimbriae, exopolysaccharides (EPS) or flagella, that favor the adherence to materials or host cell surfaces motility and pathogenicity. Food can become contaminated with *E. coli* when animals are slaughtered or processed, even if precautions are taken and also when it is handled by a person infected with *E. coli*, or from cross-contamination (BEUMER and KUSUMANINGRUM, 2003). Food residues left on food processing or handling equipment may provide a niche of microorganisms that can rapidly grow. The growth of pathogenic bacteria can result in cross-contamination from food processing surfaces such as cutting boards to food products (MONTVILLE *et al.*, 2012).

In the food industry, good manufacturing, hygienic production and regular cleaning and disinfection procedures are very important, since food safety and quality are determined by the efficacy of sanitizer agents (KROLASIK *et al.*, 2010). Bacteria have the ability to adhere to any surface including, but not limited to, glass, stainless steel, polypropylene, rubber and wood (COQUET *et al.*, 2002; TEIXEIRA *et al.*, 2008). To prevent bacterial attachment on surfaces the choosing an appropriate sanitizer is very important for achieving a satisfactory end result in microbiological indexes. Many sanitizers have been broadly used across many industries to reduce pathogenic bacterial contamination in food products or on kitchen utensils, because these compounds have been shown to effectively inactivate foodborne pathogenic bacteria (CABEÇA *et al.*, 2012; FRANK, 2003; ROSSONI and GAYLARDE, 2000). Therefore, more studies into the bactericidal properties of sanitizers at different concentrations and contact times are required to define the correct application.

Many researchers have examined materials employed in manufacturing of foods contact surfaces such as stainless steel (CABEÇA *et al.*, 2012; FRANK, 2003; KROLASIK *et al.*, 2010; ROSSONI and GAYLARDE, 2000; RYU and BEUCHAT, 2005), but few reports bacterial removal on commercial polyethylene cutting boards used in industrial food preparation have been published to date.

The objective of this study was to evaluate the *E. coli* adhesion on new and used cutting board surfaces and removal with different sanitizers used in food industry (peracetic acid, chlorhexidine, sodium hypochlorite and organic acids). For each sanitizer tested, different concentrations were evaluated over 72 h, determining the sanitizer's effectiveness on new and used polyethylene cutting boards.

MATERIAL AND METHODS

Surface material

The food processing surfaces evaluated in this study was new and used polyethylene cutting board, white high-density polyethylene (HDPE plastic). The boards were obtained from cutting room of a slaughter unit, where the used surfaces had around of 45 days of handle. Surfaces materials with 1.0 cm x 1.0 cm plates were cut, cleaned by brushing employing liquid detergent and water, and rinsed with distilled water. They were immersed in 70% ethanol, for 1 h, to fat removal, and again rinsed with distilled water and air dried. The surfaces were exposed to ultraviolet light 254 nm for 1 h to sanitize them, as described by PARIZZI (1999), before deposition of any bacterial cultures.

Adhesion of *Escherichia coli* on food processing surfaces

Bacterial strains were obtained from Seattle, USA, 1946 (American Type Culture Collection; Rockville, MD, USA). For the study of adherence was used an *E. coli* (ATCC 25922) strain, grown previously in Luria Bertani broth - LB (tryptone 10.0 g L^{-1}, yeast extract 5.0 g L^{-1}, NaCl 5.0 g L^{-1}) and incubated at 35°C (±2) for 24 h. *E. coli* was chosen as indicator organism, commonly present in industrial food plants.

The cleaned surfaces were immersed, at 25°C, in Erlenmeyer containing 100 mL of LB supplemented with a suspension of bacterial cells in order to obtain a count of 10^3 CFU mL^{-1}. The sterilized surface, for each time, was immersed in these Erlenmeyer with sterilized forceps and incubated at 35°C in LB broth. The quantities of adhered cells per square centimeter were evaluated for 72 h of contact time (0.1, 1, 3, 6, 12, 24, 48, and 72 h) on new and used surface. The initial time (0 h) corresponds to the analysis performed immediately after the immersion of the surfaces in the Erlenmeyer containing the medium culture and the bacterial suspension. Triplicates were performed for each treatment.

After the incubation, the surfaces were withdrawn from the bacterial suspension *E. coli* and transferred to tubes, containing 10 mL of peptone water 0.1% (p/v) for 1 min, to remove planktonic cells. Subsequently, immersed in tubes containing 5 mL of the same diluent solution and vortex for 1 min, to remove sessile cells (PARIZZI, 1999). The contact areas were swabbed and the adhered microorganisms in the swabs were transferred to tubes, containing 10 mL of peptone water 0.1% (p/v) sterilized at 121°C, for 15 min. The tube was stirred using a vortex for 10 s to release the bacteria from the swab. Next, 1 mL of solution was carefully plated on LB agar, incubated at 35-37°C for 24 h, to colony counting.

Efficiency of different sanitizers against *Escherichia coli* on food processing surfaces

The sanitizers used in this study were chosen to represent those used in the food industry. The following sanitizers were used: peracetic acid 15% (Johnson Diversey, São Paulo-SP, Brazil), chlorohexidine 20% (AD Foods Industry Ltda, Laguna-SC, Brazil), sodium hypochlorite 10% (CSM Chemical Products Ltda, Chapecó-SC, Brazil) and organic acids (formulated with lactic acid-30%, citric acid-3%, ascorbic acid-3%, and salts of fatty acids-7% in water). For each sanitizer, different concentrations (0, 0.2, 0.5, 0.8 and 2.0%) were investigated for 10 min of exposure, to evaluate their efficiency in removal the adhered cells. These agents were diluted in sterilized distilled water according to the supplier's instructions. After this treatment, the surfaces were immersed (separately) in 10 mL of sterilized water, for 1 min and repeated twice to removal the excess of sanitizer.

The counts of bacterial adhesion and inactivation by sanitizers were carried out using swab on cutting boards, evaluated through the standard plate count method. Then, plated on LB agar, incubated at 35-37°C for 24 h to colony counting. All determinations were performed in triplicate and the results expressed in terms of mean values (PARIZZI *et al.*, 2004).

Statistical analysis

Descriptive analyses, including the mean value and variability (standard deviation) and graphic displays were performed. Results obtained in experimental design described previously were performed considering a 95% confidence level ($p<0.05$) by the Tukey's test, using the software Statistica 8.0 (StatSoft Inc®, USA).

RESULTS

E. coli adhesion in food processing surfaces

Fig. 1 show the number of *E. coli* adhered on new and used cutting board surfaces with different contact times. Numbers of *E. coli* were estimated and expressed as $\log_{10}$ colony forming units per cm² (log CFU cm^{-2}). A fast adhesion of *E. coli* on both surfaces studied were observed for up to 12h, becoming constant after 24h on used surfaces, when the maximum population reached (6.92 log CFU cm^{-2}). A significant difference ($p<0.05$) was observed in the intensity of adhesion between the surfaces until 24h.

Effect of different sanitizers for inactivating *Escherichia coli*

Figs. 2, 3, 4 and 5 show the *data's* of inactivation *E. coli* on new and used cutting boards

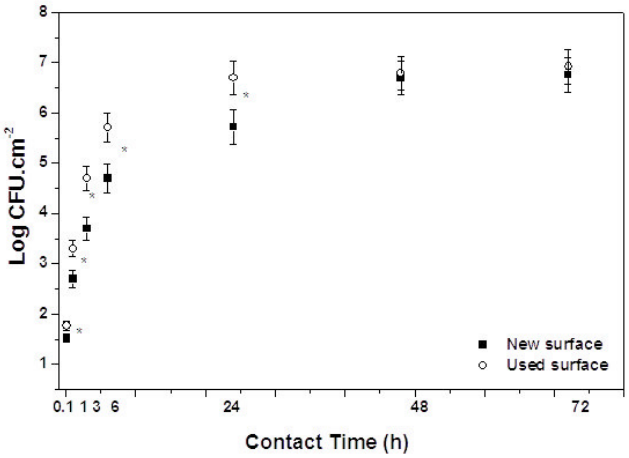

Fig. 1 - Counts of *E. coli* on new and used cutting board surfaces without the presence of sanitizers, over 72 h of contact time. Bars represent the standard errors of the mean from triplicate experiments and * simbolize significant differece ($p<0.05$).

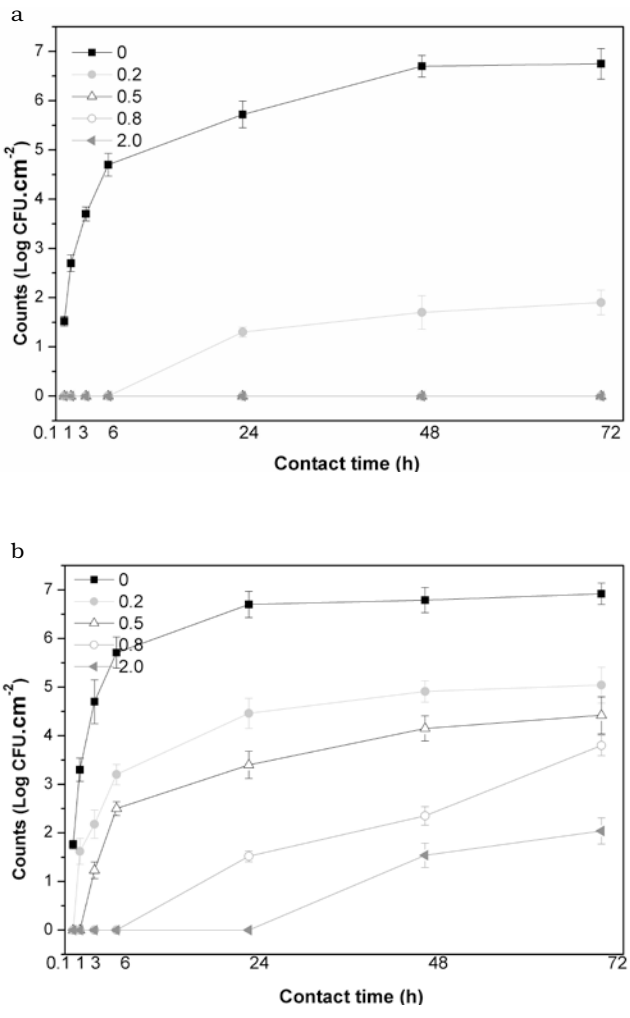

Fig. 2 - The efficacy of different concentrations of peracetic acid (0, 0.2, 0.5, 0.8 and 2.0%) on the reduction of *E. coli* on: (a) new and (b) used cutting board surfaces, over 72 h. Bars represent the standard errors of the mean from triplicate experiments.

with different concentrations (0, 0.2, 0.5, 0.8 and 2.0%) of peracetic acid, chlorhexidine, organic acid and sodium hypochlorite sanitizers, respectively, over 72 h of contact time.

Fig. 2 a and b demonstrates that the bacteria exhibited a significant decrease in the survival rate of viable cells after treatment with peracetic acid. The concentration of 0.5% peracetic acid indicated by the supplier was completely effective for inactivating *E. coli* at all times investigated on new surfaces, while 0.2% peracetic acid was effective for up to 6 h, and able to reduce the number of adhered cells of 4.4 and 5.0 log for 48 and 72 h, respectively (Fig. 2a).

In Fig. 3 a is possible to observe that only the highest concentration of chlorhexidine (2.0%) was completely effective for inactivating *E. coli* on new surfaces, for 72 h. In used surfaces this

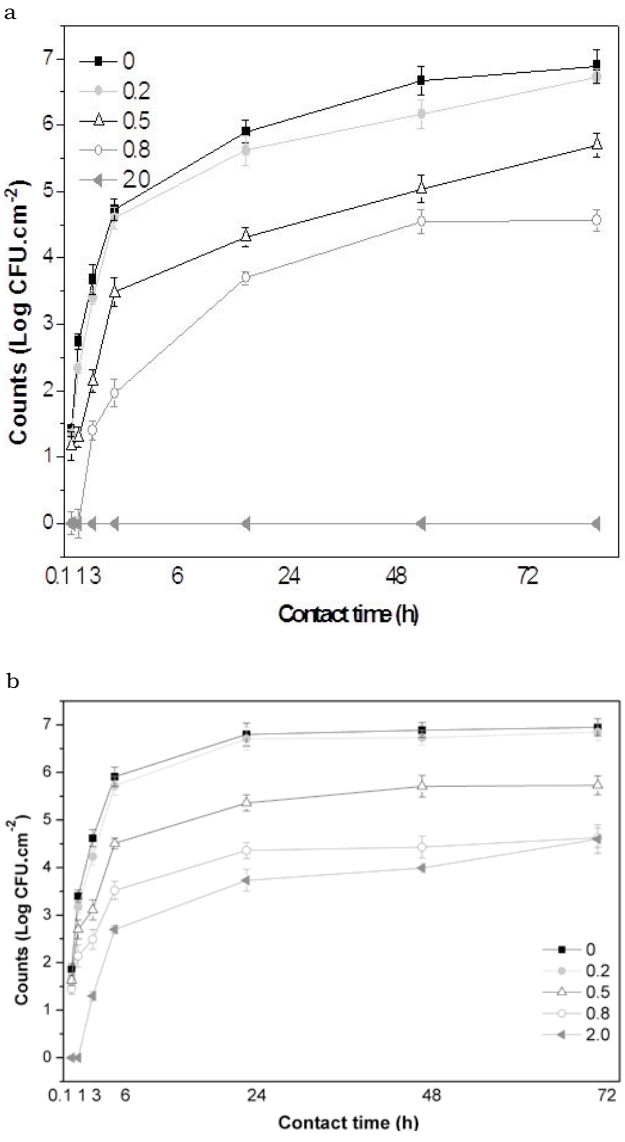

Fig. 3 - The efficacy of different concentrations of chlorhexidine (0, 0.2, 0.5, 0.8 and 2.0%) on the reduction of *E. coli* on: (a) new and (b) used cutting board surfaces, over 72 h. Bars represent the standard errors of the mean from triplicate experiments.

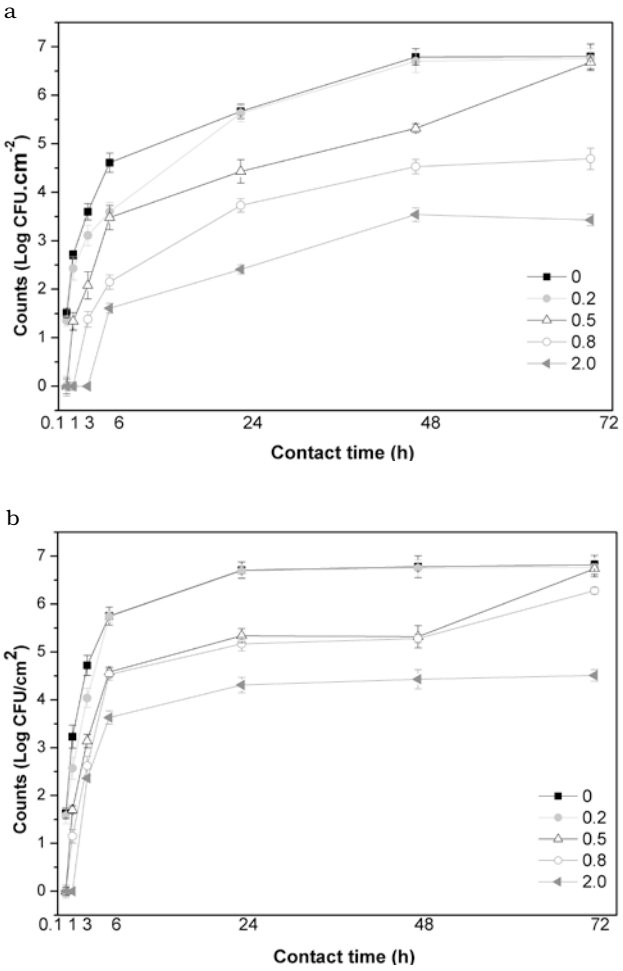

Fig. 4 - The efficacy of different concentrations of organic acid (0, 0.2, 0.5, 0.8 and 2.0%) on the reduction of *E. coli* on: (a) new and (b) used cutting board surfaces, over 72 h. Bars represent the standard errors of the mean from triplicate experiments.

sanitizer (2.0%) was effective until 1h of contact (Fig. 3 b), and reduce around 2.5 log CFU cm^{-2} of cells after 72 h. In this way, chlorhexidine sanitization had a better effect on removal attached cell on new surfaces.

For 0.8% chlorhexidine was observed completely *E. coli* removed on new boards with 1h of contact, but not was effective on the used boards. Consequently, the lower concentrations investigated (0.2 and 0.5%) not show complete inactivation. On used cutting board, all concentrations of sanitizer studied not inactive bacteria after 1 h of contact.

According to the suppliers, also organic acids are suggested in a concentration of 0.5%. In this way, this concentration showed efficiency only for 10 min, on both surfaces evaluated. Higher concentrations, 0.8 and 2.0%, were effective for removing *E. coli* up to 1 and 3 h of contact on new surfaces, respectively (Fig. 4a). The results also indicated that the amount of adherent cells reduced 2.4 log with 2.0% organic acid and was efficient for 1h on used surfaces (Fig. 4b). This

low efficiency of organic acids can be explained by the fact that the compounds are in a dissociated form at the product application moment and dilute the sanitizer, so a higher dissociation leads to lower efficiency (BELTRAME *et al.*, 2012).

Fig. 5 demonstrates the efficiency of sodium hypochlorite against *E. coli.* The concentration (0.5%) indicated by the supplier was able to remove bacteria cells, at all exposure times, on new surface (Fig 5a). On the other hand, to obtain the same effect, on the used surface, a concentration of 2.0% was required (Fig 5b).

Effectiveness correlation between different sanitizers

The sanitation in food surfaces, including cutting boards is critical for the control of microbial contamination of foods and is a significant concern of food preparation and processing industries and public health agencies. In this way, to compare the efficacy of sanitizers (peracetic acid, chlorhexidine, sodium hypochlorite and organic acids) used in the food industries was evaluated a concentration of 0.5%, after 3 h of contact, on new and used surfaces for *E. coli* removal (Fig. 6). The concentration of 0.5% correspond the minimum recommended by the supplier and 3 h of contact is the maximum time (practiced by the food industry) for disinfecting surfaces used.

Comparing the sanitizers, the peracetic acid was completely effective in removing *E. coli* on new and used surfaces ($p<0.05$), as well as for sodium hypochlorite only new surfaces. It was found that chlorhexidine and organic acids exhibit reductions on new and used cutting boards (Fig. 6), without significant difference between the sanitizers ($p>0.05$), but less effectively than other sanitizers evaluated in this work ($p<0.05$).

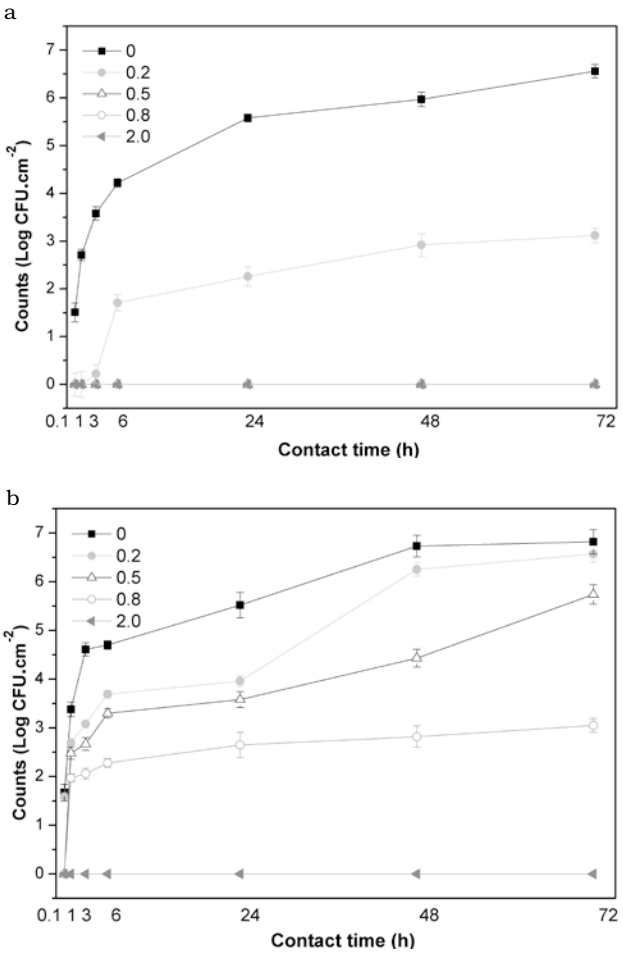

Fig. 5 - The efficacy of different concentrations of sodium hypochlorite (0, 0.2, 0.5, 0.8 and 2.0%) on the reduction of *E. coli* on: (a) new and (b) used cutting board surfaces, over 72 h. Bars represent the standard errors of the mean from triplicate experiments.

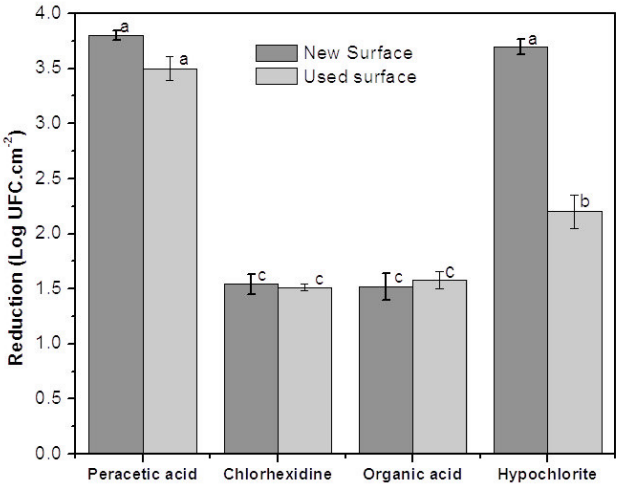

Fig. 6 - The efficacy of different sanitizers (concentration of 0.5%), over 3 h, on the reduction *E. coli* from new and used cutting board surfaces. Means (± standard deviations) followed by the same letters represents no significant difference at 5% level (Tukey's test) between the sanitizers and surfaces.

DISCUSSION

The differences of adhesion on cutting boards could be due microbiological, physical and chemical parameters related to the polyethylene. Particularly, in this study can be verify that the used surfaces have higher counts until 24 h (Fig. 1), possibly due to the surface characteristics, which visually present more cracks and wear by 45 days of use in the slaughter unit. The surface topography has been widely studied, since microorganisms adhere more easily in fissures or cracks, and can resist cleaning and disinfecting procedures (HILBERT *et al.*, 2003; PARIZZI *et al.*, 2004). Thus, macroscopic and microscopic characteristics are crucial for microbial adhesion, reflected in the food contamination by spoilage or pathogenic microorganisms (VADILLO-RODRÍGUEZ *et al.*, 2004). After 48 h the number of adherent cells remained constant over time in both surfaces. This was

also observed in surface reaches saturation level with greater numbers of planktonic cells and not result in greater number of adherent cells (HOOD and ZOTTOLA, 1997).

The results of bacteria removal demonstrate that from 48 h of contact (in used surface), even with concentrations 4 times superior than recommended by suppliers, peracetic acid was not effective. This suggests that the attachment increase during the contact time. Similar results was found by other researcher (ADETUNJI and ISOLA, 2011). MILLER *et al.* (1996) evaluated the potential of water for removal *E. coli* 0157:H7 from polyethylene cutting boards, and the microorganism was incubated for 0 to 30 h, at 37°C, to determine their inhibitory potential. The authors observed an increase in bacteria cells on the boards during the first 30 min of contact, and the water removed 2.3 log CFU cm^{-2} from the surface.

CABEÇA *et al.* (2012) carried out a study of disinfection on stainless steel surfaces, using biguanide and peracetic acid, and verified that they were able to reduce *E. coli* cells adhered of 2.2 and 2.1 log CFU cm^{-2} for 10 min, respectively, with a concentration of 0.5% (w/v). In the present work was possible reduce 3.5 log CFU cm^{-2} after for 3 h, at the same concentration of peracetic acid on new and used polyethylene cutting boards. Peracetic acid disinfectant activity is based on the release of active oxygen. It disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane and transports through dislocation or rupture of cell walls. It may also be effective on outer membrane lipoproteins, facilitating action against Gram-negative bacteria. Intracellular peracetic acid can also oxidize essential enzymes. Thus, vital biochemical pathways, transported through the membrane and intracellular solute levels of are damaged, and alterations in the DNA molecule (KITIS, 2004).

In this study, all concentrations of chlorhexidine not were effective for the removal of bacteria after 1 h. This low activity may be due mechanism action, rapid absorption of bacterial cells, resulting in several cytological modifications that affect permeability and optical properties. Studies have shown that chlorhexidine reacts with the cell from lipophobic groups, causing a disorientation of the lipoprotein membrane and generating a change in osmotic barrier function (KUDAVIDANAGE *et al.*, 2009). Chlorhexidine is a cationic molecule with a wide antimicrobial spectrum against both Gram-positive and Gram-negative bacteria (MOHAMMADI and ABBOTT, 2009). This group of biguanides differs from other cationic biocides that interact only superficially with the lipid bilayer altering fluidity through cation displacement and head group bridging (GILBERT and MOORE, 2005). In a study performed by HOUARI and DI MARTINO (2007) the authors verified that chlorhexidine diacetate (Fluka) was able to inhibit the biofilm formation of different bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* at conventional in-use concentrations.

Second Patel (2005), the bacteria resistance to antimicrobial agents begins at the attachment phase and increase with the biofilm age. Although, bacteria in biofilms are surrounded by an extracellular matrix that might physically restrict the diffusion of antimicrobial agents, this does not seem to be a predominant mechanism of biofilm-associated antimicrobial resistance.

Another indication of high counts are the surface roughness and hydrophobicity that can significantly affect the attachment, formation places for microorganism's accommodation and permanent adhesion. MOVASSAGH *et al.* (2010), showed counts of 7.69 log UFC cm^{-2} for *E. coli* O_{111} on polyethylene surfaces. Second the authors, bacteria encountered in food processing environments can be very hardy and difficult to remove. Bacterial attachment and subsequent survival involved interactions between a bacterial cell, surface and surrounding microenvironment.

The removal bacteria by sodium hypochlorite can be associated with water forms hypochlorous acid, which contains active chlorine (a strong oxidizing agent). Chlorine exerts its antibacterial action by irreversible oxidation of a sulfhydryl group of essential enzymes to microorganisms, disabling metabolic functions of the bacterial cell (POGGIO *et al.*, 2012). Sodium hypochlorite may also have a deleterious effect on the bacterial DNA, involving the formation of chlorinated derivatives of nucleotide bases. Furthermore, it has been reported that sodium hypochlorite can induce disruption of the bacterial membrane (MC DONNEL and RUSSEL, 1999).

Organic acids have an inhibitory action in the undissociated form, from 100 to 600 times greater than the dissociated form. Undissociated organic acid can permeate the cell membrane by diffusion and release protons in the cytoplasm of the cell. The influx of protons induces acidification of the cytoplasm and dissipates the membrane proton potential (KITKO *et al.*, 2009). This inhibits the transport mechanism for the substrate, energy generation and synthesis of macromolecules (STOPFORTH *et al.*, 2003).

CONCLUSIONS

In both surfaces studied it was observed a fast adhesion of *E. coli* and present lower counts in new surface when compared with used. The biofilm formed on used polyethylene cutting boards reduces significantly the action of sanitizers. Among the sanitizers evaluated, peracetic acid was the most efficient for reducing *E. coli* counts.

On the new cutting boards concentration of 0.5% peracetic acid was effective in eliminating *E. coli* adhesion during 72 h evaluated and un-

til 1 h in used surface. Hypochlorite, chlorhexidine and organic acids demonstrated similar effects until 1h, reducing the total adhesion with 0.8 and 2.0% on new and used cutting boards, respectively, although 2.0% sodium hypochlorite has been effective for total removal until 72 h. The order of efficacy in removing *E. coli* was as follows: peracetic acid, sodium hypochlorite, chlorhexidine and organic acids.

The results of the study showed the importance of hygiene procedures on surfaces that come into contact with food. It was found that biofilm formation can occur over a short time, which emphasizes the need for good cleaning procedures during food processing.

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