Original

Influence of different sanitizers on food contaminant bacteria: effect of exposure temperature, contact time, and product concentration

Influência de diferentes sanitizantes na contaminação de alimentos por bactérias: Efeito da temperatura de exposição, tempo de contato e concentração de produto

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Abstract

The efficiency of four Sanitizers - peracetic acid, chlorhexidine, quaternary ammonium, and organic acids - was tested in this work using different bacteria recognized as a problem to meat industry, *Salmonella* sp., *S. aureus*, *E. coli* and *L. monocytogenes.* The effects of sanitizer concentration (0.2, 0.5, 0.6, 1.0, 1.1 and 1.4%), at different temperatures (10 and 45 °C) and contact time (2, 10, 15, 18 and 25 minutes) were evaluated. Tests in an industrial plant were also carried out considering previously obtained results. In a general way, peracetic acid presented higher efficiencies using low concentration (0.2%) and contact time (2 minutes) at 10 °C. The tests performed in industrial scale showed that peracetic acid presented a good performance in concentration and contact time lower than that suggested by the suppliers. The use of chlorhexidine and quaternary ammonium led to reasonable results at the indicated conditions, and organic acids were ineffective under concentration and contact time higher than those indicated by the suppliers in relation to *Staphylococcus aureus*. The results, in general, show that the choice for the most adequate sanitizer depends on the microorganism contaminant, the time available for sanitizer application, and also on the process cost.

Keywords: *sanitizers; bacteria; food industries.*

Resumo

A eficiência de quatro sanitizantes, ácido peracético, clorexidina, quaternário de amônio e ácidos orgânicos, foi testada neste trabalho, usando diferentes bactérias reconhecidas como problemas na indústria de carnes, *Salmonella* sp., *S. aureus*, *E. coli* and *L. monocytogenes*. O efeito da concentração dos sanitizantes (0,2; 0,5; 0,6; 1,0; 1,1 e 1,4%) a diferentes temperaturas (10 e 45 °C) e tempo de contato (2, 10, 15, 18 e 25 minutos) foi avaliado. Testes na planta industrial foram também conduzidos considerando os resultados obtidos previamente. De uma maneira geral, o ácido peracético apresentou maior eficiência usando menores concentrações (0,2%) e tempos de contato (2 minutos) a 10 °C. Os testes em escala industrial mostraram que o ácido peracético apresentou uma boa performance em concentrações e tempos de contato inferiores aos sugeridos pelas empresas fornecedoras. Os usos da clorexidina e do quaternário de amônio levaram a resultados razoáveis nas condições indicadas e os ácidos orgânicos foram ineficientes nas concentrações indicadas em relação ao *Staphylococcus aureus*. De uma maneira geral, os resultados mostraram que a escolha do sanitizante mais adequado dependerá do micro-organismo contaminante, tempo disponível para aplicação do sanitizante e custo do processo.

Palavras-chave: *sanitizantes; bactérias; indústria de alimentos.*

1 Introduction

Recent trends in global food production, processing, distribution, and preparation have stimulated an increasing demand for food safety research in order to ensure a safer global food supply (ARVANITOYANNIS; CHOREFTAKI; TSERKEZOU, 2005; ARVANITOYANNIS; TSERKEZOU; VARZAKAS, 2006; VARZAKAS; ARVANITOYANNIS, 2008; ARVANITOYANNIS; PALAIOKOSTAS; PANAGIOTAKI, 2009). Sanitization and cleaning of equipment used for food processing is an important topic to be evaluated for controlling cross- contamination during the production process. Clean-up and disinfection can be considered regular procedures since they can remove most microorganisms that can contaminate equipment. All food processing equipment surfaces is subject to adhesion of microorganisms, which can even after proper cleaning and sanitization (ASSELT; GIFFEL, 2005; SILVA et al., 2010), and thus being a possible cause of diseases caused by contaminated food (ANDRADE; MACEDO, 1996).

One of the most effectively controlled processes in meat industries is the higienization step. Considering this statement, the choice of most appropriate antimicrobial agents should be carefully taken considering the potential contaminants as well as the types of surfaces found in industries (KUNIGK; ALMEIDA, 2001). The ideal sanitizers should be approved by competent organs, have a wide spectrum of antimicrobial activity, be able to rapidly destruct microorganisms and be stable under

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several use conditions, and present low toxicity and corrosivity (ANDRADE; PINTO; ROSADO, 2008).

The effect of sanitizers can be changed due to the characteristics of surface, temperature and contact time, product concentration, surface residues, pH, water physicochemical properties, and inactivating substances, especially organic matter.

The type and concentration of microorganism also influence the sanitizer efficiency (RUSSELL, 1992).

A model able to predict the effect of a determined sanitizer on a non-specific environment is hard to obtain. Hence, in order to perform tests under practical conditions, it is necessary to determine the effect of a certain sanitizer. Following this procedure, it is possible to determine if it will be effective based on bacteria's class, metabolic state, recovery of injured cells and microorganism biodiversity, influence of organic material/biofim, and processing conditions such as temperature and pH (ASSELT; GIFFEL, 2005).

A wide variety of chemical sanitizers is now available, including iodine compounds, quaternary ammonium, peracetic acid, and hydrogen peroxide. Other compounds can be used in sanitizer formulations, mainly aldehydes (glutaraldehyde), phenols (triclosan), biguanines (chlorhexidine), and alcohols. All cited agents are chemically distinct, but some of them present similar mechanisms of action (ASSELT; GIFFEL, 2005). The disinfectation protocols vary according to the pathogens since just a few sanitizers present a wide spectrum of activity (BLOCK, 1991).

Since several different sanitizers are available nowadays, it the necessity of knowing the functions of each product under different concentrations and their main interactions with the environment is evident. Accordingly, the objective of this work was to evaluate the efficiency of four sanitizers used in food industries (peracetic acid, chlorhexidine, quaternary ammonium, and a mixture of organic acids), against four recognized contaminant bacteria (*Salmonella choleraesuis*, *Staphylococcus aureus*, *Escherichia coli,* and *Listeria monocytogenes*). For each sanitizer tested, different product concentration, solution temperature, and contact time were also evaluated. The conditions corresponding to the most promising results were applied in industrial scale by determining the total count of mesophilic on the surface of cutting tables before and after the sanitizer application.

2 Materials and methods

2.1 Efficiency of different sanitizers used in food industries

Different sanitizer concentration, temperature, and contact time were evaluated for each of the four active principles tested, chlorhexidine (20%), ammonium quaternary (20%), peracetic acid (15%), and a mixture of organic acids (ascorbic acid 1%, citric acid 0.4%, and lactic acid 0.475%).

Four dilutions and three contact times were tested: 0.2, 0.5, 0.8 and 1.1% with contact time of 2, 10 and 18 minutes for peracetic acid and chlorhexidine; 0.2, 0.6, 1.0 and 1.4% with contact time of 2, 10 and18 minutes for quaternary ammonium; and 2, 15 and 25 minutes of contact time for organic acids under the same used for quaternary ammonium.

The temperatures tested ranged from 10 to 45 °C, which are temperature values commonly found in an industry environment and are related to those employed for solution preparations.

The microbiological efficiency of the sanitizers was measured using the methodology described by the Portary 101, 11/08/1993, Ministério da Agricultura e do Abastecimento – MAPA, Brazil. The solutions were tested based on their effectiveness against four microorganisms, *Salmonella choleraesuis*, *Escherichia coli, Staphylococcus aureus*, and *Listeria monocytogenes*.

The microorganisms were inoculated in tubes with BHI- liquid medium (brain heart infusion – Difco 0037) and incubated for 24 hours at 35 °C. After incubation, successive dilutions were performed for each culture (10^{-1} to 10^{-8}) in peptone water (0.1%), inoculation in standard counting agar (PCA - triptone 5.0 $g.L^{-1}$, yeast extract 2.5 $g.L^{-1}$, dextrose 1.0 $g.L^{-1}$, agar 15.0 $g.L^{-1}$), followed by incubation for 24 hours at 35 °C and posterior counting.

The sanitizers were aseptically diluted in 9 mL of distilled water. Immediately before the begning of the efficiency tests, 1 mL of UHT milk (source of organic material) was added. For each sanitizer dilution, 0.1 mL of culture test in stationary phase was added at the dilution of 10^{-2} $UFC.mL^{-1}$, and the mixture was then homogenized. After different exposure times, a loopful was transferred to tubes containing BHI medium and incubated at 35 °C for 96 hours. The observation of cell growth was performed visually by the turvation of the medium and formation of a film on the surface or precipitated in the tubes. The positive results were confirmed by replication and incubation in PCA considering as inefficient the sanitizer concentration or exposure time confirmed by PCA test.

2.2 Industrial scale tests

The previously defined conditions for each sanitizer were tested in an industrial plant using the cotton swab technique by measuring numbers of total aerobic heterotrophic microorganisms on the surface of cutting tables before and after the sanitizer application. The cutting room of a slaughter unit was chosen as being the key point of the process, where most of raw material used in industrialization steps is present. Swabs (3M) were used over a contact surface of 20 cm^2 in two different areas of the surface of cutting tables.

The tests were performed before (after the higienization step) and after the sanitizer application in duplicate runs considering two successive days. Table 1 presents different combination of concentration, contact time, and exposure temperature determined previously.

The contact areas were swabbed down 10 times exerting a pressure on the contact surface.

The microorganisms adhered to the swabs were transferred to tubes containing 10 mL of peptone water 0.1% (p/v) sterilized at 121 °C for 15 minutes. The tube was stirred using a vortex

Table 1. Efficiency of peracetic acid at different concentrations and exposure times at 10 and 45 °C.

Concentration (%)	Time (minutes)	*Salmonella choleraesuis*		*Staphylococcus aureus*		*Escherichia coli*		*Listeria monocytogenes*	
		10 °C	45 °C	10 °C	45 °C	10 °C	45 °C	10 °C	45 °C
0.2	2	+	–	+	+	+	+	+	–
0.2	10	+	+	+	+	+	+	+	–
0.2	18	+	+	+	+	+	+	+	+
0.5	2	+	–	+	+	+	+	+	+
0.5	10	+	+	+	+	+	+	+	+
0.5	18	+	+	+	+	+	+	+	+
0.8	2	+	–	+	+	+	+	+	+
0.8	10	+	+	+	+	+	+	+	+
0.8	18	+	+	+	+	+	+	+	+
1.1	2	+	+	+	+	+	+	+	+
1.1	10	+	+	+	+	+	+	+	+
1.1	18	+	+	+	+	+	+	+	+

+Efficient; - Non-efficient (in terms of tested microorganisms, in specific experimental conditions).

for seconds to release the bacteria from the swab. Next, 1 mL of the solution was carefully transferred to a sterile plate to which about 15 mL of PCA were added, homogenized, and solidified on a flat surface.

The aerobic heterotrophic bacterial count was based on growing 1mL of swabbing solution in PCA followed by incubation at 36 ± 1 °C for 48 hours. After complete solidification, the plates were incubated at 36 °C for 48 hours, and the colonies were then counted.

3 Results and discussion

3.1 Efficiency of different sanitizers used in food industries

Qualitative evaluation of peracetic acid

Table 1 presents the results for the efficiency of peracetic acid under different concentrations and exposure times at 10 and 45 °C. This table shows that this chemical was efficient at 10 °C for all tested concentrations, contact time, and microorganisms. At 45 °C, this active principle presented efficiency only for *S. aureus* and *E. coli* at concentrations and exposure time lower than those suggested by the supplier.

The analysis of the results of Table 1 enables us to verify that the peracetic acid demonstrated loss of efficiency starting at temperature of 45 °C for *Salmonella choleraesuis* at 0.2, 0.5 and 0.8% at 2 minutes and at 0.2% after 2 and 10 minutes for *L. monocytogenes*. Kunigk, Gomes and Forte (2001) showed that at 45 °C a rapid decomposition of the product could have occurred.

Based on the literature, one can cite the study by Rossoni and Gaylarde (2000), which showed that the peracetic acid presented good activity under *E. coli* and *Pseudomonas fluorescens* reducing the adhered cells in 90% in a concentration of 250 $mg.L^{-1}$. On the other hand, at the same experimental conditions, the number of *S. aureus* was reduced by 50%. However, it increased to 90% when a sanitizer concentration of 1,000 $mg.L^{-1}$ was used.

Another study carried out by Briñez et al. (2006) evaluated the bactericidal effect of peracetic acid under pathogenic and non-pathogenic strains of *Staphylococcus* spp., *Listeria* spp., and *Escherichia coli*. These authors verified that the sanitizer was effective at the concentration of 0.1% and 10 minutes of exposure in all evaluated systems.

Qualitative evaluation of chlorhexidine

Analyzing Table 2, it can be seen that the chlorhexidine was efficient for *E. coli* at all evaluated concentrations and exposure times at 10 and 45 °C. An increase on efficiency was observed from 45 °C. For example, for *Salmonella choleraesuis*, the solution was efficient at 45 °C and 0.2% after 18 minutes or 0.5% and 10 minutes. Table 2 also shows that at 0.2, 0.5 and 0.8% after 2 minutes of exposure, the active principle did not present efficiency at 10 and 45 °C against *S. aureus*. Bambace et al. (2003) demonstrated the efficiency of chlorhexidine at 0.5% for the control of surfaces against *S. aureus*.

For *L. monocytogenes*, the chlorhexidine did not present efficiency at 0.2% after 2 minutes and 10 and 45 °C. This sanitizer was efficient at 45 °C but not at 10 °C using a concentration of 0.2% and an exposure time of 10 minutes. Under the other evaluated concentrations, the product was efficient both at 10 and 45 °C.

Generally, chlorhexidine demonstrated efficiency at concentrations lower than those recommended by the supplier thus being a good alternative for the control of the tested microorganisms.

Qualitative evaluation of quaternary ammonium

The results obtained for the qualitative evaluation of quaternary ammonium, presented in Table 3, demonstrated the efficiency of this sanitizer for all tested bacteria in concentrations lower than 0.6% at all exposure times and temperatures.

The study published by Ioannou, Hanlon and Denyer (2007) showed the high efficiency of quaternary ammonium against *S. aureus*. The supplier recommends a concentration

Table 2. Efficiency of chlorhexidine at different concentrations and exposure times at 10 and 45 °C.

Concentration (%)	Time (minutes)	*Salmonella choleraesuis*		*Staphylococcus aureus*		*Escherichia coli*		*Listeria monocytogenes*	
		10 °C	45 °C	10 °C	45 °C	10 °C	45 °C	10 °C	45 °C
0.2	2	–	–	–	–	+	+	–	–
0.2	10	–	–	–	+	+	+	–	+
0.2	18	–	+	+	+	+	+	+	+
0.5	2	–	–	–	–	+	+	+	+
0.5	10	–	+	+	+	+	+	+	+
0.5	18	+	+	+	+	+	+	+	+
0.8	2	–	+	–	–	+	+	+	+
0.8	10	+	+	+	+	+	+	+	+
0.8	18	+	+	+	+	+	+	+	+
1.1	2	+	+	+	+	+	+	+	+
1.1	10	+	+	+	+	+	+	+	+
1.1	18	+	+	+	+	+	+	+	+

+Efficient; - Non-efficient (in terms of tested microorganisms, in specific experimental conditions).

Table 3. Efficiency of quaternary ammonium at different concentrations and exposure times at 10 and 45 °C.

Concentration (%)	Time (minutes)	*Salmonella choleraesuis*		*Staphylococcus aureus*		*Escherichia coli*		*Listeria monocytogenes*	
		10 °C	45 °C	10 °C	45 °C	10 °C	45 °C	10 °C	45 °C
0.2	2	–	–	–	–	–	–	–	–
0.2	10	–	–	–	–	–	–	+	+
0.2	18	+	–	+	+	+	+	+	+
0.6	2	+	+	+	+	+	+	+	+
0.6	10	+	+	+	+	+	+	+	+
0.6	18	+	+	+	+	+	+	+	+
1.0	2	+	+	+	+	+	+	+	+
1.0	10	+	+	+	+	+	+	+	+
1.0	18	+	+	+	+	+	+	+	+
1.4	2	+	+	+	+	+	+	+	+
1.4	10	+	+	+	+	+	+	+	+
1.4	18	+	+	+	+	+	+	+	+

+Efficient; - Non-efficient (in terms of tested microorganisms, in specific experimental conditions).

of 0.6% as appropriate for the higienization of surfaces in contact with food. In the present study, it was observed that this recommendation was confirmed at a contact time of 2 minutes, lower than that recommended by the supplier (10 minutes).

The resistance of some microorganisms to quaternary ammonium is has been reported in some studies present in the literature (MCBAIN et al., 2004; SIDHU; SORUM; HOLCK, 2002). The focus on food security and production of refrigerated food prepared to consumption led to an increase in the use of this sanitizer in food industries.

Qualitative evaluation of organic acids

Table 4 shows the low efficiency of organic acids against the bacteria tested in this study. In general, for *S. choleraesuis*, *E. coli* and *L. monocytogenes*, the temperature of 10 °C demonstrated higher efficiency at different concentrations and exposure times. At 45 °C, this sanitizer demonstrated efficiency at 0.6% and 25 minutes of exposure time, but only for *S. choleraesuis* and *L. monocytogenes* . *E. coli* can be identified as a good indicator microorganism since it presented high resistance at all concentrations and exposure times tested in the present study. According to the suppliers, organic acids are suggested in a range of concentration from 0.6 to 1.0% and exposure time of 15 minutes. However, the results obtained here demonstrated that this sanitizer was efficient only against *S. choleraesuis*, and *L. monocytogenes*. For *E. coli,* the organic acids' efficiency was observed only at 25 minutes in 0.6% of.

The low efficiency of organic acids related to the tested microorganisms can be explained by the fact that the compounds are in the dissociated form at the moment of product application and the more diluted the sanitizer, the higher the dissociation and lower the efficiency. The inhibitory action of organic acids in non-dissociated form is 100 to 600 times higher than in the dissociated form. In the non-dissociated form, this compound can permeate the cell membrane by diffusion and liberate protons in the cell cytoplasm (EKLUND, 1983).

The antimicrobial activity of organic acids is related to pH reduction and the ability of carboxyl dissociation (CHERRINGTON; HINTON; CHOPRA, 1991). In their non-dissociated form, these acids can penetrate in a passive way in microbial cell, in which protons and anions are released

Table 4. Efficiency of organic acids at different concentrations and exposure times at 10 and 45 °C.

Concentration (%)	Time (minutes)	*Salmonella choleraesuis*		*Staphylococcus aureus*		*Escherichia coli*		*Listeria monocytogenes*	
		10 °C	45 °C	10 °C	45 °C	10 °C	45 °C	10 °C	45 °C
0.2	2	–	–	–	–	–	–	–	–
0.2	15	–	–	–	–	–	–	+	–
0.2	25	–	–	–	–	–	–	+	–
0.6	2	–	–	–	–	–	–	–	–
0.6	15	+	–	–	–	–	–	+	–
0.6	25	+	+	–	–	+	–	+	+
1.0	2	–	–	–	–	–	–	–	–
1.0	15	+	–	–	–	+	–	+	–
1.0	25	+	+	–	–	+	–	+	+
1.4	2	–	–	–	–	–	–	–	–
1.4	15	+	–	–	–	+	–	+	–
1.4	25	+	+	–	–	+	–	+	+

+Efficient; - Non-efficient (in terms of tested microorganisms, in specific experimental conditions).

resulting in a reduction in the intracellular pH inhibiting the enzyme action and provoking the death of the microorganism. The microbial activity, however, can also depend on the anions accumulation in the intracellular content (RUSSELL, 1992).

Industrial scale tests

The analysis performed in the industrial plant before and after the sanitization with different agents in general proved effective in reducing heterotrophic bacteria. Peracetic acid at 0.2% and 2 minutes led to good results from 300 UFC.20 cm^{-2} to 5 and 2 UFC.20 cm^{-2}. Chlorhexidine, from an initial concentration of 120 UFC.20 cm^{-2}, led to a final concentration of 5 UFC.20 cm^{-2}. The use of ammonium quaternary at a concentration of 0.6% and contact time of 2 minutes presented a reduction from 270 to 11 UFC.20 cm^{-2}. These sanitizers proved efficient (10 UFC.cm^{-2} or 200 UFC.20 cm^{-2}) in accordance with Act Nº 471/2001 of European Union legislation.

The analysis using organic acids demonstrated low efficiency of this active principle at 1% and exposure time of 15 minutes. In spite of the low initial cell count (from 1 to 11 UFC.20 cm^{-2}), this value was kept after sanitization. These results are in agreement with those obtained earlier indicating the lower performance of this active principle compared to others tested in this work.

Final remarks

Peracetic acid at 0.2% and 2 minutes at 10 °C was efficient against the microorganisms tested, condition referred to concentration and contact time lower than that indicated by the supplier. Another alternative, using the same active principle, could be the use of a concentration of 0.2% and contact time of 18 minutes for 10 and 45 °C or 0.5% and 10 minutes.

Chlorhexidine proved efficient when applied at a concentration of 0.2% and contact time of 18 minutes at 45 °C for all tested microorganisms. The indication made by the supplier was confirmed, and when a concentration of 0.5% for 10 minutes was applied, it also proved efficient against the four microorganisms tested.

The quaternary ammonium proved efficient at 10 °C against all microorganisms at 0.2% and 18 minutes or 0.6% and 2 minutes, both at 10 and 45 °C.

The best concentrations of the organic acids were those indicated by the supplier, 0.6 and 1% at a contact time of 25 and 15 minutes, respectively, at a temperature of 10 °C against *S. choleraesuis*, *E. coli* and *L. monocytogenes*.

Based on the results obtained in this study and discussed above, it can be concluded that the choice for the most adequate sanitizer will depend on the microorganism contaminant, the time available for sanitizer application, and also on the process cost. It is worth to mention that the survival of bacteria after the sanitization step represents a potential risk for food industry and consumers. The lost of microbial activity in the presence of organic material is presented in the literature, varying as a function of the sanitizer and contaminant (BEST; KENNEDY; COATES, 1990), demonstrating the importance of performing specific tests for choosing the most adequate products for the higienization step. Therefore, the bacteria strains can increase their resistance (bacterial resistance) as a result of stress and biofilm formation.

4 Conclusions

Comparing the results obtained in the evaluation of efficiency of the four tested active principles used in food industries with those from a study conducted in industrial scale make possible to carry out simulations aiming at showing alternatives, such as the use of sanitizers, which contributed decisively to the production of food following the microbiological standards recommended by the legislation and reducing the process costs.

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