PAPER

LISTERIA MONOCYTOGENES ADHESION TO FOOD PROCESSING SURFACES (BONING KNIVES) AND THE REMOVAL EFFICACY OF DIFFERENT SANITIZERS

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ABSTRACT

The adhesion and biofilm formation of *Listeria monocytogenes* on new and used boning knives (handle and blade) were observed. The number of pathogens on both surfaces increased with the contact time, forming a biofilm after 2 h. Peracetic acid or hot water effectively removed adhered *L. monocytogenes* at each of the contact times and concentrations evaluated. Biguanide showed lower removal efficacy on used surfaces, but had increased effectiveness at concentrations of 2.0%. The new knife blades had a lower roughness and flattened morphology in comparison with used surface

Keywords: bacterial adhesion, sanitizer, *Listeria monocytogenes*, nactivation, boning knives

1. INTRODUCTION

Food contact surfaces constantly must pass a microbiological evaluation for efficiency control and sanitizing procedures, in order to avoid contamination of the food produced (PINHEIRO *et al*., 2010; ALMEIDA *et al*., 2013). The exposure of surfaces to pathogens may take place either by direct contact with contaminated objects or indirectly through airborne particles. According to KUSUMANINGRUM *et al*. (2003), many bacteria, including *L. monocytogenes* may survive on utensils for hours or days after the initial contamination and can persist on industrial equipment and installations with high potential for adhesion and biofilm formation at low temperatures (GIAOURIS *et al*., 2014; CARPENTIER and CERF, 2011).
Cell surface hydrophobicity and production of extracellular polymeric substances influence the rate and extent of attachment of microbial cells. Another factor that can also influence bacterial adhesion is the surface roughness (RODRIGUEZ *et al*., 2008; SKOVAGER *et al*., 2013).
Bacterial adhesion to stainless steel, glass, rubber and polypropylene surfaces is a potential source of contamination that may lead to disease transmission (CHAVANT *et al*., 2007). The adhered cells are highly resistant to acids present in sanitizers, to desiccation and to heat. Tolerance to increased sub-lethal concentrations of disinfectants or resistance to lethal concentrations are documented, because sessile cells produce exopolysaccharides that protect against chemical agents (JOSEPH *et al*., 2001; CARPENTIER and CERF 2011; SILVA *et al*., 2010). Several sanitizers are available with different uses, but they do not always eliminate bacteria to the expected level (BELTRAME *et al*., 2012). In this way, it is very important to know the factors involved in the adherence of biofilms to surfaces, which could be useful to improve methods for disinfection of food processing surfaces (boning knives). Therefore, the purpose of this study was to investigate adhesion and biofilm formation of *L. monocytogenes* on the handle and blade of new and used boning knives. This study also evaluated the removal of bacteria on the surface of the knife using different contact times and concentrations of chemical sanitizers (peracetic acid and biguanide) or hot water.

2. MATERIALS AND METHODS

In this study, bacterial adhesion and inactivation were evaluated on new and used boning knives using Gram-positive *L. monocytogenes* (ATCC 7644). The bacterial culture was purchased from the Instituto Oswaldo Cruz, mantained at -80°C and revitalized in Luria Bertani broth (LB Merck, Darmstadt, Germany) at 30°C, 24 hours before the experiments.

2.1. Bacterial adhesion and quantification of adhered cells

In order to assess bacterial adhesion, the polypropylene (PP) handle and stainless steel (SS) blade of new boning knives (Professional line Mundial® model 5315-6) and the same model of used knives (use in a cattle slaughterhouse for 45 to 60 days) were studied.
The entire knife surface (handle and blade) was prepared according to the follows steps: cleaning by manual rubbing with water and neutral liquid detergent, rinsing with water and then by sterile distilled water and air drying. For sanitization, the surfaces were exposed to ultraviolet light (254 nm) for one hour. After cleaning, the entire knife surface was swabbed in order to confirm the absence of initial contamination (negative control), before carrying out the experiments.

The bacteria were incubated in LB broth for 24 h at 35-37°C. Next, 10 mL of inoculum (3 log CFU/mL) was inoculated into 1.5 L LB broth that had been previously poured into a polyvinylchloride (PVC) tube with a 10 cm diameter to accommodate the entire knife, and the samples were incubated for 24 h at 35-37°C. Afterward, the knives (new or used) were removed from the PVC tube with sterile forceps and rinsed with water to remove the planktonic cells. The entire surface of the knife handle and knife blade were swabbed separately to assess contamination. Dilutions were performed in peptone water, plated onto LB agar, and incubated at 35-37°C for 24 h. The number of adherent cells was assessed in intervals of 0.1, 0.5, 1, 2, 6, 24 and 48 h at 35°C. These times were selected in order to simulate the factory production time, which is the period that knives remain in contact with products without sanitization.

2.2. Characterization of the boning knife surface by contact angle

The hydrophobicity and hydrophilicity of the new and used blades with and without *L. monocytogenes* adhesion for 6 h, were determined by contact angle with a drop of water using a contact angle metre (KSV Instruments, Helsinki, Finland). The measurements were performed at 25°C and 45% humidity, by depositing 4.0 μL of water with a Hamilton syringe. The handles were not evaluated in this analysis because the texture of the handle produces differences in surface that did not permit the stable formation of a drop on the surface.

2.3. Characterization of the boning knife surface by Atomic Force Microscopy (AFM)

The morphology and average roughness (Ra) of the new and used blades with and without *L. monocytogenes* adhesion for 6 h were analyzed with a Dimension V (Veeco Instruments Inc.) AFM, using a silicon nitride tip, with a spring constant of 42 N/m and resonance frequency of 285 kHz. All images were obtained in tapping™ mode at a scan rate of 1 Hz. The images were processed with the aid of Gwydion© 2.1 data analysis software. The handles were not evaluated due to the characteristics of the non-slip coating that had very large differences in height, which did not permit a surface scan.
The Ra value (arithmetic mean deviation of the profile) is the most common measure used to define the surface roughness (VERRAN *et al*., 1991). AFM scans were performed in 500 x 500 nm^2 areas on each surface. The roughness was calculated from three scans in different areas.

2.4. *L. monocytogenes* inactivation

In this study, peracetic acid (Pluron 461 AP®) and biguanide (Pluron 463 AP®) sanitizers were studied; they were prepared in sterilized water immediately prior to testing, according to the supplier's instructions. In inactivation experiments, the entire knife was analyzed at intervals of 1, 2 and 6 h, simulating industrial conditions. After rinsing with deionized water, the knives were immersed in beakers containing 500 mL of the respective sanitizer solution at concentrations of 0, 0.2, 0.5, 1.0 or 2.0% (v/v) for 10 min at 25°C, to evaluate their efficacy against cell attachment.
The hot water treatment was performed by immersing the knife surface in a hot water bath (82.2°C) for 15 s, according to 175/2005/MAPA method 2 (BRASIL 2005).
Bacterial presence was quantified by the enumeration method as previously described (KIM *et al*., 2008) using swabs of the knives.

2.5. Statistical analysis

The results of *L. monocytogenes* counts were converted to decimal logarithmic values (log CFU/cm^2) and subjected to Tukey's test at a 5% significance level using Statistica 8.0 software (StatSoftInc®, USA). All experiments were run in triplicate and repeated with three separate Knives.

3. RESULTS AND DISCUSSIONS

3.1. *Listeria monocytogenes* adhesion

The adhesion of *L. monocytogenes* on new and used boning knives (handles and blades) is shown in Fig. 1. A rapid bacterial growth was observed for the first 6 h, with a tendency to stabilize at 48 h. Adherence occurred on handles and blades and the adhesion velocities were similar for used and new materials. However, statistical analysis showed a significant difference in adherence to new and used polyethylene handles between 6 and 48 h (Fig. 1a). The knives a non-slip coating on the surface of the handle, which conveys firmness and grip during handling, however, this feature promoted easy adhesion of bacteria on used surfaces.

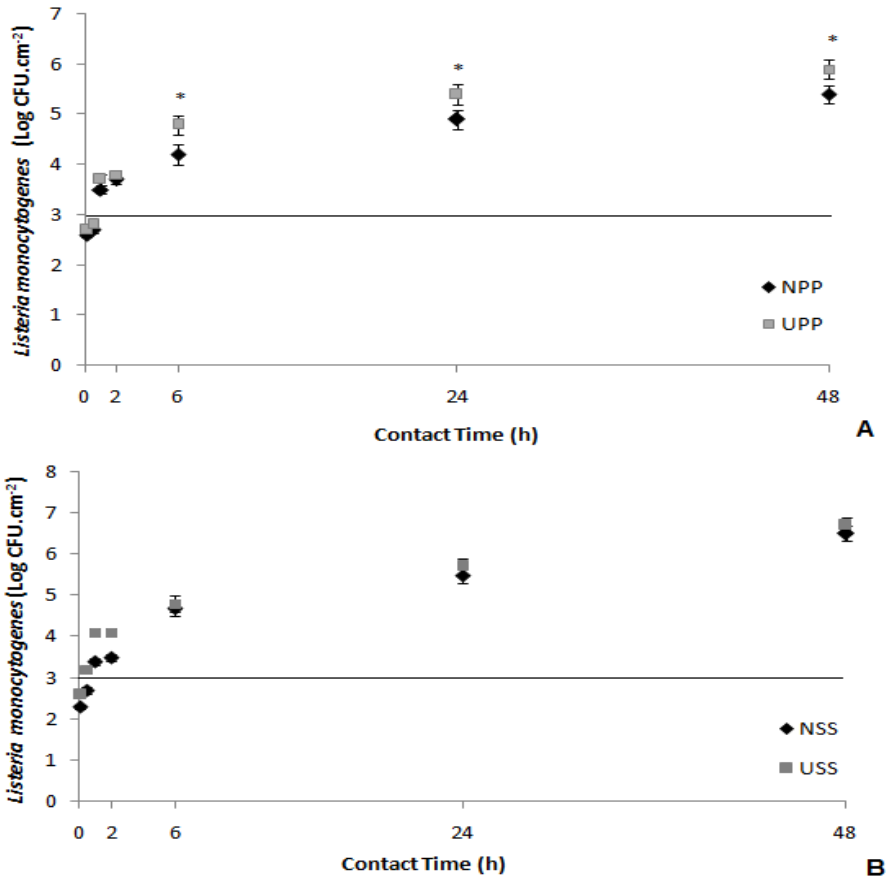

Figure 1: Adhesion kinetics of *L. monocytogenes*on(A) new polyethylene handles (NPP) and usedpolyethylene handles (UPP) and (B) newstainless steel blades (NSS) and usedstainless steel blades(USS). Biofilm formation with 3.0 Log CFU/cm^2. Means (± standard deviations), for each time tested, who having the * symbol are significantly different ($p<0.05$).

L. monocytogenes showed a greater capacity for adhesion onto SS than to PP (Fig. 1) from 6 h in new, and 24 h in used knives. These results confirm those by Teixeira *et al*. (2008), who observed greater adhesion on SS as compared with the extent of adhesion on PP (cutting board).
RONNER and WONG (1993) defined biofilm formation as a recovery of greater than 3.0 log CFU/cm^2 adhered cells. Thus, according to this criterion, *L. monocytogenes* biofilm growth occurred after 1 h of contact on the handles (new and used), and from 30 min (new) to 1 h (used) on the SS surface.

3.2. Characterization of the surface of the boning knife by contact angle

Water contact angle is a quantitative measurement of surface wettability, and also can be used to evaluate the cleanliness of the material surface. Throughout this study, a contact angle of 70±1.0 and 82±1.0 degrees was obtained for new stainless steel (NSS) and used stainless steel (USS) surfaces without microbial adhesion, respectively. These results agree with those found by BERNARDES *et al*. (2010), who found a value of 70±7.9 on SS surfaces. After 6 h of bacteria exposure, contact angles of 19±1.4 and 30±2.1 were obtained on NSS and USS respectively, showing that the surfaces became more wettable after microbial adhesion. These results corroborate with those of CHAVANT *et al*. (2007), who observed better adhesion and biofilm formation of *L. monocytogenes* on hydrophilic (SS) rather than on hydrophobic (PP) surfaces. These decreases in the value of contact angle from 70 down to 19 (NSS) and 80 down to 30 (USS) may be attributed to the *L. monocytogenes* surface composition (molecules such as proteins, teichoic and lipoteichoic acids) (Bereksi *et al*., 2002).

3.3. Morphological and roughness characterization

The morphology of new and used blades was analyzed through use of AFM before and after *L. monocytogenes* adhesion (Fig. 2).
Roughness values (Ra) of 5.1±2.3, 14.3±1.7, 17.4±2.4 and 28.8±2.1 nm were obtained from new and used blades without and with *L. monocytogenes* adhesion, respectively. New blades had a low roughness and flattening morphology compared with used surfaces, which is very important to note because studies have shown that an increase in Ra value will cause a corresponding increase in microbial adhesion on surfaces (Whitehead *et al*., 2004). This increase may be due to protective cells present in microscopic niches.
Roughness values of 800 nm or less are generally used to describe a hygienic surface (FLINT *et al*., 1997). These values were found for all surfaces evaluated in this work (Fig. 2). Teixeira *et al*. (2008), observed that SS is a material with high surface roughness, similar to what was measured in this study for the surface of the knife blade (Mundial®).
TAYLOR *et al*. (1998) observed a significant increase in the attachment of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* on polymethyl methacrylate when the surfaces had a small increase in roughness (40-1240 nm). Bacterial adhesion has been found to increase numerically with surface roughness, by comparing bacterial adhesion on used versus abraded SS and for Ra value increases from 602 to 706 nm or from 484 to 698 nm (Holah and Thorpe, 1990). Thus, it was found that the roughness data obtained after the adherence of *L. monocytogenes* are in agreement with those from the literature, but no correlation was demonstrated between Ra and the number of adhering cells. From this evaluation of the surface characteristics of boning knives, it can be observed that with an increase of the surface roughness, there was a decrease in the contact angle.

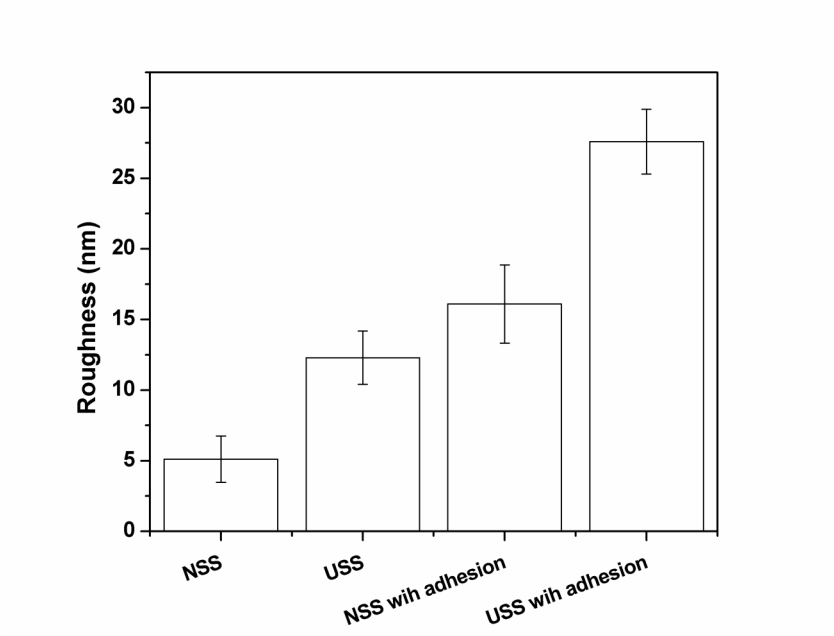

Figure 2: Roughness values on NSS and USS with and without*L. monocytogenes* adhesion. Mean values and standard deviations (error bars) are indicated. Observations correspond to on 500 x 500 nm scan area.

3.4. Efficacy of different sanitizers on food processing surfaces

To evaluate their ability to sanitize new and used knives, biguanide and peracetic acid sanitizers were used at different concentrations (0.2, 0.5, 1.0 and 2.0% v/v) for 10 min, or hot water (82.2°C) was used for 15 s. The boning knives remained in contact with *L. monocytogenes* for different contact times (1, 2 and 6 h) to simulate industrial conditions for adhesion. *L. monocytogenes* was resistant to biguanide sanitizer under the different contact times studied (Table 1).

Similar efficacy levels have been obtained by other researchers. MARTÍN-ESPADA *et al*. (2014) observed that 1.61% peracetic acid was effective against *P. aeruginosa* biofilms formed on polystyrene surfaces, inhibiting almost 100% of the microbial population. Similarly, BELTRAME *et al*. (2015) evaluated the efficacy of 0.5% peracetic acid at inactivating *L. monocytogenes* cells adhered to cutting boards and observed that it was able to reduce the amount of adhered cells by 100%, after 3 h of contact time with bacteria. Cabeça *et al*. (2012) observed adhesion of *L. monocytogenes* on SS surfaces and studied the inactivation after treatment with 0.5% biguanide and 0.5% peracetic acid. The authors verified an initial count of 6.2 log CFU/cm^2 with reduction to 2.9 log CFU/cm^2 and 1.1 log CFU/cm^2 using biguanide and peracetic acid, respectively, showing that peracetic acid was more effective against *L. monocytogenes* cells.

For the sanitizer evaluated, the suppliers recommend a maximum disinfection concentration of 0.5% for both biguanide and peracetic acid. Thus, there was a variation in the effectiveness of the procedures, based on decimal reductions, in microbial effect conveyed by the different sanitization process investigated, as demonstrated in Table 2.

In the maximum contact time (6h) between the knife and the microorganism, the low concentration of peracetic acid (0.2%), lower than that recommended by the supplier, showed reliable results from the point of microbiology safety. Similar results were observed for hot water, where the presence of surviving *L. monocytogenes* was not observed. On the other hand, the maximum concentration of biguanide recommended by

the supplier was not able to remove adherent cells, with maximum efficacy of only 50% on NPP (Table 2).

Table 1: Reduction of the *L. monocytogenes* count (Log CFU/cm²) as a function of contact time (1, 2 and 6 h) and the Biguanide sanitizer concentration (0.2, 0.5, 1.0 and 2.0%).Results in Log CFU/cm².

Contact time	Boning knives	Initial count	Biguanide Concentration (%) R* 0.2%	%	R* 0.5%	%	R* 1.0%	%	R* 2.0%	%
1h	NPP	3.5	2.0±0.2^{c}	57,1	2.7±0.1^{b}	77.1	3.3±0.1^{a}	94,3	3.5±0.3^{a}	100
	UPP	3.7	0.5±0.1^{c}	14.3	1.7±0.1^{b}	45,9	1.8±0.2^{b}	48.6	3.0±0.1^{a}	81.1
	NSS	3.4	0.6±0.1^{c}	17.6	1.3±0.1^{b}	38.2	3.2±0.1^{a}	94.1	3.4±0.1^{a}	100
	USS	4.1	0.2±0.1^{d}	4.9	1.7±0.1^{c}	41.5	2.8±0.1^{b}	68.3	4.0±0.1^{a}	97.6
2h	NPP	3.7	1.7±0.1^{b}	4.6	2.4±0.2^{ab}	64.9	2.8±0.2^{a}	75.7	2.7±0.1^{a}	73.0
	UPP	3.8	0.8±0.1^{c}	21.0	1.8±0.1^{b}	47.4	1.8±0.1^{b}	47.4	2.7±0.1^{a}	71.0
	NSS	3.5	0.2±0.1^{d}	5.7	0.9±0.1^{c}	25.7	1.9±0.1^{b}	54.3	3.3±0.1^{a}	94.3
	USS	4.1	0.6±0.1^{d}	14.6	1.6±0.1^{c}	39.0	2.1±0.1^{b}	51.2	3.3±0.1^{a}	80.5
6h	NPP	4.2	1.1±0.1^{c}	26.2	2.1±0.1^{b}	50.0	2.5±0.2^{a}	59.5	2.8±0.1^{a}	66.7
	UPP	4.8	0.1±0.1^{c}	2.1	2.3±0.2^{b}	47.9	2.7±0.2^{ab}	56.2	3.1±0.2^{a}	64.6
	NSS	4.7	1.0±0.1^{d}	21.3	2.0±0.1^{c}	42.6	2.7±0.1^{b}	57.4	3.9±0.1^{a}	83.0
	USS	4.8	0.7±0.1^{c}	14.6	0.5±0.1^{c}	10.4	2.4±0.1^{b}	50.0	3.7±0.1^{a}	77.1

NPP: new handles; UPP: used handles, NSS: new blades; USS: used blades.
*R Decimal reduction (Log CFU initial population-Log CFU end population submitted to the sanitizers application). Values followed for the same letters in the lines do not differ statistically according to theTukey Test, with 95% confidence range.

Table 2: Reduction of the *Listeria monocytogenes* count (Log CFU/cm²) in boning knives after hot water and sanitizer treatments (6 h of contact time, sanitizer concentrations - 0.5% biguanide and 0.2% peracetic acid).

Boning Knives	Initial Count	Biguanide R*	%	Peracetic acid R*	%	Hot water R*	%
NPP	4.2	2.1±0.1	50.0	4.2±0	100	4.1±0.1	100
UPP	4.8	2.3±0.2	47.9	4.8±0	100	4.8±0	100
NSS	4.7	2.0±0.1	42.6	4.7±0	100	4.7±0	100
USS	4.8	0.5±0.1	10.4	4.8±0	100	4.8±0	100

NPP: new handles; UPP: used handles; NSS: new blades; USS: used blades.
* Decimal reduction (initial population Log CFU - end population Log CFU).

Thus, this work confirms that the most effective treatments were the hot water and peracetic acid sanitizer, whereas biguanide showed lower performance. Additional studies should be performed to improve the action of biguanide on removal of *L. monocytogenes* from food preparation surfaces.
The treatments with hot water and peracetic acid were shown to be effective for the inactivation of bacteria that had initially adhered. This result can be associated to the disinfectant activity of peracetic acid based on the release of active oxygen, which may

disrupt the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport through the dislocation or rupture of cell walls. This can also be effective against outer membrane lipoproteins, facilitating its action against Gram-negative cells. The intracellular peracetic acid may also oxidize essential enzymes, which may damage the bases of DNA molecules (KITIS, 2004). Moreover this sanitizing agent has low environmental hazards and does not produce toxic compounds upon reaction with organic materials.

Likewise, the application of hot water was also an effective means of bacterial inactivation. The mechanism of action of hot water treatment is multifactorial. The exposure to this high temperature is likely to affect most components of the bacterial cell including the cell wall, cell membrane, enzymes and proteins, DNA and RNA (PHUA *et al*., 2014).

Differences between the bactericidal effect of biguanide solutions and peracetic acid were observed, corroborating data obtained by SILVA *et al*. (2010), who showed that peracetic acid was a more effective bactericidal agent than the quaternary ammonium compound (whose mechanism of action is similar to biguanide).

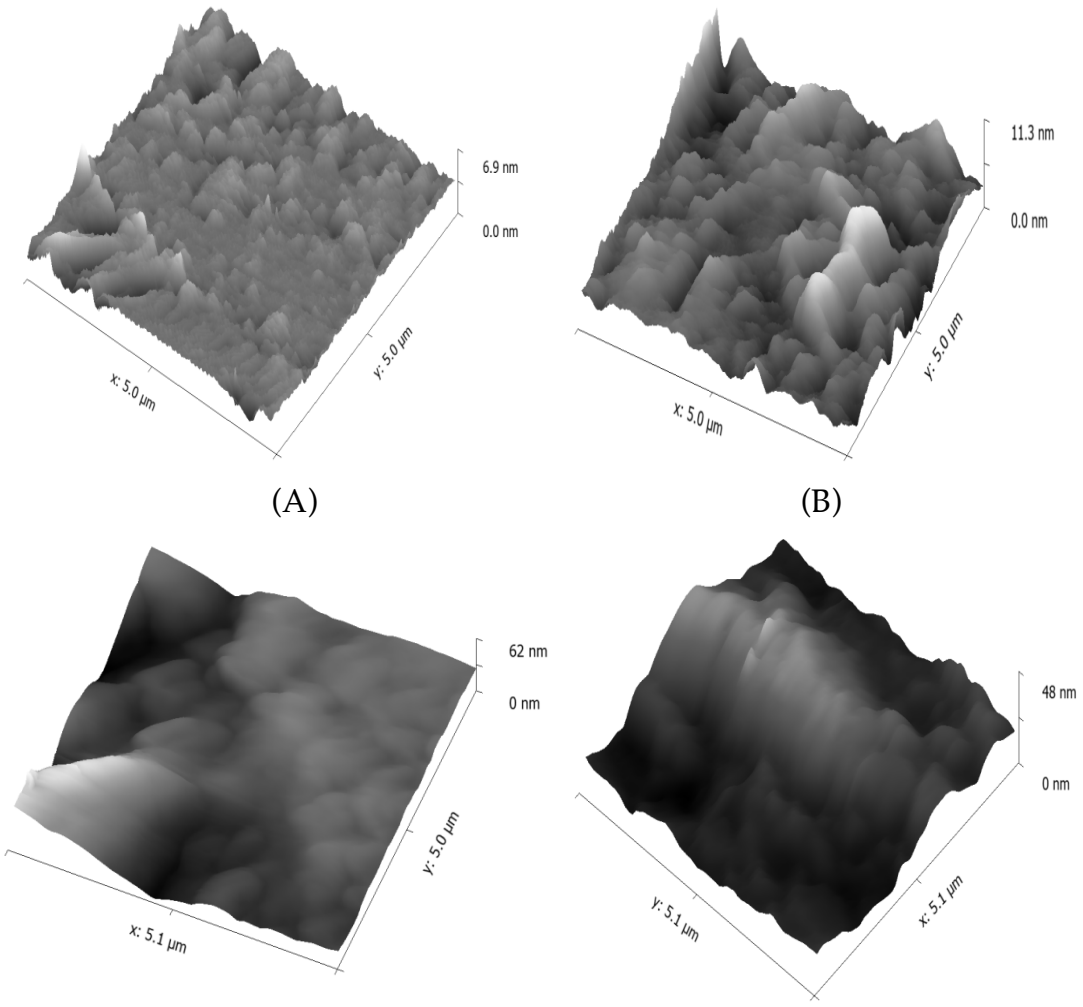

Figure 3: AFM characterization of the knives surface in 3D (A) new without bacterial adhesion, (B) used without bacterial adhesion, (C) new with bacterial adhesion and (D) used with bacterial adhesion.

Sodium dichloroisocyanurate, hydrogen peroxide and peracetic acid have been evaluated for their abilities to inactivate the biofilm formed by *S. aureus* on SS and glass surfaces; peracetic acid showed a significant difference ($p < 0.05$) compared with the three disinfectants used (MARQUES *et al*., 2007). The higher efficacy of peracetic acid was explained by its high capacity to oxidize cellular molecules.
The survival of bacteria after cleaning and sanitizing is a potential danger to the food industry and the consumer (RODGERS *et al*., 2001). This study demonstrates the need for specific tests in order to select products to be used to clean surfaces that come into contact with food. Furthermore, bacteria can obtain high resistance through adaptation, genetic elements, stress response and biofilm formation (BRIDIER *et al*., 2011).
It should be noted that an appropriate and effective cleaning process is extremely important, since the American Public Health Association (APHA) recommends a maximum tolerated limit of 2 CFU/cm^2 in order to consider a food contact surface appropriate (VANDERZANT and SPLITTSSTOESSER, 1992), whereas the World Health Organization (WHO) suggests limits of 30 CFU/cm^2. Based on the obtained results, this study confirms that hot water and peracetic acid was fully effective in removing *L. monocytogenes* cells, which had adhered onto new and used boning knives, whereas biguanide was not efficient in removing the bacterial cells.

4. CONCLUSIONS

Adherence of *L. monocytogenes* occurred on handles and blades, and the adhesion velocities were similar between the used and new materials. Regarding the morphology and contact angle of the surfaces, an increase of the wettability and roughness on the used stainless steel surface in relation to the new stainless steelsurface was observed.
Disinfection with peracetic acid was effective at all contact times and concentrations evaluated; no surviving bacteria was found after sanitizer application in all conditions investigated. The hot water treatment (82.2°C for 15 s) also was effective in reducing *L. monocytogenes* adhesion on the surfaces tested. Biguanide showed lower efficacy on new and used handles and blades, but had increased effectiveness at concentrations of 2.0% with 1 h of contact time.

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REFERENCES

Almeida G. *et al*. 2013. Foci of contamination of *Listeria monocytogenes* in different cheese processing plants. Int. J. Food Microbiol. 167:303-309 doi:http://dx.doi.org/10.1016/j.ijfoodmicro.2013.09.006

Beltrame C.A. *et al*. 2012. Influence of different sanitizers on food contaminant bacteria: effect of exposure temperature, contact time, and product concentration Ciência Tecnol. Alim. 32:228-232.

Beltrame C.A. *et al*. 2015. Adhesion of *Listeria monocytogenes* to cutting board surfaces and removal by different sanitizers. J. Verbr. Lebensm. 10:41-47 doi:10.1007/s00003-014-0923-7

Bereksi N., Gavini F., Benezech T. and Faille C. 2002. Growth, morphology and surface properties of *Listeria monocytogenes* Scott A and LO28 under saline and acid environments. J. Appl. Microbiol. 92:556-565.

Bernardes P.C., Andrade N.J.d., Ferreira S.O., Sá JPNd, Araújo EA, Delatorre DMZ and Luiz LMP. 2010. Assessment of hydrophobicity and roughness of stainless steel adhered by an isolate of *Bacillus cereus* from a dairy plant. Braz. J. Microbiol. 41:984-992.

Brasil. 2005. Departamento de Inspeção de Produtos de Origem Animal. Circular Nº 175/2005/CGPE/DIPOA, de 16 de maio de 2005. Procedimentos de Verificação dos Programas de Autocontrole. Brasilia

Bridier A., Briandet R., Thomas V. and Dubois-Brissonnet F. 2011. Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27(9):1017-32.

Cabeça T.K., Pizzolitto A.C., Pizzolitto E.L. 2012. Activity of disinfectants against foodborne pathogens in suspension and adhered to stainless steel surfaces. Braz. J. Microbiol. 43:1112-1119.

Carpentier B., Cerf O. 2011. Review-Persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145:1-8 doi:10.1016/j.ijfoodmicro.2011.01.005
Chavant P., Gaillard-Martinie B., Talon R., Hebraud M. and Bernardi T. 2007. A new device for rapid evaluation of biofilm formation potential by bacteria. J. Microbiol. Meth. 68:605-612 doi:10.1016/j.mimet.2006.11.010

Flint S.H., Bremer P.J. and Brooks J.D. 1997. Biofilms in dairy manufacturing plant-description, current concerns and methods of control. Biofouling 11:81-97 doi:10.1080/08927019709378321

Giaouris E. *et al*. 2014. Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. Meat Sci. 97:298-309 doi:http://dx.doi.org/10.1016/j.meatsci.2013.05.023

Holah J.T. and Thorpe R.H. 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. J. Appl. Bact. 69:599-608.

Joseph B., Otta S.K., Karunasagar I. and Karunasagar I. 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol. 64:367-372.

Kim H., Bang J., Beuchat L.R. and Ryu J.H. 2008. Fate of *Enterobacter sakazakii* attached to or in biofilms on stainless steel upon exposure to various temperatures or relative humidities. J. Pood Prot. 71:940-945.

Kitis M. 2004. Disinfection of wastewater with peracetic acid: a review. Environ. Int. 30:47-55 doi:http://dx.doi.org/10.1016/S0160-4120(03)00147-8

Kusumaningrum H.D., Riboldi G., Hazeleger W.C. and Beumer R.R. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int. J. Food Microbiol. 85:227-236.

Marques S.C., Rezende JdGOS, Alves LAdF, Silva B.C., Alves E., Abreu LRd and Piccoli R.H. 2007. Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. Braz. J. Microbiol. 38:538-543.

Martín-Espada M.C., D'Ors A., Bartolomé M.C., Pereira M. and Sánchez-Fortún S. 2014. Peracetic acid disinfectant efficacy against *Pseudomonas aeruginosa* biofilms on polystyrene surfaces and comparison between methods to measure it LWT - Food Sci Technol 56:58-61 doi:http://dx.doi.org/10.1016/j.lwt.2013.11.013

Phua L.K., Neo S.Y., Khoo G.H. and Yuk H.-G. 2014. Comparison of the efficacy of various sanitizers and hot water treatment in inactivating inoculated foodborne pathogens and natural microflora on mung bean sprouts. Food Control 42:270-276 doi:http://dx.doi.org/10.1016/j.foodcont.2014.02.013

Pinheiro M.B., Wanda T.C. and Pereira C.A.M. 2010. Análise microbiológica de tábuas de manipulação de alimentos de uma instituição de ensino superior em São Carlos, SP.Rev Simbio-Logias 3:115-124.

Rodgers J.D., McCullagh J.J., McNamee P.T., Smyth J.A. and Ball H.J. 2001. An investigation into the efficacy of hatchery disinfectants against strains of *Staphylococcus aureus* associated with the poultry industry. Veterinary Microbiol. 82:131-140.

Rodriguez A., Autio W.R. and McLandsborough L.A. 2008. Effect of surface roughness and stainless steel finish on *Listeria monocytogenes* attachment and biofilm formation. J. Food Prot. 71:170-175.

Ronner A.B. and Wong A.C.L. 1993. Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella typhimurium* on stainless steel and Buna-n rubber. J Food Protection

Silva I.D., Careli R.T., Lima J.C. and Andrade N.J 2010. Effectiveness of cleaning and sanitizing procedures in controlling the adherence of *Pseudomonas fluorescens*, *Salmonella Enteritidis*, and *Staphylococcus aureus* to domestic kitchen surfaces. Food Sci. Technol. (Campinas) 30:231-236.

Skovager A., Whitehead K., Wickens D., Verran J., Ingmer H. and Arneborg N. 2013. A comparative study of fine polished stainless steel, TiN and TiN/Ag surfaces: Adhesion and attachment strength of *Listeria monocytogenes* as well as anti-listerial effect Colloids Surfaces B: Biointerfaces 109:190-196 doi:http://dx.doi.org/10.1016/j.colsurfb.2013.03.044

Taylor R.L., Verran J., Lees G.C. and Ward A.J.P. 1998. The influence of substratum topography on bacterial adhesion to polymethyl methacrylate J Mater Sci: Mater Medicine 9:17-22 doi:10.1023/A:1008874326324

Teixeira P., Lima J., Azeredo J. and Oliveira R.M. 2008. Adhesion of *Listeria monocytogenes* to materials commonly found in domestic kitchens. Int. J. Food Sci. Tech. 43:1239-1244.

Vanderzant C. and Splittstoesser D.F. 1992. Compendium of methods for the microbiological examination of foods. 3.ed. Washington: American Public Health Association, 1219 p.

Verran J., Lees G. and Shakespeare A.P. 1991. The effect of surface roughness on the adhesion of *Candida albicans* to acrylic. Biofouling 3:183-191 doi:10.1080/08927019109378173

Whitehead K.A., Colligon J.S. and Verran J. 2004. The production of surfaces of defined topography and chemistry for microbial retention studies, using ion beam sputtering technology. Int. Biodeterioration and Biodegradation 54:143-151 doi:http://dx.doi.org/10.1016/j.ibiod.2004.03.010

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