Antimicrobial Activity of Nisin Adsorbed to Surfaces Commonly Used in the Food Industry

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ABSTRACT

The adsorption isotherms of nisin to three food contact surfaces, stainless steel, polyethyleneterephthalate (PET), and rubber at 8, 25, 40, and 60°C, were calculated. For all surfaces, the increase in temperature led to a decrease in the affinity between nisin and the surface. The rubber adsorbed a higher amount of nisin (0.697 μg/cm²) in comparison with PET (0.665 μg/cm²) and stainless steel (0.396 μg/cm²). Adsorption of nisin to the stainless steel surface described L-2 type curves for all temperatures assayed. However, for PET and rubber surfaces, the isotherms were L-2 type (at 40 and 60°C) and L-4 type curves (at 8 and 25°C). Nisin retained its antibacterial activity once adsorbed to the food contact surfaces and was able to inhibit the growth of Enterococcus hirae CECT 279 on Rothe agar medium. The attachment of three Listeria monocytogenes strains to the three surfaces was found to be dependent on the surface, the strain, and the initial bacterial suspension in contact with the surface. The adsorption of Nisaplin on surfaces reduced the attachment of all L. monocytogenes strains tested. The effect of PET-based bioactive packaging in food was very encouraging. When applied to a food system, nisin-adsorbed PET bottles reduced significantly (P < 0.05) the levels of the total aerobic plate counts in skim milk by approximately 1.4 log units after 24 days of refrigerated storage (4°C), thus extending its shelf life.

Bacterial adhesion to solid surfaces is a general phenomenon that is recognized as the first step in the development of biofilms. In particular, the growth of biofilms on metal surfaces results in a variety of problems for the food industry, including decreased heat transfer efficiency and both increased corrosion rates and fluid frictional resistance (5, 13, 14). Consequently, biofilm formation can increase the production costs and reduce the service life of industrial devices such as tanks, pipelines, and heat exchangers, among others (33, 37). However, the most serious implication of biofilms in the food industry is their potential for food contamination, which can produce health risks or high economic losses or both (5, 12, 23, 28, 49).

It has also been documented that bacteria attached to surfaces persist after control measures and are difficult to inactivate or remove with chemical sanitizers and heat (36, 46). Then, the analysis of factors affecting the adherence process is an important step in identifying new methods to prevent bacterial attachment in the food industry (19).

In general, bacterial adhesion depends on environmental factors (such as pH, temperature, or ionic strength), properties of bacterial cell surface, the culture state, and the chemical and physical characteristics of the surfaces, including elemental composition, hydrophobicity, hydration, charge, free energy, roughness, and the presence of pores (41, 47).

Listeria monocytogenes is one of the pathogenic bacteria that can appear on food contact surfaces. This bacterium may grow at refrigeration temperatures, in high salt concentrations, and at a pH range of 5.0 to 9.0. In addition, it is capable of adhering to a variety of food contact surfaces, floors, walls, drains, and critical control point areas (26, 30, 31, 35, 44).

Owing to recent outbreaks of listeriosis caused by L. monocytogenes, interest in investigating the behavior of this bacterium in foods (10) is increasing. The mortality rate following an infection by L. monocytogenes is much greater than that by other pathogens, including Escherichia coli O157:H7 (9).

Researchers have focused on preventing initial adhesion of microbial contaminants by applying an antimicrobial substance to the surface rather than trying to remove the undesirable bacteria once they are adhered (25). Various kinds of preservatives can be incorporated to the food contact surfaces or packaging materials or both to improve their functionality (38). These bioactive packages provide antimicrobial activity by releasing the preservative at a controlled rate (6, 18). Several compounds, such as enzymes (lysozyme), organic acids (sorbate, propionate, and benzoate), metals, fungicides, and bacteriocins, have been used for antimicrobial activity in food packaging. However, some of these antimicrobial substances could have a safety risk to consumers if the release is not tightly controlled by some mechanisms within the packaging material (35, 48). For example, excessive release of SO2 from pads of sodium metabisulphite-incorporated microporous material to the foods could cause toxicological problems (32, 48). On the other hand, the use of butylated hydroxytoluene in contact

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with foods (43, 48) has been questioned due to its tendency to accumulate in human adipose tissue (48).

In addition, some of these antimicrobial compounds have disadvantages because the food may not be considered “natural” or the level required for inhibition may introduce a strong flavor to the food. For these reasons, investigators are now focusing on the production of antimicrobial films using natural preservatives such as bacteriocins, mainly nisin. This antibacterial peptide is produced by Lactococcus lactis, and it is bactericidal against a variety of gram-positive bacteria, including spoilage organisms and foodborne pathogens such as L. monocytogenes, Clostridium botulinum, and Staphylococcus aureus (16). In addition, nisin is heat stable, nontoxic, and sensitive to the action of digestive proteases. Nisin, in conjunction with other inhibitory factors, can provide a barrier for the growth of unwanted contaminating bacteria, thereby reducing the amount of chemicals added to the food, decreasing the intensity of the processing conditions, and contributing to the development of hurdle technologies (12).

Studies dealing with the development of bioactive food packaging materials using bacteriocins and antioxidants for use in the food packaging industry have been reported before. Some examples of these studies include the production of cellulose-based bioactive inserts and antimicrobial polyethylene-polyamide pouches using nisin and lacticin (37), and the production of an antimicrobial and antioxidant paperboard coated with nisin and α-tocopherol (24).

The adsorption of nisin to silica surfaces (4–6) or to hydrophilic and hydrophobic silicon surfaces (11, 12) at a fixed temperature has been described before. From the nisin adsorption isotherms obtained by these researchers, clear differences as a function of the type of surface (hydrophilic or hydrophobic) were observed. However, little is known about the influence of temperature on the adsorption of biologically active proteins, such as nisin, to food contact surfaces including stainless steel (SS), polyethylene-terephthalate (PET), and rubber. In addition, reports dealing with the influence of temperature on the shape of the adsorption isotherms of nisin are lacking.

In this investigation, we studied the effect of temperature on the adsorption of nisin to three food contact surfaces, SS, PET, and rubber, which are commonly used in the food industry. Second, the stability of the Nisaplin-adsorbed surfaces was ascertained using an activity retention test with Enterococcus hirae as a target bacterium. Third, we also studied the effect that Nisaplin adsorbed to these food contact materials had on the adhesion of L. monocytogenes strains isolated from foods. Finally, the functionality of adsorbed Nisaplin on a PET surface was determined in the presence of pasteurized skim milk by checking its effectiveness in reducing populations of natural microbiota.

MATERIALS AND METHODS

Bacterial strains. Enterococcus hirae CECT 279, the target organism used in the bacteriocin photometric activity assay, was obtained from the Spanish Type Culture Collection (CECT). These bacteria were maintained at 4°C on Rothe agar (Cultimed, Madrid, Spain) slants.

The three L. monocytogenes strains (ES15, ES24, and ES25) used in this study were isolated from foods (frozen hake, cheese, and meat, respectively) in the course of routine food testing in the Food Control Services (Consellería de Sanidade) of Galicia (Spain). All the strains were stored at −20°C in skim milk (Fluka, Madrid, Spain), and during the experiments the strains were routinely maintained at refrigerated temperatures on Trypticase soy agar (Cultimed, Madrid, Spain).

Bacteriocin preparation and quantitation. Nisaplin was obtained from Aplin and Barrett, Ltd. (Danisco Cultor, England). According to the suppliers, the typical composition (in percentages) of this antimicrobial agent is nisin, 2.5; sodium chloride, 77.5; milk protein, 12.0; carbohydrates, 6.0; and moisture, 2.0. Sodium phosphate buffer solutions were prepared using chemicals of analytical grade and distilled, deionized water. Nisaplin was added to 0.01 M monobasic sodium monophosphate (pH 4.5) to assure complete solubilization of nisin. This suspension was then centrifuged (5,000 × g for 10 min) to remove insoluble impurities. Subsequently, 0.01 M dibasic sodium monophosphate (pH 9.0) was added until a pH of 6.0 and a final concentration of 12 mg/ml of Nisaplin (0.3 mg of nisin per ml) were obtained. Varying concentrations of Nisaplin solutions were obtained by dilution of stock solution with 0.01 M sodium phosphate buffer (pH 6.0).

The antimicrobial activities of these solutions of Nisaplin were determined against Enterococcus hirae CECT 279 with a turbidimetric bioassay (7). Briefly, the method is based on the determination of growth inhibition (at 700 nm) of a target bacterium (Enterococcus hirae CECT 279) caused by serial dilutions of bacteriocin samples. First, Nisaplin samples were diluted as needed in distilled and sterile water. Second, equal volumes (2.5 ml) of diluted bacteriocin samples and a culture of E. hirae CECT 279 (diluted to an absorbance of 0.2 at 700 nm with sterile buffered Rothe broth [pH 6.3]) were added in sterile culture tubes. Finally, the tubes were incubated for 6 h at 30°C. Controls consisted of three culture tubes in which the diluted bacteriocin samples were substituted with distilled sterile water. Growth inhibition was measured spectrophotometrically at 700 nm. Dose-response curves were obtained from these data. Bacteriocin activity was calculated as bacteriocin units (BU) per milliliter (1 BU/ml = amount of bacteriocin needed to obtain 50% growth inhibition compared with control tubes).

Preparation of solid surfaces. SS type 304 was provided by Gamelsa, S.A. (Santiago de Compostela, Spain) as rectangular plates (7.9 by 2.5 by 0.1 cm) and disks (0.8 cm in diameter and 0.1 cm in thickness). PET was provided as rectangular plates (18.4 by 3.0 by 0.02 cm) by Catalana de Polímeros (El Prat de Llobregat, Barcelona). Plates (5.4 by 5 by 0.2 cm) of rubber type 158 (a blend of styrene butadien rubber) were also used in this study.

These materials were cut into pieces of convenient dimensions for each experiment. The pieces were washed with 1% (wt/vol) sodium dodecyl sulphate (Sigma, Madrid, Spain); rinsed with distilled, deionized water; and washed again with ethanol (Panreac, Barcelona, Spain) during 10 min under shaking conditions each time. Finally, materials were rinsed with distilled, deionized water and air dried.

Kinetics of nisin adsorption. The necessary times to reach the adsorption equilibrium of nisin to the three surfaces were determined using two Nisaplin solutions (1.2 and 12 g/liter) prepared as described above. First, rectangular plates of SS, PET, and rubber surfaces with surface areas of 39.5, 110.4, and 60.0 cm², respectively (as they were provided by the suppliers), were cleaned as described above. Each surface was placed into individual sterile
glass bottles (50 ml) and covered with 20 ml (in case of SS and rubber) or 25 ml (in case of PET) of the corresponding Nisaplin solution. Then they were incubated at four different temperatures (8, 25, 40, and 60°C) under static conditions to allow adsorption. Triplicate samples were run simultaneously. Control samples (20 and 25 ml) of each Nisaplin solution were placed into sterile glass bottles (50 ml) and subjected to the above adsorption conditions, without the presence of any surface. At preestablished times (6, 9, 12, 24, and 36 h), the surfaces were removed and both the remaining Nisaplin solutions and the control solutions of Nisaplin were analyzed for antibacterial activity using the above-mentioned photometric assay (7). This procedure eliminated the need to quantify the amounts of nisin adsorbed to the walls of the glass bottles.

Then, the individual concentrations of nisin (grams per liter) were determined from a standard curve of nisin concentration (grams per liter) versus bacteriocin activity units (bacteriocin units per milliliter), which was prepared by determining the antibacterial activity of varying concentrations of Nisaplin solutions. The amounts of nisin adsorbed to each material were determined from the difference between nisin concentrations in the controls and in the remaining solutions (29, 38). To be consistent, the results were divided by the area of each surface and expressed as micrograms of nisin adsorbed per square centimeter of surface.

**Nisin adsorption isotherms.** To obtain the adsorption isotherms, the SS, PET, and rubber surfaces were placed into individual sterile glass bottles (50 ml) and exposed to the different Nisaplin solutions under static conditions by contacting each surface with one volume of protein solution (as described above). The samples were then incubated for 12 h at four different temperatures: 8, 25, 40, and 60°C. Triplicate samples of each material were run simultaneously. For each surface, control samples of each Nisaplin solution were placed into sterile glass bottles (50 ml) and subjected to the above adsorption conditions, without the presence of surface. The amounts of nisin adsorbed to each surface (expressed as micrograms of nisin per square centimeter) were calculated as described in the previous section.

**Antibacterial activity of nisin-adsorbed surfaces.** Bacteriocin activity of surfaces with adsorbed nisin was determined using the agar diffusion assay (16). Molten Rothe agar was cooled to 45°C and subsequently seeded with 0.1 ml of an overnight culture of the indicator strain, *E. hirae* CECT 279. Inoculated medium was dispensed in sterile petri dishes and allowed to solidify in a laminar flow hood.

To prepare a material with adsorbed nisin, cleaned rectangular plates (2.5 by 1.8 cm) of SS (0.20 cm thick), PET (0.05 cm thick), and rubber (0.18 cm thick) were placed into individual sterile glass bottles (50 ml) and covered with 20 ml of a 10-g/liter Nisaplin buffered solution (0.01 M NaH₂PO₄, 0.01 M Na₂HPO₄, pH = 6.0) during 12 h at 25°C under static conditions. Then the surfaces with adsorbed nisin were rinsed twice with distilled and deionized water and placed on the agar surface. Cleaned surfaces contacted with phosphate buffer (0.01 M NaH₂PO₄, 0.01 M Na₂HPO₄, pH = 6.0) without Nisaplin were used as controls. All agar plates were held at 4°C for 4 h to allow nisin diffusion prior to incubation at 30°C for 48 h.

**Attachment of *L. monocytogenes* to surfaces treated and untreated with Nisaplin.** In this assay, cleaned and dried disks of SS (100.4 mm²), PET (58.4 mm²), and rubber (97.0 mm²) were used. Half of the disks of each material were placed into individual sterile glass tubes (12 ml) with screw caps and covered with 10 ml of a 10 g/liter Nisaplin buffered solution as described above. Then the disks were rinsed twice with distilled and deionized water. Bacterial suspensions were prepared from overnight cultures of *L. monocytogenes* strains in Trypticase soy broth (TSB; cultimed, Barcelona, Spain) at 37°C and 90 rpm. These cultures were centrifuged at 10,000 × g for 5 min at 4°C and washed twice with phosphate buffered saline (PBS: 0.33 M NaCl, 3 mM KCl, 8.4 mM Na₂HPO₄, 1.6 mM KH₂PO₄, pH = 7.2). The cells were finally resuspended in sterile PBS and spectrophotometrically (530 nm) adjusted with sterile PBS to a concentration of 10⁸ CFU/ml. The spectrophotometric reading was converted into CFU per milliliter by using a standard growth curve (CFU per milliliter versus optical density) previously prepared. Tenfold serial dilutions were made to obtain other bacterial suspensions.

Aliquots (3 ml) of two initial bacterial suspensions (10⁶ and 10⁷ CFU/ml), prepared as described above, were added to each screw-cap glass tube and incubated with a piece of each material (treated and untreated with Nisaplin) for 1 h (25°C at 90 rpm). After incubation, materials were washed twice with PBS to remove poorly adhered bacteria, immersed in 2 ml of cooled TSB, and treated ultrasonically at 30 W for 20 s in a sonifier (model 250, Branson Ultrasonics Corporation, Danbury, Conn.). Then, tenfold serial dilutions of TSB in PBS were made, and 0.1-ml triplicate portions of appropriate dilutions were plated on *Listeria* Oxford agar (Oxoid, Madrid, Spain). Agar plates were incubated at 37°C for 24 to 48 h. Colonies were counted, and results were expressed as number of attached CFU per square millimeter of surface (39). Each adhesion assay was repeated three times, and adequate control assays were also carried out to test that cell viability was not affected by the procedure.

**Effect of Nisaplin solution on *L. monocytogenes* suspensions.** In this assay, *L. monocytogenes* strains were examined for their ability to survive in presence of Nisaplin solutions containing those amounts of nisin adsorbed to each surface (previously determined after obtaining the adsorption isotherms). The three Nisaplin solutions were prepared by appropriate dilution of the stock solution (12 g/liter) with 0.01 M sodium phosphate buffer (pH 6.0) to achieve the amounts of Nisaplin double those determined to be adsorbed to each surface. Then, 1.5 ml of each Nisaplin solution was added in sterile culture tubes. Each tube was inoculated with 1.5 ml of an appropriate *L. monocytogenes* suspension to obtain six different initial cell populations (from 10⁸ to 10⁶ CFU/ml). After mixing, the tubes were incubated for 15 min at 25°C and 90 rpm. Final cell suspensions were serially diluted in sterile PBS, and triplicates were plated on *Listeria* Oxford agar. Finally, plates were incubated as described above and results were expressed as the number of surviving CFU per milliliter.

**Antibacterial activity of the Nisaplin-adsorbed PET surfaces in a food system.** Five-hundred-milliliter PET bottles (cleaned as described above for pieces of materials) of the type used for mineral water (Cabreiroá, S.A., Ourense, Spain) were filled with 500 ml of a 10 g/liter Nisaplin buffered solution and maintained at 25°C for 12 h. After adsorption, the bacteriocin solution was removed under sterile conditions and PET bottles were rinsed and dried as described above. Subsequently, the bottles were filled with 500 ml of pasteurized skim milk, which was obtained from a local supermarket 24 h after production. Controls consisted of clean PET bottles without adsorbed Nisaplin. These samples were stored at 4°C and analyzed daily over a 24-day period. At selected intervals, samples (1 ml) of skim milk were withdrawn for bacterial enumeration. Each sample was serially diluted with sterile buffered peptone water. Total aerobic plate counts (TAPC) were determined by the spread plate technique on plate count agar (Merck, Darmstadt, Germany). Plates were in-
cubated at 30°C for 48 h. Colonies were counted and results expressed as CFU per milliliter of milk. All assays were carried out in triplicate, and only mean values are presented.

Statistical methods. Individual experiments were performed in triplicate. All data points are represented by the mean, with the standard error indicated by error bars. Data sets were analyzed by analysis of variance on SPSS 8.0 for Windows.

RESULTS AND DISCUSSION

Nisin adsorption isotherms. To determine the potential protective activity of SS, PET, and rubber surfaces with adsorbed nisin against the attachment of pathogen bacteria, the nisin adsorption capacity of these materials was determined throughout the construction of their adsorption isotherms.

The results of nisin adsorption kinetics from 1.2 and 12 g/liter Nisaplin solutions to the SS, PET, and rubber surfaces at 8, 25, 40, and 60°C are shown in Figure 1. The maximum of adsorption was detected after 12 h of contact and remained constant thereafter, thus showing that this was the time needed to reach equilibrium in all cases. Therefore, this incubation time was chosen to obtain the nisin adsorption isotherms for all the surfaces at 8, 25, 40, and 60°C.

The nisin adsorption isotherms to SS, PET, and rubber are presented as plots of amounts of adsorbed nisin (micrograms per square centimeter) versus nisin concentration (milligrams per liter) in Figure 2. As can be observed, the temperature influenced the shape of the isotherms obtained. Thus, according to the system of isotherm classification (15), adsorption of nisin to the SS surface described L-2 type curves for all temperatures assayed (Fig. 2). Nevertheless, for PET and rubber surfaces (Fig. 2), the isotherms were L-2 type (40 and 60°C) and L-4 type curves (8 and 25°C). For all temperatures, nisin adsorption to the SS surface exhibited a steep initial slope followed by a steady increase to the concentration of 250 mg/liter, from which the adsorbed nisin amounts decreased slightly (Fig. 2). This same behavior was observed for both PET and rubber surfaces for the isotherms obtained at 40 and 60°C (Fig. 2). This suggests that nisin formed a monolayer when adsorbed on SS (at all temperatures) and on PET and rubber surfaces at 40 and 60°C (15).
However, at 8 and 25°C, adsorption of nisin to these last surfaces increased with the increase in nisin concentration in solution, displaying three phases. The first exhibited a steep slope in the nisin concentration range of 0 to 30 mg/liter, followed by a second (from 30 to 200 mg/liter) in which the slope was less pronounced as concentration raised. And, in the third phase, the adsorption of nisin increased rapidly from 200 to 250 mg/liter and remained constant thereafter. This suggests that nisin adsorbs onto both surfaces in more than one layer. So the first plateau (at a nisin concentration of 30 mg/liter) could be identified with the completion of the first monolayer, and the second one (at a nisin concentration of 250 mg/liter) could be identified with the completion of the second layer. In addition, the slope in the first phase was higher than that of the second phase because the forces generating the second and subsequent layers are weaker than those generating the first (15).

In addition, the increase in temperature led to a decrease in the amount of nisin adsorbed in all surfaces (Fig. 3). This finding is in agreement with a previous observation (22) in which the increase in temperature produced a reduction in the adsorbed amounts of both cellobiohydrolases I and II on microcrystalline cellulose. This phenomenon could be a result of an increase in the excitation state of protein molecules at higher temperature that could decrease the attractive forces between them and the solid surfaces. On the other hand, the increase in the amounts of nisin adsorbed at lower temperatures may be related to a reduction in translational energy of nisin, which could favor the positioning of this bacteriocin for adsorption or to a reduction in energy available for desorption of nisin once adsorbed (22).

The adsorption capacity of the three surfaces was significantly different (P < 0.05) and followed the order rubber > PET > SS for all temperatures. Thus at 8°C, the maximal amounts of nisin adsorbed on SS, PET, and rubber surfaces were 0.396, 0.665, and 0.697 µg/cm², respectively (Fig. 3). These differences in the adsorption capacity could be related to the different chemical and physical characteristics of the surfaces used, mainly the surface elemental composition, hydrophobicity, roughness, and the amounts of pores on the surface, as well as their charge and free energy (17).

**Antibacterial activity of the nisin-adsorbed surfaces.** The antibacterial activity of the nisin-adsorbed surfaces against *E. hirae* CECT 279, a nisin-sensitive strain (16), was detectable on all tested surfaces (SS, PET, and rubber) pretreated with Nisaplin. Thus, the thicknesses of the zones of inhibition observed around the periphery of the treated surfaces were 0.3 mm (in case of SS), 0.6 mm (in case of PET), and 0.8 mm (in case of rubber). These observations may result from the diffusion of nisin into the medium (12), or they may be due to the fact that nisin retained its antibacterial activity in the adsorbed state (6, 40). Other researchers have observed that both nisin and pediocin retained their antibacterial activity when adsorbed onto different surfaces (4, 5, 11, 27). In addition, it has been observed that nisin incorporated into a polyethylene-based plastic retained its antibacterial activity against *Lactobacillus helveticus* and *Brochothrix thermosphacta* (40).

**Attachment of bacterial cells to surfaces treated and untreated with Nisaplin.** The attachment of the three *L. monocytogenes* strains (ES15, ES24, and ES25) to the three surfaces depended on the type of surface, the strain, and the initial bacterial suspensions used (Fig. 4). Incubation of untreated surfaces with an initial bacterial suspension of 10^8 CFU/ml resulted in higher levels (P < 0.05) of attached cells compared with those levels of adhesion observed when an initial bacterial suspension of 10^4 was used (Fig. 4). Thus, for an initial bacterial suspension of 10^8 CFU/ml (Fig. 4), attachment of ES15 and ES25 strains was highest on the untreated PET surface, whereas the attachment of ES24 strain was highest on the SS surface. In the same way, the attachment of the three *L. monocytogenes* strains was lowest on the untreated rubber surface. Differences in the number of adhered cells between *L. monocytogenes* strains have been previously observed by different re-

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**FIGURE 3. Maximum adsorbed mass of nisin on SS, PET, and rubber surfaces as a function of temperature.** Data of adsorbed mass of nisin were obtained after immersing each surface in a 12 g/liter Nisaplin solution for 12 h. Data reported are means ± standard deviations of three replicates.
FIGURE 4. Effect of bioactive surfaces (stainless steel, PET, and rubber) treated with Nisaplin and untreated surfaces on the attachment of two initial bacterial suspensions: $10^4$ and $10^8$ CFU/ml, of three Listeria monocytogenes strains (ES15, ES24, and ES25). Data reported are means ± standard deviations of three replicates.

searchers (8, 21, 39). This fact has been attributed to the different bacterial cell surfaces or to the different cell physiology of the three L. monocytogenes strains (39).

Nisin adsorption on the different treated materials resulted in a statistically significant reduction ($P < 0.05$) in L. monocytogenes attachment compared with the results obtained with the untreated surfaces. In general, this reduction was higher when $10^4$ CFU/ml initial suspension was used (Fig. 4). The highest reduction in the final numbers of adhered cells was observed for the treated rubber surface, which adsorbed the highest amounts of nisin. However, it has been shown that milk proteins (without antibacterial activity) adsorb to surfaces, forming a "conditioning" layer that inhibits the initial attachment of bacteria to surfaces (2, 3, 20, 42). Therefore, the decrease of L. monocytogenes viable counts in the Nisaplin-treated surfaces could be related to both the inactivation of attached cells by residual nisin on the surfaces (4) and the inhibition of initial attachment of bacteria by adsorbed milk proteins without antibacterial activity initially present in the Nisaplin preparation (2, 3, 20, 42).

Because of the risk derived from the presence of L. monocytogenes in food industries, some investigations about disinfectants’ efficacy against this pathogen were conducted, concluding that the efficacy of disinfectants is limited by the ability of bacteria to attach to solid surfaces (1, 34, 39, 45). Since Nisaplin-treated surfaces reduced L. monocytogenes attachment, it would be expected that the effect of these active surfaces was higher in a food-processing plant because the number of bacterial cells adhered to these surfaces (when adequate cleaning and disinfecting procedures are used) would be lower than those used in this work.

On the other hand, no significant differences ($P > 0.05$) in cell viability were observed, when different suspensions (from $10^3$ to $10^8$ CFU/ml) of the three L. monocytogenes strains were treated with Nisaplin solutions containing the same amounts of nisin as those adsorbed to the three surfaces. But, as it was demonstrated with the experiment of cell attachment to treated and untreated surfaces, those same amounts were able to reduce the viability when adsorbed on surfaces (Fig. 4). This means that nisin once adsorbed to surfaces is more concentrated than in the solutions used in this assay, thus increasing the probability that the L. monocytogenes cells were killed by the bacteriocin.

Antibacterial activity of Nisaplin-adsorbed PET in a food system. Fresh pasteurized skim milk packaged in the control PET bottles (untreated with Nisaplin) showed a continued increase in the TAPC until the 10th day of incubation at 4°C (Fig. 5). Subsequently the TAPC increased slightly, and by the end of the experiment (24 days) it had increased by approximately 1.1 log units ($P < 0.05$). In contrast to this, in PET bottles to which Nisaplin had been adsorbed, there was an initial drop of 0.65 log unit, which was followed by a slight increase until the end of the incubation (24 days). However, at this point the final TAPC was lower than the initial level ($P < 0.05$). In addition, it can be noted that PET bottles prepared with Nisaplin re-
produced levels of TAPC on skim milk by 1.44 log units ($P < 0.05$), compared with the controls (PET bottles untreated with Nisaplin) after 24 days of incubation. Thus, it is reasonable to suppose that the use of bioactive PET bottles would provide good protection against the outgrowth of nisin-sensitive strains, including species of *Listeria*, *Clostridium*, *Micrococcus*, and *Staphylococcus* that result from postpasteurization contamination. If so, the use of PET bottles treated with Nisaplin could contribute to the extension of the shelf life of skim milk.

When bacteria are attached to food contact surfaces, high levels of disinfectants (chlorine, iodine, anionic acid, and quaternary ammonium sanitizers) are needed to eliminate them. However, increasing the concentrations of sanitizers may pose health risks to personnel using these agents, probably resulting in unacceptable chemical residuals in foods and increased production costs (12). Therefore, treating food contact surfaces with Nisaplin to inhibit bacterial attachment could be an alternative to using high amounts of sanitizers. In addition, the use of packaging materials treated with Nisaplin could contribute to reduced amounts of chemicals added to foods and to a decrease in the intensity of the processing conditions. In consequence, the application of Nisaplin in the control of bacterial adhesion could be a way to maintain the stability of perishable foods, as well as prevent surface contamination (18).

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