Food Microbiology 27 (2010) 940–944

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Potential of nisin-incorporated sodium caseinate films to control *Listeria* in artificially contaminated cheese

Lan Cao-Hoang [a,b,c,*], Aline Chaine [a], Lydie Grégoire [a], Yves Waché [a,b,*]

[a] DSAN, UP Biotechnologie et Microbiologie Alimentaire, AgroSup Dijon, 1 Esplanade Erasme, F-21000 DIJON, France
[b] Laboratoire de Génie des Procédés Microbiologiques et Alimentaires, AgroSup Dijon, Université de Bourgogne, 1 Esplanade Erasme, F-21000 DIJON, France
[c] Laboratory of Post-harvest Technology, School of Biological and Food Technology, Hanoi University of Science and Technology, 1 Dai Co Viet Road, Hanoi, Viet Nam

ARTICLE INFO

Article history:
Received 13 May 2009
Received in revised form
20 May 2010
Accepted 25 May 2010
Available online 2 June 2010

Keywords:
Antimicrobial packaging
Sodium caseinate
Nisin
Listeria innocua
Mini Babybel®
Food matrix

ABSTRACT

A sodium caseinate film containing nisin (1000 IU/cm^2) was produced and used to control *Listeria innocua* in an artificially contaminated cheese. Mini red Babybel® cheese was chosen as a model semi-soft cheese. *L. innocua* was both surface- and in-depth inoculated to investigate the effectiveness of the antimicrobial film as a function of the distance from the surface in contact with the film. The presence of the active film resulted in a 1.1 log CFU/g reduction in *L. innocua* counts in surface-inoculated cheese samples after one week of storage at 4 °C as compared to control samples. With regard to in-depth inoculated cheese samples, antimicrobial efficiency was found to be dependent on the distance from the surface in contact with the active films to the cheese matrix. The inactivation rates obtained were 1.1, 0.9 and 0.25 log CFU/g for distances from the contact surface of 1 mm, 2 mm and 3 mm, respectively. Our study demonstrates the potential application of sodium caseinate films containing nisin as a promising method to overcome problems associated with post-process contamination, thereby extending the shelf life and possibly enhancing the microbial safety of cheeses.

1. Introduction

The growing consumer's concern for health and natural product is becoming a driving force for research and innovation in many fields of the food industry. One important problematic is the replacement of ingredients from chemical synthesis or having a bad health image by natural ones. This movement is now reaching the field of food preservatives. These compounds are employed for food that cannot be sterilized due to heat sensitivity but they include a great number of compounds with a chemical image. As a result, a growing attention is put on natural antimicrobial agents by scientists as well as food industries. These compounds can be incorporated directly into the product formulation to inhibit growth of undesirable microorganisms in food during storage. However, if no inhibiting interaction occurs between the antimicrobial compound and the food matrix, this will result in an immediate and short-term reduction of bacterial populations. If a long-term reduction is needed, antimicrobials may be directly coated onto the surface of food or incorporated into packaging materials. These antimicrobial films can maintain their activity, thus slowing down bacterial growth during extended storage after packaging (Hoffman et al., 2001).

Nisin is probably, with chitosan, the commercially available antimicrobial the most used in the food industry as food biopreservative worldwide. Nisin is a cationic peptide belonging to the bacteriocin group that is produced by certain strains of *Lactococcus lactis*. It is the only bacteriocin recognized as safe for the food industry by the World Health Organization. It has antimicrobial properties, especially against many Gram positive bacteria, such as the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus* or *Bacillus cereus* (Brewer et al., 2002; Lopez-Pedemonte et al., 2003; Sobrino-Lopez and Martin-Belloso, 2006). However, it exhibits little or no activity against Gram negative bacteria, yeasts and moulds.

Due to its effect on the important Gram positive foodborne pathogens, many studies have focused on the incorporation of nisin into various kinds of films made of cellulose, nylon, whey protein isolate, hydroxypropyl methylcellulose, zein etc. and their use as nisin delivery systems packaging films to reduce undesirable bacteria in foodstuff (Chollet et al., 2008; Coma et al., 2001; Gadang et al., 2008; Ko et al., 2001; Kristo et al., 2008; Natrajan and Sheldon, 2000; Neetoo et al., 2008; Nguyen et al., 2008; Teerakarn et al., 2002). Nisin

* Corresponding authors at: Laboratoire de Génie des Procédés Microbiologiques et Alimentaires, AgroSup Dijon, Université de Bourgogne, 1 Esplanade Erasme, F-21000 DIJON, France.
E-mail addresses: lanch-ibft@mail.hut.edu.vn (L. Cao-Hoang), yves.wache@u-bourgogne.fr (Y. Waché).

0740-0020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2010.05.025

effectiveness in food products depends on its diffusion throughout the food matrix, which depends on several parameters such as the composition and physico-chemical properties of food, the storage temperature, etc. (Carnet Ripoche et al., 2006).

Cheese is a ready-to-eat product which is considered as a "potentially hazardous food". The probability of consuming a contaminated cheese has even been estimated at 65.3% for soft cheese made with raw milk (Bemrah et al., 1998). Depending on the type of cheese and especially on the physico-chemical properties of the matrix (density of the gel, moisture…), of the origin of the contamination (from raw materials, during the cheese making or ripening), and of the contaminant species (*L. monocytogenes*, *Salmonella* spp., *Escherichia coli*, *S. aureus*…), pathogenic bacteria can develop more or less toward the interior of the product. Among pathogenic microorganisms of most concern to cheese makers, *L. monocytogenes* represents the highest risk. Indeed, in comparison with other foodborne diseases associated with cheese, listerioses occur with both higher severity and probability. Furthermore, *Listeria* survives if not thrives under the conditions of cheese making and it is able to grow at refrigeration temperatures corresponding to the cheese ripening and storage temperatures. Cheese storage is thus a step important to control. However, to our knowledge, this problematic has generated relatively little work on the utilization of antimicrobial films to inhibit undesirable microorganisms in cheese during storage. Zottola et al. (1994) demonstrated that addition of nisin in Cheddar cheese resulted in significant reductions on the numbers of pathogenic bacteria in cheese after eight weeks of storage. Scannell et al. (2000) investigated the immobilization of nisin and lacticin 3147 within packaging materials. The authors reported that the combination of antimicrobial packaging with modified atmosphere conditions and refrigeration temperatures reduced the levels of *Listeria innocua* and *S. aureus* by more than 2 log and 1.5 log units, respectively. Chollet et al. (2008) pointed out interactions between nisin and Emmental cheese slurry with antimicrobial activity when adding nisin directly to cheese. However, information on the effectiveness of nisin-coated films in inhibiting bacteria in cheese and the migration of nisin from active films to cheese matrix is still lacking. In dairy products, contamination can occur in the liquid milk, resulting in the inoculation of the whole product, or after milk gelation, in this case, contaminating cells remain near the contact area (Ly et al., in press). However, cell movement can depend on the physico-chemical structure of the matrix although this parameter has not been much investigated in real food products. Depending on the cell movement, a surface antimicrobial treatment can be more or less efficient. It is thus particularly important to evaluate the depth of both the efficiency of the treatment and the cell development. The purpose of this study was to investigate the effect of sodium caseinate films containing nisin on the inhibition of *L. innocua* from artificially contaminated cheese. Mini red Babybel® cheese was chosen as a model of semi-soft and non-cooked cheese. This cheese has been used as a model of food mixture for development of microorganisms such as *Listeria*, *Salmonella* (Hallier-Soulier et al., 2005) or *Staphylococcus* (Meyrand et al., 2000).

The antimicrobial activity produced by the films against the evolution of *L. innocua* in experimentally contaminated cheese was determined during a seven-day storage at 4 °C under aerobic conditions. The influence of the food matrix on the growth of microorganism and also on the antimicrobial properties of the nisin film has been evaluated. For this purpose, cheeses were artificially contaminated by two different methods, surface- and in-depth contamination, to evaluate the effect of active bacterial film against bacteria on the surface and in the interior of the packaged product.

2. Materials and methods

2.1. Preparation of nisin standard solution

Nisin, from Sigma–Aldrich (Sigma Chemical, St. Louis, MO, USA), was prepared by dissolving 0.1 g of a 2.5% nisin powder in 2 ml of a 0.01 M HCl solution (pH = 2). The resulting solution was filtered through a 0.2 μm-pore-size Millipore filter (Nalgene, Rochester, New York, USA) and stored at 4 °C before use.

2.2. Preparation of nisin-coated sodium caseinate films

Films were prepared as previously described by Kristo et al. (2008). Sodium caseinate (6% w/v) (Sigma–Aldrich) was dissolved in distilled water under continuous stirring. After total dissolution, sorbitol (Sigma–Aldrich) was added as the plasticizer at a concentration of 25% (w/w) which is necessary to overcome the brittleness and to improve the flexibility and extensibility of sodium caseinate films (Kristo et al., 2008). The nisin solution was then added to the sodium caseinate solution to reach a final concentration of bacteriocin of 500 μg/ml. After vacuum-degassing to remove air bubbles, films were cast with 12.5 g portions of sodium caseinate solutions, poured into 90 mm plastic Petri dishes and then dried at 37 °C for 24 h. The amount of pure nisin in the resulting nisin-coated films was of 6.25 mg/g, corresponding theoretically to 0.04 mg (or 1000 IU)/cm^2 surface area of active film (Jin and Zhang, 2008).

2.3. Evaluation of the antimicrobial activity of the film

Before each experiment, the antimicrobial activity of the films (control- and nisin-containing-films) was determined with the zone inhibition assay using *L. innocua* as the test microorganism. In that goal, an overnight culture of *L. innocua* was prepared in Fraser broth (Sigma–Aldrich) at 37 °C. For the antimicrobial test, films were cut into 4 mm diameter discs before being brought into contact with the Plate Count Agar (PCA) medium previously inoculated with 1 ml culture of *L. innocua* (containing 10^{10} CFU/ml). The Petri dishes were incubated at 37 °C for 48 h. The antimicrobial activity of the film was evaluated through the presence of inhibition zones around the disc.

2.4. Packaging of surface-contaminated cheese with sodium caseinate films

Commercially-packaged mini red Babybel® (23.5% fat, 22% protein, 0.67% calcium, 0.67% sodium, pH = 5.2, water content 48%, a_w 0.97 (Canac-Arteaga et al., 1999)) was locally purchased. Cylindric slices of cheese (5 mm diameter and 1 mm thickness) were obtained with a sterile pastry cutter and then piled up. The suspension of *L. innocua* was prepared by transferring and diluting one colony of *L. innocua* maintained on Tryptic Soy Agar (Sigma–Aldrich) plates into 100 ml of sterile physiological water. 20 μl of this inoculating suspension was spread on the first slice (slice 1) of each pile of cheese slices (Fig. 1). The final concentration of *L. innocua* on the cheese surface was about 10^5 CFU/cm^2. After inoculation, the piles of cheese slices were placed upside down in Petri dishes containing the control or nisin-coated films placed on the bottom of the dish. The contaminated side of cheese slices was thus in contact with films (Fig. 1). This experiment was carried out to evaluate the proliferation of *L. innocua* from the surface to the interior of cheese samples (from slice 1 to the next slices) and the efficiency of the active films to control *L. innocua* in the cheese product depending on the distance from the nisin-containing film. Petri dishes with piles of cheese slices were then stored at 4 °C and bacterial viability in cheese slices was periodically analyzed.

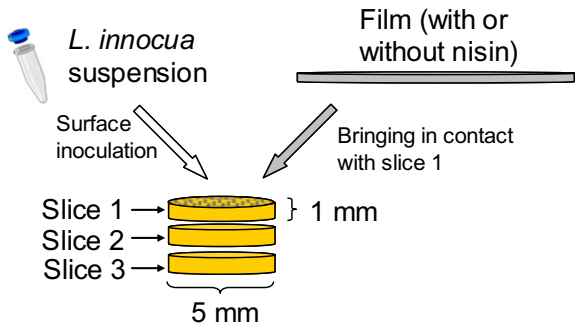

Fig. 1. Schematic representation of experiments performed to assess the antimicrobial film effect on *L. innocua* inhibition on the cheese slice surface.

2.5. Packaging of in-depth contaminated cheese with sodium caseinate films

In the first experiment, the inoculation of cheese slices with *L. innocua* was done in surface and then the proliferation of *L. innocua* in the cheese matrix was evaluated by counting bacteria in cheese slices. This second experiment was designed with the objective of testing the efficiency of the active films against *L. innocua* present in the surface and also in the interior of the cheese matrix. For this purpose, Babybel® cheeses were in-depth contaminated with *L. innocua*. Cheese samples were mixed with a *L. innocua* suspension and were then reconstituted using sodium alginate as the gelling agent. For this experiment, cheese slurries were obtained by homogenizing 40 g of Babybel® for 10 min at room temperature with 3 ml of 0.5% calcium chloride with an IKA Ultra-Turrax T25 System (IMLAB, Lille, France) set to 24,000 rpm. The inoculum was prepared by diluting one colony of *L. innocua* into 1 ml of sterile physiological water. An aliquot of this suspension (408 μl) was added to cheese slurries to obtain a final concentration of *L. innocua* of $3–5 \times 10^5$ CFU/g of cheese. Thereafter, 15 ml of 1% sodium alginate solution were added to cheese slurries (40 g) to permit gel formation and cheese reconstitution. This method resulted in a reconstituted cheese with 70% w/w of Babybel®. The mixture was homogenized again for 10 min then placed in sterile Petri dishes (40 g of reconstituted cheese slurries per dish) in which the bottom was covered with control or nisin-coated film. These Petri dishes were stored at 4 °C and bacterial viability in reconstituted cheese slurries was periodically analyzed. In that goal, discs of reconstituted cheese slurries with dimensions similar to the one of the discs of the first experiment (5-mm diameter and 1-mm thickness) were obtained by cutting with a sterile pastry cutter (cut from the contact surface with the nisin-coated film).

2.6. Microbiological analyses

To fit more with reality, the inoculation amounts used in this study were generally lower than those used in other studies (Coma et al., 2002; Nguyen et al., 2008; Sanjurjo et al., 2006). However, they were sufficient to obtain *Listeria*'s development in less than 7 days. Cheese samples were analyzed after 1, 3 and 7 days of storage at 4 °C. For microbial analyses, cheese slices obtained from both surface- and in-depth inoculation samples were mixed with 10 ml of sterile physiological water and homogenized with the Ultra-Turrax homogenizer. Appropriate serial decimal dilutions were made in sterile physiological water. Counts of *L. innocua* were determined in triplicate using the drop-plate method (Bremer et al., 1998) by plating cell suspension on a selective medium for enumeration of *Listeria* (PALCAM, Difco Laboratories, Detroit, USA). Colonies were counted after a 48-h incubation at 37 °C.

2.7. Statistical analysis

The counts of *L. innocua* were converted to CFU/g and analyzed by a one-way ANOVA procedure. Differences in CFU/g means among treatments were determined using Tukey test ($\alpha = 0.05$).

3. Results and discussion

3.1. Zone inhibition assay

The antibacterial activity of the sodium caseinate films containing nisin produced in this study against *L. innocua* on PCA plates was determined by measuring the diameter of inhibition zones around the films after incubation. The diffusion assay showed that there was no inhibition zone around the sodium caseinate films without nisin, indicating that the films alone had no antimicrobial activity. On the contrary, an inhibition zone of 13 mm diameter was observed around the nisin-coated film disc, corresponding to a total diameter of inhibition of 17 mm for an initial disc diameter of 4 mm. This indicated diffusion of nisin from the active films to the solid medium which induced consequently growth inhibition of *L. innocua* in the medium, suggesting the effectiveness of the nisin-coated film fabrication method.

3.2. L. innocua inhibition on the cheese slice surface

In this experiment, piles of cheese slices (1-mm thickness) were surface-inoculated with *L. innocua* then brought in contact with sodium caseinate films containing nisin (c.f. Fig. 1). *L. innocua* counts were determined after 0, 1, 3 and 7 days of storage at 4 °C. Results showed that in both control (in contact with the nisin-free films) and treated cheese samples (in contact with the nisin-coated films), bacteria were only detected in slice 1 which was directly surface inoculated with *Listeria* but not in other cheese slices during the storage period ($P < 0.05$). It indicated that in our experimental conditions, the inoculated bacteria developed only on the surface of slice 1 and did not penetrate up to 1 mm (thickness of each cheese slice) of the cheese matrix. This may be attributed to the structure of the cheese model chosen which does not necessarily favor the movement of the bacteria toward the interior of the cheese matrix. Hallier-Soulier et al. (2005), when studying the development of *Listeria* and *Salmonella* in food using Babybel® as food mixture model, have shown that this cheese is an appropriate environment for the development of both studied strains. However, the proliferation of the bacteria toward the interior of the food matrix has not been studied yet.

As shown in Fig. 2, after 24-h incubation at 4 °C, it was found that the number of *Listeria* in slice 1 decreased sharply in both control samples (~50% population reduction) and treated samples (~90%). At day 3, difference in the bacteria behavior was observed between two samples: the bacterial population in untreated samples increased to the initial level then remained stable until day 7 of storage, while no increase in the number of *Listeria* was observed in treated samples until day 7 (Fig. 2). These results suggested in one hand that there was no growth of *Listeria* in cheese at 4 °C. The same results were obtained with *L. monocytogenes* growth in semi-soft cheeses during refrigerated storage (Genigeorgis et al., 1991; Morgan et al., 2001; Rogga et al., 2005). On the other hand, our microbial analyses confirmed the results obtained with the diffusion assay that the sodium caseinate films alone did not affect the survival of *L. innocua*. This observation was in agreement with that reported by Kristo et al. (2008), who showed that antimicrobial-free sodium caseinate films were not effective by themselves to inhibit growth of *L. monocytogenes*.

With regard to slice 1 of treated samples which was directly in contact with the nisin film, more than 90% of the bacterial

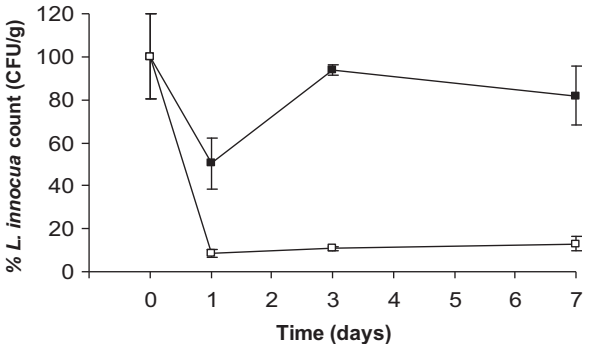

Fig. 2. Changes in *L. innocua* counts in slice 1 during storage at 4 °C. (■): surface-inoculated cheese sample in contact with nisin-free film; (□): surface-inoculated cheese sample in contact with nisin-coated film.

population were inactivated at day 1 of storage at 4 °C (Fig. 2), corresponding to 1.1 log CFU/g of inactivation. However, no significant variation in bacterial counts was recorded between day 1 and day 7 (Fig. 2). Since there was no growth of *L. innocua* in control sample, it could be said that 1.1 log CFU/g reduction is the maximum inactivation level reached with contaminated cheese samples in contact with the active films produced at 4 °C. This inactivation level obtained was similar to that obtained by Maisnier-Patin et al. (1992) when working with Camembert cheese containing the nisin-producing starter artificially contaminated with *L. monocytogenes*.

In order to verify the diffusion of nisin into cheese slice samples in contact with nisin films, after one day storage at 4 °C, three first cheese slices were cut into 4 mm diameter discs and placed on PCA agar inoculated with *L. innocua*. The diffusion assay showed that there was no inhibition zone around the cheese slices deposited on agar medium inoculated with *L. innocua*, even for slice 1 which was in direct contact with the active films. It is known that nisin diffused through distances varying from 1 to 2 mm and its antimicrobial activity is less effective in food with high fat content (Aasen et al., 2003; Carnet Ripoche et al., 2006). It has been shown that the hydrophobic nature of nisin could favor bonds with the high fat level containing in Emmental cheese (Liu and Hansen, 1990). Chollet et al. (2008) reported that nisin interacted with the cheese matrix, likely milk fat globules. Aasen et al. (2003) also showed that 80% of the added nisin were lost due to its interaction with protein in food. Thus, two hypotheses may explain the absence of inhibition zone in our assay of evaluation of nisin bioactivity in cheese slice samples: (i) There was no or low diffusion of nisin from the film into cheese slices, thus dilution of nisin concentration below active concentration due to migration into the food matrix. (ii) Nisin antimicrobial activity may be lost due to inactivation of nisin by food components, particularly proteins and fat.

3.3. *L. innocua* inhibition on the in-depth contaminated cheese

In this second experiment, to study the effect of antimicrobial films on in-depth contaminated food products, Babybel® cheese slurries were obtained then contaminated with *L. innocua* before being reconstituted, forming an homogenously contaminated cheese slurry. The homogeneity of bacterial concentration in the reconstituted cheese slurry obtained was verified by taking randomly three discs of cheese with the same dimensions from the reconstituted cheese slurry to determine the number of *L. innocua* in each slice. No significant difference was observed in *L. innocua* populations in three discs ($P < 0.05$), bacterial counts varied in a range of $4.2 \times 10^5 \pm 1.2 \times 10^5$ CFU/g (results not shown), indicating that the reconstituted cheese slurries obtained were homogenously in-depth contaminated.

To investigate the nisin effectiveness with respect to the distance from the surface contact, three slices were cut from control (in contact with nisin-free film) and treated (in contact with active film) samples, each 1 mm deep: slice 1 (1 mm from the surface of the film), slice 2 (2 mm) and slice 3 (3 mm). Fig. 3a–c display changes in *L. innocua* counts in each during storage at 4 °C. After one day at 4 °C, it was found that the number of *L. innocua* increased about 150% and 50% CFU/g in all three slices of control and treated samples, respectively (Fig. 3a–c). However, bacterial counts remained generally stable in control samples during the storage period at 4 °C, whereas at day 3, decrease in the number of *L. innocua* was observed in all three slices of treated samples covered with films containing nisin. This decrease was found to be dependent on the distance from the surface contact, as bacterial concentration was the lowest in slice 1, then slices 2 and 3 (Fig. 3a–c). It is important to note that slice 1 was in direct contact with the active film, while slices 2 and 3 were 1 and 2 mm of distance from the contact surface with the film, respectively. It indicated that the concentration of nisin released from the active film was higher in slice 1 than that in slices 2 and 3, giving consequently higher inactivation rate of *L. innocua* in slice 1 in comparison with the other slices. Our results were in agreement with those of Sebti et al. (2003) who found that film effectiveness

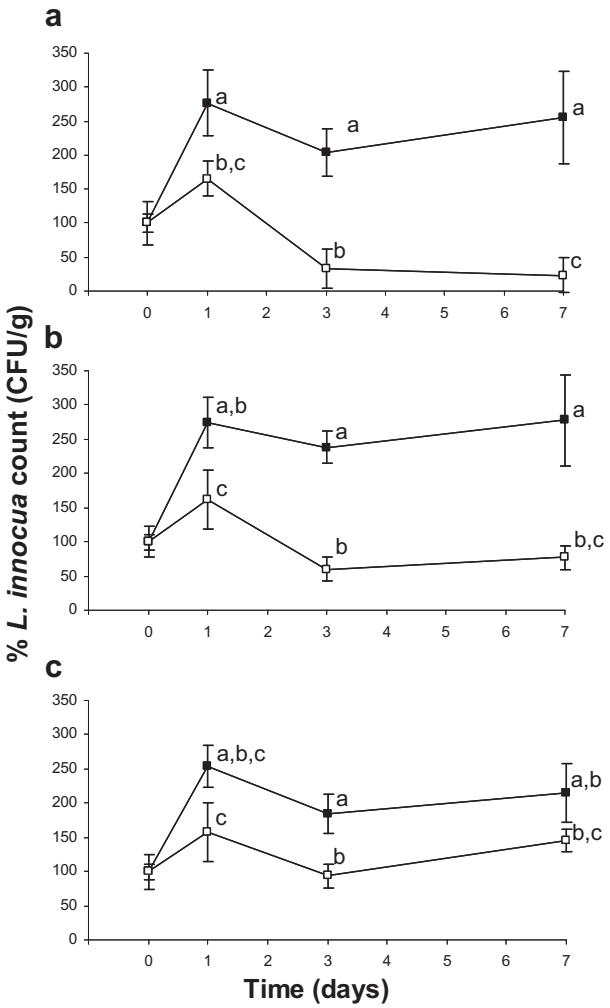

Fig. 3. Changes in *L. innocua* counts in (a) slice 1 ($d = 1$ mm), (b) slice 2 ($d = 2$ mm) and (c) slice 3 ($d = 3$ mm) obtained from in-depth contaminated-reconstituted cheese samples during storage period at 4 °C. (■): control samples in contact with nisin-free film; (□): treated samples in contact with nisin-coated film. [a, b, c]Indicate significant differences between samples ($P < 0.05$).

Table 1
Inactivation of *L. innocua* (log CFU/g) in treated cheese during storage at 4 °C.

	Day 1	Day 3	Day 7
Slice 1	0.4	0.8	1.1
Slice 2	0.4	0.8	0.9
Slice 3	0.4	0.3	0.25

depended on both nisin desorption from the film and diffusion through food matrix. Chollet et al. (2009), who developed model of nisin desorption from polyethylene-based films and diffusion in agarose gel, also reported that concentration of nisin in agarose gel decreased with increasing the distance from the surface of contact with the antimicrobial films.

Overall, during the storage period study, the nisin-containing sodium caseinate films were shown to be effective in reducing growth of *L. innocua* in cheese samples. After 7 days of refrigerated storage, the inactivation level converted in log CFU/g was 1.1, 0.9 and 0.25 with slices 1, 2 and 3, respectively (Table 1). This inactivation rate was comparable with that obtained by Nguyen et al. (2008) while studying the effect of nisin-containing bacterial cellulose films (2500 IU/ml) on *L. monocytogenes* on the surface of smoked salmon and of frankfurters. Kristo et al. (2008) found that nisin-coated sodium caseinate films completely inhibited pathogen growth on agar for the first three days of storage, since the *L. monocytogenes* numbers were below the detection limit. They also reported that nisin films were the most effective against *Listeria* among the tested films (nisin, K sorbate and Na lactate-containing sodium caseinate films) as nisin may be released more slowly from the sodium caseinate matrix than the two salts Na and K, thus maintaining an effective concentration on the contact surface (Kristo et al., 2008).

In conclusion, our study demonstrates the effectiveness of the nisin-coated sodium caseinate films against *L. innocua* in cheese during storage at refrigerated temperatures, indicating that combining nisin into sodium caseinate films is a promising method to enhance the safety and extend the shelf life of processed cheeses. Particularly, it was shown that although nisin did not migrate much inside the cheese matrix, its effect was sufficient for surface-contaminated products since results showed that cells were not encountered at more than 1-mm depth but developed mostly in the surface. It would be interesting to produce active films with higher nisin concentration and to analyze the effectiveness of the antimicrobial films for longer storage period to determine the maximum food shelf life extension as well as the efficiency against other undesirable microorganisms such as mould and yeast.

Acknowledgments

We acknowledge the financial support from Bourgogne region (France). We also thank Frédéric Debeaufort, Pierre-André Marechal, Jean François Cavin for their scientific support, Christine Rojas for her technique assistance and Michelle Kelleher for revising and editing the English of the manuscript.

References

Aasen, I.M., Markussen, S., Møretrø, T., Katla, T., Axelsson, L., Naterstad, K., 2003. Interactions of the bacteriocins sakacin P and nisin with food constituents. Int. J. Food Microbiol. 87, 35–43.
Bemrah, N., Sanaa, M., Cassin, M.H., Griffiths, M.W., Cerf, O., 1998. Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. Prev. Vet. Med. 37, 129–145.
Bremer, P.J., Osborne, C.M., Kemp, R.A., Smith, J.J., 1998. Survival of *Listeria monocytogenes* in sea water and effect of exposure on thermal resistance. J. Appl. Microbiol. 85, 545–553.
Brewer, R., Adams, M.R., Park, S.F., 2002. Enhanced inactivation of *Listeria monocytogenes* by nisin in the presence of ethanol. Lett. Appl. Microbiol. 34, 18–21.
Canac-Arteaga, D., Viallon, C., Berdagué, J.L., 1999. Effect of a dry purge step on the analysis by dynamic headspace – GC-MS of the volatile fraction of a cheese. Analusis 27, 780–785.
Carnet Ripoche, A., Chollet, E., Peyrol, E., Sebti, I., 2006. Evaluation of nisin diffusion in a polysaccharide gel: influence of agarose and fatty content. Innov. Food Sci. Emerg. Technol. 7, 107–111.
Chollet, E., Sebti, I., Martial-Gros, A., Degraeve, P., 2008. Nisin preliminary study as a potential preservative for sliced ripened cheese: NaCl, fat and enzymes influence on nisin concentration and its antimicrobial activity. Food Control 19, 982–989.
Chollet, E., Swesi, Y., Degraeve, P., Sebti, I., 2009. Monitoring nisin desorption from a multi-layer polyethylene-based film coated with nisin loaded HPMC film and diffusion in agarose gel by an immunoassay (ELISA) method and a numerical modeling. Innov. Food Sci. Emerg. Technol. 10, 208–214.
Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F., Deschamps, A., 2002. Edible antimicrobial films based on chitosan matrix. J. Food Sci. 67, 1162–1169.
Coma, V., Sebti, I., Pardon, P., Deschamps, A., Pichavant, F.H., 2001. Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. J. Food Prot. 64, 470–475.
Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G., Owens, C., 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. J. Food Sci. 73, M389–M394.
Genigeorgis, C., Carniciu, M., Dutulescu, D., Farver, T.B., 1991. Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4 to 30 °C. J. Food Prot. 54, 662–668.
Hallier-Soulier, S., Leborgne, G., Batisse-Debitte, Festoc, G., Badiche, 2005. Method and apparatus for extracting nucleic acids from a complex mixture.
Hoffman, K.L., Han, I.Y., Dawson, P.L., 2001. Antimicrobial effects of corn zein films impregnated with nisin, lauric acid, and EDTA. J. Food Prot. 64, 885–889.
Jin, T., Zhang, H., 2008. Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging. J. Food Sci. 73, M127–M134.
Ko, S., Janes, M.E., Hettiarachchy, N.S., Johnson, M.G., 2001. Physical and chemical properties of edible films containing nisin and their action against *Listeria monocytogenes*. J. Food Sci. 66, 1006–1011.
Kristo, E., Koutsoumanis, K.P., Biliaderis, C.G., 2008. Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on *Listeria monocytogenes*. Food Hydrocol. 22, 373–386.
Liu, W., Hansen, J.N., 1990. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl. Environ. Microbiol. 56, 2551–2558.
Lopez-Pedemonte, T.J., Roig-Sagues, A.X., Trujillo, A.J., Capellas, M., Guamis, B., 2003. Inactivation of spores of *Bacillus cereus* in cheese by high hydrostatic pressure with the addition of nisin or lysozyme. J. Dairy Sci. 86, 3075–3081.
Ly-Chatain, M.H., Le, M.L., Belin, J.-M., Waché, Y., 2010. Cell surface properties affect colonisation of raw milk by lactic acid bacteria at the microstructure level. Food Res. Int., in press. doi:10.1016/j.foodres.2010.04.019.
Maisnier-Patin, S., Deschamps, N., Tatini, S.R., Richard, J., 1992. Inhibition of *Listeria monocytogenes* in Camembert cheese made with a nisin-producing starter. Lait 72, 249–263.
Meyrand, A., Folio, P., Giraudon, N., Hadj-amar, D., Cavaud, M.C., Reynaud, A., Rozand, C.V., 2000. Comparaison d'une nouvelle technique d'extraction-concentration des entérotoxines staphylococciques par rapport à la méthode de référence dans les produits laitiers. Rev. Méd. Vét. 151, 205–211.
Morgan, F., Bonnin, V., Mallereau, M.P., Perrin, G., 2001. Survival of *Listeria monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. Int. J. Food Microbiol. 64, 217–221.
Natrajan, N., Sheldon, B.W., 2000. Inhibition of Salmonella on poultry skin using protein- and polysaccharide-based films containing a nisin formulation. J. Food Prot. 63, 1268–1272.
Neetoo, H., Ye, M., Chen, H., Joerger, R.D., Hicks, D.T., Hoover, D.G., 2008. Use of nisin-coated plastic films to control *Listeria monocytogenes* on vacuum-packaged cold-smoked salmon. Int. J. Food Microbiol. 122, 8–15.
Nguyen, V.T., Gidley, M.J., Dykes, G.A., 2008. Potential of a nisin-containing bacterial cellulose film to inhibit *Listeria monocytogenes* on processed meats. Food Microbiol. 25, 471–478.
Rogga, K.J., Samelis, J., Kakouri, A., Katsiari, M.C., Savvaidis, I.N., Kontominas, M.., 2005. Survival of *Listeria monocytogenes* in Galotyri, a traditional Greek soft acid-curd cheese, stored aerobically at 4 °C and 12 °C. Int. Dairy J. 15, 59–67.
Sanjurjo, K., Flores, S., Gerschenson, L., Jagus, R., 2006. Study of the performance of nisin supported in edible films. Food Res. Int. 39, 749–754.
Scannell, A.G., Hill, C., Ross, R.P., Marx, S., Hartmeier, W., ElkeArendt, K., 2000. Development of bioactive food packaging materials using immobilised bacteriocins lacticin 3147 and nisaplin. Int. J. Food Microbiol. 60, 241–249.
Sebti, I., Delves-Broughton, J., Coma, V., 2003. Physicochemical properties and bioactivity of nisin-containing cross-linked hydroxypropylmethylcellulose films. J. Agric. Food Chem. 51, 6468–6474.
Sobrino-Lopez, A., Martin-Belloso, O., 2006. Enhancing inactivation of *Staphylococcus aureus* in skim milk by combining high-intensity pulsed electric fields and nisin. J. Food Prot. 69, 345–353.
Teerakarn, A., Hirt, D.E., Acton, J.C., Rieck, J.R., Dawson, P.L., 2002. Nisin diffusion in protein films: effects of film type and temperature. J. Food Sci. 67, 3019–3025.
Zottola, E.A., Yezzi, T.L., Ajao, D.B., Roberts, R.F., 1994. Utilization of cheddar cheese containing nisin as an antimicrobial agent in other foods. Int. J. Food Microbiol. 24, 227–238.