Review

Use of nisin and other bacteriocins for preservation of dairy products

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Abstract

Non-thermal treatments are attracting interest of the food industry due to their capability of assuring the quality and safety of food. Among them, bacteriocins from lactic acid bacteria, such as nisin, pediocin PA-1, lacticin 3147 and enterocins, may be potentially useful for the dairy industry. Although cheese manufacturers have used bacteriocins for years, the combination of bacteriocins with heat and non-thermal treatments, such as high pressure, pulsed electric fields and other antimicrobials, opens innovative possibilities for application in other dairy products in hurdle-type approach. Bacteriocins alone, or combined with other treatments, could represent a promising advance for the microbiological safety and maintenance of sensory properties in dairy products. However, more research is needed to identify drawbacks out that may hinder their future application, such as their complete characterization, influence of food media on their effectiveness and their microbiological spectra.

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1. Introduction

Consumer demand today is for natural and minimally processed foods, with a fresh appearance and taste, ease-to-eat and high safety. As a result, research and development of new products is leading to the reduction or even displacement of heat treatments and traditional preservatives by treatments capable of assuring the sensory and nutritional properties of the product without reducing food safety. Non-thermal preservation methods are thus of growing interest as alternative treatments, especially high-intensity pulsed electric fields (HIPEF), high pressure (HP) and the addition of natural antimicrobial substances (Bendicho, Espachs, Aránguiz, & Martín, 2002).

Natural antimicrobial compounds have been exploited unknowingly for ages due to their effect against several food spoilage microorganisms and pathogens. Common spices and aromatic plants have been used in cooking not only for their taste, but also for their antibacterial effect. The practical application of these compounds generates changes, in the sensory and textural properties of foods, when they are added. On the other hand, lactic acid bacteria (LAB) have been used in food production as an effective method for extending safe storage of foodstuffs by simple fermentation. Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus and Carnobacterium are the genera most commonly used as starter cultures in the fermentation processes of milk, meat and vegetable products (Stiles & Hastings, 1991).

The preservative effect of those bacteria is mainly due to the production of one or more active metabolites with antimicrobial properties, such as organic acids (lactic and acetic acid), that intensify their action by reducing the pH of the media. However, another mechanism was suspected to be involved in killing or at least inhibiting the growing of other related bacteria and even pathogens by LAB. As a result, a large number of bacteriocins produced by LAB have been identified, although their potential application as biopreservatives has not been fully developed.

Bacteriocins are gaining interest because of their wide antibacterial spectrum with feasible application in foods, such as meat and fish products, fruits and vegetables, cereals and beverages (Cleveland, Montville, Nes, & Chikindas, 2001). Moreover, LAB-derived bacteriocins are generally recognized as safe (GRAS) and are attractive to the food industry because of their activity against key Gram-positive pathogens involved in food-borne illnesses, such as Listeria monocytoogenes or Staphylococcus aureus.

These compounds may be used in three ways (Schillinger, Guisens, & Holzapfel, 1996): (i) as purified or semi-purified antimicrobial additives, (ii) as bacteriocin-based ingredients from fermented foods, and (iii) through bacteriocin-producing starter cultures. The use of bacteriocins as purified powders and, consequently, as food additives, demands an exhaustive evaluation for toxico logical effects before legal acceptance. For that reason, nisin and pediocin PA-1 are the only bacteriocins commercially exploited to date. Unfortunately, although nisin is applied worldwide in dairy products (especially cheese making) as well as sausages, canned and packaged meat and brewing, there is no widespread agreement on the maximum level of bacteriocin allowed among those countries where nisin has been approved as a preservative. For instance, nisin can be added to cheese without limit in United Kingdom, while a maximum concentration of 12,500 mg g⁻¹ in that food is allowed in Spain. Currently, new techniques in bacteriocin application are being steadily directed towards spread of bacteriocin-containing powder (Morgan, Galvin, Ross, & Hill, 2001), bacteriocin-producing strains as fermentation starter cultures (Martinez-Cuesta, Peláez, & Requena, 2001) or biofilms incorporating the bacteriocin (Mauriello, De Luca, La Storia, Villani, & Ercolini, 2005).

Furthermore, recent strategies for controlling spoilage and pathogenic microorganisms tend to apply hurdle technology, whereby different preservation methods are combined to inhibit microbial growth and improve food safety. Syneresis has been reported between bacteriocins and traditional and novel treatments: mixtures of LAB bacteriocins (O’Sullivan, Ryan, Ross, & Hill, 2003) or LAB bacteriocins combined with other antibacterial compounds (Kozákóvá, Holubová, Plocková, Chumchalová, & Curda, 2005) may enhance their antibacterial effect. An increase in microbial inactivation has been also reported by adding bacteriocins prior to a mild thermal (Penna & Moraes, 2002) or non-thermal (Sobrino-López, Raybaudi-Massilia, & Martín-Belloslo, 2006) treatment.

Although LAB are the main source of bacteriocin-producing bacteria and bacteriocin breakthroughs, few have been studied as potentially applicable in dairy products. Therefore, many bacteriocins have not been fully characterized yet and, consequently, they are not extensively used in food industry. To date, different studies have highlighted particular features of nisin, as well as pediocin PA-1 and lacticin 3147, that make them suitable for promising uses. However, potential uses of nisin and other novel bacteriocins are being studied in a wide variety of food products; meanwhile, their dairy origin also suggests the possibility of improving and enhancing features of those dairy products from which they have been isolated. Hence, the objective of this review is to compile research, applications and drawbacks of LAB-derived bacteriocins, as well as the interactions of bacteriocins of LAB with thermal and non-thermal treatments in dairy products.

2. Nisin

Nisin is a peptide composed of 34 amino acid residues, with a molecular mass of 3.5 kDa, and is classified as a class-Ia bacteriocin or lantibiotic (Hurst, 1981). It is produced by strains of Lactococcus lactis subsp. lactis isolated from milk and vegetable-based products and its importance is due to its wide spectrum of activity against Gram-negative and Gram-positive bacteria.
The use of nisin as a biopreservative has been widely investigated in a large variety of fresh and processed foods (Jung, Bodyfelt, & Daeschel, 1992). Consequently, it was admitted into the European food additive list, where it was assigned the number E234 (EEC, 1983). Because it was thoroughly studied and formed part of the human diet, nisin was also approved by the Food and Drug Administration (1988) in the USA as GRAS; to date, it is the only bacteriocin that has been approved by the World Health Organization for use as a food preservative and it is commercialized as a dried concentrated powder.

2.1. Applications of nisin for fermented dairy products

Nisin has been shown to be effective in the microbial control of a number of dairy products and its use has been widely assessed in cheese manufacturing at low pH. The use of nisin-producing and nisin-resistant starter cultures appears to be a viable means of incorporating and maintaining this bacteriocin, through the cheese-making process, to control food-borne pathogenic and spoilage bacteria. Lc. lactis subsp. lactis TAB50 and its lactase-negative proteinase-negative mutant strain TAB50-M4 have been tested and selected as useful starter cultures or adjuncts in semi-hard cheese from raw or pasteurized milk, providing protection against contamination of milk or curd by S. aureus (Rodriguez et al., 2000). However, the environmental conditions and processing factors, such as pH and water activity, required to stimulate nisin production by transconjugant or natural producers should be defined for further and future implementation of this technique. In Cheddar cheese, strains of Lc. lactis ssp. cremoris and Lc. lactis ssp. lactis grown in pH-controlled reconstituted skim milk (RSM) produced 2–5 times more nisin than bulk cultures incubated in RSM, although differences in nisin production depended on bacterial strains (Yezzi, Ajao, & Zottola, 1993). When those bulk starter cultures prepared by pH control of the starter media were used to make Cheddar-type cheese, the concentrations of nisin increased by approximately 20% (Yezzi et al., 1993). A threshold of 400 IU nisin g−1 was sufficient to protect against spoilage by C. sporogenes for more than 90 days in cheese spreads (Roberts & Zottola, 1993). In contrast, the use of a nisin-producing strain to control L. monocytogenes in Feta and Camembert cheeses has shown variable results, ranging from partial to non-inhibitory effects (Ramsaran, Chen, Brunke, Hill, & Griffiths, 1998).

The addition of nisin powder to milk for the production of cheese made without a starter culture can control microbial contamination, while nisin concentration remains unaltered after pasteurization. Addition of nisin at 100 or 500 mg kg−1 suppressed total plate and anaerobic spore counts in processed cheese during 3 months of storage at 5 or 21°C, and even the growth of Bacillus stearothermophilus, Bacillus cereus and Bacillus subtilis were inhibited by 5 mg kg−1 nisin (Plockova, Stepanek, Demnerova, Curda, & Svirakova, 1996). Shelf life analysis of Ricotta-type cheese demonstrated that 2.5 mg L−1 nisin inhibited the growth of L. monocytogenes for more than 8 weeks, while cheese made without nisin contained unsafe levels of the bacteria within 1–2 weeks. In addition, the residual levels of nisin in the cheese after 10 weeks of incubation at 6–8°C indicated a high level of retention, with only 10–32% loss of nisin activity (Davies, Bevis, & Delves-Broughton, 1997).

2.2. Inclusion of nisin in active packaging

Nisin is a highly surface-active molecule that can bind to different compounds, such as fatty acids of phospholipids; this feature makes it suitable for adsorption to solid surfaces and killing bacterial cells that subsequently adhere. Therefore, nisin adsorption may represent a promising advance in the development of active packaging, where the classical protective function of packaging is supported by the antimicrobial action of nisin. Furthermore, the efficacy of the bacteriocin activity could be improved by control of migration of the bacteriocin into the packaged media, enabling its antimicrobial effect to be preserved beyond consumer purchase. To achieve this purpose, packaging materials, such as those made of polymers (e.g., cellulose-based packaging or polypropylene), may incorporate, or be coated with, the bacteriocin.

Mauriello et al. (2005) successfully tested a low-density polyethylene film coated with nisin for inhibition of Micrococcus luteus as an indicator strain during the storage of milk. The antimicrobial package retarded microbial growth and lowered the maximum growth levels in raw, pasteurized and UHT milk, although the activity and the release of nisin from the film strongly depended on pH and temperature. Since the solubility and stability of nisin decreases from the optimal pH 2.0 to 6.0 (Hurst, 1981), a lower pH and a higher temperature favoured the migration of the bacteriocin from the film. These results agree with those of Lee, Park, and Lee (2004), whereby virgin paperboard coated with nisin and/or chitosan in a binder of vinyl acetate–ethylene copolymer was assessed during storage of pasteurized milk at different temperatures. The antimicrobial paperboards retarded the microbial growth of aerobic bacteria and yeasts at 3 and 10°C, whereas the effect was marginal at 20°C. Cross-linked hydroxypropylmethylcellulose (HPMC) films containing nisin also have been found to be active against M. luteus (Sebti, Delves-Broughton, & Coma, 2003). However, the temperature reached in the process of cross-linking meant that the heat stability of the bacteriocin, if this was added previously, needed to be taken into account.

Further applications of packaging materials have been focused on the production of inserts placed between portions of sliced products, such as cheese. Cellulose-based packaging, including nisin, was assayed as an insert interleaved between slices of Cheddar cheese packaged under a modified atmosphere (Scannell et al., 2000). In that
case, the population of *L. innocua* and *S. aureus* dropped dramatically in the first week of refrigeration conditions and, as a result, the shelf life of Cheddar cheese was significantly extended.

### 2.3. Use of nisin in combination with thermal treatments

Milk is commonly heated to provide stability during storage and assure microbiological safety to consumers. From a hurdle approach, nisin is known to influence the thermal resistance of microorganisms. The D value of *B. cereus* in milk was reduced in the presence of nisin by up to 40% at temperatures in the range 80–100 °C (Penna & Moraes, 2002), while the apparent D values of *B. stearothermophilus* at 130 °C were reduced by 21% due to the presence of 4000 IU nisin mL⁻¹ (Rao and Mathur, 1996). Consequently, the use of nisin in combination with those heat treatments extended the shelf life of milk, even with poor refrigeration conditions, and, as a result, the shelf life of Cheddar cheese was dramatically in the first week of refrigeration conditions and, moreover, milk treated in this way was easily distinguishable and preferred to a UHT-heated control in sensory analysis trials (Wirjantoro, Lewis, Grandison, Williams, & Delves-Broughton, 2001).

### 2.4. Use of nisin in combination with non-thermal treatments

Current thermal treatments are known to cause undesirable changes in the sensory, nutritional and/or technological properties of milk. Taking advantage of the antimicrobial action of nisin against several spoilage and pathogenic microorganisms, innovative non-thermal food preservation offer the inactivation of microorganisms with minimal impact on quality and nutritional factors. Combining nisin addition with other antimicrobial agents or non-thermal treatments, such as HP or HIPEF, has acted synergistically in reducing the population of different microorganisms, including bacterial spores (Table 1). Therefore, each treatment can be optimized by setting

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Table 1

<table>
<thead>
<tr>
<th>Nisin dose (IU mL⁻¹)</th>
<th>Combined treatment Product</th>
<th>Target microorganism</th>
<th>Inactivation (log units)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>751</td>
<td>Heat, 117 °C, 2 s</td>
<td>Milk</td>
<td>Natural flora</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>751</td>
<td>Monolaurin, 250 µg mL⁻¹</td>
<td>Skim milk</td>
<td><em>B. cereus</em></td>
<td>1.5</td>
</tr>
<tr>
<td>1000</td>
<td>Reuterin, 8.4 U mL⁻¹</td>
<td>Milk</td>
<td><em>L. monocytogenes</em></td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>LPS, HPb, 500 MPa, 30 min, 25 °C</td>
<td>Milk</td>
<td><em>S. aureus</em></td>
<td>2.5-7</td>
</tr>
<tr>
<td>1000</td>
<td>CNQc HPb, 500 MPa, 30 min, 20 °C</td>
<td>Cheese</td>
<td><em>E. coli</em></td>
<td>4</td>
</tr>
<tr>
<td>201</td>
<td>HIPEFd, 550 MPa, 15 min, 20 °C</td>
<td>Skim milk</td>
<td><em>S. aureus</em></td>
<td>6</td>
</tr>
<tr>
<td>100</td>
<td>HIPEFd, 50 kV cm⁻¹, 50 pulses</td>
<td>Skim milk</td>
<td><em>L. innocua</em></td>
<td>4</td>
</tr>
<tr>
<td>381</td>
<td>Lysozyme and HIPEFd, 1638 IU mL⁻¹, 80 kV cm⁻¹, 50 pulses, 52 °C</td>
<td>Raw skim milk</td>
<td>Natural flora</td>
<td>7</td>
</tr>
</tbody>
</table>

**a**LPS: lactoperoxidase system.
**b**HP: high-pressure.
**c**HIPEF: high-intensity pulsed electric fields.
**d**CNQ: concentration not quantified.
lower values of the controlled variables, while the goal of microbial reduction is improved.

2.4.1. Use of nisin in combination with other antimicrobial substances

Combining nisin with other antimicrobial compounds, such as monolaurin, the lactoperoxidase system (LPS) or other bacteriocins, can induce the sensitization of resistant spoilage and food-borne microorganisms. Monolaurin, the monoester of lauric acid, has received special attention because of its antimicrobial properties (Wang & Johnson, 1997), which may be intensified when combined with nisin. The combination of monolaurin and nisin has been found to be active against bacilli in milk; in particular, the inhibition by both antimicrobial substances of *B. licheniformis* increased with increasing pH when they were added simultaneously to milk (Mansour, Amri, Boutefroy, Linder, & Milliere, 1999). In addition, the combination of both compounds successfully exerted a bactericidal effect against different *Bacillus* species in skim milk, and also inhibited their regrowth and sporulation (Mansour & Milliere, 2001). However, a high concentration of monolaurin may produce an unpleasant soapy odour and taste (Bell & de Lacy, 1987), which is undesirable in dairy products.

The LPS system in raw milk increases the storage stability of raw milk at ambient temperature (Wolfson & Summer, 1993). The combination of LPS and nisin had a synergistic and long-lasting inhibitory effect on *L. monocytogenes* in reconstituted skim milk and, in addition, its effectiveness did not depend on pH (Boussouel et al., 1999). Curiously, higher antibacterial activity was observed when the inhibitors were added to skim milk in two steps; the effect of the LPS-nisin combination was enhanced when nisin was added to skim milk inoculated with *L. monocytogenes* after the addition of LPS (Zapico, Medina, Gaya, & Nunez, 1998). Clearly, the order of addition for maximum inactivation has received insufficient attention.

Although the mechanisms of action are different for LPS and nisin, both antimicrobial agents cause damage to the cytoplasmic membrane, which could explain their synergistic action (Zapico et al., 1998). The primary reaction product of the LPS, hypothiocyanite, is known to react with the thiol groups of various proteins and inactivate crucial enzyme and protein systems (Boots & Floris, 2006). On the other hand, nisin forms a wedge-like pore composed of multiple nisin molecules bound on to the phospholipids of the membrane (Moll et al., 1997; Fig. 1). Both antimicrobials play an important role in the depletion of proton motive force, since the bacterial respiration chain is targeted by the LPS (Boots & Floris, 2006), and nisin pores lead to a collapse of the membrane electrical potential and the pH gradient (Bruno, Kaiser, & Montville, 1992).

Lysozyme, which may be added to cheese milk to prevent blowing and the activity of *Bacillus* spp., displayed an inhibitory effect on different strains of lactobacilli when mixed with nisin, while it did not have any influence on nisin-producing lactococci when added as starter (Kozáková et al., 2005). Reuterin is a broad-spectrum antimicrobial compound produced by some strains of *Lactobacillus reuteri* (Axelsson, Chung, Dobrogosz, & Lindgren, 1989). The combination of reuterin and nisin acted synergistically against *L. monocytogenes* and additively against *S. aureus* in milk (Arqués et al., 2004). Recently, activity of nisin and a cell-free supernatant of *B. licheniformis* ZJU12 have also been found to be synergistic against three food-borne bacteria: *M. flavus, B. cereus* and *S. aureus* (He & Chen, 2006). The flora of minimally processed dairy products mainly consists of bacteria, but moulds and yeasts are commonly associated with special ingredients, such as wild blueberries added to yoghurt (Penney, Henderson, Blum, & Johnson-Green, 2004). For that reason, yoghurt containing minimally processed wild blueberries has an extremely short shelf life; thus, some phytopreservatives, such as vanillin, have been tested efficiently for fungal inactivation in yoghurt with blueberries, while added nisin did not

![Fig. 1. General mode of action of nisin: lipid II serves as a docking molecule which energetically facilitates the formation of pores by binding the molecule of nisin and allowing to adopt the correct position for pore opening (adapted from Brötz & Sahl, 2000).](image-url)
prevent spoilage (Penney et al., 2004). In the case of yoghurt containing fresh peaches, nisin actually hastened the growth of spoilage microorganisms (Penney et al., 2004). This fact may represent a drawback in nisin use since, particularly; yeast growth might be stimulated by added nisin through two mechanisms, i.e., the addition of new carbohydrate substrates, and the suppression of lactic acid bacterial competitors (Penney et al., 2004).

2.4.2. Use of nisin in combination with high pressure

Nisin has been shown to enhance the microbial reduction achieved by HP, due to sublethal injury and sensitization caused to target cells. Studies carried out on milk demonstrated that Gram-negative bacteria, such as *Pseudomonas fluorescens* or *Escherichia coli*, and Gram-positive bacteria, as *L. innocua*, are inactivated by HP treatment, although Gram-positive bacteria seemed to be rather resistant (Black, Kelly, & Fitzgerald, 2005). Hence, the degree of inactivation achieved by the combined use of HP and antimicrobials such as nisin should be higher than that achieved by the sum of the inactivation achieved by the individual treatments. Treatment at 500 MPa for 5 min in the presence of 500 IU mL\(^{-1}\) nisin completely inactivated *Ps. fluorescens* and *E. coli* and reduced *L. innocua* by more than 8.3 log, whereas those treatments, when applied separately, produced a decrease of only 3.8 and 1.5 log units, respectively (Black et al., 2005). Working from the hypothesis that the conditions used to destroy pressure-resistant strains would be sufficient to kill less-resistant pathogens, Alpas and Bozoglu (2000) found that *S. aureus* was the most resistant to HP among the pathogens considered in their study. After that, a biopreservative powder consisting of nisin and pediocin PA-1 at a final level of 5000 Au mL\(^{-1}\) was added to milk inoculated with *S. aureus* followed by HP treatment (345 MPa, 50 °C, 5 min); a reduction in cell population of *S. aureus* of more than 8-log cycles was found and no growth was observed for up to 30 days in samples stored at 25 °C. Nevertheless, Garcia-Graells, Masschalck, and Michiels (1999) found that the complex environment of milk exerts a strong protective effect on microorganisms against HP inactivation. In the latter study, an increase in the lethality of pressure-resistant *E. coli* strains in milk was achieved by the addition of lysozyme (400 µg/mL\(^{-1}\)) and nisin (400 IU mL\(^{-1}\)) before HP treatment. As a result, the population decreased by 3 log units in skim milk at 550 MPa, which represented an additional log reduction. However, that reduction level was significantly lower in 1.55% fat and whole milk.

Owing to the microbial reduction achieved by the combination of HP and nisin, particularly on pathogenic bacteria such as *S. aureus* or *L. monocytogenes*, the microbiological safety of cheese made from raw milk could also be improved while producing little or no change in its sensory quality. Arqué’s et al. (2005a) reported 6.7 log units of *S. aureus* on day 1 in cheese made from milk inoculated with LAB excluding bacteriocin-producing LAB. In that study, synergistic effects on *S. aureus* were recorded in cheese made from milk inoculated with a commercial lactic acid bacterial culture and a nisin-producing LAB submitted to HP treatment after manufacture. When nisin-containing cheese was treated at 500 MPa and 10 °C for 5 min on day 2, counts of *S. aureus* dropped sharply by up to 2.5 log cycles on day 3 and no growth was detected between 20 and 60 days later. Similarly, Arqués, Rodríguez, Gaya, Medina, and Nuñez (2005b) evaluated the combined effect of HP and nisin-producing LAB in cheese on *L. monocytogenes*; HP treatment at 500 MPa and 10 °C for 5 min proved to be more effective in killing *L. monocytogenes* when applied on day 51 than on day 2. In agreement with the results of Arque’s et al. (2005b), applying HP treatment at 500 MPa and 10 °C for 5 min in raw-milk cheese manufactured with nisin-producing LAB led to undetectable counts of *E. coli* on day 50 (Rodriguez, Arques, Nuñez, Gaya, & Medina, 2005). Capellas, Mor-Mur, Gervilla, Yuste, and Guamis (2000) applied those combinations of HP and nisin that caused the lowest impact on the sensory characteristics of cheese, and measured more than 2 log reductions in the viability of aerobic mesophilic bacteria when 7 IU mL\(^{-1}\) nisin and 500 MPa were combined for 30 min at 25 °C. Furthermore, HP treatment may also improve the efficacy of nisin for inactivation of some spores by increasing the permeability of the spore coat after the germinating process; counts of spores of *B. cereus* in traditional cheese curd were dramatically reduced when the addition of nisin was followed by two HP cycles, a cycle to induce spore germination and a second to destroy vegetative cells (López-Pedemonte, Roig-Sagués, Trujillo, Capellas, & Guamis, 2003).

Two hypotheses may explain the synergism of combining HP and nisin. The first step of the wedge model of nisin pore formation (Moll et al., 1997) is a parallel orientation of the molecule and subsequent binding to the membrane, which could increase sensitization of the microorganisms to pressure by local immobilization of phospholipids (Ter Steeg, Hellemons, & Kok, 1999). Secondly, synergistic effects have been attributed to sublethal damage by the permeabilization effect of HP on the cell wall and/or outer membrane for Gram-negative microorganisms that could facilitate the access of bacteriocins to the cytoplasmic membrane (Hauben, Wuytac, Soontjens, & Michiels, 1996). Mechanisms involved in permeabilization of the cell envelope and its subsequent sensitization to nisin seem to be dependent on process variables such as pressure and treatment time. Diels, Taeye, and Michiels (2005) studied the effect of nisin on *E. coli* under low pressure and short exposure time conditions and concluded that the outer membrane was permeabilized transiently. This transience only occurred during the treatment within the pressure range of 150–300 MPa by mechanical damage, rather than physiological or metabolic damage and, thus, the outer membrane was immediately repaired after the process.
In contrast, HP treatment can cause permanent membrane damage due to a higher pressure applied or a longer exposure time. Black et al. (2005) observed that, although part of the damage sustained during HP treatment (200 MPa for 5 min at 20°C) is rapidly reversed on depressurization, a portion of the cells of *Ps. fluorescens* remained permeabilized and susceptible to nisin, demonstrating that significant cell damage is sustained during and after pressure.

Permeabilization due to HP has been evidenced by leakage of the periplasmic enzyme β-lactamase of *E. coli* (Hauben et al., 1996). This phenomenon may be explained by changes in membrane fluidity following HP treatment (Ter Steeg et al., 1999). HP treatment induces a phase transition of the lipid bilayer membrane, shifting the natural crystalline phase to an initial reversible gel phase and finally to an irreversible integrated phase, as well as reduced thickness of the bilayers (Kato & Hayashi, 1999).

Membrane composition has been shown to influence the efficacy of nisin-HP treatment with regard to membrane fluidity, as well as treatment temperature. An increased degree of unsaturation of membrane fatty acids was correlated with protection against pressure inactivation, while higher content of lysylphosphatidylglycerol and diphosphatidylglycerol play a key role in increased susceptibility to nisin and/or HP, respectively (Ter Steeg et al., 1999). With respect to temperature, cell membranes far below their growth temperature are in a semicrystalline gel state, which is more rigid and HP sensitive than those of cells closer to their growth temperature (Ter Steeg et al., 1999). The effect of such changes and damage in the cell envelope results in the disruption of H bonds, ionic bonds and hydrophobic interactions of the macromolecules (Hoover, 1993), protein denaturation (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989) and losses in transmembrane proton gradient and proton motive force (Kalchayanand, Dunne, Sikes, & Ray, 2004).

Nevertheless, treatment variables and proposed mechanisms for modes of action of HP action seem to be strongly interrelated. Surprisingly, the effect of adding nisin to milk, which was made into cheese then treated by HP, was rapidly manifested in killing aerobic mesophilic bacteria within the pH range 6–7, although pH 4–5 is apparently the most favourable for nisin activity (Capellas et al., 2000). Moll et al. (1997) found that acidity of media affected nisin-induced pores in terms of dissipation of the transmembrane pH gradient (ΔpH) and the transmembrane electrical potential (Δψ). The efficiency of nisin in collapsing the Δψ decreases markedly when the pH is lowered from pH 7.0 to 6.0. In addition, dissipation of the ΔpH is only marginally lower at pH 6.0 than at 7.0, ΔpH being the primary target for nisin action at an acidic pH. Thus, pore dissipation changes with regard to pH but pores can keep their functionality. Moreover, higher doses of nisin were incapable of increasing synergism with HP for inactivation of germinated spores of *B. cereus* (López-Pedemonte et al., 2003) and, in addition, poor effectiveness of nisin in reducing *E. coli* population in milk was observed, despite the higher concentration of nisin (400 IU mL⁻¹) and HP applied (600 MPa for 15 min at 20°C) (García-Graells et al., 1999). The antagonism between nisin activity and nisin concentration could be due to competition for membrane binding sites, to the formation of heterooligomeric pore complexes, or both (Moll et al., 1997). In contrast, Christ, Wiedemann, Bakowsky, Sahil, and Bendas (2007) concluded that nisin concentration is not a limit for pore formation. Therefore, the interaction of nisin and HP in causing cell death suggests that mechanisms involved in synergism may need further research for a better understanding.

### 2.4.3. Use of nisin in combination with high-intensity pulsed electric fields

HIPEF has generated interest as a feasible non-thermal technology for processing liquid foods. Although only a few studies have been focused on microbial inactivation in milk by this treatment, the results of inactivation by combining HIPEF with other antimicrobials, particularly nisin, bode well for future development. Calderon-Miranda, Barbosa-Cánovas, and Swanson (1999a) found that combining the electric field intensity, the number of pulses and the nisin concentration acted synergistically for inactivation of *L. innocua* in skim milk. Up to 3.8 log units inactivation of *L. innocua* was observed in that study after exposure to 100 IU nisin mL⁻¹ and a HIPEF treatment of 32 pulses at 50 kV cm⁻¹, over 0.6 log units more than an additive effect. The same behaviour was observed for natural flora of raw milk, when nisin added prior to a HIPEF treatment at 80 kV cm⁻¹ for 100 μs produced an extra inactivation of 4.42 log cycles (Smith, Mittal, & Griffiths, 2002). Similarly, the loss of viability and cell death of *S. aureus* in skim milk caused by both treatments applied simultaneously resulted in a remarkable synergistic effect, compared with those treatments applied individually (Sobrino-López et al., 2006), with over 4.0 additional log units inactivation when HIPEF treatment time, pH and nisin were 2400 μs, 6.8 and 20 IU mL⁻¹, respectively. However, the combined effect was found to be pH dependent, since only 2 extra log cycles inactivation was achieved at pH 5.0 under the same treatment conditions (Fig. 2). Based on those results, nisin seemed to be inactivated by HIPEF, although either the presence of bacterial cells (Terebiznik, Jagus, Cerrutti, de Huergo, & Pilosof, 2000) or insolubilization at neutral pH may exert a protective effect (Sobrino-López et al., 2006). Thus, the interaction between nisin and HIPEF may be affected in a complex manner by treatment variables and food properties.

Synergy between HIPEF treatment and nisin may be further enhanced by a third hurdle, such as a mild thermal treatment or the addition of other antimicrobials. The efficacy of a HIPEF-nisin treatment against vegetative cells of *B. cereus* in milk may be intensified by adding carvacrol, although only when used at a high concentration.
be an effective method for the pasteurization of skim milk (Smith et al., 2002).

However, variables and conditions affecting synergism of the combined treatment or mechanisms by which the cell membranes of microorganisms become sensitized are not yet fully understood. From observations of *L. innocua* by transmission electron microscopy, Calderon-Miranda, Barbosa-Cánovas, and Swanson (1999b) noticed marked morphological differences between nisin-HIPEF-treated cells and those treated with nisin and HIPEF alone as electric field intensity increased. Cells treated by nisin-HIPEF exhibited lack of cytoplasmic or holes, cytoplasmic clumping, an increase in the cell wall surface roughness and cell membrane thickness, blebs or separation of the cytoplasm from the cell membrane, and, finally, a slight increase in the cell length. Therefore, the combination of nisin and HIPEF treatment induced damage to the cell wall and cell membrane that inhibited their functionality as a barrier.

As synergism exhibited by the combination of nisin and HP, formation of non-lethal pores in the cell wall or outer membrane by HIPEF could ease the reach of the cytoplasmic membrane to nisin (Terebiznik et al., 2000) and, conversely, using nisin as an adjunct to HIPEF treatment may alter the HIPEF resistance of microorganisms (Dutreux, Notermans, Góngora-Nieto, Barbosa-Cánovas, & Swanson, 2000) by reducing the critical field strength required for cell lysis (Ho, Mittal, Cross, & Griffiths, 1995). However, contradictory results obtained in different studies apparently suggest that the proposed hypotheses need to be detailed or even rewritten. Dutreux et al. (2000) showed that there was no sublethal injury in *M. luteus* as a result of HIPEF treatment. In agreement with those results, Gallo, Pilosof, and Jagus (2007) studied the sequence of application of nisin and HIPEF and concluded that applying nisin before HIPEF treatment enhanced their simultaneous effect, whereas nisin addition after HIPEF did not modify the final effect. This behaviour suggests that changes in the media and those inflicted to the cell envelope by HIPEF may impede the action of nisin. In this respect, modification of the soluble components of whey protein concentrate by HIPEF would favour interaction of nisin with the medium instead of the bacteria, and changes in the cell envelope, loss of osmotic response and reduction of cell surface hydrophobicity may induce cellular resistance to nisin (Gallo et al., 2007). These results partially agree with the mode of action of nisin and its amphipathic character, which allows nisin to interact with phospholipid membranes (Moll et al., 1997), since membrane phospholipids act as target molecule of nisin where it is bound by electrostatic attraction of their negative charges. In addition, and consistent with the wedge model for pore formation of nisin (Moll et al., 1997), Terebiznik et al. (2000) claimed that no additional effect of nisin would be expected when HIPEF has lethal effects. The shrinkage of the outer and cytoplasmic membranes could allow entrance of nisin into the cytoplasm and,
consequently, internalized nisin would not be able to form pores from the cytoplasm due to inadequate Δψ (negative inside) and ΔpH (alkaline inside). Since little research has been performed on membrane changes due to HIPEF and their influence on mode of action of nisin, interaction between nisin and HIPEF in cell inactivation has not been fully explained.

2.5. Limitations of using nisin in dairy products

Several limitations curb the use of nisin in dairy products, such as its adsorption to fat and the surface of protein globules, a heterogeneous distribution in dairy product matrices, the inhibition of non-resistant starter cultures, or flavour alteration on incorporation of nisin-producing strains as starters. To overcome these limitations, microencapsulation of nisin in phospholipid vesicles has been tested in Cheddar cheese (Laridi et al., 2003). Vesicle-binding nisin could successfully withstand the Cheddar cheese-making temperature cycle and improve nisin stability, efficacy and distribution, although the stability of liposome vesicles is affected by the fat content of milk (Laridi et al., 2003). The appearance of resistant cells in strains sensitive to nisin may constitute another limitation to its use. Str. thermophilus INIA 463 is a nisin-sensitive strain, although it has been shown to become nisin-resistant after exposure in skim milk to subminimal inhibitory concentrations of nisin (1–3 IU mL⁻¹) for less than 2 h, by the induction of a resistance mechanism based on changes in the cell wall (Garde, Ávila, Medina, & Nuñez, 2004). In a similar way, nisin-resistant variants of wild-type Listeria isolated from hand-made cheeses commercialized in Spain were able to survive and grow in milk fermented by a nisin-producing Lactococcus (Martínez, Bravo, & Rodríguez, 2005). The exposure of L. monocytogenes to acidic conditions in milk enhanced its long-term survival in the presence of nisin in refrigerated conditions (Bonnet & Montville, 2005).

The development and efficacy of the use of nisin as a biopreservative in processed or minimally processed foods may depend on a wide range of physicochemical properties of the molecule itself and its behaviour in the medium. Milk is a complex mixture of different substances, such as water, proteins and fat, so that the effectiveness of nisin in processed milk may be affected by composition. Several studies have reported an interaction between milk fat and nisin activity, which may limit its application in fat-containing dairy products. Jung et al. (1992) found that activity of nisin against L. monocytogenes decreased as the milk fat concentration increased. Bhatti, Veeramachaneni, and Shelef (2004) also found a maximum antilisterial effect of nisin in skim milk and a reduced effect in milk with ≥2% fat. Moreover, Bhatti et al. (2004) verified that homogenization of milk reduced the antilisterial activity of nisin. Zapico, de Paz, Medina, and Nuñez (1999) reported a loss of up to 64% in the effectiveness of the bacteriocin against L. innocua in homogenized whole milk, which may be due to the binding of nisin to milk fat globules, and thus may be prevented by minimizing its adsorption to the globules’ surface. For example, emulsifiers have been shown to be useful in maintaining nisin activity; polyoxethylene sorbitan monooleate, known as Tween 80, was tested in half-whole milk and shown to retain 43.4% of the original activity of nisin after 2 h at 37 °C, while only 19.6% was detected without the emulsifier (Jung et al., 1992). Although the mechanism by which emulsifiers reverse the loss of the antilisterial effects of nisin in homogenized milk is not known at this time, Bhatti et al. (2004) suggested that peptides such as nisin can be displaced from an interface by the Tween 80 and be available to adhere to bacterial cells.

3. Pediocin PA-1

Pediocin PA-1, a plasmid-encoded peptide produced by Pediococcus acidilactici, is commercially exploited as a bacteriocin-containing fermentate powder. Although this antimicrobial compound is mainly used in meat products, the extension of its application to dairy products is being evaluated due to its antilisterial activity, but also due to its stability in aqueous solutions, its wide pH range for activity and the fact that it is unaffected by heating or freezing (Nes et al., 1996). However, as far as is known, the effect of adding the pediocin-containing powder in milk has not yet been studied. Instead, the production of pediocin in heterologous hosts is currently considered an attractive alternative in milk and dairy products, since P. acidilactici is not suitable for the production of dairy products from both metabolic and technological points of view. The antilisterial activity of recombinant L. lactis MM217 as a pediocin-producing starter culture has been successfully tested in Cheddar cheese without affecting its technological properties (Buyong, Kok, & Luchansky, 1998). In a recent study, strains of L. lactis ESI 153 and L. lactis ESI 515, isolated from hand-made raw milk cheese and transformed into pediocin producers, were identified as likely candidates for food-grade bacteriocin-producing strains (Reviriego et al., 2005). In addition, Lactobacillus plantarum WHE 92, which grows particularly well in cheese, especially Munster type, has been revealed as a spontaneous pediocin producer at a high enough concentration and pH range to be used in the industrial manufacture of cheeses (Ennahar et al., 1996).

4. Lacticin 3147

Although lacticin 3147 has not been commercially exploited, many studies have suggested this bacteriocin as being potentially suitable for many applications. More particularly, interest in lacticin 3147 has steadily risen owing to its activity against a broad range of organisms of importance in foods. Lacticin was isolated from an Irish kefir grain used for making buttermilk; Lc. lactis subsp. lactis DPC3147, identified as this bacteriocin producer
(McAuliffe et al., 1998), is not a nisin producer. Lacticin 3147 is a two-component bacteriocin that is hydrophobic in nature (McAuliffe et al., 1998) even though it has been classified as belonging to class I (Cintas, Casaus, Herranz, Nes, & Hernández, 2001). Like nisin, properties of lacticin 3147 are pH dependent; its activity increases in acidic media, as well as its stability to thermal treatments. Lacticin 3147 was inactivated when treated at 121 °C for 10 min at 9.0 pH, while it only lost 50% of its activity at pH 5.0 (Ryan, Rea, Hill, & Ross, 1996).

4.1. Potential use of lacticin 3147 in fermented dairy products

Research on use of lacticin 3147 has led to both a commercial lacticin-3147 powder and the generation of lacticin 3147-producing transconjugant starters. Since production of, and immunity to, lacticin 3147 are plasmid-encoded traits, this plasmid can be conjugally transferred to commercial starters (Ryan et al., 1996). To date, more than 30 lacticin-3147-producing transconjugant starters have been generated for possible application in cheese making (Coakley, Fitzgerald, & Ross, 1997). From a technological point of view, lacticin 3147 has shown antimicrobial effects, but it may be applied to achieve control of cell lysis of adjuncts or LAB and flavour formation in cheese as a lytic compound. Martínez-Cuesta et al. (2001) proved that the constructed lacticin-3147-producing transconjugant, *Lc. lactis* IFPL3593, improved sensory properties of semi-hard cheese, such as taste, after 27 days of maturation. However, transconjugants, such as *Lc. lactis* DPC4275, are generally less efficient producers of lacticin 3147, and, as a result, a sufficiently high bacteriocin concentration to inhibit spoilage by NSLAB is absent (Ryan, Ross, & Hill, 2001).

The industrial viability of lacticin 3147-based powder is being evaluated as a biopreservative, so that it may be applied as a food ingredient in a variety of foods. Morgan et al. (2001) found that a 10% lacticin 3147 powder was extremely effective for the inhibition of *Listeria* in yoghurt and cottage cheese. Within 60 min of adding lacticin 3147 powder, no viable cells of *Listeria* remained in the yoghurt and, in the case of cottage cheese, counts showed 85% of non-viable cells after 120 min; the kill rate was more rapid at higher temperature. In spite of lacticin 3147 powder being effective as an antimicrobial, two serious setbacks may prevent its commercial use: its stability in food media and heat sensitivity. A concentrated powdered product containing lacticin 3147 lost 75% of its activity after 5 months at room temperature, while full activity was retained for 5 months at 4 °C (Morgan et al., 2001). The effect of autoclaving (121 °C for 15 min) a lacticin 3147 powder and a resuspended preparation in whey also resulted in considerable loss of activity (Morgan et al., 2001). In contrast, Morgan, Galvin, Kelly, Ross, and Hill (1999) reported that pasteurization had no effect on a lacticin 3147-enriched demineralized whey powder. Assessment of the inhibitory activity of the bioactive powder demonstrated that it is capable of inhibiting both *L. monocytogenes* and *S. aureus* at pH 5 and 7 and effectively inactivated 99% of *L. monocytogenes* and *S. aureus* added to infant formula within 3 h.

4.2. Use of lacticin 3147 in combination with other bacteriocins

From a hurdle concept, a cheap method of introducing bacteriocins to foods could be the use of cultures that produce multiple bacteriocins. Although coproduction of some bacteriocins, such as pediocin PA-1 and nisin, does not improve their antimicrobial activity, others, such as lacticin 3147 and 481, have a more inhibitory effect than either of the individual bacteriocins. O’Sullivan et al. (2003) demonstrated that it is relatively straightforward to construct food-grade lactococcal strains that coproduce the lantibiotics lacticin 3147 and lacticin 481 by conjugating the lacticin 3147 genetic determinants into a 481-producing recipient. As an alternative to inefficient transconjugant strains, resistant starter strains of *Lactobacillus* have been isolated to evaluate their potential use as adjuncts of bacteriocin producers. *Lactobacillus paracasei* DPC5337, incorporated in combination with bulk starters and lacticin 3147-producing starter culture, improved Cheddar cheese flavour up to commercial grade in comparison addition of the bacteriocin producer alone (Ryan et al., 2001).

4.3. Use of lacticin 3147 in combination with non-thermal treatments

The combination of two or more antimicrobial treatments at a suboptimal concentration is more effective than one at the optimal level. In the same way as nisin, adding lacticin 3147 following HP treatment at 150–275 MPa acted synergistically on the inactivation of *S. aureus* in reconstituted skim milk (Morgan, Ross, Beresford, & Hill, 2000); interestingly, lacticin 3147 activity remained stable and even increased after the HP treatment. Although lacticin 3147 has been mainly used in cheese production by incorporation of the starter producer, its characteristics open new possibilities for its application in minimally processed or refrigerated food products and for additional strategies for control of growth and survival of pathogens. Since lacticin 3147 should be broken down easily by digestive enzymes, it does not represent a threat to humans, making it a safe and economically viable alternative to chemical preservatives currently used in the food industry (Guinane, Cotter, Hill, & Ross, 2005). Furthermore, industrial exploitation of this bacteriocin is expected to be given legislative approval in different countries.

5. Enterocins

Several bacteriocin-producing strains of enterococci have been isolated from sausage, fish, vegetables and dairy
products, specifically cheese, where they naturally occur. Enterococcal bacteriocins have been characterized as substances with strong activity towards \textit{L. monocytogenes}, although many of these bacteriocin-like compounds have not yet been identified. Enterocin CCM 4231, produced by \textit{Enterococcus faecium} CCM 4231, has shown great inhibitory effects against \textit{S. aureus} in skim milk and yoghurt and \textit{L. monocytogenes} in yoghurt, indicating possibilities for further application in dairy products (Lauková, Čziková, Dobransky, & Burdova, 1999). Alvarado, Garcia-Almendarez, Martin, and Regalado (2005) attributed the antilisterial activity of \textit{Ent. faecium} UQ31, a strain isolated from hand-made Mexican-style cheese, to one bacteriocin-like inhibitory substance. That compound has not yet been completely characterized, although \textit{Ent. faecium} UQ31 has been suggested as a feasible culture for the preservation of dairy products. In an extensive study, the bacteriocin of \textit{Ent. faecium} 7C5, added as an adjunct with a thermophilic culture in soft cheese, led to complete death of \textit{L. monocytogenes} and \textit{L. innocua} without altering the acidifying activity of the starter culture (Giraffa, Carminati, & Tarelli, 1995). Recently, a bacteriocinogenic \textit{Ent. faecium} F58 strain was isolated from Jben goats' milk cheese and, when added as adjunct culture, caused a sharp decrease in the number of viable \textit{L. monocytogenes}, which were undetectable after 1 week of cheese storage at 22°C (Achemchem, Abrini, Martinez-Bueno, Valdivia, & Maqueda, 2006). The strains \textit{Ent. faecium} M241 and 249 obtained from raw goat milk produced bacteriocins especially active towards \textit{L. monocytogenes} and \textit{C. butyricum}, while other species of LAB were not affected (Cocolin, Foschino, Comi, & Fortina, 2007). The production of enterocin AS-48 by \textit{Ent. faecalis} A-48-32 as an adjunct in milk or fermented cheese was also found to be persistent and effective against \textit{B. cereus} without modifying the growth of starter cultures (Munoz et al., 2004). Incubation of \textit{L. monocytogenes} in raw milk in the presence of \textit{Ent. faecalis} INIA 4, which releases enterocin 4, also revealed low counts of the pathogen after 24 h (Rodriguez, Gayà, Medina, & Nunez, 1997).

### 6. Other bacteriocins

Little research has been focused on the use of other bacteriocins in dairy products; even so, their technological properties, their bactericidal or bacteriostatic effect, and their implication on some sensory mechanisms suggest both potential usefulness and promising advances in dairy product. Among them, pediocin \( \gamma \), a bacteriocin produced by \textit{P. acidilactici} UL5, sharply reduced viable counts of \textit{L. monocytogenes} in milk (Huang, Lacroix, Daba, & Simard, 1994). Reuterin, which is produced by \textit{Lb. reuteri}, inhibited growth of \textit{L. monocytogenes} and \textit{E. coli} in both cottage cheese and milk when added as a lyophilized powder (El-Ziney & Debevere, 1998); in contrast to nisin, efficacy of reuterin activity did not depend on fat content.

Most research on the application and the effectiveness of new bacteriocins is being directed towards the use of the bacteriocinogenic strains as protective cultures in different milk products. Hence, the activity of the bacteriocin-producing strain and its suitability for the fermented milk product should also be evaluated. Recently, a proteinaceous compound produced by a \textit{Lb. paracasei} subsp. \textit{paracasei} strain used as a starter for Bulgarian yellow cheese was shown to be active against some yeast species, such as \textit{Candida albicans} and \textit{Saccharomyces cerevisiae} (Atanassova et al., 2003). The non-identified antimicrobial peptide of the thermophilic starter \textit{Str. thermophilus} was active against \textit{L. monocytogenes} and \textit{S. aureus} during fermentation and refrigerated storage (Benkerroum, Oubel, & Mimoun, 2002). \textit{Str. macedonicus} ACA-DC 198, a strain isolated from Greek Kasseri cheese, produces an antilisterial bacteriocin called macedocin. This strain is of particular interest for the production of hard-cooked cheeses, due to its thermophilic character, but also for its contribution to aroma and flavor development (Van den Berge, Skourtas, Tsakalidou, & de Vuyst, 2006). Moreover, the persistence of macedocin on prolonged incubation suggested that the protective effect may be present during the maturation period of cheese (Van den Berge et al., 2006).

Alternatively, the use of bacteriocins to accelerate cheese maturation and to control flavour development could represent a novel approach to their potential usefulness. A \textit{Lc. lactis} subsp. \textit{lactis} strain encoding production of lactococcins A, B and M was used to satisfactorily enhance the sensory characteristics of Cheddar cheese (Morgan, Ross, & Hill, 1997). Proteolysis and flavour in semi-hard cheese was likewise increased by a bacteriocin-producing strain of \textit{Ent. faecalis} INIA 4 (Martinez-Cuesta et al., 2000). Lacticin 481, a bacteriocin produced by \textit{Lc. lactis} subsp. \textit{lactis} strain DPC5552, was shown to cause membrane permeabilization of starter cultures in Cheddar cheese (O’Sullivan, Morgan, Ross, & Hill, 2002); lacticin-481-producing strains allowed the target strain to continue to grow, with a simultaneous release of intracellular enzymes involved in the development of cheese flavour. Therefore, strain DPC5552 may provide improved adjuncts for delivering intracellular lactococcal enzymes into the cheese matrix and thus improve cheese quality and flavour. In a later study, the lacticin-481-producing strain \textit{Lc. lactis} CNRZ481, used as an adjunct for Cheddar cheese manufacture, achieved improved flavour and a reduction in defects in the final product owing to the prevention of NSLAB proliferation (O’Sullivan et al., 2003).

Unfortunately, many bacteriocin producers are poorly adapted to the milk environment, such as probiotic lactobacilli in yoghurt, which may hinder their use as starter cultures. Avons, Van Uytten, and De Vuyst (2004) found that some probiotic lactobacilli of intestinal origin showed only slight growth in milk. \textit{Lb. casei} strains showed the best growth in milk, whereas best growth and bacteriocin production was observed with \textit{Lb. acidophilus}.
when milk was supplemented with yeast extract. The addition of a growth factor, cocultivation or fermentation in another medium and subsequent addition to the milk were suggested in order to overcome the problem.

Recently, a number of studies have pointed out the antimicrobial activity of new substances from bacteria, especially potential bacteriocins that still remain to be defined, classified or characterized, although their incidence in the dairy industry may be also promising. Rodriguez et al. (2000) studied the natural flora of milk and found non-identified bacteriocins to be produced by 16 LAB strains isolated from raw milk. Those bacteriocins were, in general, heat resistant, and in some cases showed a broad inhibitory spectrum, especially those from LaC lactis subsp. lactis biovar diacetylactis or some strains of Ent. faecalis. Additionally, an antimicrobial substance from B. subtilis has exhibited a broad inhibitory spectrum against Gram-positive, Gram-negative bacteria and moulds (Bie, Lu, & Lu, 2006). This antimicrobial compound enhanced milk preservation and the sensory acceptance of pasteurized milk. B. cereus isolated from raw milk and other dairy products, ice cream or milk powder, has been found to be a producer of bacteriocins, which were stable at pH 3–10 and after heating at 75°C for 2 min (Torkar & Matijasic, 2003).

7. Conclusions

Studies of the antimicrobial activity and behaviour of LAB bacteriocins in the milk environment suggest that they have the potential to ensure microbiological safety and to control quality of dairy products. Their practical use in dairy products may lie both in their incorporation into the finished product, such as adhered bacteriocins on active packaging, and in their addition or inclusion during food manufacture. In the latter case, great effort is being directed towards selecting bacteriocin producers as potential adjuncts in fermentation processes or even as starter cultures. Moreover, bacteriocins have been shown to improve synergistically the inhibitory effects of thermal and non-thermal treatments, such as HIPEF and HP. In this way, the combination of bacteriocins with heat may facilitate the application of mild thermal treatments, which may diminish the typical cooked flavour of milk and reduce the cost of the heating operation. In particular, the enhancement of the lethal effect of HIPEF and HP, when combined with bacteriocin addition, may provide a suitable alternative for traditional thermal treatments, since it causes minimal alteration to sensory properties of the product. However, a few drawbacks may curd the use and practical application of bacteriocins. Firstly, the use of bacteriocin producers in fermented products demands sensory evaluation of such products, while the commercialization of bacteriocins as food preservatives needs to be regulated by strict requirements of food legislation, with nisin being the only bacteriocin exploited as a food additive to date. Secondly, results in the laboratory have to be scaled up to a food industry process to evaluate the effectiveness of bacteriocins during processing and storage. The use of bacteriocins is thus still limited in dairy products, although their potential applications suggest they may be industrially exploited in the medium term.

References


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